

**THE USE OF DOPANTS IN ATMOSPHERIC PRESSURE IONIZATION SOURCES  
OF MASS SPECTROMETERS**

by

Faezeh Dousty

M.Sc, The University of Tehran, 2009

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

in

THE COLLEGE OF GRADUATE STUDIES

(Chemistry)

THE UNIVERSITY OF BRITISH COLUMBIA

(Okanagan)

May 2015

© Faezeh Dousty, 2015

## Abstract

Dopants, as “ionization assisting” chemicals, have been used in different ionization techniques. However, they have been used most frequently in the field of Atmospheric Pressure Photoionization (APPI). These chemicals that have high photoabsorption and photoionization cross sections easily get photoionized and “preserve” to a certain extent the energy of the photon flux, which otherwise is significantly lost due to the absorption by matrix components. Subsequently, these photo-dopant ions ionize analyte molecules through ion-molecule chemical reactions.

Obtaining a profound understanding of ion formation mechanisms is a crucial step in successful applications of mass spectrometry. The first part of this thesis focuses on comprehensively describing ionization mechanisms and specifically the role of dopants. With the understanding obtained from in depth research on ion formation mechanisms, two novel dopants for APPI are proposed, i.e., carbon disulfide and isoprene. The potential of these chemicals as dopants, alongside their underlying ionization mechanisms, were investigated with a commercial APPI source coupled to a liquid chromatograph and a custom-built APPI source coupled to a gas chromatograph. Carbon disulfide was proven to be an effective charge transfer reagent for the positive ion APPI (PI-APPI) and promoted the ionization of non-polar compounds, for which the proton transfer route was not possible. The advantage of carbon disulfide over commonly used dopants is its high ionization energy (10 eV), which enables the ionization of analytes with high IEs through a charge transfer route, when other commonly used dopants with less IEs suppressed their ionization. Isoprene, which is considered a green chemical, gave effective results in the negative ion APPI (NI-APPI). In contrast to LC, in which mobile phases can interfere with the ionization of analytes and complicate the ionization matrix, GC provides a very simple matrix for photoionization. Thus, underlying ion formation mechanisms can be more reliably studied and interpreted. The investigation of ionization mechanisms in both LC- and GC-based/MS analyses in parallel contributed to better formulating the role of all chemicals present in the source. Last but not least, the role of dopants in enhancing ionization responses in Atmospheric Pressure Laser Ionization (APLI) for GC/MS applications was investigated. In APLI, instead of a one-step VUV ionization event used in APPI, a two-step UV ionization event is

employed. APLI has only been recently introduced; thus, the utilization of dopants in this technique has not been explored as extensively as for APPI. The potential of carbon disulfide and isoprene as dopants were also examined for this technique. The short lifetime of the excited transition states of carbon disulfide suppressed its laser ionization. Therefore, laser ionization of carbon disulfide did not increase the total ion production in order to assist the ionization in the PI mode. Positive electron affinity of carbon disulfide disqualifies its applications for negative ionization. Therefore, dopant-assisted studies in PI-APLI were limited to using toluene as dopant, which produced an abundant intensity of toluene radical cations. Laser ionization of isoprene produced a range of radical cations. Thus, the elevated chemical noise and the presence of many ions at the low  $m/z$  range of the isoprene spectrum can interfere with ion products of small molecules, which render its application as a dopant for PI-APLI problematic, similar as for PI-APPI. In NI APLI, toluene offered more effective results than isoprene. Therefore, toluene was chosen to investigate the role of dopants in enhancing the ionization responses and to study the corresponding ionization mechanisms in PI/NI APLI.

## Preface

The author has performed all experiments and data analysis in this thesis.

The research project presented in Chapter 2 and 3 have been previously published as follows:

- (i) Dousty, F., O'Brien, R., Gahler, R., Kersten, H., & Benter, T. Carbon disulfide as a dopant in photon-induced chemical ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* 2013, 27, 1969.
- (ii) Dousty, F., O'Brien, R. The use of isoprene as a novel dopant in negative ion atmospheric pressure photoionization mass spectrometry coupled to high-performance liquid chromatography. *Rapid Commun. Mass Spectrom.* 2015, 29, 1–8.

In addition, some of the findings of the research presented in Chapters 2 and 3 contributed to a multi-university, international collaboration publication as follows:

- (iii) S. Klee, V. Derpmann, W. Wißdorf, S. Klopotoski, H. Kersten, K. J. Brockmann, T. Benter, S. Albrecht, A. P. Bruins, F. Dousty, T. J. Kauppila, R. Kostianen, R. O'Brien, D. B. Robb, et al. Are clusters important in understanding the mechanisms in atmospheric pressure ionization? Part 1: Reagent ion generation and chemical control of ion populations. *J. Am. Soc. Mass Spectrom.* 2014, 25, 1310.

The following is a list of poster presentations related to the work presented in this thesis:

- (1) Kersten, H., Haberer, K., Kroll, K., **Dousty F.**, Benter, T; "Progress in the development of a GC-APPI source with femtogram sensitivity". Presented at 62nd ASMS Conference on MASS Spectrometry and Allied Topics, Baltimore, MD, June 4-9, 2014

(2) **Dousty F.**; O'Brien R.; "The Use of Isoprene as a New Dopant in Negative Ion Atmospheric Pressure Photo Ionization (NI-APPI) Mass Spectrometry" Presented at 61st ASMS Conference on Mass Spectrometry and Allied Topics, Minneapolis Convention Center, Minneapolis, MN, June 11, 2013.

(3) **Dousty F.**; O'Brien R.; Benter T, Kersten H. "The Use of Carbon Disulfide as a New Dopant in Atmospheric Pressure Photo Ionization Mass Spectrometry" Presented at 60th ASMS Conference on Mass Spectrometry and Allied Topics, Vancouver Convention Centre, Vancouver, Canada, June 23rd, 2012, WP 746.

(4) **Dousty F.**; O'Brien R.; Benter T, Kersten H. "An atmospheric pressure photo-ionization source based on a window-less atmospheric pressure spark discharge" Presented at Vancouver Post- ASMS Mass Spectrometry Symposium, Vancouver, BC, Canada, September 29th, 2011.

(5) **Dousty F.**; O'Brien R.; Benter T, Kersten H. "An atmospheric pressure photo-ionization source based on a window-less atmospheric pressure spark discharge" Presented at 59th ASMS Conference on Mass Spectrometry and Allied Topics, Colorado Convention Center, Denver, CO, June 08, 2011.

# Table of Contents

<b>Abstract.....</b>	<b>ii</b>
<b>Preface.....</b>	<b>iv</b>
<b>Table of Contents .....</b>	<b>vi</b>
<b>List of Tables .....</b>	<b>xi</b>
<b>List of Figures.....</b>	<b>xiii</b>
<b>List of Abbreviations .....</b>	<b>xix</b>
<b>Acknowledgements .....</b>	<b>xxi</b>
<b>Dedication .....</b>	<b>xxiii</b>
<b>1 Chapter: Introduction .....</b>	<b>1</b>
1.1 Atmospheric Pressure Photoionization (APPI) and Dopant Assisted-Atmospheric Pressure Photoionization (DA-APPI).....	11
1.2 Dopant-free APPI (Direct photoionization) Applications.....	12
1.3 Methods of dopants introduction into the APPI source .....	13
1.4 Advantages of APPI over ESI and APCI.....	14
1.5 Ionization mechanisms in APPI and DA-APPI .....	17
1.6 Chemicals utilized as dopants in dopant assisted positive ion- atmospheric pressure ionization sources.....	18
1.6.1 Benzene .....	20
1.6.1.1 The ranges of application for benzene as a dopant .....	20
1.6.1.2 Ionization mechanisms of analytes with benzene-Assisted APPI.....	20
1.6.1.3 Advantages of utilizing benzene as a dopant .....	21
1.6.1.4 Limitations of utilizing benzene as a dopant.....	21
1.6.2 Acetone .....	22
1.6.2.1 The range of applications for acetone as a dopant .....	22
1.6.2.2 Ionization mechanisms of analytes with acetone-Assisted APPI.....	23
1.6.2.3 Advantages of utilizing acetone as a dopant .....	23
1.6.2.4 Limitations of utilizing acetone as a dopant .....	24
1.6.3 Toluene .....	25

1.6.3.1	The ranges of application for toluene as a dopant.....	25
1.6.3.2	Ionization mechanisms of analytes with toluene-Assisted APPI.....	28
1.6.3.3	Advantages of utilizing toluene as a dopant .....	29
1.6.3.4	Limitations of utilizing toluene as a dopant.....	29
1.6.4	Anisole.....	30
1.6.4.1	The ranges of application for anisole as a dopant .....	30
1.6.4.2	Ionization mechanisms of analytes with anisole-Assisted APPI .....	30
1.6.4.3	Advantages of utilizing anisole as a dopant .....	31
1.6.4.4	Limitations of utilizing anisole as a dopant .....	31
1.6.5	Tetrahydrofuran .....	32
1.6.5.1	The ranges of application for tetrahydrofuran as a dopant .....	32
1.6.5.2	Ionization mechanisms of analytes with Tetrahydrofuran-Assisted APPI.....	33
1.6.5.3	Advantages of utilizing Tetrahydrofuran as a dopant .....	33
1.6.5.4	Limitations of utilizing Tetrahydrofuran as a dopant .....	33
1.6.6	Substituted benzene compounds: Chloro- and bromobenzene .....	34
1.6.6.1	The ranges of application for chloro- and bromobenzene as dopants.....	34
1.6.6.2	Ionization mechanisms of analytes with chloro/ bromobenzene-Assisted APPI...	35
1.6.6.3	Advantages of utilizing chloro/ bromobenzene as dopants.....	35
1.6.6.4	Limitations of utilizing chloro/ bromobenzene as dopants .....	35
1.6.7	Utilization of mixture of dopants to enhance the ionization efficiencies .....	36
1.7	Negative Ion- Atmospheric Pressure Photo Ionization (NI-APPI) and Dopant Assisted Negative Ion- Atmospheric Pressure Photo Ionization (DA-NI-APPI) .....	37
1.7.1	NI-APPI versus PI-APPI .....	39
1.7.2	Chemicals utilized as dopants in dopant assisted negative ion- atmospheric pressure ionization sources .....	40
1.8	APPI and DA-APPI used in GC-based MS analysis versus LC-based MS analysis .....	41
1.9	Atmospheric Pressure Laser Ionization (APLI).....	42
1.9.1	APLI applications .....	45
1.9.2	Use of dopants in APLI .....	47
1.10	Theoretical and practical contribution of thesis to the field of photoionization .....	48
<b>2</b>	<b>Chapter: Carbon Disulfide as a Dopant in Atmospheric Pressure Photoionization Mass Spectrometry Coupled to a HPLC .....</b>	<b>50</b>
2.1	Abstract .....	50

2.1	Experimental .....	50
2.1.1	Chemicals and Reagents .....	50
2.1.2	Sample preparation .....	54
2.1.3	Instrumentation .....	54
2.2	Results and discussion .....	55
2.2.1	Suitability of CS <sub>2</sub> as dopant .....	55
2.2.2	Reagent chemistry generated by CS <sub>2</sub> .....	63
2.3	Conclusion .....	65
<b>3</b>	<b>Chapter: The Use of Isoprene as a Novel Dopant in Negative Ion Atmospheric Pressure Photo Ionization (NI-APPI) Mass Spectrometry Coupled to HPLC .....</b>	<b>66</b>
3.1	Abstract .....	66
3.2	Experimental .....	67
3.2.1	Materials and Methods .....	67
3.2.2	Instrumentation .....	70
3.3	Results and discussion .....	70
3.3.1	Reagent ions produced by isoprene in the NI-APPI with ACN as eluent .....	73
3.3.2	Capability of isoprene as a dopant for NI-APPI .....	76
3.4	Conclusion .....	81
<b>4</b>	<b>Chapter: Performance of carbon disulfide as a charge transfer dopant in GC-PI-APPI-MS .....</b>	<b>82</b>
4.1	Abstract .....	83
4.2	Experimental .....	84
4.2.1	Chemicals and sample preparations .....	84
4.2.2	Instrumentation .....	84
4.2.2.1	Gas chromatograph .....	84
4.2.2.2	Ionization source .....	86
4.2.2.3	Mass spectrometer .....	89
4.3	Results and discussion .....	89
4.3.1	GC Direct APPI-MS .....	89
4.3.2	GC Dopant Assisted APPI MS .....	91
4.3.2.1	Reactant ion profiles with toluene and carbon disulfide as dopants. ....	91
4.3.3	Comparison of direct APPI, toluene, and carbon disulfide assisted APPI .....	93
4.3.3.1	Linear dynamic range and detection limits. ....	93

4.3.3.2	Range of ionizable compounds by each method.....	98
4.4	Conclusion .....	105
<b>5</b>	<b>Chapter: Performance of Isoprene as a dopant in GC-NI-APPI-MS.....</b>	<b>107</b>
5.1	Abstract .....	107
5.2	Experimental .....	108
5.2.1	Chemicals and sample preparations.....	108
5.2.2	Instrumentation .....	108
5.2.2.1	Gas chromatograph .....	108
5.2.2.2	Ionization source .....	110
5.2.2.3	Mass spectrometer .....	110
5.3	Results and discussion .....	111
5.3.1	GC Direct NI-APPI-MS .....	115
5.3.2	GC Dopant Assisted NI-APPI MS .....	118
5.3.2.1	Reactant ion profiles for toluene and isoprene as dopants.....	118
5.3.2.2	Comparison of direct APPI, toluene, and carbon disulfide assisted APPI.....	120
5.3.2.2.1	Linear dynamic range and detection limits.....	120
5.3.2.2.2	Range of ionizable compounds obtained by each method.....	125
5.3.2.2.3	Comparison of sensitivity and selectivity of Dopant Assisted APPI in NI and PI approaches.....	127
5.4	Conclusion .....	130
<b>6</b>	<b>Chapter: Evaluation of direct and dopant-assisted APLI in GC-MS applications</b>	<b>132</b>
6.1	Abstract .....	132
6.2	Experimental .....	133
6.2.1	Chemicals and sample preparations.....	133
6.2.2	Instrumentation .....	133
6.2.2.1	Gas chromatograph .....	133
6.2.2.2	Ionization source .....	135
6.2.2.3	Mass spectrometer .....	137
6.3	Results and discussion .....	137
6.3.1	Dopant-free APLI .....	139
6.3.2	Dopant-assisted APLI.....	145
6.3.3	Comparison of direct APLI and toluene-assisted APLI .....	153
6.3.3.1	Linear dynamic range and detection limits.....	153

6.3.3.2	Range of ionizable compounds obtained by each method. ....	158
6.4	Conclusion .....	160
<b>7</b>	<b>Chapter: Conclusion.....</b>	<b>161</b>
7.1	Overall conclusions and contributions of the dissertation .....	161
7.2	Future research directions .....	164
	<b>Bibliography .....</b>	<b>167</b>
	<b>Appendices.....</b>	<b>203</b>
	Appendix A : List of compounds in the EPA mixture with their available gas-phase ion energetic data from NIST Chemistry WebBook <sup>154</sup> .....	203
	Appendix B Optimization of GC column position in the ionization source in order to obtain the maximum ionization. ....	209
	Appendix C Optimization of the make-up gas flow rate .....	212
	Appendix D The preliminary results obtained using synthetic air instead of nitrogen as the make-up gas in negative ionization to examine the possible dopant function of oxygen in promoting negative ionization. ....	213

## List of Tables

Table 1.1	Ionization pathways in positive mode APPI and DA-APPI .....	18
Table 1.2	Molecular properties and relative gas-phase ion energetics data of dopants.....	19
Table 1.3	The main ionization routes in NI-APPI and DA-NI-APPI.....	38
Table 2.1	Ion energetic properties of the solvents and compounds studied .....	52
Table 3.1	Ion energetics of the studied solvent, dopants, and compounds. ....	69
Table 3.2	The enhancement factor of analytical responses obtained with using toluene and isoprene compared to dopant free NI-APPI. In addition, repeatability of the analysis in terms of percent RSDs of the peak areas is shown. ....	80
Table 5.1	Compounds detected in NI-Direct APPI with no dopant .....	117
Table 5.2	The R <sup>2</sup> values of linearity, LODs, and %RSDs obtained with Direct NI-APPI, Toluene Assisted NI-APPI, and Isoprene-Assisted NI-APPI. ....	124
Table 5.3	Compounds detected only with dopant assisted NI-APPI and not with Direct NI-APPI.....	126
Table 6.1	Analytes ionized by dopant free APLI. ....	140
Table 6.2	The list of labeled peaks shown in TICs of dopant-free PI APLI and toluene-assisted PI APLI.....	151
Table 6.3	The list of labeled peaks shown in TICs of dopant-free NI APLI and toluene-assisted NI APLI. ....	152
Table 6.4	The R <sup>2</sup> values of linearity, LODs, and %RSDs obtained with Direct PI-APLI and toluene assisted PI-APPI.....	156
Table 6.5	The R <sup>2</sup> values of linearity, LODs, and %RSDs obtained with direct NI APLI and toluene-assisted NI APLI. ....	158
Table A.1	List of PAHs in the EPA mixture	203
Table A.2	List of oxygen-containing compounds in the EPA mixture.....	204
Table A.3	List of nitro-compounds in the EPA mixture.....	205
Table A.4	List of halogenated compounds in the EPA mixture .....	206
Table A.5	List of nitrogen-containing compounds in the EPA mixture .....	207
Table A.6	List of esters in the EPA mixture.....	207

Table A.7 List of ethoxy esters in the EPA mixture ..... 208

## List of Figures

Figure 1.1	Two commercial designs of APPI; PhotoSpray (a), PhotoMate (b) .....	14
Figure 1.2	The two-step APLI ionization mechanism versus the one-step ionization mechanism in APPI.....	44
Figure 2.1	Structures of the studied compounds.....	53
Figure 2.2	Absolute abundance of the total ion currents and relative proportions of $M^{++}$ and $[M+H]^+$ for benzamide (a), melamine (b), caffeine (c), and vanillin (d) in CAN in the positive mode. Experiments were done in five replicates. Repeatability was assessed by calculating percent RSDs between areas of all five replicates, which was less than 5% for analytes.....	56
Figure 2.3	Absolute abundance of the total ion currents and relative proportions of $M^{++}$ and $[M+H]^+$ for 2-naphthol (a), phenanthrene (b), 9-methylanthracene (c), and BHA (d) in ACN in the positive mode. Experiments were done in five replicates. Repeatability was assessed by calculating percent RSDs between areas of all five replicates, which was less than 7% for analytes. ....	58
Figure 2.4	Absolute abundance of the total ion currents and relative proportions of $M^{++}$ and $[M+H]^+$ for biphenyl (a), diphenylmethane (b), 1,4-dibromobenzene (c), 1,2,4,5-tetrachlorobenzene (d), 3-methoxybenzoic acid (e), and 2,4,6-trichloroanisole (f) in ACN in the positive mode. Experiments were done in five replicates. Repeatability was assessed by calculating percent RSDs between areas of all five replicates, which was less than 10% for analytes. ....	60
Figure 2.5	Mass Spectra of ACN with carbon disulfide as dopant.....	64
Figure 3.1	Structures of the studied compound .....	68
Figure 3.2	A preliminary investigation of the ability of monoterpenes (isoprene, limonene, $\alpha$ -pinene, and p-cymene) to enhance the ionization efficiency in the NI-APPI by comparing the results obtained for seven analytes with those obtained by APPI with no dopant and toluene assisted NI-APPI. The analytical responses are in terms of the total ion currents of the dominant negative ion for each analyte. List of abbreviations for analytes can be found in Table 3.1. ....	72
Figure 3.3	Tentatively identified reagent ions formed from isoprene (flow rate =20 $\mu$ l/min)	

in acetonitrile (flow rate = 200  $\mu\text{l}/\text{min}$ ) in the NI-APPI. tBoBQ, 4-tBC, and NBBZS stand for tert-Butyl-o-benzoquinone, 4-tert-butylcatechol, and n-butyl benzene sulfonamide, respectively. The ion at  $m/z$  212 is a background ion existing in the spectra of acetonitrile and toluene as well and is likely the deprotonated molecular ion of n-butyl benzene sulfonamide (NBBZS), which could enter the source as a water contaminant.<sup>344,345</sup> The corresponding protonated molecular radical cation of this compound was seen in the positive ion mode..... 75

Figure 3.4 Comparison of the absolute abundances of the total ion currents of  $M^+$  and substitution products in APPI without a dopant and with toluene and isoprene as dopants. These compounds all possess positive electron affinities; therefore, they were ionized by electron capture, charge transfer, and substitution reactions. List of abbreviations for analytes can be found in Table 3.1. .... 77

Figure 3.5 Comparison of the absolute abundances of the total ion currents of  $[M-H]^-$  in APPI without a dopant and with toluene and isoprene as dopants. These compounds all possess high gas-phase acidities; therefore, they were mainly ionized by proton transfer, forming deprotonated analyte molecular ions. List of abbreviations for analytes can be found in Table 3.1. .... 78

Figure 4.1 Ramp chart of the GC oven temperature program. Total Ion Chromatograph (TIC) was obtained by 1  $\mu\text{L}$  injection of 100  $\text{pg}/\mu\text{L}$  of EPA mix. The TIC was accordingly scaled to be shown in parallel to the oven program. .... 85

Figure 4.2 (a) Schematic of API interface showing GC and make up gas entries, and the exit hole to the MS. (b) the entire GC-API interface and the custom-made lamp holder that positions the lamp on the  $\text{MgF}_2$  window of the interface. Because of the matched size of lamp and cone bases, the entire source volume is irradiated by photons. The small size of the cone assures the closest distance of photons and analyte stream..... 88

Figure 4.3 The retention time windows of different classes of compounds during a GC run. .... 90

Figure 4.4 The reactant ion profiles obtained by using toluene (a) and carbon disulfide (b) as dopants. The spectra were obtained by flow injection of dopants' vapor into the

	ionization source at a flow rate of 300 $\mu\text{L}/\text{min}$ . Prior to the ionization source the dopant vapour was mixed with the make-up gas ( $\text{N}_2$ at a flow rate of 700 $\text{mL}/\text{min}$ ) in a tee junction.....	92
Figure 4.5	Calibration curves for (a) naphthalene, (b) carbazole, (c) fluorene, (d) 4-bromophenyl phenyl ether, (e) indeno(1,2,3-cd)pyrene, (f) benzo(g,h,i)perylene, (g) Phenanthrene, and (h) anthracene. Analytical response was obtained from absolute abundance of peak area for single ion chromatography (SIM) of molecular radical cation of each analyte.....	95
Figure 4.6	Comparison of LODs obtained by direct APPI, Toluene and Carbon disulfide Assisted APPI for eight compounds. Spectral LODs were calculated for the molecular radical cation of analytes based on S/N of 3.....	97
Figure 4.7	Comparison of absolute abundances of SIM of molecular radical cations for compounds with high IE. In toluene assisted APPI, ionization of compounds having higher IE than that of the toluene were completely suppressed or decreased drastically. ....	101
Figure 4.8	Comparison of range of ionizable compounds with direct APPI, toluene, $\text{CS}_2$ , anisole assisted APPI. As seen, toluene and anisole with the IEs of 8.8 and 8.2 eV, reduces the range of ionizable compounds, since their radical cations do not have sufficient energies to ionize compounds with high IE. On the contrary, $\text{CS}_2$ with IE of 10 eV, provides with the broadest range of ionizable compounds...	102
Figure 4.9	The sensitivity of the three ionization approaches to different classes of compounds. The analytical responses for all compounds were normalized against the maximum response in each method and then for each class of compounds the average of responses was calculated for comparison. This shows a comparative general degree of ionization obtained with each method for each class of compounds. ....	104
Figure 5.1	Ramp chart of the oven temperature program of GC. Total Ion Chromatograph (TIC) was obtained by 1 $\mu\text{L}$ injection of 500 $\text{pg}/\mu\text{L}$ of the EPA mix. The TIC was accordingly scaled to be shown in parallel to the oven program. ....	109
Figure 5.2	The reactant ion profiles obtained by using toluene (a) and isoprene (b) as dopants in the NI APPI mode. The insets display the reactant ion profile of each	

	dopant in the positive mode. The letter D stands for dopant. ....	119
Figure 5.3	Calibration curves for eight compounds in the negative mode with direct APPI-Toluene Assisted APPI and Isoprene Assisted APPI. For each analyte, the absolute abundance of the peak area of single ion chromatography (SIM) for its dominant anion, as shown in each graph, has been considered. ....	123
Figure 5.4	A comparison of the range of ionizable compounds with direct APPI, toluene Assisted APPI, and isoprene assisted APPI. The comparison was made with the results obtained by injecting 1 $\mu\text{L}$ of the EPA mixture with a concentration of 100 $\text{pg}/\mu\text{L}$ into the GC. ....	127
Figure 5.5	The general selectivity of $\text{CS}_2$ Assisted PI APPI and Isoprene Assisted NI APPI obtained with the EPA mixture (which can be regarded as a relatively comprehensive selection of various analytes). The comparison was made when 1 $\mu\text{L}$ of the EPA mixture at a concentration of 100 $\text{pg}/\mu\text{L}$ was injected into the GC. ....	129
Figure 6.1	Ramp chart of the GC oven temperature program. Total Ion Chromatograph (TIC) was obtained by 1 $\mu\text{L}$ injection of 50 $\text{pg}/\mu\text{L}$ of EPA mix, which was then ionized by direct PI APLI. The TIC was accordingly scaled to be shown in parallel to the oven program. ....	134
Figure 6.2	(a) The APLI probe, which directs laser radiation to the $\text{MgF}_2$ window of the API interface. (b) The entire GC APLI MS system. ....	136
Figure 6.3	A comparison of the range of ionizable compounds by dopant-free (direct) APLI and APPI in both PI and NI modes obtained by injection of 1 $\mu\text{L}$ of the mixture at a concentration of 100 $\text{pg}/\mu\text{L}$ . APLI in positive mode offers exceptionally high selectivity toward polycyclic aromatic compounds, while PI APPI provides a universal selectivity, ionizing all compounds that were able to make stable positive ions. The <i>ratio</i> of ionized compounds to non-ionized compounds in NI APPI and NI APLI are comparable; however, the <i>types</i> of ionized compounds are not similar. ....	143
Figure 6.4	The reagent ion profiles of isoprene (a) and toluene (b) as dopants for PI-APLI. The spectra were obtained by flow injection of dopant' headspace into the ionization source at a flow rate of 300 $\mu\text{L}/\text{min}$ , which was mixed with the make-	

	up gas (N <sub>2</sub> ) at a flow rate of 700 mL/min. ....	147
Figure 6.5	Total Ion Chromatographs (TICs) that were obtained by (a) dopant-free APLI (red) and (b) toluene-assisted APLI (blue) in PI mode. TICs were obtained by 1 μL injection of 100 pg/μL of EPA mix. The labeled peaks were listed in Table 6.2.....	149
Figure 6.6	Total Ion Chromatographs (TICs) that were obtained by (a) dopant-free APLI (red) and (b) toluene-assisted APLI (blue) in NI mode. TICs were obtained by 1 μL injection of 100 pg/μL of EPA mix. The labeled peaks were listed in Table 6.3.....	150
Figure 6.7	Calibration curves for eight compounds in PI mode with direct APLI and toluene-assisted APLI. For each analyte, the absolute abundance of the peak area of single ion chromatography (SIM) for the molecular radical cation has been considered. ....	155
Figure 6.8	Calibration curves for four compounds in NI mode with direct APLI and toluene-assisted NI APLI. For each analyte, the absolute abundance of the peak area of single ion chromatography (SIM) of the dominant anion, as shown in each graph, has been considered.....	157
Figure 6.9	The range of ionizable compounds obtained by direct APLI and toluene-assisted APLI in both PI and NI modes. ....	159
Figure B.1	Four tested positions for GC column inside the ionization source. Positions 1, 2, 3, and 4 refer to the positions right at the entrance, 1 mm before the tip of the cone, right above the cone tip, and 1 mm after the tip of the cone, respectively.....	209
Figure B.2	The results obtained for four compounds in the EPA mixture with each ionization position, when 1 μL of EPA mixture at a concentration of 100 pg/μL was injected and ionized with direct PI APPI. The results show that the optimized position is position number 2 (1 mm before the cone tip), which gives the best sensitivity towards vortex flow and radiation.....	210
Figure B.3	The optimized GC column position inside the ionization source, which was used in all experiments with GC-APPI/APLI MS. ....	211
Figure C.1	The results obtained for three compounds in the EPA mixture with each N <sub>2</sub> flow	

rate, when 1  $\mu\text{L}$  of EPA mixture at a concentration of 100  $\text{pg}/\mu\text{L}$  was injected and ionized with direct PI APPI. The results show that the optimum flowrate is 700  $\text{ml}/\text{min}$ , which gives the best vortex flow and in turn, best ionization efficiencies. Therefore, the flowrate of 700  $\text{ml}/\text{min}$  was kept for all the experiments with GC-APPI /APPI MS..... 212

- Figure D.1 TICs obtained with NI APPI using  $\text{N}_2$  (a) and synthetic air (b), when 1  $\mu\text{L}$  of EPA mixture at a concentration of 100  $\text{pg}/\mu\text{L}$  was injected. As seen in the figure, utilization of synthetic air increased the number of detected compounds, which confirmed the potential dopant function of oxygen for NI. .... 213
- Figure D.2 A comparison of calibration curves obtained with NI-APPI using  $\text{N}_2$  and synthetic air as the make-up gas. As seen, oxygen offers a dopant function in the lower concentrations. .... 214
- Figure D.3 A comparison of ionization responses obtained for seven compounds, when 1  $\mu\text{L}$  of the EPA mixture at a concentration of 25  $\text{pg}/\mu\text{L}$  was injected and ionized with NI APPI. As seen, oxygen offers a dopant function and enhanced the ionization responses of almost all studied compounds. .... 215
- Figure D.4 A comparison of ionization responses obtained for seven compounds, when 1  $\mu\text{L}$  of the EPA mixture at a concentration of 200  $\text{pg}/\mu\text{L}$  was injected and ionized with NI APPI. As seen, the dopant function of oxygen becomes less efficient in this higher concentration. .... 216
- Figure D.5 The background spectra obtained with  $\text{N}_2$  (a) and synthetic air (b) as make-up gas with NI-APPI. In the background spectra, carbonate anion ( $\text{CO}_3^{2-}$ ,  $m/z$  60) was observed with a considerable intensity, which originates from  $\text{CO}_2$  impurity in the synthetic air bottle.  $\text{CO}_2$  acts as a sink for thermal electrons and reduces the accessible electrons for analytes. Therefore, removing  $\text{CO}_2$  from the synthetic air can further increase the efficiency of the negative ionization. Despite successful preliminary attempts to remove  $\text{CO}_2$ , due to time restrictions, I could not finish these series of experiments. This path can be further explored and could lead to much more effective ionization in the negative mode. .... 217

## List of Abbreviations

AcA-APPI	Acetone- Assisted APPI
AnA-APPI	Anisole -Assisted APPI
APCI	Atmospheric Pressure Chemical Ionization
APLI	Atmospheric Pressure Laser Ionization
API	Atmospheric Pressure Ionization
APPI	Atmospheric Pressure Photoionization
BHA	Butylated Hydroxy Anisole
BFRs	Brominated Flame Retardants
CE	Capillary Electrophoresis
CID	Collision Induced Dissociation
DA-	Dopant-Assisted
DAPCI	Desorption Atmospheric Pressure Chemical Ionization
DESI	Desorption Electrospray Ionization
DOM	Dissolved Organic Matter
EA	ElectronA
ECD	Electron Capture Detector
ESI	Electrospray
ETD	Electron Transfer Dissociation
FID	Flame Ionization Detector
FT-ICR	Fourier Transform Ion Cyclotron Resonance
GC	Gas Chromatography
HFRs	halogenated Flame Retardants
HAFID	Hydrogen Atmosphere Flame Ionization Detector
FAIMS	High- field Asymmetric Ion Mobility Spectrometry
IMS	Ion Mobility Spectrometry
IE	Ionization Energy
LC	Liquid Chromatography
LOD	Limit Of Detection
LOQ	Limit Of Quantification

MALDI	Matrix-Assisted Laser Desorption Ionization
MS	Mass Spectrometry
NI	Negative Ionization
PA	Proton Affinity
PAHs	Polycyclic Aromatic Hydrocarbons
PASHs	Polycyclic Aromatic Sulfur Heterocycles
PBDEs	Polybrominated Biphenyl Ethers
PCBs	Polychlorinated Biphenyls
PDECD	Pulsed-Discharge Electron-Capture Detector
PI	Positive Ionization
PID	Photoionization Detector
REMPI	Resonantly Enhanced Multiphoton Ionization
RSD	Relative Standard Deviation
SIM	Single Ion Chromatograph
TA-APPI	Toluene-Assisted APPI
THF	Tetrahydrofuran
TIC	Total Ion Chromatograph
UV	Ultraviolet
VUV	Vacuum Ultraviolet
$\mu$ APPI	microchip APPI
$\Delta_{\text{acid}} G$	Gas-phase Acidity

## **Acknowledgements**

I would like to acknowledge my previous supervisor, Dr. Rob O'Brien, for his continuous encouragement and mentorship throughout the course of my degree. Thank you for believing in me, initiating this journey, and supporting me fully throughout. .

I would like to thank my supervisor, Dr. Karen Perry, for her involvement with mentorship of my work. Your ongoing encouragement and guidance has helped me manage this endeavor.

I would like to thank my committee members, Dr. James Bailey and Dr. Stephen McNeil, for their commitment and time invested in my research. Your valuable input, insightful comments and dedicated instruction have made this work achievable.

I have deep gratitude for my co-supervisor from the University of Wuppertal in Germany, Prof. Thorsten Benter, who has been the biggest inspiration and a role model for me as a person and as a researcher. I feel extremely fortunate to have been a part of your research group. Thank you for your support and instruction. Thank you for making the research process an enjoyable experience for all. Thank you for showing me how to take the lead in my own life. You have taught me much and I will forever remember the lessons learned.

I would like to give a special thanks to Dr. Hendrik Kersten, for being a great inspiration, a patient mentor and most importantly a valuable friend. Thank you for inspiring me to be a better person and a better researcher. I am a true admirer of your personality.

I would like to sincerely thank the past and present members of Prof. Benter's research group in Germany for all the support and love that they have given me, during my six-month research visit at the University of Wuppertal in Germany.

I would like to express my deepest gratitude to the technicians, staff, and my fellow researchers in the Department of Chemistry at the University of British Columbia's Okanagan campus. Sharing this journey with all of you has been an incredible experience. Special thanks to Dr. Sandra Mecklenburg for her friendship, support, and inspiring personality. I admire your passion and devotion. You are invaluable to the chemistry department.

A special thanks to my family. Words cannot express how grateful I am to my parents and my siblings, for emotionally and spiritually supporting me and encouraging me all the way. Thank you for giving me unconditional love.

I would also like to thank all of my friends who have supported me throughout the years and have made my graduate years memorable and exciting. You are my family in Canada.

At the end, I would like to express my deepest appreciation to my husband, Hamed Ghannad, for his endless support, patience, love, and genuine friendship. You have always supported me in everything that I have ever done. You have always fought to keep your dreams alive and inspired me to do so. Thank you for standing beside me on this long journey.

**Dedication**

**To you**

## 1 Chapter: Introduction

The first description of dopants as a new concept dates back to a patent (Woodyard, 1944) filed in 1944, which was granted to Dr. John Woodyard, an experimental physicist at the radiation laboratory of Berkeley, in 1950. He observed that some naturally occurring crystals that contain impurities make more sensitive radio detectors compared to pure germanium. This was the first introduction of the concept, which was later called “doping.” To our best knowledge, the word dopant, which denotes "intentionally added impurities", appeared initially in the literature in 1962 in the field of photoelectric solar energy convertors (Zaitseva & Gliberman, 1962). In this field, a dopant or a doping agent is an element, which intentionally and in a very low concentration is added to a substance in order to change the electrical or optical characteristics of the substance.

The idea of using dopants as a way to increase ionization efficiency was first introduced in the mid-1950s when Jesse and Sadauskis reported that the addition of trace quantities of gaseous impurities including Argon, CO<sub>2</sub>, Kr, Xe, H<sub>2</sub>, N<sub>2</sub>, and C<sub>2</sub>H<sub>4</sub> significantly increased the alpha-particle ionization efficiency in helium: Neutral metastable helium atoms ionize impurities forming a radical cation and an electron ( $\text{He}^* + \text{X} \rightarrow \text{He} + \text{X}^+ + \text{e}^-$ , if  $\text{IE}_\text{x} < \text{E}_{\text{He}^*}$ , X represents impurity atom or molecule) (Jesse & Sadauskis, 1955). According to this finding, the addition of a foreign gas in order to increase the ionization efficiency of a detector for gas chromatography was utilized by different research groups. For example, Deal et al. in their radiological detector for gas chromatography using a  $\beta$ -emitter radioactive ionization source introduced one mole % heptane into the nitrogen stream (Deal, Otvos, Smith, & Zucco, 1956).

In 1958, Dr. James E Lovelock invented the Electron Capture Detector (ECD) for GC in order to detect trace amounts of volatile organic compounds (J. Lovelock, 1958). Although, he did not use the term dopant, methanol vapor was used as a foreign gas, which was added to pure argon flowing into the detector. They found that the addition of a trace amount of a foreign gas into rare gases under constant radiation would lead to an increase in the ionization efficiency. Later, Lovelock realized that many strong electron-capturing compounds show the maximum cross section for capturing electrons when electrons carry thermal energy levels, which is lower than 4 eV (LOVELOCK, 1962). In 1963, Dr.

Lovelock incorporated this concept in a new design of ECD involving a pulse sampling technique (J. E. Lovelock, 1963). This method created thermal electrons between pulses when no voltage was applied. Similar to his first ECD, methane was utilized to improve the ionization, but this time methane was assumed to serve another function in enhancing the ionization efficiency as well as its known primary role of reacting with metastable carrier rare gas molecules. Methane acted as a slow-down medium and reduced the kinetic energy of free electrons to a constant thermal level through non-elastic collisions, which positively affected the electron capture process. Siu and Aue in 1980, in their study on dopants' effect on the linearity of a d.c. ECD, summarized the roles of dopants in improving the performance of an ECD (Siu & Aue, 1980). In addition to the previously established roles, which were to react with excited states of carrier gas to produce ionized species and in turn, give additional ionization, and to remove transitional energy from electrons to maintain a thermal distribution; Siu and Aue also stated that dopants may donate hydrogen to product radicals, mediate the transfer of energy and impact the mobility of all species present including electrons.

In this time period, alternative ways to produce electrons for ECD instead of the conventional  $^{63}\text{Ni}$  radioactive source were explored as well (Kapila, Bornhop, Manahan, & Nickell, 1983; Neukermans, Kruger, & McManigill, 1982; Simmonds, 1987; W.E Wentworth, Limero, Batten, & Chen, 1988, 1989; W.E Wentworth, Tishbee, Batten, & Zlatkis, 1975).

Interestingly, in some non-radioactive sources, dopants were also utilized to enhance the analytical response. For example, in 1983, Kapila et al. reported the construction of a photoionization detector (PID) for GC utilizing an ultraviolet lamp, which worked at both photoionization and electron capture modes (Kapila et al., 1983). Operating in the ECD mode, a readily photoionizable dopant gas was introduced into the carrier stream and ionized by the lamp to produce large quantities of electrons which made the baseline current. Then this baseline current was reduced after electron-capturing analytes absorbed electrons and created a capture signal. The reason to add a readily ionizable species was that the photon's energy from the UV lamp was not enough to ionize the carrier gas (in this case, nitrogen). Therefore, to produce electrons needed for ECD from photoionization, they added naphthalene and tri-n-propylamine as dopants to act as the source of electrons. To the best of our knowledge, this is the first use of dopants in photoionization. Wentworth et al. introduced

another non-radioactive detector, which they initially patented in several forms between 1992 (Wayne E. Wentworth & Stearns, 1992) and 1997 (Stearns & E., 1997), then published, (W.E Wentworth et al., 1988, 1989) and subsequently named as the pulsed-discharge electron-capture detector (PDECD) (W. Wentworth, Dsa, Cai, & Stearns, 1992). In their detector, the initial source of ionization was high energy-photons originating from a pulsed high-voltage discharge in pure helium. These photons ionized dopant molecules, which were added upstream of the discharge. The addition of readily photoionizable dopants was required in order to supply free electrons, which created the necessary constant standing current for electron capture. Additionally, dopants lowered the average kinetic energy of electrons to thermal level through inelastic collisions, which made them more capturable by analytes. In a review paper (W.E Wentworth et al., 1999) on their PDECD, Wentworth et al. stated three criteria for selecting a chemical as a dopant for ECD. First, a dopant must not have a positive electron affinity; otherwise, dopant molecules capture electrons and reduce the number of accessible electrons to analytes. Second, a dopant should have low ionization energy and a large cross-sectional area so that the extra energy needed to ionize a dopant molecule can be imparted to the electron, which reduces the rate of electron-positive ion recombination. Third, a dopant should possess internal and vibrational modes in order to thermalize the electron energy by removing the translational energy of electrons. This may be the first more comprehensive published description of requirements for a good dopant in the literature. In this study, new chemicals were tested as potential dopants including isooctane, propene, 1-butene, benzene, and toluene. Their preliminary results showed that saturated hydrocarbons such as isooctane and heptane as dopants gave comparable results to the ones obtained by methane as a dopant. Therefore, regardless of the expectation that larger molecules may be more effective in thermalization of electrons, size of molecule did not impact the functionality of dopants.

The Flame ionization detector (FID), which is mostly coupled to GC, was introduced in 1958 in New Zealand (I.G., R.A., & Desty, 1958). In the FID, hydrocarbons eluted from a GC column burn in a flame and produce ions. The corresponding current creates compounds' responses (McWilliam & Dewar, 1958). In the literature, the terminology of doping and dopants in the FID field similarly represents the concept of deliberately adding a chemical to the ionization source (flame, here) to enhance the electrical conductivity of the flame and

promote the ionization of specific classes of compounds. In this field, dopants have been used with a modified version of the FID named as “hydrogen atmosphere flame ionization detector (HAFID)”, which is simply constructed from a commercial standard FID with slight modifications (Hill, 1975). In the HAFID, the flame is fed with oxygen-enriched air and burns in a hydrogen atmosphere, which is doped with a volatile metal or volatile alkali metal. The resulting flame, containing metal vapors, shows an enhanced selective response towards organometallic and heteroatom-containing compounds over hydrocarbons (Roberts & Hill, 1979). For example, in 1964, Karmen and Giuffrida developed a HAFID, doped with sodium, to selectively increase the detector response towards halogen and phosphorus-containing compounds (Karmen & Giuffrida, 1964). The dopant in this case was a platinum wire, which was dried after immersion into a 1N sodium hydroxide solution. Then this wire was suspended above the flame jet in the detector. Compounds containing halogens or phosphorus burned in the flame and generated products, which increased the release rate of sodium from the wire probe. The released sodium was ionized by the flame and significantly increased the electrical conductivity of the flame compared to an un-doped flame and this is the principle of distinguishing halogen or phosphorus compounds from other compounds.

Earlier designs of PIDs for GCs appeared in the literature in the 1960s (D. C Locke & Meloan, 1965; J. E. Lovelock, 1961; LOVELOCK, 1960; Price, Fenimore, David C Simmonds, & Zlatkis, 1968; Roesler, 1964; Yamane, 1962). Although these works established the potential value of photoionization as a detector for GC, none of the designs achieved commercial success. In these designs, the ultraviolet radiation as the source of ionization was generated in a glow discharge in an inert gas at reduced pressures and illuminated an open ionization chamber containing the effluent from a GC (carrier and analyte gases). The discharge gases were selected to produce photons with energies higher than the ionization energies of most organic compounds and lower than those of the common GC carrier gases. Those PIDs were more sensitive than FIDs but because of no physical separation between the ionization chamber and the radiation source, it was impossible to simultaneously optimize an analysis for both GC and photon source conditions (D. C Locke & Meloan, 1965). In addition, operating at low pressures made them prone to contamination problems from column bleed. The commercial generation of PIDs for GC was introduced in

1976-1977 by Driscoll (J. N. Driscoll, 1976; John N Driscoll, 1977) and Driscolet al. (J. N Driscoll & Clarici, 1976; J. Driscoll & Spaziani, 1976) due to availability of sealed UV lamps. Separating the discharge compartment from the ionization chamber by a window allowed the ionization chamber to remain at atmospheric pressure and eliminated the deficiencies of the previous window-less UV lamp generation such as instable responses. By this development, different lamps with different photon energies from 9.5 eV to 11.7 eV were examined (Adamiya, Budovich, & Shlyakhov, 1994; J. Driscoll, 1982; John N Driscoll, Ford, Jaramillo, & Gruber, 1978) and the interest in the detector's potential for a variety of applications (Castello, Benzo, & Gerbino, 1995; Cavalcante, de Andrade, Márcia V.F Marins, & Oliveira, 2010; Clark II & Delfino, 2003; Cutter, Cutter, & San Diego-McGlone, 1991; John N Driscoll et al., 1978; Hester & Meyer, 1979; Hunter & Kuykendall, 2004; Kok & Ong, 1994; M. L. Langhorst & Nestricks, 1979; M. Langhorst, 1981; Q. Liu, Liu, & Wang, 2011; Ogino, Aomura, & Komuro, Masatugu Kobayashi, 1989; Oyler et al., 1978; Puig & Sacks, 1991; Vien & Fry, 1988) and modifications (Lewis et al., 2010; Norlander, Carlsson, & Bertler, 1986; Peng, Luoa, Yuan, & Qiu, 2010; Sacks, 2007; Sinha, Nölscher, Bockisch, Klüpfel, & Williams, 2012; K. Song, Ahn, Jung, Lee, & Ko, 2007) increased. During the same time period, using the knowledge gained in the development of GC-PID, liquid-phase PID was utilized as a detection method for LC by Locke et al. (David C Locke, Dhingra, & Baker, 1982; J. Schmermund & Locke, 1975), and later the vapor-phase PID for LC was introduced by Driscoll et al (J.N Driscoll, Conron, Ferioli, Krull, & Xie, 1984). In the latter, a heated oven containing an adjustable ratio eluent splitter interfaced HPLC to PID, which vaporized the LC eluents and mixed it with a carrier gas prior to PID introduction. This modification diminished the quenching of the analyte photo-ions by mobile-phase molecules in the detector which was the problem with the liquid-phase designs. Although the vapor-phase PID performed well for aromatics and compounds with elevated electron affinity, it did not have wide applications and only a few studies have been reported (de Wit & Jorgenson, 1987; J.N Driscoll et al., 1984; J. T. Schmermund & Locke, 1990). According to the present literature review, dopants have not been utilized either with these generations of PID-GC or with PID-LC.

The first use of PI at atmospheric pressure (APPI) in a field related to mass spectrometry was introduced by Baim et al. in 1983 (Baim, Eatherton, & Hill, 1983). In their newly developed

ion mobility spectrometer (IMS) as a GC detector, they replaced the  $^{63}\text{Ni}$  ionization source with a routinely used vacuum ultraviolet (VUV) lamp in a commercial PID-GC. APPI enabled selective detection of low molecular compounds, which was restricted by the  $^{63}\text{Ni}$  due to depletion of ions by reactant ions. At that time, various studies of APPI coupled to IMS were proposed (G. A. Eiceman & Vandiver, 1986; G. Eiceman, Fleischer, & Leasure, 1987; Leasure, Fleischer, Anderson, & Eiceman, 1986; Spangler, Roehl, Patel, & Dorman, 1992). It appears as if, dopants have not been examined extensively in the early studies of APPI-IMS (Doering, Arnold, Adler, Roebel, & Riemenschneider, 1996; Spangler et al., 1992). However, recently, with the development of High-field Asymmetric Ion Mobility Spectrometry (FAIMS) (Guevremont, 2004; Shvartsburg, 2009), the use of APPI and DA-APPI as the ionization methods has become an area of interest (Y. Liu et al., 2014; Tang et al., 2011).

More effective analysis of large nonvolatile molecules, for which gas chromatographic separations are not effective or feasible at all, has been a goal for years. Although many approaches have been developed, it was the development of atmospheric pressure ionization (API) sources for coupling to liquid chromatography that achieved this objective (Niessen, 1999). The first API source by Horning et al. in the 1970s contained a  $^{63}\text{Ni}$  foil operated at atmospheric pressure, external to the high vacuum chamber of a quadrupole mass spectrometer (Carroll, Dzidic, Stillwell, Horning, & Horning, 1974; Horning, Horning, Carroll, Dzidic, & Stillwell, 1973; Horning, Carroll, et al., 1974) and the next designs utilized a corona discharge electrode as the ionization source (Carroll, Dzidic, Stillwell, Haegele, & Horning, 1975; Horning, Carroll, et al., 1974). The latter design is the base design for the current commercially available APCI (Carroll et al., 1975). In the meantime, the second API interface, Electrospray Ionization (ESI), was introduced by Whitehouse et al. in 1985 (Whitehouse, Dreyer, Yamashita, & Fenn, 1985). With further improvements and introduction of pneumatically assisted electrospray (Bruins, Covey, & Henion, 1987), API sources gained more widespread use and during the late 1980s and early 1990s, many brands of API sources were commercially available. The last API source, atmospheric pressure photoionization (APPI), was introduced in 2000 simultaneously by Robb et al. (Robb, Covey, & Bruins, 2000) and Syage et al. (Syage, Evans, & Hanold, 2000) with a motivation to

broaden the range of LC/MS amenable compounds to include non-polar compounds, since both ESI and APCI favor the ionization of polar compounds.

Among these three API sources, ESI has become a dominant technique in biochemical analysis; since it enables the ionization of large, non-volatile molecules directly from the liquid phase. In ESI, analyte containing fluid is introduced into the atmospheric pressure ionization chamber through a capillary, whose tip is held at a potential of several kilo volts. The fluid is electrostatically sprayed out of the capillary forming a fine aerosol of charged liquid droplets. While moving toward the mass analyzer, these charged droplets undergo evaporation within potential and pressure gradients, which finally leads to signal ions (Iribarne & Thomson, 1976; Thomson & Iribarne, 1979; Wilm & Mann, 1994). ESI can ionize fragile macromolecules with low internal energies without in-source fragmentation. Also, by producing multiple charged ions, ESI expands the mass range of the analyzer to Mega Dalton range and unlike other ionization sources, uniquely provides an unlimited ionization range in terms of mass (Siuzdak et al., 1996).

The concept of dopants as chemicals that are deliberately added to the analysis to assist the ionization does apply to ESI as well but is usually termed as nonvolatile adduct-forming agents, which facilitate the formation routes of adduct ions and/or salt cluster complexes of target analytes. In addition to autoadduct formation in ESI—in which indigenous adduct-forming reagents driven from glassware, mobile phases' impurities, biological sources, etc. assist ionization—these reagents can be deliberately added to spray solvent—post-column or as LC buffers—to facilitate the formation of both positive and negative ions for samples that do not generate stable ions. For example, in the negative mode, chlorinated solvents promote the formation of adduct ions with chloride anions and assist the ionization of neutral or very weakly acidic molecules incapable of deprotonation (Cole & Zhu, 1999). On the other hand, in the positive mode, metal salts are deliberately added to LC mobile phase and advance the formation of positive ions for weakly basic or neutral analytes (Ackloo, Smith, Terlouw, & McCarry, 2000; Saf, Mirtl, & Hummel, 1994; Sudha, Panda, Chandrasekhar, & Balaram, 2002). Although addition of dopants to the ionization source is a common approach, a survey of the literature shows that the terminology of doping in the ESI-based methods has only been used a few times (Cotte-Rodríguez, Hernandez-Soto, Chen, & Cooks, 2008; Koch, Forcisi, Lehmann, & Schmitt-Kopplin, 2014; Meier, Garrard, & Muddiman,

2014; Tam & Hill, Jr, 2004). For example, Cotte-Rodríguez et al. (Cotte-Rodríguez et al., 2008) developed a desorption electrospray ionization (DESI) mass spectrometry method to directly detect peroxide-based explosives on ambient surfaces without any sample preparation. They doped the spray solvent with alkali metals or ammonium ions, which led to observing the analytes as alkali metal or ammonium ion complexes, which in turn, increased the signal intensity by an order of magnitude. Additionally, they developed a desorption atmospheric pressure chemical ionization (DAPCI) method, in which they doped the DAPCI gas with ammonium acetate vapor as dopant. The dopant gas became ionized by DAPCI providing the ammonium reagent ions, which assisted the ionization of analytes similarly to their proposed DESI method (Cotte-Rodríguez et al., 2008). In brief, (a) lithium, potassium, ammonium (Cotte-Rodríguez et al., 2008; Deery et al., 1997), silver (Deery et al., 1997; Meier et al., 2014), sodium, copper, cobalt ions (Deery et al., 1997), tetramethylurea (TMU) as the precursor of the reactant acylium ion (Meurer, Sabino, & Eberlin, 2003) in the positive ion ESI and (b) chloride (Evans, Sleeman, Luke, & Keely, 2002; Gapeev, Sigman, & Yinon, 2003; Reid Asbury, Klasmeier, & Hill Jr, 2000; Tam & Hill, Jr, 2004; Zhao & Yinon, 2002), nitrate (Tam & Hill, Jr, 2004; Zhao & Yinon, 2002), acetate (Casetta & Garofolo, 1994; Gapeev et al., 2003; Schreiber, Efer, & Engewald, 2000; Wu, Hendrickson, Rodgers, & Marshall, 2002), formate (Cassada, Monson, Snow, & Spalding, 1999; Gapeev et al., 2003; Suni et al., 2011), propanoate (Zhao & Yinon, 2002) ions in the negative ion ESI have been used as indigenous and axillary adduct-forming reagent (dopants).

The next most commonly used API source is APCI, in which ionization initiates by a corona discharge (Carroll et al., 1975; Horning, Horning, et al., 1974; Horning, Carroll, et al., 1974). The analyte solution is pneumatically nebulized into a tube kept at 350-500 °C, where the solvent is evaporated. A high-voltage is applied to a needle located at the exit of the nebulizer, which creates or captures electrons according to the polarity of the applied voltage, leading to a typical point to plane discharge (corona). The electrons initiate a series of chemical reactions with the surrounding gas-phase molecules and produces reagent ions, which further ionize the analyte molecules through ion-molecule chemical reactions. APCI ionizes low molecular compounds with medium to low polarities. This API interface exerts more thermal stress on the sample due to the high temperature of the probe and ideally, the

sample must be thermally stable and volatile (Terrier, Desmazières, Tortajada, & Buchmann, 2011).

Dopants have been utilized much more in APCI and it is not surprising, since DA-APPI can be considered as chemical ionization method, comparable to APCI. In DA-APPI, after production of dopant-photo ions, the analytes are ionized through the same gas-phase ion/molecule reactions as those underlying APCI and the only difference is how the primary reagent ions are generated in each method. Therefore, in APCI, addition of an easily ionizable chemical can enhance the ionization efficiency and promote one ionization route over others.

In the earliest design of corona discharge ion sources for LC/MS analysis (Carroll et al., 1975; Horning, Horning, et al., 1974), which are the basis of APCI designs today, chloroform (in NI), isooctane, benzene, and hexane (in PI) were used to generate the reactant ions. Later, in 1976, Dzidic et al. used nitrogen, argon, isobutane, ammonia, and nitric oxide to produce reagent ions in a modified design of APCI. In their design, they used an adjustable discharge needle to change its distance from the sampling aperture in order to control the residence times of ions including reagent ions. This modification enabled them to detect the initial, intermediate, and product ions engaged in APCI process and for the first time, they published a more detailed definition of the sequence of ion-molecule reactions in APCI (as to be: carrier gas → reagent ions → product ions), which was found to be similar to what was already suggested for a conventional CI/MS condition at a pressure of about 1 Torr, at that time (Dougherty & Roberts, 1974; Field, 1972; Hunt & Ryan, 1972; B L Jelus, Munson, & Fenselau, 1974; Barbara L Jelus, Munson, Babiak, & Murray, 1974; Milne, Fales, & Axenrod, 1971). Moreover, they defined the adequate concentration of reagents in order to produce efficient reagent ions as 0.01-0.1% of the carrier gas (Dzidic, Carroll, Stillwell, & Horning, 1976).

Generally, ionization mechanisms underlying APCI favor ionization of polar compounds, therefore, desired analytes' signals could be suppressed in the presence of compounds with higher ionization efficiencies when competing for reaction with the reagent ions present. The key motivation to use dopants in APCI is to promote the charge transfer route by creating high quantities of dopant ions as reagent ions and drive the ionization toward non-polar compounds, for which proton transfer fails (Hourani & Kuhnert, 2012a, 2012b). For

example, several researchers have applied DA-APCI technique for the analysis of PHAs, as they represent non-polar compounds and their characterization by GC, especially for the larger ones is limited (Anacleto, Ramaley, Benoit, Boyd, & Quilliam, 1995; Lafleur, Taghizadeh, Howard, Anacleto, & Quilliam, 1996). For example, a dopant assisted APCI (DA-APCI) method was introduced in 2011 for analysis of PHAs. In this study, chlorobenzene, toluene, and anisole were examined as dopants. Anisole, which improved the signal to noise ratio, gave the best results. Dopants mainly promoted the formation of radical cations for most analytes, since all three dopants mainly generated  $D^{*+}$  as reagent ions. However, anisole also produced a noticeable amount of  $[D+H]^+$  and in the toluene spectra, both  $[D+H]^+$  and  $[D-H]^+$  ions were observed. The authors hypothesized that these reagent ions can be responsible for proton transfer routes, which were followed by an in-source fragmentation. This work demonstrated the potential of a dopant assisted-APCI as an alternative technique for the analysis of non-polar compounds (L. Song et al., 2011).

A detailed chemical characterization of sulfur-containing analogues of the PAHs, polycyclic aromatic sulfur heterocycles (PASHs), in a sample is very useful and informative in environmental analytical analysis, because PASHs can be used as markers for oil pollution, thermal processes and vehicular traffic (Andersson, Hegazi, & Roberz, 2006). Among other mass-selective detection techniques used for this purpose such as NCI/MS (Becker, Nilsson, Colmsjö, & Östman, 1998), ICP-MS (Bouyssiére et al., 2004) MALDI-MS, (Müller & Andersson, 2004), ESI-MS (Rudzinski, Zhang, & Luo, 2003), and ESI-FTICR-MS (Müller, Andersson, & Schrader, 2005), APCI also has been utilized to detect PASHs (Gimeno, Altelaar, Marcé, & Borrull, 2002; Herrera, Ramaley, & Grossert, 2009; Hourani et al., 2013; Thomas, Crain, Sim, & Benoit, 1995). Among them, in one work, the post-column addition of tropylium cations prior to APCI/MS was used to enhance the sensitivity of the ionization toward larger PASHs containing three and more rings. Similar to PAHs, non-polar PASHs are not ionized through proton transfer, which is the dominant ionization route in APCI; but the addition of tropylium cations provides charge transfer reagents and enhances the formation of PASHs' molecular cations via charge transfer reactions (Rudzinski & Rai, 2005).

Last but not least is APPI, in which dopants have been used the most compared to any other ionization technique, much better and more consistently termed DA-APPI and is almost considered the standard and common way of photoionization at atmospheric pressure. Utilization of dopants in APPI is the main focus of this thesis; therefore, in the following sections APPI and DA-APPI in combination with both LC/MS and GC/MS are discussed in detail. Finally, Atmospheric Pressure Laser Ionization (APLI), which is based on resonantly enhanced multiphoton ionization (REMPI), offering unique sensitivity and selectivity toward non-polar aromatic hydrocarbons, is reviewed.

### **1.1 Atmospheric Pressure Photoionization (APPI) and Dopant Assisted-Atmospheric Pressure Photoionization (DA-APPI)**

In an APPI source, a high frequency, low pressure krypton lamp is used as the ionization source, which generates vacuum ultraviolet (VUV) photons with energies of 10.0 and 10.6 eV. In LC/APPI, the LC eluent evaporates into the gas-phase state by heat-aided nebulization, is then irradiated by photons, and becomes partly ionized (Robb et al., 2000). The lamp's photon energy is typically greater than the IEs of most analytes, which are in the range of 7 to 10 eV. The principal ionization mechanism in APPI is the formation of molecular radical cation of analytes as a result of photoabsorption and ejection of an electron. Since the ionization energies of the most commonly used LC solvents, atmospheric gases, and background water are higher than 10 eV, direct photoionization of these species is not possible. Therefore, considering the trace amount of analytes compared to the nebulized eluent from LC and atmospheric gases, the total ion production in the source is quite low. However, the dense mixture of gases still absorbs the photons and suppresses APPI signals of analytes due to non-deliberate depletion of photons, which renders the direct photoionization of trace analyte statistically unlikely. Robb et al. utilized this finding in a positive way and rationalized that if an easily ionizable compound (dopant) is deliberately added to the LC eluent or to the vapor generated from LC eluent, the total number of photo-ions significantly increases and the high collision rate in the atmospheric pressure source assures ample reaction events of the photoion-reagents with the surrounding molecules including trace analyte. In this way, the probability of charge transfer to an analyte significantly increases.

To make this happen, they hypothesized that dopant photo-ions must have a relatively high recombination energy, or a low proton affinity in order to transfer their charge to the surrounding molecules. Two dopants utilized in their study were toluene and acetone. They observed a significant increase in the ionization efficiency of studied compounds with dopants (at a factor of 25 to 100 fold increase). The new APPI method, utilizing dopant, was named Dopant Assisted-Atmospheric Pressure Photo Ionization (DA-APPI). Since in DA-APPI, the total ion production increases significantly compared to direct APPI, the ionization efficiency will be governed by the ion-molecule reactions initiated by photo-dopant ions rather than by the difference in photoionization cross sections of different analytes. In that same paper, comparing the results obtained by APPI to the ones obtained by APCI ionization (Henion, Thomson, & Dawson, 1982), Robb et al, suggested that the ionization mechanisms underlying atmospheric pressure ionization sources may follow similar paths regardless of how the primary reagent ions are formed in the source (Robb et al., 2000).

## **1.2 Dopant-free APPI (Direct photoionization) Applications**

Since it is well established that dopants significantly increase the ionization efficiency of APPI, nowadays DA-APPI is the prevailing way of photoionization and dopant-free APPI (hereafter referred to as APPI) is rarely used. The literature review indicates three fields of applications where APPI has been favored over DA-APPI. In the first field, where fragmentation or pyrolysis is not favored, direct APPI has been found to be a method of choice. For example, in 2002 (Richeter et al., 2002), direct APPI was proven to be a powerful method to confirm the structure of fragile synthetic porphyrin oligomers, by determining their molecular weight. For the same reason, in 2004, dopant-free APPI was applied to determine the molecular weight of asphaltene (Mullins, Cunico, & Sheu, 2004). In 2008, dopant-free APPI was successfully applied to analysis of some per-O-methylated oligosaccharides of the d-xylan type which led to no cross ring cleavages in contrast to the results obtained by ESI and MALDI (Aïcha Bagag, Laprévote, Hirsch, & Kováčik, 2008). In the second field, the addition of dopants did not, or did not significantly, enhance the ionization of some specific analytes compared to dopant-free APPI; therefore, dopant-free APPI, which has comparably fewer background ions, has been favored. For example, in

analysis of patulin in apple juice (Takino, Daishima, & Nakahara, 2003c) carbamate pesticide residues in vegetables and fruits (Takino, Yamaguchi, & Nakahara, 2004), and fat-soluble vitamin standards (Miller, Cormia, & Fischer, 2001), the addition of dopants did not promote the ionization of the analytes of interest.

The third reason to prefer dopant-free APPI in some applications is because direct APPI has resulted in a desirable limit of detection; for example this occurred in analysis of aldehydes based on 2,4-dinitrophenylhydrazine (DNPH) derivatization in automobile exhaust and cigarette smoke (van Leeuwen, Hendriksen, & Karst, 2004), analysis of fungicides in citrus fruits (Yoshioka, Akiyama, & Teranishi, 2004), determination of acetylated ambiphilic polyaromatic amines (E. Straube, Dekant, & Völkel, 2005), analysis of fatty acid ester and acylglycerol lipids and natural fish oil (S.-S. Cai & Syage, 2006a, 2006b), and analysis of kava lactones in food and drinks (Diachenko, Perfetti, & de Jager, 2004). Another example of successful application of dopant-free APPI is the characterization of extremely complex crude oils along with ESI, APCI, atmospheric pressure laser ionization (APLI) ionization techniques. In that study, APPI enabled the detection of the broadest range of compounds compared to ESI, APCI, and APLI which allowed classification of different compounds (Panda, Andersson, & Schrader, 2009).

However, the above studies are significantly fewer than the number of reports with dopant assisted APPI and it is safe to say that DA-APPI is practically the standard operation mode in APPI applications.

### **1.3 Methods of dopants introduction into the APPI source**

In DA-APPI, generally the dopant is added as a liquid. How the liquid dopant is mixed with the sample stream depends on the design of APPI. Commercially, two APPI designs are available. Robb et al. designed a "linear" flow tube type APPI Source (PhotoSpray®) in which the spray is in line with the mass spectrometer's inlet (Figure 1.1 (a)) (Robb et al., 2000). This design is based on dopant-assisted photoionization. On the other hand, in the design by Syage et al. (PhotoMate®), which is described as an "orthogonal" source, the spray is at a 90° degree angle to the mass spectrometer's inlet (Figure 1.1 (b)) (Syage et al., 2000). Although, a dopant can be added to the source, this design is based on direct photoionization.

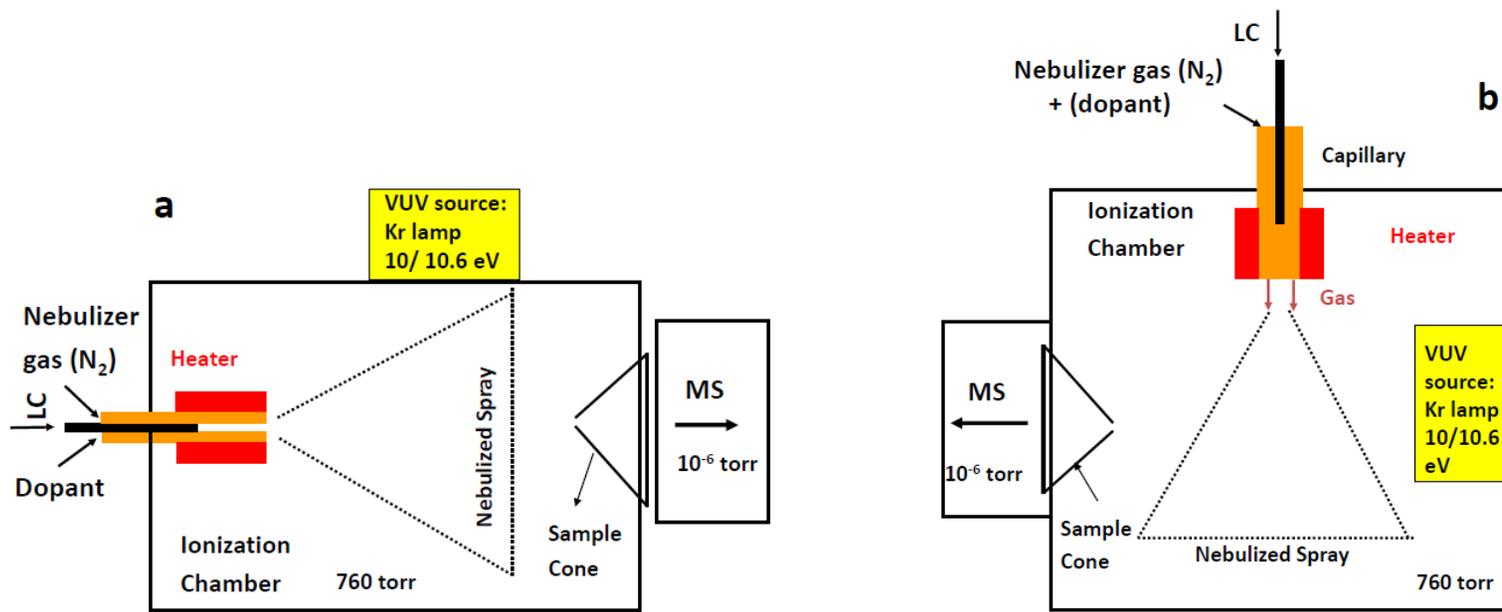


Figure 1.1 Two commercial designs of APPI; PhotoSpray (a), PhotoMate (b)

PhotoSpray APPI sources are equipped with a means to deliver the dopant to the vaporizer separately from the solvent. However, when a PhotoMate APPI source is used, the liquid dopant is mixed with the sample stream in a T upstream from the source. Therefore, with PhotoMate APPI sources, the degree of miscibility of dopant and LC mobile phases can impact the results.

With the orthogonal design (PhotoMate®), different methods have been utilized for dopant addition. In the most common way the dopant is infused post column and directly into the ionization source using a syringe pump (Yanxuan Cai, Kingery, McConnell, & Bach, 2nd, 2005; Hanold, Fischer, Cormia, Miller, & Syage, 2004).

The other way of delivery to the ionization source is to use the dopant as a mobile phase or to introduce the dopant through a mobile phase as an organic modifier (Bacaloni et al., 2009; Yanxuan Cai, McConnell, & Bach, 2nd, 2009; Cavaliere, Foglia, Pastorini, Samperi, & Laganà, 2006; Letcher & Chu, 2010; Marteau et al., 2012; Mascher, Mascher, & Zech, 2008; McClaine, Zhang, & Wornat, 2006; Riu, Zalko, & Debrauwer, 2006). If the dopant is used in the mobile phase, it may improve the chromatography separations as well as enhance the ionization (Yanxuan Cai et al., 2009). For example, recently (Black, Sun, Zhao, Gänzle, & Curtis, 2013), a normal phase LC method coupled to APPI MS/MS has been proposed to elucidate the structure of antifungal hydroxy fatty acid produced by lactobacilli. In this study a hexane/ethyl acetate/methanol/water solvent system was utilized for LC. Hexane, which has a low ionization energy (10.13 eV) (NIST Standard Reference Database Number 69), acted as a dopant eliminating the need for the post-column addition of a dopant. In addition, introducing dopants through the mobile phase makes their distribution inside the source homogeneous and consistent, which in turn, decreases the background noise and improves the signal to noise ratios (Bacaloni et al., 2009; Letcher & Chu, 2010).

Furthermore, a dopant can be introduced with sample as the solvent media (Chiaberge et al., 2013; Y Cho et al., 2013; Andras Gaspar, Zellermann, Lababidi, Reece, & Schrader, 2012; Tiina J Kauppila, Ostman, et al., 2004; Osborne et al., 2013).

Last but not least, Ehrenhauser et al., proposed the introduction of dopants in the gas-phase. This method addresses problems associated with liquid-dopant addition, such as (a) limited capacity of syringes—which precludes non-stop analysis—and insufficient immiscibility in case of post column addition and (b) the consequence of affecting chromatographic

separation, in case of dopant addition through mobile phase (Ehrenhauser, Wornat, Valsaraj, & Rodriguez, 2010).

#### **1.4 Advantages of APPI over ESI and APCI**

Although APPI bears some limitations especially in the scope of bioanalytical LC/MS applications, due to its unique nature of ionization this technique offers some distinctive advantages over other atmospheric pressure ionization methods, specifically APCI and ESI. The main advantages are briefly discussed here:

- The key advantage of APPI as well as the major motivation for its primary development is its capability to ionize less polar and non-polar compounds, which are not well addressed by ESI and APCI (Yanxuan Cai et al., 2005; Imbert et al., 2012; Moriwaki, Ishitake, Yoshikawa, Miyakoda, & Alary, 2004). The role of ESI in analytical and bioanalytical fields for highly sensitive and selective ionization of complex mixtures is indispensable (Keski-Hynnälä et al., 2002; Louw, Njoroge, Chigorimbo-Murefu, & Chibale, 2012); however, ESI favors analytes with basic or acidic groups that make ionic species in the solution; since ESI ionizes analytes directly from the liquid phase. APCI is also an affinity method that relies on acid-base chemistry and favors polar analytes. In contrast to ESI and APCI, photoionization of a compound is based on the interaction of the compound with a photon of sufficient energy. Therefore, APPI is not strongly dependent on the polarity of analytes and has the potential to provide a more general ionization than APCI and ESI. Although the complementary profile that can exclusively be obtained by APPI may be small compared to that obtained by ESI, the role of APPI cannot be underestimated because it enables detection of important classes of non-polar compounds such as polycyclic aromatic hydrocarbons, steroids, hydrophobic peptides, and non-polar polymers (Bae, Na, Chung, Kim, & Kim, 2010; S.-S. Cai & Syage, 2006b; Delobel, Halgand, Laffranchise-Gosse, Snijders, & Laprévote, 2003; González-Domínguez, García-

- Barrera, & Gómez-Ariza, 2015; Headley et al., 2014; Panda et al., 2009; Terrier et al., 2011; A.-L. Zheng, Fan, Liu, et al., 2014; A.-L. Zheng, Fan, Wang, et al., 2014).
- Compared to ESI and APCI, APPI has been proven to be less sensitive to ion suppression in biological samples, caused by matrix components coeluted with target analytes (H.-C. Chen, Kuo, & Ding, 2009; Keski-Rahkonen et al., 2013; Nakahara, Yamaguchi, Takino, & Tanaka, 2004; Rodil, Schrader, & Moeder, 2009; Ross & Wong, 2010; Sanchís, Oliveira, de Leão, Farré, & Barceló, 2015; Suni et al., 2011; Takino et al., 2004; Hendrik B Theron, van der Merwe, Swart, & van der Westhuizen, 2007). This leads to obtaining more consistent analytical results and validated statistics with minimal sample clean-up requirements. The main reason is the nature of ionization in these methods; while polar matrix components in ESI and APCI, usually present in large quantities compared to target analytes, are more successful in the so-called competition for ionization (King, Bonfiglio, Fernandez-Metzler, Miller-Stein, & Olah, 2000). In APPI these interfering constituents, which usually possess much higher IEs than ionization power (10 eV), cannot even take part in the competition.
  - APPI's reduced susceptibility to matrix effects also results in less background chemical noise, which in turn results in higher signal-to-noise ratios, improved detection limits, and larger dynamic ranges (S.-S. Cai & Syage, 2006a, 2006b; Hakala et al., 2003; Himmelsbach, Buchberger, & Reingruber, 2009; Rodil et al., 2009; Takino, Daishima, & Nakahara, 2003a, 2003b). Moreover, a lack of adduct ions with sample and matrix constituents and cleaner spectra make the interpretation of APPI spectra more reliable and straightforward.
  - APPI offers a greater tolerance toward salts and buffers. This feature becomes crucial when techniques such as capillary electrophoresis (CE) is coupled to MS (Axén, Malmström, Axelsson, Petersson, & Sjöberg, 2010; Hommerson, Khan, & Bristow, 2007; Mol, de Jong, & Somsen, 2005; Nilsson et al., 2003; Rizvi, Shamsi, Zheng, & Hou, 2007; Schappler, Guillarme, Prat, Veuthey, & Rudaz, 2007). In CE, using non-volatile background electrolytes such as sodium phosphate significantly improves the chromatographic behavior in terms of resolution and peak shape symmetry. ESI,

- which has been the major ionization method for on-line coupling of CE and MS, is not compatible with CE separation buffers and undergoes considerable ion suppression in the presence of such buffer salts. Therefore, ESI limits the choice and quantity of buffers to volatile buffers at their lowest practical concentration, which is a major drawback especially for method developments. On the other hand, APCI requires higher flow rates compared to APPI to achieve comparable sensitivities (Takada, Sakairi, & Koizumi, 1995; Takeda et al., 2001; Tanaka, Otsuka, & Terabe, 2003). Advantageously, non-volatile salts in CE buffers do not suppress the APPI response. In addition, a suitable dopant can be simply added to the sheath liquid in order to enhance the PI efficiency (Nilsson et al., 2003).
- Unlike ESI and APCI, APPI is compatible with normal phase LC conditions. APPI does not use a high voltage discharge (as in APCI) and is not exposed to one (as in ESI probe tip). Therefore, in contrast to ESI and APCI, in normal phase LC applications, in which flammable solvents like hexane are used as mobile phases, APPI provides no discharge explosion hazard at high flow rates (S.-S. Cai, Hanold, & Syage, 2007; J. Chen, Korfmacher, & Hsieh, 2005).
  - Specifically compared to APCI, APPI offers some advantages, such as higher source stability (S.-S. Cai & Syage, 2006b), superior sensitivities at low flow rates (Yang & Henion, 2002), and less thermal degradation of thermally unstable compounds due to requiring less heat for desolvation (Korfmacher et al., 1997), as well as avoidance of deposit formation and source contamination due to the replacement of the APCI corona needle with an external Kr lamp. In APCI, “carbon deposit” could be formed at the tip of corona needle and on the entrance face of the mass analyzer, which could cause unstable signals and reduce the sensitivity over time (S.-S. Cai, Hanold, et al., 2007; S.-S. Cai, Short, Syage, Potvin, & Curtis, 2007). In addition, APCI leads to more in-source fragmentation than APPI, due to the high voltage gradient that is applied to the tip of the corona needle (Korfmacher et al., 1997).

## **1.5 Ionization mechanisms in APPI and DA-APPI**

The ionization mechanisms underlying APPI and DA-APPI have been studied in detail by different researchers (Tiina J Kauppila, Kostianen, & Bruins, 2004; Tiina J Kauppila et al., 2002; Marotta, Seraglia, Fabris, & Traldi, 2003; Robb & Blades, 2005, 2006b; Tubaro, Marotta, Seraglia, & Traldi, 2003) and four review papers on APPI (Karst, Leeuwen, van, & Bos, 2006; Marchi, Rudaz, & Veuthey, 2009; Raffaelli & Saba, 2003; Robb & Blades, 2008) have thoroughly reported and discussed the proposed ionization mechanisms. Therefore, here I will describe the most universally suggested ionization pathways in Positive Ion APPI (PI-APPI) and DA-PI APPI in Table 1.1.

**Table 1.1 Ionization pathways in positive mode APPI and DA-APPI**

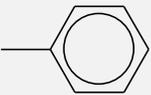
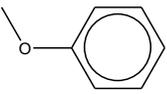
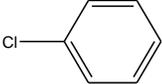
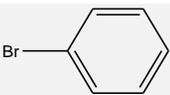
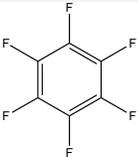
Ionization and quenching mechanisms in direct PI-APPI			
Reaction			Thermodynamical requirement
1	Formation of an electronically excited analyte	$M + h\nu \rightarrow M^*$	If a photon is absorbed by the analyte
2	Direct ionization of an analyte	$M + h\nu \rightarrow M^{*+}$	If IE (M) $\leq$ 10 eV
3	Photon emission as a result of a de-excitation	$M^* \rightarrow M + h\nu$	If IE (M) $>$ 10 eV
4	Photo-dissociation as a result of a de-excitation process	$M^* \rightarrow A + B$	If IE (M) $>$ 10 eV
5	Collisional quenching with a non-excited molecule	$M^* + C \rightarrow M + C^*$	If IE (M) $>$ 10 eV
Ionization mechanisms in dopant-assisted PI-APPI			
Reaction			Thermodynamical requirement
6	Dopant photoionization	$D + h\nu \rightarrow D^{*+}$	If IE (D) $\leq$ 10 eV
7	Direct proton transfer with dopant	$D^{*+} + M \rightarrow [D-H]^+ + [M+H]^+$	If PA (M) $\geq$ PA [D-H] $^+$
8	Formation of protonated solvent or solvent clusters	$D^{*+} + nS \rightarrow [D-H]^+ + [S_n+H]^+$	If PA (S) $\geq$ PA [D-H] $^+$
9	Proton transfer with solvent	$[S_n+H]^+ + M \rightarrow [M+H]^+ + nS$	If PA (M) $\geq$ PA (S)
10	Charge transfer	$D^{*+} + M \rightarrow M^{*+} + D$	If IE (M) $\leq$ IE (D)

M, D, and S donate analyte, dopant, and solvent respectively. IE and PA represent ionization energy and proton affinity respectively.

## **1.6 Chemicals utilized as dopants in dopant assisted positive ion- atmospheric pressure ionization sources.**

Molecular properties and gas-phase ion energetics data for different dopants, which were obtained from NIST Chemistry WebBook (NIST Standard Reference Database Number 69), are shown in Table 1.2. Following, areas of application, engaged ionization mechanisms, advantages and limitations are reviewed for each dopant.

**Table 1.2 Molecular properties and relative gas-phase ion energetics data of dopants.**

Dopant	Formula	Structure	Molecular Weight	IE (eV)	PA (kJ/mol)	Gas basicity (kJ/mol)	$\Delta_{\text{acid}}G$ (kJ/mol)
Benzene	C <sub>6</sub> H <sub>6</sub>		78.1118	9.24	750.4	725.4	1641.8
Acetone	C <sub>3</sub> H <sub>6</sub> O		58.0791	9.70	812.0	782.1	1514
Toluene	C <sub>7</sub> H <sub>8</sub>		92.1384	8.83	784.0	756.3	1557
Anisole	C <sub>7</sub> H <sub>8</sub> O		108.1378	8.20	839.6	807.2	1648
THF	C <sub>4</sub> H <sub>8</sub> O		72.1057	9.40	822.1	794.7	
Chlorobenzene	C <sub>6</sub> H <sub>5</sub> Cl		112.557	9.07	753.1	724.6	1584
Bromobenzene	C <sub>6</sub> H <sub>5</sub> Br		157.008	9.00	754.1	725.8	1611
Hexafluorobenzene	C <sub>6</sub> F <sub>6</sub>		186.0546	9.90	648.0	642.4	

IE, PA, and  $\Delta_{\text{acid}}G$  present ionization energy, proton affinity, and gas-phase acidities respectively

## **1.6.1 Benzene**

### **1.6.1.1 The ranges of application for benzene as a dopant**

The use of benzene as a dopant dates back to 199 (Ketkar, Dulak, Dheandhanoo, & Fite, 1991) when it was used as a charge transfer reagent in APCI to enhance the ionization of low proton affinity compounds in air. Later, benzene was used as a dopant to improve the sensitivity and selectivity of a PI-IMS (Spangler et al., 1992). However, its high toxicity, strictly limited its further applications.

It appears as if benzene has appeared in the literature as a dopant to increase the ionization efficiency of APPI only twice. In 2006, McClaine et al. used benzene in a HPLC/UV/APPIMS technique as one constituent of the mobile phase and applied it to separate and identify the products of supercritical pyrolysis of toluene. These products are mainly large polycyclic aromatic hydrocarbons (PAHs), which are formed under supercritical pyrolysis. In this case, benzene enhanced the ionization of large PAHs present in low concentrations and improved the mass spectral results (McClaine et al., 2006). The second time was in 2010, when Ehrenhauser et al. introduced a new gas-phase dopant-delivery system for use with HPLC/MS in order to overcome some of the drawbacks of delivering dopants as a liquid. The performance of the new device was evaluated with benzene as the dopant for the analysis of standard polycyclic aromatic hydrocarbons (PAHs) (Ehrenhauser et al., 2010).

### **1.6.1.2 Ionization mechanisms of analytes with benzene-Assisted APPI**

The photoionization of benzene and identification of photo-induced benzene reagents has been studied by Marotta et al. in 2003. Their data confirmed that photoionization of benzene initially led to the formation of its molecular radical cations; however, at higher partial benzene pressure, other ionization products such as phenol ions dominated the spectra, which were produced as the result of benzene radical cations' attack on oxygen molecules present in

APPI source. They hypothesized that these phenol ions are mainly responsible for the formation of protonated analytes through proton transfer route (Tubaro et al., 2003). However, it should be further noted that in that case, mass spectra were obtained when benzene was directly admitted into the source via a head-space injection and a liquid injection without the presence of any LC eluent. Therefore, their observed photo-ions do not reflect dopants' background reagent ions expected in the presence of LC eluents, since with the practical utilization of APPI-MS as the detection method for liquid chromatography, LC eluents usually affect the reagent background profile of dopants. In 2010, Ehrenhauser et al. introduced benzene as a dopant in the APPI source with their newly designed gas-phase dopant delivery system, while the mobile phase composition from LC was 80:20 methanol/water (v/v). The main background reagent ions from benzene in the positive mode were benzene radical cations which means benzene mainly favors charge transfer ionization route for analytes that have less IEs than that of the dopant (Ehrenhauser et al., 2010).

#### **1.6.1.3 Advantages of utilizing benzene as a dopant**

Because of the low molecular weight of benzene ( $78.11 \text{ g mol}^{-1}$ ), its background photo-ions are in the relatively low  $m/z$  range and do not interfere with the analysis of most target analytes, while other dopants' background ions may interfere (Ehrenhauser et al., 2010; Traldi & Marotta, 2004).

#### **1.6.1.4 Limitations of utilizing benzene as a dopant**

The principal downside of using benzene as a dopant is its toxicity and carcinogenicity (Cogliano, Baan, & Straif, 2011). Therefore its application necessitates taking precautions, such as using a low-temperature trap to condense the out-going gas mixture of the mass spectrometer (Perazzolli, Mancini, & Guella, 2005). The other issue with benzene is its immiscibility with water, which in turn makes it incompatible with highly aqueous mobile phases. With the PhotoMate APPI design this aspect may affect the even distribution of benzene inside the source; since the dopant and LC stream could not be homogeneously mixed

ionization efficiencies are adversely affected by increasing background noise and inefficient charge/proton transfer.

## **1.6.2 Acetone**

### **1.6.2.1 The range of applications for acetone as a dopant**

As a dopant, acetone was first reported in a patent in 1992 for photoionization enhancement of a PI coupled to ion mobility spectrometer (PI-IMS) (Spangler et al., 1992). In 2000, Robb et al. introduced acetone and toluene as dopants to enhance the ionization of the first commercially available APPI source (Robb et al., 2000).

Acetone- Assisted APPI (AcA-APPI) has been mostly used in the field of environmental analysis to improve the detection of estrogenic compounds (H.-C. Chen et al., 2009) surfactants, (Takino et al., 2003b) in water samples, antibiotics (chloramphenicol residues) in fish (Takino et al., 2003a), microbial respiratory ubiquinones and menaquinones (Geyer, Peacock, White, Lytle, & Van Berkel, 2004), halogenated flame retardants (HFRs) in fish (Zhou et al., 2010) and polycyclic aromatic hydrocarbons (PAHs) (Ehrenhauser et al., 2010) in edible oils (Hollosi & Wenzl, 2011). Also, AcA-APPI has improved the detection of brominated flame retardants in water samples, industrial effluents (Bacaloni et al., 2009), Gull eggs (Letcher & Chu, 2010), and human hepatocytes (Marteau et al., 2012).

AcA-APPI has also been utilized in the fields of pharmaceutical and clinical analysis. It has improved the analysis of free anabolic steroids in human urine (Leinonen, Kuuranne, & Kostianen, 2002), endogenous salsolinol and catecholamines in brain tissue (Starkey, Mechref, Muzikar, McBride, & Novotny, 2006), corticosteroids in human serum (Mascher et al., 2008), and 27-hydroxycholesterol in plasma (Karuna, von Eckardstein, & Rentsch, 2009). In the field of food analysis, the investigation of standard vitamins (Miller et al., 2001), mycotoxin in apple juice (Takino et al., 2003c), ergosterol (a fungus-specific marker) in *Fusarium*-infected wheat (Varga, Bartók, & Mesterházy, 2006), triacylglycerol lipids in tuna

oil (S.-S. Cai, Short, et al., 2007), and aflatoxins in food (Nakahara et al., 2004) has been improved using AcA-APPI.

In the field of biochemistry, AcA-APPI has been utilized to improve the ionization of biological molecules like nucleobases, nucleotides and nucleosides (A Bagag, Giuliani, & Laprévotte, 2007). In addition, Ac-APPI has been used as a tool to characterize and study the photo-induced fragmentation of peptides (Debois, Giuliani, & Laprévotte, 2006; Delobel et al., 2003), and Lipid peroxidation (Ronsein et al., 2010).

In the field of agrochemicals, AcA-APPI has been applied to the detection of pesticide residues in vegetables, fruits (Takino et al., 2004), and unpolished rice (Itoh, Otake, Aoyagi, Matsuo, & Yarita, 2009), and to the detection of avermectin and moxidectin residues in milk (Turnipseed, Roybal, Andersen, & Kuck, 2005).

#### **1.6.2.2 Ionization mechanisms of analytes with acetone-Assisted APPI**

Acetone's radical cations become solvated in the presence of background water and polar solvents of reversed-phase LC and/or neutralized with neutral acetone molecules (self-protonation), which finally leads to the formation of protonated dopant cations, which can further enhance the proton transfer route. Therefore, molecular radical cations of acetone do not remain in the system to promote charge transfer and it has been reported that acetone is not a suitable dopant to enhance the ionization of compounds having low proton affinities through charge transfer; while, it can promote the ionization of compounds having high proton affinities through proton transfer (Hanold et al., 2004; Leinonen et al., 2002; Robb et al., 2000). In terms of its ability to promote proton transfer routes, acetone is less effective compared to toluene (Fredenhagen & Kühnöl, 2014; Hanold et al., 2004; Leinonen et al., 2002; Robb et al., 2000).

#### **1.6.2.3 Advantages of utilizing acetone as a dopant**

Among commonly used dopants, acetone possesses the lowest mass, which creates less background ions and masking in the mass range of most interest. It is especially beneficial

where background ions with low  $m/z$  are desired in the analysis of small molecules. Although toluene is more effective in proton transfer reactions, acetone has been reported to create less background noise (S.-S. Cai, Short, et al., 2007; Hanold et al., 2004; Kamel, Jeanville, Colizza, & J-Rivera, 2008) and, even in some cases, suppress the background noise levels of analytes, which leads to a better signal to noise ratios, compared to that obtained by toluene (S.-S. Cai, Hanold, et al., 2007). Miscibility of acetone with water adds a unique advantage to the analysis; in addition to externally introducing the acetone to the ionization source, it can be internally added as a LC mobile phase component, leading to a homogeneous distribution of dopant inside the source. This miscibility improves the proton transfer likelihood, reduces the noise, and in turn, enhances signal to noise ratios (Bacaloni et al., 2009; Cavaliere et al., 2006; Marteau et al., 2012; Mascher et al., 2008). The choice of internally adding the dopant enables the utilization of a higher percentage of dopant and increases the sensitivity without increasing the noise level (Letcher & Chu, 2010). It is also important to keep in mind that acetone has the same elution power as acetonitrile and can facilitate separation as well (Mascher et al., 2008).

#### **1.6.2.4 Limitations of utilizing acetone as a dopant**

The main limitation of using acetone as a dopant is its high proton affinity ( $812 \text{ kJ.mol}^{-1}$ ); therefore, the majority of its radical cations become easily protonated in the presence of LC mobile phases and/or neutral acetone molecules (self-protonation), leaving no radical cations to act as charge transfer reagents and promote the ionization of nonpolar compounds having low proton affinities. Therefore, ionization of non-polar compounds which is the target of APPI is not effective or even impossible with acetone-Assisted APPI. In some cases, acetone can even hinder the ionization of non-polar compounds compared to direct APPI by consuming all the photons to produce protonated reagents, which are not able to transfer their proton to non-polar compounds (Yanxuan Cai et al., 2009; Karst et al., 2006; Tiina J Kauppila, Kostianen, et al., 2004; Mol, de Jong, et al., 2005; Mol, Jong, & Somsen, 2005; Robb et al., 2000; J. Zheng & Shamsi, 2006; Zhou et al., 2010).

### **1.6.3 Toluene**

#### **1.6.3.1 The ranges of application for toluene as a dopant**

Toluene, which was introduced by Robb et al. (Robb et al., 2000) in 2000 as a dopant for APPI, has been the most applied dopant for both qualitative and quantitative analysis and also has been used as a tool for mechanistic studies (Allegrand, Touboul, Giuliani, Brunelle, & Laprévotte, 2012; Tiina J Kauppila et al., 2002; Tiina J Kauppila, Bruins, & Kostainen, 2005; Robb & Blades, 2005, 2006b).

Toluene assisted APPI (TA-APPI) has been utilized mostly in the fields of pharmaceutical and clinical analyses. In clinical analysis, TA-APPI has been used to improve the detection of neurotransmitters (T J Kauppila, Nikkola, Ketola, & Kostainen, 2006), steroids such as ergosterol in wheat grains (Varga et al., 2006), corticosteroids (Greig, Bolaños, Quenzer, & Bylund, 2003) in serum and plasma (Kushnir, Neilson, Roberts, & Rockwood, 2004), lanosterol in inhibition buffer (Trösken, Straube, Lutz, Völkel, & Patten, 2004), and free and esterified phytosterols in human serum (Lembcke et al., 2005). It also has been used to assist steroid profiling in human serum or plasma (Guo, Chan, & Soldin, 2004). In addition, in 2010 Toluene was utilized to increase the ionization efficiency obtained by a microchip APPI interfacing GC to MS in order to analyze underivatized anabolic steroids in urine samples (Hintikka et al., 2010).

In the field of pharmaceutical analysis, this method has been applied to improve the detection of standard small drug molecules (Yanxuan Cai et al., 2005, 2009; Robb et al., 2000); drugs in different biological matrices such as levonorgestrel in human plasma (H.B Theron, Coetzee, Sutherland, Wiesner, & Swart, 2004); apomorphine, dobutamine, and entacapone in urine, rat hepatocyte, and human liver microsomes (Keski-Hyynilä et al., 2002); drug mixtures in Caco-2 cells (Hakala et al., 2003) and human and rat plasma (Hsieh, Merkle, Wang, Brisson, & Korfmacher, 2003; Hsieh, Merkle, & Wang, 2003); idoxifene and its metabolites in human plasma (Yang & Henion, 2002); cyclosporin A in rat plasma (Wang, Hsieh, & Korfmacher, 2005); and 27-hydroxycholesterol in plasma (Karuna et al., 2009).

In the field of biochemistry, TA- APPI has been utilized to improve the ionization of hydrophobic peptides (Delobel et al., 2003); to characterize lipid peroxidation (Ronsein et al., 2010); and to monitor the fragmentation patterns of biological molecules such as nucleobases, nucleotides, nucleosides (A Bagag et al., 2007), and small oligonucleotides (di- and trimers) (A Bagag, Giuliani, & Laprévotte, 2008). In the field of phytochemical analyses, TA-APPI has been used to aid the detection of flavonoids (Rauha, Vuorela, & Kostianen, 2001), and phytosterols in human serum (Lembcke et al., 2005). In addition, TA-APPI has been applied to obtain a comprehensive molecular structural identification of wheat straw lignin polymer (Banoub, Benjelloun-Mlayah, Ziarelli, Joly, & Delmas, 2007).

In the field of environmental analyses, TA-APPI has been applied to improve the detection of estrogenic compounds (H.-C. Chen et al., 2009); natural and synthetic steroids in water and solid samples (Snow, Damon-Powell, Onanong, & Cassada, 2013; Zavitsanos, 2003); phenylbenzotriazole-type (PBTA) mutagens in water samples (Moriwaki, Harino, et al., 2004); microbial respiratory ubiquinone and menaquinone isoprenologues in environmental samples and cell cultures (Geyer et al., 2004); dinitropyrene and aminonitropyrene in rat plasma (E. A. Straube, Dekant, & Völkel, 2004); polycyclic aromatic hydrocarbons (PAHs) (Tiina J Kauppila et al., 2002; Smith, Robb, & Blades, 2009) in sediment (Moriwaki, Ishitake, et al., 2004); pyrrolic and pyridinic nitrogen heterocyclic compounds in petroleum samples (J. M. Purcell, Hendrickson, Rodgers, & Marshall, 2007); polybrominated diphenyl ethers (PBDEs) (Cariou et al., 2006; Debrauwer et al., 2005; Riu et al., 2006) in house dust (Lagalante & Oswald, 2008), environmental water, and industrial effluents (Bacaloni et al., 2009); and aldehydes in atmospheric aerosol particles (Ruiz-Jiménez et al., 2013).

In the field of agrochemicals, TA-APPI has improved the analysis of neonicotinoid pesticides in river water samples (Yamamoto, Terao, Hisatomi, Kawasaki, & Arakawa, 2012).

Moreover, TA-APPI has improved the analysis of fullerenes, perfluorinated compounds, and pentafluorobenzyl derivatives (L. Song, Wellman, Yao, & Adcock, 2007). TA-APPI has also been applied to the qualitative and quantitative analysis of toxic and/or environmentally prevalent polychlorinated biphenyls (PCBs) congeners in water (Moukas, Thomaidis, & Calokerinos, 2014) and hair extract samples (Barbounis et al., 2012).

In the field of food analyses, TA APPI has been utilized to improve the detection of standard vitamins (Miller et al., 2001) and antibiotics (sulfonamides) in honey (Mohamed et al., 2007)

to obtain anthocyanins fingerprinting in wine (Gómez-Ariza, García-Barrera, & Lorenzo, 2006), and to determine patulin in apple-based food (Zhang, Wong, Mai, & Trucksess, 2014).

In addition, TA-APPI has been utilized in the field of polymer characterization for the analysis of nonpolar polymers like polyisobutylene (PIB) derivatives (Kéki, Török, Nagy, & Zsuga, 2008; Nagy et al., 2009). In addition, it has been used as a tool for investigation of the hydrolytic pathway of phosphite antioxidants (M Papanastasiou et al., 2008), polymer characterization of polyethylene waxes (Kéki, Nagy, Kuki, & Zsuga, 2008), and structural composition of coals (A.-L. Zheng, Fan, Liu, et al., 2014; A.-L. Zheng, Fan, Wang, et al., 2014).

Recently, DA-APPI coupled to Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) (Comisarow & Marshall, 1974; Marshall, Hendrickson, & Jackson, 1998) has been found to be a promising complementary tool to ESI ionization in order to expand the identification and quantification of chemical composition in complex natural mixtures. In this area, toluene has been dominantly applied as the dopant. For example, TA-APPI has been applied to obtain the total lipid profiling of ham in order to classify different types based on pig diet for fraud prevention (González-Dominguez, García-Barrera, & Gómez-Ariza, 2012). Other examples of this application describe the molecular composition of marine dissolved organic matter (DOM) (D'Andrilli et al., 2010), profile steroidal hormones in river and estuarine water samples (Yamamoto, Kakutani, Yamamoto, Kamiura, & Miyakoda, 2006) and provide compound-level assessments of marine dissolved organic matter (DOM) (Mopper, Stubbins, Ritchie, Bialk, & Hatcher, 2007), and reactive and refractory dissolved natural organic nitrogen compounds (Osborne et al., 2013).

In the field of petroleomics, TA-APPI FT-ICR MS has been successfully applied to obtain a detailed chemical characterization of heavy crude oils (YJ Cho, Na, Nho, & Kim, 2012), and a nonpolar sulfur speciation of higher-boiling fractions from petroleum crude oil without chemical derivatisation (J. M. Purcell, Hendrickson, Rodgers, & Marshall, 2006; J. M. Purcell, Juyal, et al., 2007); to study the chemical composition of the unconventional oil, shale oil (Bae et al., 2010; Y Cho et al., 2013); to find and quantify markers characteristic of the crude oil in order to identify its geochemical characterization and group the samples according to their geographical origin (Chiaberge et al., 2013); and to analyze the asphaltene

fraction of a crude oil (Andras Gaspar et al., 2012). In this last case, asphaltene was dissolved in toluene as a solvent which additionally served as the dopant to promote ionization. This study compared the profiles obtained by different API sources in order to describe the potential discrimination effects of different ionization techniques. In a previous work by the same research group, TA-APPI FT-ICR MS was applied to monitor the chemical transformations and mechanisms of asphaltenes hydrotreatment (J. Purcell et al., 2010). Marteau et al. recently developed a TA APPI to simultaneously analyze PBDEs, hydroxylated PBDEs (OH-PBDEs), and other PBDE metabolites (Marteau et al., 2012). The suggested method was proved to effectively monitor the metabolism process of BDE-47- incubated with human primary cultures of hepatocytes- through identification of parent and expected hydroxylated metabolites. In addition, the analysis led to characterization of other non-targeted metabolites. In the suggested method, using acetone and anisole as dopants led to similar results in terms of ionization intensities and patterns.

### **1.6.3.2 Ionization mechanisms of analytes with toluene-Assisted APPI**

Similar to acetone, toluene radical cations tend to enter water/solvent clusters in the presence of reversed-phase LC solvents. In this way, the resulting protonated clusters enhance the ionization of compounds having high proton affinities through proton transfer route. However, depending on the ratio of organic solvent in a LC system, some part of toluene radical cations may remain non-solvated in the system which promotes the ionization of non-polar compounds through charge transfer ionization route (Allegrand et al., 2012; Haapala et al., 2007; Hintikka et al., 2010; Itoh, Yarita, et al., 2009; Karst et al., 2006; Tiina J Kauppila, Kostiainen, et al., 2004; Tiina J Kauppila et al., 2002; McCulloch, Robb, & Blades, 2008; Mol, de Jong, et al., 2005; Mol, Jong, et al., 2005; Moriwaki, Ishitake, et al., 2004; Robb & Blades, 2006a; Robb et al., 2000; Ronsein et al., 2010; Smith et al., 2009). Therefore, toluene mainly enhances the ionization of polar compounds through a proton-transfer route and to some extent can promote a charge transfer route for ionization of non-polar compounds; however, it cannot provide the best efficiency for non-polar compounds.

### **1.6.3.3 Advantages of utilizing toluene as a dopant**

The main advantage of toluene as a dopant is its universality compared to other dopants in a way that it can promote the sensitivity of APPI toward compounds having both high and low proton affinities. However, the ionization of polar compounds through proton transfer route is much more dominant. This feature has made toluene the most commonly used dopant with the widest applications.

### **1.6.3.4 Limitations of utilizing toluene as a dopant**

The main disadvantage of utilizing toluene as a dopant, as discussed earlier, is that the ionization of compounds with low proton affinities are achieved in solvents with low PAs. It means that for ionization of non-polar compounds in a reversed-phase LC system, toluene is not efficient and other dopants may give better results. Another disadvantage for toluene is its poor miscibility with water; therefore, it is not the best fit for highly aqueous mobile phases. In addition, It has been reported that toluene increases the chemical noise along with increasing the signal intensity of analytes, leading to lower signal to noise ratios compared to acetone (S.-S. Cai, Hanold, et al., 2007; Miller et al., 2001; Sioud, Amad, & Al-Talla, 2012). For example, for the analysis of fat-soluble vitamins, not only toluene did not enhance the signal, but also led to an increased chemical noise. On the other hand, acetone did not increase the noise while it enhanced the signal intensities compared to free dopant analysis (Hanold et al., 2004). However, in a study by Cai. et al, the addition of both toluene and acetone did not change the baseline noise meaningfully, when a Waters Micromass Q-Tof-1 was used, but with using a Waters Micromass ZQ mass spectrometer, toluene caused an increased chemical noise. In that study, it was pointed out that many other factors may be involved in creating baseline noises, such as source geometry, lamp position, cone voltage, analyte, and mobile phase composition, which all need to be considered for an definitive conclusion (S.-S. Cai, Short, et al., 2007).

## 1.6.4 Anisole

### 1.6.4.1 The ranges of application for anisole as a dopant

Anisole as a dopant for APPI was introduced in 2004 by Kauppila et al (Tiina J Kauppila, Kostianen, et al., 2004) to enhance the photoionization of compounds with low proton affinity in the presence of reversed-phase LC mobile phases (Tiina J Kauppila, Kostianen, et al., 2004).

Anisole -assisted APPI (AnA-APPI) has been mostly utilized in the field of environmental analyses to improve the detection of polycyclic aromatic hydrocarbons (PAHs) (Ehrenhauser et al., 2010; Smith et al., 2009) in edible oils (Holloosi & Wenzl, 2011), polybrominated diphenyl ethers (PBDEs) and their metabolites in human hepatocytes (Marteau et al., 2012), and aldehydes in atmospheric aerosol particles (Ruiz-Jiménez et al., 2013).

In the fields of pharmaceutical and clinical analyses, AnA-APPI has been utilized to improve the determination of endogenous salsolinol and major catecholamines in brain tissue (Starkey et al., 2006).

In the field of biochemistry, AnA-APPI has been used to improve the ionization of biological molecules such as nucleobases, nucleotides and nucleosides (A Bagag et al., 2007), and to investigate the fragmentation behaviors of peptides under APPI conditions (Debois et al., 2006).

In the field of food analyses, AnA-APPI has been utilized to improve the detection of the fungus-specific marker, ergosterol, in *Fusarium*-infected wheat (Varga et al., 2006).

### 1.6.4.2 Ionization mechanisms of analytes with anisole-Assisted APPI

Due to anisole's high proton affinity, its photo-radical cations do not enter water and/or solvent clusters and remain unaltered in the presence of reversed phase LC solvents and can act as charge transfer reagents (Tiina J Kauppila, Kostianen, et al., 2004). Therefore, anisole is an effective dopant to promote the charge transfer ionization route for compounds having

lower IEs than that of anisole (Fredenhagen & Kühnöl, 2014; Kamel et al., 2008; Tiina J Kauppila, Ostman, et al., 2004).

#### **1.6.4.3 Advantages of utilizing anisole as a dopant**

Anisole radical cations are not depleted by background water and/or solvent molecules. Therefore, anisole is considered a good dopant for compounds with low PAs and relatively low IEs, for which the proton transfer route is not effective; these compounds could undergo direct charge transfer with anisole radical cations, if their IE is lower than that of anisole.

#### **1.6.4.4 Limitations of utilizing anisole as a dopant**

In a DA-APPI system and in the presence of LC solvents, a proportion of photo-dopant radical cations enter water/ solvent clusters and further form protonated analyte molecules through a proton transfer route and some remain stable and act as charge transfer reagents forming analytes' molecular radical cations. Therefore, most dopants can promote both ionization routes, i.e. proton transfer (for compounds with high PAs) and charge transfer (for compound with low PAs. However, anisole's radical cations do not enter water/ solvent clusters; therefore, with anisole a proton transfer route is not possible. On the other hand, its relatively low ionization energy, suppresses the ionization of compounds with higher IEs. In brief, anisole can suppress the ionization of compounds with high IEs and PAs; consequently, when a wide range of analytes are expected, mixing anisole with other dopants like toluene can compensate for this drawback (Fredenhagen & Kühnöl, 2014; A.-L. Zheng, Fan, Liu, et al., 2014; A.-L. Zheng, Fan, Wang, et al., 2014). In addition, it has been reported that anisole increased the proportion of fragment ions compared to other dopants and led to more complexity in the spectra, which may make the interpretation of unknown spectra more difficult. This fragmentation was ascribed to the instability of analyte molecular radical cations produced by anisole compared to protonated analytes (Yanxuan Cai et al., 2009; Tiina J Kauppila, Kostiainen, et al., 2004; Varga et al., 2006).

## 1.6.5 Tetrahydrofuran

### 1.6.5.1 The ranges of application for tetrahydrofuran as a dopant

In 2001, Rauha et al. tested tetrahydrofuran (THF) as a dopant in their report on the effect of eluent on the ionization efficiency of flavonoids by different API techniques including ESI, APCI, and APPI. Although the sensitivity of flavonoids analysis using toluene as dopant was about 1.3-2 times better than that obtained by THF, their results proved the promising potential of THF as a dopant for APPI (Rauha et al., 2001).

In 2005, Cai et al. reported the APPI MS analyses of 100 Wyeth proprietary drug candidates, representing different classes of analytes, with THF as the dopant (YX Cai, Herold, McConnell, & Bach, 2005). The enhanced signal intensity, up to 33-fold with THF compared to controlled direct APPI, confirmed the suitability of THF as a dopant. The same research group published a more detailed report on the suitability of THF as a dopant for APPI in 2009 (Yanxuan Cai et al., 2009). THF was delivered to the ionization source in two different ways: THF was added through the syringe pump at 1/10 of flow rate of the mobile phase which led to a 33-fold increase in the signal intensity of studied compounds. In addition, THF was introduced through the mobile phase at the flow rate of 100  $\mu\text{L}/\text{min}$ , which resulted in a 114-fold enhancement of the studied compounds. Compared to commonly used dopants, THF proved to work better than anisole, comparable to acetone and slightly less effective than toluene for the majority of studied compounds.

THF was evaluated as a dopant for simultaneous APPI MS analysis of endogenous salsolinol and major catecholamines in brain tissue of experimental rats along with acetone, anisole and toluene (Starkey et al., 2006). However, toluene and THF both proved to be ineffective in enhancing the photoionization efficiency and anisole, even worse, decreased the ionization efficiency compared to direct APPI. On the other hand, acetone significantly improved the ionization efficiency of the studied compounds.

### **1.6.5.2 Ionization mechanisms of analytes with Tetrahydrofuran-Assisted APPI**

The gas-phase ion energetic properties of THF are similar to those of acetone. Therefore, THF is also more effective for ionization of polar compounds through proton transfer events and may decrease or suppress the ionization of low polarity compounds (Yanxuan Cai et al., 2009; Rauha et al., 2001).

### **1.6.5.3 Advantages of utilizing Tetrahydrofuran as a dopant**

In addition to the classical method of post-column introduction of dopants, THF, which is a polar aprotic solvent, can also be added through the LC system as a mobile phase (Qian et al., 2002; Sanwald et al., 1995) or as a stronger organic modifier in the mobile phase (Andrés, Villanueva, & Tenorio, 2014; Miyabe, Sotoura, & Guiochon, 2001), because it has been shown that THF can improve the chromatographic separation of low-polarity (Bonfanti, Careri, Mangia, Manini, & Maspero, 1996) and weakly acidic compounds (Horváth, Gergely, Mazák, Kökösi, & Szász, 2013). In this way, THF acts as a dopant in the ionization source as well, and it improves the ion signals. This THF introduction method also addresses the limited capacity of syringes in post-column additions and enables non-stop addition of dopants.

### **1.6.5.4 Limitations of utilizing Tetrahydrofuran as a dopant**

THF radical cations do not remain stable in the presence of reversed-phase LC solvents and therefore, cannot promote the ionization of low-polarity compounds through charge transfer events. In addition, for proton transfer route, toluene was reported to give better results than THF (Yanxuan Cai et al., 2009; Rauha et al., 2001).

## 1.6.6 Substituted benzene compounds: Chloro- and bromobenzene

### 1.6.6.1 The ranges of application for chloro- and bromobenzene as dopants

In 2008, Robb et al. introduced a new class of dopants, which can promote the charge transfer ionization of non-polar compounds in the presence of common reversed-phase LC solvents (Robb, Smith, & Blades, 2008). They pointed out that for an effective charge transfer, dopant radical photo-cations must have a low reactivity with solvent and/or neutral dopant molecules in order to remain free and accessible in the source. In addition, dopants must have a high ionization energy to be able to ionize a broad range of analytes including the ones with high IEs. Several substituted-benzene compounds and fluoroanisole compounds were evaluated as potential candidates and chloro- and bromo-benzene were found to be promising dopants for ionization of non-polar compounds.

Later, Chloro- and bromobenzene were proven to be more effective in ionization of polycyclic aromatic hydrocarbons (PAHs) compared to toluene and anisole (Smith et al., 2009). Addition of 0.5% of either 2,4-difluoroanisole, and 3-(trifluoromethyl)anisole to both chloro- and bromobenzene slightly improved the overall sensitivity of the ionization.

Hexafluorobenzene ( $C_6F_6$ ) alongside toluene and anisole was used as a dopant to study the photo-induced fragmentation of peptides (Debois et al., 2006). According to this study, protonated peptides captured photo-electrons released upon photoionization of dopants leading to an electron capture dissociation/electron transfer dissociation (ECD/ETD) fragmentation pattern. Therefore, high ionization efficiency of dopant molecules led to intense photo-induced peptide fragmentation.

Among suggested substituted-benzene compounds used as dopants, chlorobenzene seems to show the highest effectiveness in enhancing photoionization; therefore, chlorobenzene has more applications according to the literature. For example, chlorobenzene was utilized as a dopant for the analysis of polycyclic aromatic hydrocarbons (PAHs) standards (S.-S. Cai, Syage, Hanold, & Balogh, 2009; Ramirez, Wang, & Gardinali, 2014) and in oyster samples (S.-S. Cai, Stevens, & Syage, 2012).

Recently, chlorobenzene was shown to effectively assist a microchip APPI interfacing GC to MS (GC- $\mu$ APPI MS/MS) for the analysis of anabolic androgenic steroids in urine samples. The steroids were identified as their trimethylsilyl (TMS) derivatives. Photo-ionized chlorobenzene radical cations promoted the charge transfer ionization route while inducing minimal fragmentation (Hintikka, Haapala, Kuuranne, Leinonen, & Kostiainen, 2013). Previous to this work, the same research group combined GC and MS through a modified commercially available APPI interface and applied the DA-APPI GC/MS in the analysis of trimethylsilylated neurosteroids in human urine samples. Toluene, anisole, and chlorobenzene were evaluated as dopants and since chlorobenzene led to the highest ionization intensity through charge transfer, it was adapted as the dopant (Suominen, Haapala, Takala, Ketola, & Kostiainen, 2013).

#### **1.6.6.2 Ionization mechanisms of analytes with chloro/ bromobenzene-Assisted APPI**

Under reversed-phase LC conditions, photo-radical cations of chloro- and bromobenzene remain partially stable in the source and therefore can act as charge transfer reagents (Fredenhagen & Kühnöl, 2014; Robb et al., 2008).

#### **1.6.6.3 Advantages of utilizing chloro/ bromobenzene as dopants**

Chloro- and bromobenzene possess relatively high IEs compared to other dopants; therefore, their radical cations can cover the ionization of a broader range of analytes through charge transfer routes (Fredenhagen & Kühnöl, 2014; Ramirez et al., 2014).

#### **1.6.6.4 Limitations of utilizing chloro/ bromobenzene as dopants**

Chloro- and bromobenzene are not effective in promoting the ionization of polar compounds, since these dopants do not promote the proton transfer route.

### 1.6.7 Utilization of mixture of dopants to enhance the ionization efficiencies

As generally accepted, there has not been a universal dopant that can effectively promote both charge transfer and proton transfer ionization routes. Therefore, multicomponent mixtures of different dopants have been proposed by different research groups in the hope of exploiting complementary features of dopants in order to achieve the most efficient possible ionization. For example, toluene has been proven to be the most effective dopant for proton transfer and it is known that anisole favors the charge transfer ionization route. For that reason, a mixture of these dopants can be capable of promoting both ionization routes and covering a broader range of analytes in a single run.

The mixture of toluene/anisole has been utilized in environmental analyses to improve the detection of polycyclic aromatic hydrocarbons (PAHs) (Itoh, Aoyagi, & Yarita, 2006), to investigate the ionization mechanism of PAHs, (Itoh, Yarita, et al., 2009) and to characterize coals' components (A.-L. Zheng, Fan, Liu, et al., 2014; A.-L. Zheng, Fan, Wang, et al., 2014). In addition 0.5% anisole in toluene was used to detect synthetic musks in real air samples (Lung & Liu, 2011).

A multicomponent dopant that contained 99% ethanol and 1% mixture of chlorobenzene, bromobenzene and anisole was applied to the non-targeted rapid analysis of PAHs (Sioud et al., 2012). The mixture not only increased the ionization efficiency of compounds by 2-10 fold compared to the results obtained by pure chlorobenzene, but also provided a less toxic dopant solution.

Recently, an anisole/toluene-assisted APPI was proven to give the best results for the analysis of aldehydes in atmospheric aerosol particles compared to results obtained when each of the dopants was used individually (Ruiz-Jiménez et al., 2013). Similarly (Chiaia-Hernandez, Krauss, & Hollender, 2013), a mixture of toluene/anisole (95:5, v/v) was used as dopant in order to include the ionization of less polar compounds within a method developed to screen lake sediments for emerging contaminants. The method aimed at a comprehensive inspection of targeted and suspected pharmaceuticals, personal care products, pesticides, biocides, additives, corrosion inhibitors, musk fragrances, UV light stabilizers, and industrial chemicals in sediments. DA-APPI coupled to a high resolution Orbitrap mass spectrometer (HRMS/MS), served as a complementary ionization approach to ESI and led to

characterization of two isomeric musk fragrances due to their different photoionization pathways.

### **1.7 Negative Ion- Atmospheric Pressure Photo Ionization (NI-APPI) and Dopant Assisted Negative Ion- Atmospheric Pressure Photo Ionization (DA-NI-APPI)**

In each ionization event, negative charges are formed along with positive ions and can be detected with appropriate polarity changes of the instrument. The use of APPI in the negative mode (NI-APPI) has been limited, simply because there are fewer analytes forming stable negative ions. However, analytes that effectively form negative ions generally lead to good quality results. In the negative ion mode, a logical argument is that thermalized electrons are the source of analyte ionization. Thermalized electrons could induce analyte ionization by direct capture or by ionizing oxygen molecules to form the highly reactive superoxide anions. In direct NI-APPI, electrons can be produced as a result of photoionization, and can also be ejected from metallic surfaces of the source as a result of the photo electric effect, especially in the "orthogonal" design (PhotoMate®, Figure 1.1), in which VUV lamp is directed toward the metallic surface (Basso, Marotta, Seraglia, Tubaro, & Traldi, 2003). In NI-DA-APPI, in which a high quantity of dopants are added, the photo-electrons released during the photoionization of dopants are considered the main source of electrons and the contribution of photoelectrons from metal surfaces is less important.

The ionization mechanisms in the NI-APPI and DA-NI-APPI (Basso et al., 2003; Delobel et al., 2006; Derpmann, Albrecht, & Benter, 2012; Desmazières, Legros, Giuliani, & Buchmann, 2014; Dzidic, Carroll, Stillwell, & Horning, 1974, 1975; Tiina J Kauppila, Kotiaho, Kostianen, & Bruins, 2004; Klee et al., 2014; Roy et al., 2006; L. Song, Dykstra, Yao, & Bartmess, 2009; L. Song, Wellman, Yao, & Adcock, 2007; L. Song, Wellman, Yao, & Bartmess, 2007; Takino et al., 2003b) have been studied thoroughly. Below, a summary of ionization pathways in the NI-APPI and DA-NI-APPI is presented in Table 1.3.

**Table 1.3 The main ionization routes in NI-APPI and DA-NI-APPI**

Reaction	Thermodynamical requirement
1 Formation of thermal electrons	$D + h\nu \rightarrow D^{*+} + e^{-}$ If IE (D) < 10 eV
2 Electron capture	$M + e^{-} \rightarrow M^{\bullet-}$ If EA (M) > 0
3 Formation of superoxide ions	$O_2 + e^{-} \rightarrow O_2^{\bullet-}$ None <sup>a</sup>
4 Charge Transfer	$O_2^{\bullet-} + M \rightarrow M^{\bullet-} + O_2$ If EA (M) > EA (O <sub>2</sub> ) = 0.45 eV
5 Proton abstraction via superoxide ion	$M + O_2^{\bullet-} \rightarrow [M-H]^{-} + HO_2^{\bullet}$ If $\Delta_{acid} G(M) < \Delta_{acid} G(HO_2^{\bullet})$ <sup>b</sup>
6 Deprotonation of solvent molecules	$S + O_2^{\bullet-} \rightarrow [S-H]^{-} + HO_2^{\bullet}$ If $\Delta_{acid} G(S) < \Delta_{acid} G(HO_2^{\bullet})$
7 Proton transfer via deprotonated solvent	$M + [S-H]^{-} \rightarrow [M-H]^{-} + S$ If $\Delta_{acid} G(M) < \Delta_{acid} G(S)$
8 Dissociative electron capture <sup>c</sup>	$M^{\bullet-} \rightarrow [M-H]^{-} + H^{\bullet}$ If $\Delta_{acid} G(M) < \Delta_{acid} G(H^{\bullet})$
9 Formation of oxidation products <sup>d</sup>	$M + O_2^{\bullet-} \rightarrow [M-X+O]^{-} + OX^{\bullet}$ $M + O_2 \rightarrow [M-X+O]^{-} + OX^{\bullet}$ X = H, F, Cl, Br, NO <sub>2</sub>
10 Halid-anion attachment: (a)dissociative electron capture,(b) anion attachment	(a) $CH_zX_{4-z} + e^{-} \rightarrow CH_zCl_{3-z}^{\bullet} \rightarrow CH_zX_{3-z}^{\bullet} + X^{-}$ (b) $X^{-} + M \rightarrow [M+X]^{-}$ X = Cl, Br

<sup>a</sup> oxygen, with a positive EA of 0.45 eV, can easily capture an electron forming a superoxide ion, which plays an important role in the negative mode.

<sup>b</sup> O<sub>2</sub><sup>•-</sup> is a relatively strong gas-phase base ( $\Delta_{acid} G(HO_2^{\bullet}) = 1451 \text{ kJmol}^{-1}$ )

<sup>c</sup> Directly following process 2. Dissociative electron capture could also play a role and enhance the role of O<sub>2</sub><sup>•-</sup> ions in the formation of [M-H]<sup>-</sup> ions. This process involves a hydrogen atom loss, which leads to a [M-H]<sup>-</sup> ion.

<sup>d</sup> The products of these reactions may undergo further oxidation and produce other oxidation products. For example, if X = H, further oxidation reactions can produce ions such as [M-2H+O]<sup>-</sup> and [M-2H+2O]<sup>-</sup>.

### 1.7.1 NI-APPI versus PI-APPI

- Although the negative mode has been far less popular compared to the positive mode, its unique dependence on the electron capture event adds another dimension of selectivity to the mass spectrometry-based analysis and can reveal specific profiles of samples that cannot be described by PI-APPI. This complementary role of NI-APPI is crucial when complex samples are analyzed. Therefore, using both positive and negative polarities could assist in obtaining a more comprehensive profile of the content of complex mixtures. An example of this application is the examination of both NI and PI spectra in the analysis of complex mixtures by APPI/FT-ICR/MS in order to obtain a more comprehensive image of their key components. Ultrahigh resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR/MS) is the current technique of choice to obtain molecular composition information on natural organic matter mixtures. Among other API techniques, the combination of APPI with FT-ICR/MS, and specifically investigating NI and PI spectra alongside each other, has been reported to give the most extensive molecular characterization of non-polar compounds in complex mixtures such as crude oil samples (Pereira et al., 2014; J. M. Purcell et al., 2006) and oil sands (Headley et al., 2014).
- For the analysis of fragile non-polar compounds like polymers, NI-APPI has been reported to produce intact ions, versus PI-APPI that has led to an extensive fragmentation. These intact stable anions are often halogenated adducts, which are produced in the presence of a halogenated solvent and a dopant like toluene through a halid-anion attachment route (Table 1.3). It has been shown that these anions are stable enough to be detected in the recorded spectra, in contrast to products of PI-APPI (protonated or cationized species, or radical-cations) that could undergo undesirable decompositions after their production in the source. In a mixture analysis, these decompositions cannot be ascribed to an individual compound and render the estimation of the average molecular masses and the correct assignments of ions

unreliable. It is worth noting that analysis of non-polar polymers is not possible or efficient by MALDI or ESI techniques, due to the lack of effective ionization sites for adduct formation, protonation, or deprotonation (Desmazières et al., 2014; Kéki et al., 2008; Kéki et al., 2008; Nagy et al., 2009; Terrier et al., 2011).

- NI-APPI usually offers far less background noise compared to PI-APPI, which could lead to lower limits of detection (Rauha et al., 2001).

### **1.7.2 Chemicals utilized as dopants in dopant assisted negative ion- atmospheric pressure ionization sources**

As mentioned earlier, in DA-NI- APPI, effective photoionization of a dopant, which produces a dopant radical cation and an electron, drastically increases the number of accessible thermal electrons that initiate all the ionization routes in the NI mode. Therefore, an efficient dopant for NI-APPI must meet two main criteria: (a) An efficient dopant cannot have a positive electron affinity, otherwise it would act as a sink for electrons, decrease the quantity of thermal electrons, and suppress the ionization of analytes and (b) An efficient dopant must have a high photoionization cross section, which leads to a high initial concentration of electrons.

Toluene, acetone and to some extent chlorobenzene are the dopants that have been utilized mainly in NI-APPI:

Toluene Assisted NI-APPI has been utilized in polymer analysis (Desmazières et al., 2014; Kéki et al., 2008; Kéki et al., 2008; Nagy et al., 2009) and in the determination of polychlorinated biphenyls in water samples (Moukas et al., 2014), underivatized estradiol in female serum and endometrium tissue samples (Keski-Rahkonen et al., 2013), indigo dyestuffs of historical importance (Malvina Papanastasiou et al., 2012), fullerenes (Núñez, Gallart-Ayala, Martins, Moyano, & Galceran, 2012), and halogenated flame retardants (Debrauwer et al., 2005) in fish (Zhou et al., 2010), in house dust (Lagalante & Oswald, 2008), in sewage sludge (Mascolo, Locaputo, & Mininni, 2010), and in environmental water

and industrial effluents (Bacaloni et al., 2009). In addition, Toluene Assisted NI-APPI has also been applied in detection of chlorothalonil in aqueous environment and food samples (Yamamoto et al., 2009), patulin in apple juice (Takino et al., 2003c), and analysis of organic explosives (L. Song & Bartmess, 2009).

Acetone Assisted NI-APPI has been applied for the determination of halogenated flame retardants (BFRs) in gull eggs (Letcher & Chu, 2010), in fish (Zhou et al., 2010), and in environmental water and industrial effluents (Bacaloni et al., 2009), avermectin and moxidectin residues in milk (Turnipseed et al., 2005), and patulin in apple juice (Takino et al., 2003c).

Chlorobenzene has been reported to provide good results for analysis of non-polar compounds in NI-APPI (Fredenhagen & Kühnöl, 2014).

### **1.8 APPI and DA-APPI used in GC-based MS analysis versus LC-based MS analysis**

As in the case for modern generations of liquid chromatography/ mass spectrometry (LC/MS) instrumentation that utilize API interfaces to perform ionizations under atmospheric pressure (Carroll et al., 1975; Robb et al., 2000; Syage et al., 2000; Whitehouse et al., 1985) innovative generations of GC/MS instruments are currently being developed facilitating the API techniques (Bristow, Harrison, & Sims, 2010; Carrasco-Pancorbo et al., 2009; Haapala, Suominen, & Kostianen, 2013; Hintikka et al., 2013; Luosujärvi et al., 2008; McEwen & McKay, 2005; Suominen et al., 2013). This groundbreaking generation of GC/MS-based analytical approaches combines all three outstanding analytical strategies together in the hope of achieving a comprehensive analysis. First, the new generation of atmospheric pressure ionization methods, which are primarily intended for LC/MS applications, can now be exploited with any GC. Second, using GC as the chemical separation domain, adds all of its superior advantages over LC to the analysis, including its better chromatographic resolution, higher peak capacities, versatility, comparative low cost of purchase and maintenance, ease of routine operation and maintenance, absence of matrix-dependent ion suppression, and green nature (Fritz, 1981). Third, with custom-built API interfaces, any high

resolution mass spectrometer can be easily coupled to GCs, and in turn, all of its impressive capabilities such as,  $MS^n$ , high mass resolution, and accurate mass measurements can benefit an analysis.

In addition to the general superior chromatographic performance of GC compared to LC, using GC as a separation technique for APPI brings some valuable advantages for the ionization itself: Unlike LC solvents, which often interfere with the ionization by inducing competing photo-induced side reactions and suppressing reactant ions (Tiina J Kauppila et al., 2005), the GC carrier gas simplifies the nature of eluting system offering a clean controllable ionization matrix.

Therefore, in GC/PI-APPI techniques, a significant increase in the probability of direct ionization of analytes is expected. This increase stems from eliminating radiation loss and interferences caused by LC eluents, which may diminish the crucial role of dopants in enhancing the ionization of analytes through saving the number of available photons. Moreover, because of the elimination of LC eluents, and in turn, solvent clusters, the proton transfer route is significantly limited and can only be accessible through background water molecules in the atmospheric volume of ionization chamber and/or dopant-function of some analytes. These fundamental differences make the charge transfer route the main ionization mechanism in PI-APPI coupled to a GC, and in turn, molecular radical cations of analytes become the dominant ions.

On the other hand, similar to LC/NI-APPI, in GC/NI-APPI thermal electrons are still the primary initiatives of all ionization routes and dopants could still play a crucial role in obtaining high sensitivities, because the addition of high quantity of dopants drastically enhances the quantity of thermal electrons, which are very limited otherwise. The utilization of dopants in GC/APPI-based analysis are reported in chapters 4 and 5.

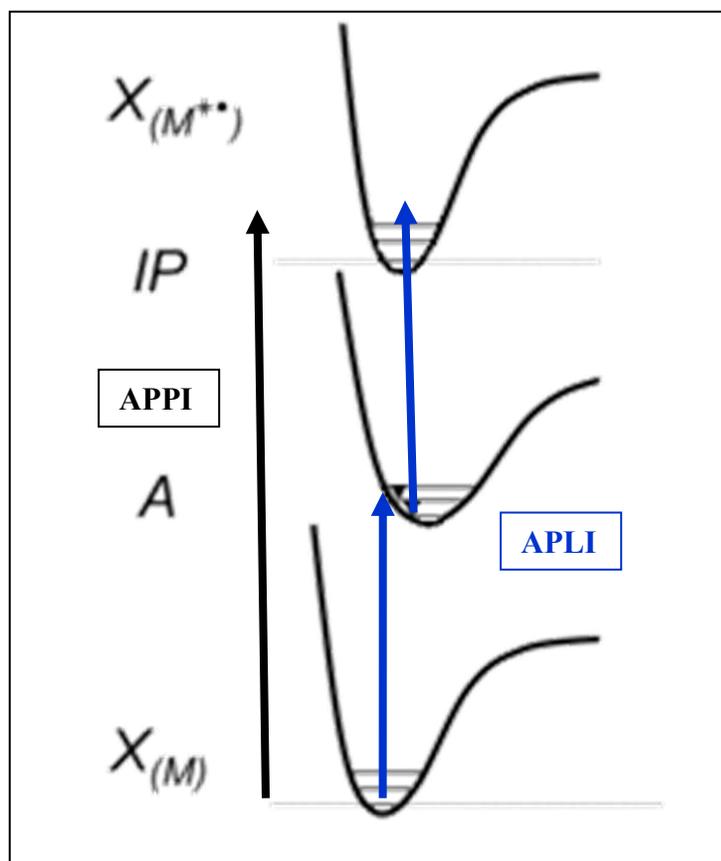
## **1.9 Atmospheric Pressure Laser Ionization (APLI)**

Laser ionization is a photoionization-based technique, but instead of one-step VUV approach (APPI), resonantly-enhanced multi-photon ionization (REMPI) is utilized as the primary ion-production mechanism. Excitation pathways in REMPI and other possible interfering processes, such as deactivation and photo-dissociation events have been thoroughly

explained (Kühlewind, Boesl, Weinkauff, Neusser, & Schlag, 1983; ROBIN, 1980). Equation 1 and Equation 2 present a classical (m+n) stepwise excitation in REMPI:



Although higher-order processes have been reported for specific applications, for common analytical purposes, usually  $m = 1$  and  $n = 1$ , that is, one-color (1+1) REMPI meets the requirements and provides sufficient efficiencies (Figure 1.2). To have a strong signal under laser ionization, a compound must have: (a) high linear absorption coefficients in the applied wavelength region, (b) long-lived intermediate electronic states at the energy level of the first photon, so that the subsequent absorption of further photon(s) and ionization from this level is highly probable, and (c) a high threshold fragmentation energy, so that the decomposition of generated ions under absorption of further photons, i.e., at high photon density conditions, is not possible (Kühlewind et al., 1983; ROBIN, 1980). The latter however is far less restrictive in APLI {i.e., (1+1) REMPI} in comparison to the general (m+n) REMPI scheme since the employed laser power densities in APLI are comparably low and fragmentation is generally no issue.



**Figure 1.2** The two-step APLI ionization mechanism versus the one-step ionization mechanism in APPI

For the first time, in 2005, Constapel et al. reported an *analytical* application of the resonant two-photon ionization at *atmospheric pressure* utilizing a commercially available API interface (Atmospheric Pressure Laser Ionization (APLI)). In their design, ionization occurred in an API chamber, which was upstream from the sampling cone of the mass spectrometer. Then by applying an external electrical field, the laser-generated ions were directed toward the sampling cone of the mass spectrometer (Constapel et al., 2005).

Two-step ionization process in APLI offers some advantages over one-step VUV ionization in APPI: (a) Atmospheric gases and commonly used LC solvents show very small linear absorption cross sections within the near-UV wavelength range used for excitation in APLI and are thus safely considered transparent in this region. Therefore, in APLI applications with LC or flow injection analysis, significant photon loss such as that which occurs in LC/APPI techniques does not occur. This transparency of LC solvents also eliminates the

possible parallel unwanted reactions, such as solvent isomerization, which may adversely affect the probability of direct ionization of analytes.

Moreover, due to high IEs of atmospheric gases and LC solvents, at least three photons are required for their ionization, which is unlikely with the laser power densities usually used in APLI, because the laser power density in APLI is adjusted close to the threshold of (1+1) REMPI excitation. These conditions minimize the excitation and ionization of matrix components, and therefore, regardless of the matrix composition, almost the entire photon flux becomes accessible for direct ionization of analytes.

(b) Two-step ionization provides a highly selective ionization towards compounds having stable electronic states at the energy level of the first photon such as PAHs. This selectivity, which is tied in with greatly reduced chemical noise, leads to significantly reduced limits of detection. (Constapel et al., 2005) (c) In addition, due to a highly increased photon flux in APLI, the ionization efficiencies and sensitivities are greatly increased.

Polycyclic aromatic hydrocarbons (PAHs), which possess unique spectroscopic features with respect to (1+1) REMPI, are effectively ionized by APLI (Boesl, 2000; Lubman, 1990). In PAHs, the aromatic ring, which acts as the chromophore, facilitates the resonant (1+1) ionization in near-UV absorption bands, forming long-lived intermediate states and highly vertical ionization transitions. IEs of PAHs are often lower than the sum of the energy of two photons applied; therefore, ionization easily occurs from the intermediate states. The absorption coefficients for these transitions are large enough, thus relatively low laser power densities lead to the production of molecular ions without fragmentations. Very few other compounds show these features; therefore, APLI offers exceptional sensitivity and selectivity for PAHs.

### **1.9.1 APLI applications**

APLI is considered the superior technique among all API methods for the analysis of non-polar polymeric and polycyclic aromatic compounds such as PAHs. Therefore, the analysis of these compounds establishes the principal field of applications for APLI. For the analysis of PAHs, APLI has been coupled to LC, GC (Arthen-Engeland & Dunsbach, 2008; R

Schiewek et al., 2007; Stader, Beer, & Achten, 2013), and capillary electrochromatography (CEC) (Droste et al., 2005). In the latter, a combination of ESI and APLI was used as a multimode source; sample stream was first volatilized by ESI and subsequently was ionized by APLI. This design enabled the analysis of thermally labile non-polar aromatic compounds without decomposition; because instead of using the heated APCI probe, ESI was used for volatilization (Droste et al., 2005).

Although the selectivity of APLI toward non-polar polymeric and polycyclic aromatic compounds could limit its analytical applications; this selectivity could be employed in favor of analyses of complex mixtures and reduces the errors associated with ionization of competing matrix components. This quality of APLI could address the necessity of hyphenated techniques such as two-dimensional chromatographic separations or stable-isotopic standards, which are used to achieve a higher degree of separation of sample components and correct for competing ionization processes, respectively. One example of these complex mixtures is crude oil, which also contain high amounts of non-polar aromatic hydrocarbons. Different API methods, separately or in hybrid as multimode sources, have been coupled to ultrahigh-resolution FT-ICR-MS in order to obtain best representation of the key components of crude oil. Among all techniques, APLI has also been proven to provide unique complementary quantitative and qualitative information about non to very low-polar aromatic hydrocarbons, sulfur species and low-polarity oxygen species in crude oils with the minimum amount of sample preparation work-up (A Gaspar, Zellermann, Lababidi, Reece, & Schrader, 2012; Lababidi, Panda, Andersson, & Schrader, 2013; Panda, Brockmann, Benter, & Schrader, 2011; Schmitt-Kopplin et al., 2008; Schrader, Panda, Brockmann, & Benter, 2008).

In 2008, Schiewek et al. used a derivatisation strategy to broaden the analytical applicability of APLI for compounds whose spectroscopic features do not favor laser ionization. In their work, anthracene-9-ylmethoxyacetic acid and anthracene-9-ylmethanol were used as APLI ionization labels to derivatize alcohols, amines, and organic acids into complexes that are active toward ionization mechanism of APLI. The APLI-labeled complexes showed spectroscopic features and ionization yields equivalent to those of the label (Ralf Schiewek et al., 2008).

### 1.9.2 Use of dopants in APLI

Application of dopants in APLI has been reported only in a few studies, because the technique has been only used recently. In 2008, Schmitt-Kopplin et al (Schmitt-Kopplin et al., 2008). reported on the application of a multimode ion source consisting of a chip-ESI (cESI) in combination with an APLI (cESILI) coupled to FT-ICR/MS for the analysis of crude oil samples. cESI ionization mode (laser off) enabled the ionization of polar compounds and cESILI ionization mode (laser on) facilitated the ionization of non-polar aromatic compounds; therefore, with simultaneous application of these ionization techniques, polar and non-polar aromatic compounds were ionized alongside each other. In the sample preparation process, toluene, in a concentration of 7%, was used to assist the dissolving the sample. Similar to one-photon ionization of toluene in APPI, two-photon ionization of toluene with cESILI generated the same ion reagents, i.e. toluene radical cations. Therefore, it was hypothesized that these reagent ions could evolve the same ion-chemistry ionization mechanisms as in APPI and develop proton and charge transfer ionization routes, which could facilitate the ionization of aliphatic non-polar compounds. The detection of additional signals by the multimode ion source (cESILI), which were assigned to protonated analyte molecules, verified this hypothesis. These compounds either possess sufficient PAs, which make their ionization possible through a proton transfer route under DA-APLI or have strongly high gas-phase basicities, in the level of PAHs, which makes them ionizable. In addition, they investigated DA-cESILI in the NI mode to ionize a nitro-PAH compound, 1-nitrocoronen, which was not ionized in PI-DA-cESILI. With the laser off, the compound was not ionized by ESI mode, while with DA-cESILI, both  $[M]^+$  and  $[M-H]^+$  were observed, which could have been produced through the same ionization mechanisms described for NI-APPI (Table 1.3) (Schmitt-Kopplin et al., 2008).

## 1.10 Theoretical and practical contribution of thesis to the field of photoionization

The key advantage of APPI, which was the major motivation for its primary development, is its ability to ionize less polar and non-polar compounds, which are not well addressed by ESI and APCI. In both ESI and APCI, the ionization mechanisms favor ionization of polar analytes. Therefore, both techniques are not very successful in ionizing non-polar compounds, such as polycyclic aromatic hydrocarbons, steroids, hydrophobic peptides, and non-polar polymers.

In contrast to ESI and APCI, photoionization of a compound is based on the interaction of a compound with a photon of sufficient energy; as long as the compound absorbs a photon and its IE is less than the energy of the photon, ionization typically occurs regardless of the polarity of the compound.

With the addition of dopants, ionization efficiencies of analytes significantly increases; however, ionization mechanisms changes from direct photoionization to ion-molecule chemical reactions, which can lead to losing the initially proposed most favorable feature of APPI, which is ionization that does not bias against polarity. Practically this was found to be the case in DA-APPI, as some dopants form proton bound clusters, which favor the ionization of polar compounds. This led to questioning APPI. *Is APPI really able to cover the gap and ionize non-polar compounds that cannot be achieved by other API techniques, as was promised?* My thesis contributed to answering this question and confirmed that APPI is able to cover the gap and ionize non-polar compounds.

Between the two main ionization routes in DA-APPI in PI mode, i.e. charge transfer and proton transfer routes, charge transfer route is only governed by the IEs of involved species, while proton transfer route, through the formation of proton-bound clusters, favors the ionization of polar compounds. Therefore, the key in keeping the ion-molecule chemical reactions in DA-APPI sensitive toward ionization of non-polar compounds is to maximize the charge transfer route and minimize the proton transfer route.

In the first phase of my PhD research, dopant applications were modified in order to maintain the charge transfer route as the main ionization route in DA-APPI and minimize the proton transfer route.

In the second phase of my PhD research, the contributions of other analysis factors to maximize the charge transfer route in DA-APPI were explored. These factors included (a) chemical separation technique (using a gas chromatograph vs. a liquid chromatograph) and (b) ionization source (using an airtight custom-built APPI source vs. a commercial non-sealed APPI source). Using a GC as the separation technique simplified the nature of eluting system by removing LC eluents and using the custom-built airtight APPI minimized the presence of background water in the source. These elements minimized the formation of proton-bound clusters, which led to establishing the charge transfer as the main ionization route in DA-APPI.

In addition, the knowledge obtained through the comprehensive review on dopants, their characteristics, their role in enhancing the ionization efficiencies in different ionization techniques, and ionization mechanisms led to the introduction of two novel dopants: carbon disulfide for PI-APPI and isoprene for NI-APPI. The suitability of these chemicals as dopants for APPI coupled to either a LC or a GC was investigated and is presented in chapters 2-5. Finally, the utilization of dopants in APLI coupled to a GC was investigated and is presented in chapter 6.

## **2 Chapter: Carbon Disulfide as a Dopant in Atmospheric Pressure Photoionization Mass Spectrometry Coupled to a HPLC**

### **2.1 Abstract**

Carbon disulfide (CS<sub>2</sub>) is introduced as a novel APPI dopant based on its absorption cross section at the commonly used VUV radiation wavelength used and its unique gas phase ion chemistry, notably the fact that it does not contain a proton. The ability of CS<sub>2</sub> to enhance the ionization effectiveness of APPI was tested by using a group of compounds that have different proton affinities (PAs) and ionization energies (IEs). These results were compared to results obtained using the most commonly used dopants, toluene and anisole. Particular attention was paid to the formation of M<sup>+</sup> ions relative to [M+H]<sup>+</sup> ions. Mass spectra were collected using a Waters Quattro Premier LC/MSMS system equipped with a commercial Photomate™ Photoionization source. The results show that CS<sub>2</sub> increases the ionization efficiency of most of the analytes studied in this work comparably to toluene and anisole. CS<sub>2</sub> promotes both ionization routes of M<sup>+</sup> and [M+H]<sup>+</sup>. In addition, due to the higher IE of CS<sub>2</sub> (10.01) compared to the IE of toluene (8.83) and anisole (8.20), CS<sub>2</sub> can enhance the ionization efficiency of analytes that cannot be enhanced with toluene and anisole. In this work it is shown that CS<sub>2</sub> is a viable dopant for use in APPI sources. For some analytes, significant [M+H]<sup>+</sup> ion signals are observed; therefore, the donated proton must come from either water clusters or solvents. In addition, CS<sub>2</sub> promotes the ionization of analytes with low PAs and higher IEs than that of toluene and anisole.

### **2.1 Experimental**

#### **2.1.1 Chemicals and Reagents**

The structures of the compounds studied in this work are shown in Figure 2.1. Some relevant thermodynamical data for the solvent, dopants, and compounds used in this study are presented in Table 2.1. The ionization energy (IE) and proton affinity (PA) values were obtained from the NIST Chemistry WebBook (NIST Standard Reference Database Number

69). However, full gas-phase energetic data for all the compounds studied were not available and some estimates had to be made using experimental data for structural analogs.

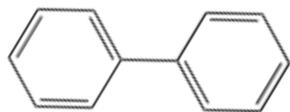
Acetonitrile (ACN), Methanol (MeOH), carbon disulfide, toluene, and anisole (all HPLC grade) were purchased from Fisher Scientific Canada. All other chemicals were of the highest purity available, and were used as received.

**Table 2.1 Ion energetic properties of the solvents and compounds studied**

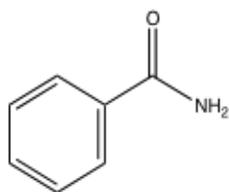
Compound	IE <sup>a</sup> (eV)	PA <sup>a</sup> (kJ/mol)
Toluene	8.83	784.0
Anisole	8.20	839.6
Carbon Disulfide	10.01	681.9
Acetonitrile	12.2	779.2
Water	12.62	691
Biphenyl	10.84	754.3
Benzamide	9.25	892.1
Benzocaine	7.42 <sup>b</sup>	993.6 <sup>b</sup>
Butylated hydroxyanisole (BHA)	7.94 <sup>b</sup>	>840 <sup>b</sup>
Phenanthrene	7.89	825.7
Vanilin	7.76 <sup>b</sup>	990.1 <sup>b</sup>
Caffeine	7.95	~ 920 <sup>b</sup>
Creatinine	8.62 <sup>b</sup>	>942.8 <sup>b</sup>
2-Naphthol	7.89	870 <sup>b</sup>
9- Methylanthracene	7.31	896.5
Diphenylmethane	9.4	802.0
1,4-Dibromobenzene	8.82	758 <sup>b</sup>
1,2,4,5-Tetrachlorobenzene	9.0	761 <sup>b</sup>
3-Methoxybenzoic acid	9.1	910.3 <sup>b</sup>
2,4,6-Trichloroanisole	8.69 <sup>b</sup>	848 <sup>b</sup>

<sup>a</sup> IE, ionization energy; PA, proton affinity

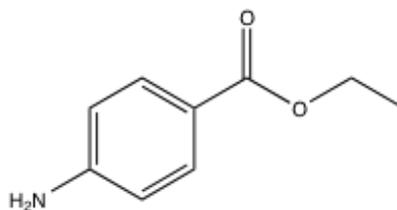
<sup>b</sup> The PA and IE of compounds which for NIST data are not available, has been estimated considering the PA and IE of the structural analogs, with data from the reference. (NIST Standard Reference Database Number 69) For example, PA of 3-methoxybenzoic acid has been estimated using the data available for benzoic acid, benzene and anisole.



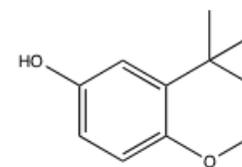
Biphenyl (1)  
MW = 154



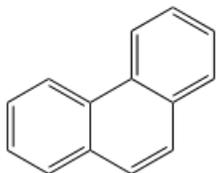
Benzamide (2)  
MW = 121



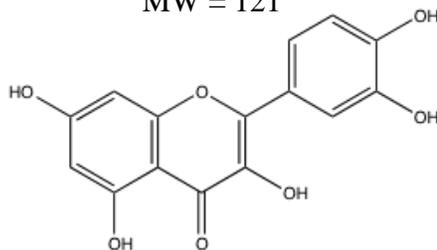
Benzocaine (3)  
MW = 165



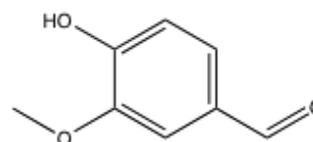
Butylated hydroxyanisole (4)  
MW = 180



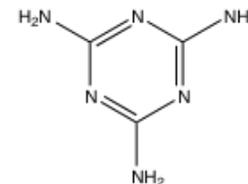
Phenanthrene (5)  
MW = 178



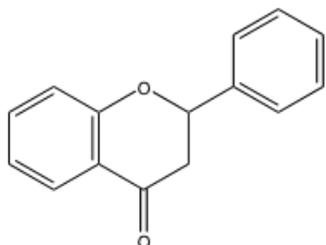
Quercetin (6)  
MW = 302



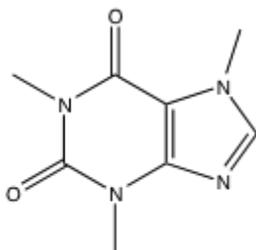
Vanillin (7)  
MW = 152



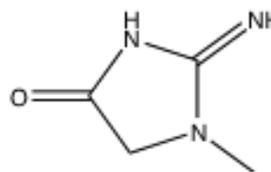
Melamine (8)  
MW = 126



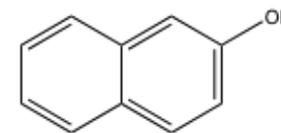
Flavanone (9)  
MW = 224



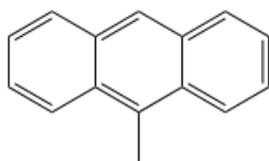
Caffeine (10)  
MW = 194



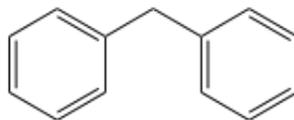
Creatinine (11)  
MW = 113



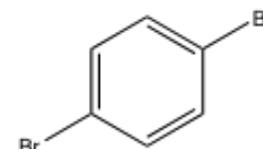
2-Naphthol (12)  
MW = 144



9-Methylanthracene (13)  
MW = 192



Diphenylmethane (14)  
MW = 168



Dibromobenzene (15)  
MW = 234

Figure 2.1 Structures of the studied compounds

### 2.1.2 Sample preparation

All standard compounds were prepared at 100-200 mg/L in acetonitrile as stock solutions. The stock solutions were diluted in acetonitrile to final concentrations of 5-10 mg/L.

### 2.1.3 Instrumentation

Mass spectra were collected on a Waters Quattro Premier LC/MSMS system. This system is equipped with a Photomate™ Photoionization source. The Photomate ion source utilizes a low-pressure krypton VUV lamp. High-purity nitrogen was produced from a Parker-Balston Analytical gas generator (model 75-72) and was used as nebulizer, cone, and desolvation gas. High purity argon was obtained from a compressed gas cylinder of ultra-high purity Argon (XRP grade) from Air Liquide and was used as collision gas. The APPI source was operated in the positive ion mode and experimental parameters of repeller voltage, cone voltage, extractor voltage, and RF lens voltages were optimized based on the relative abundances of the most intense peak for each compound. Desolvation and cone gas flow rates were kept constant for all experiments at 50 and 150 L/h respectively. The temperature of the nebulizer was 400°C for all the experiments and mass spectra were collected in a full scan mode with a mass range of  $m/z$  2–800.

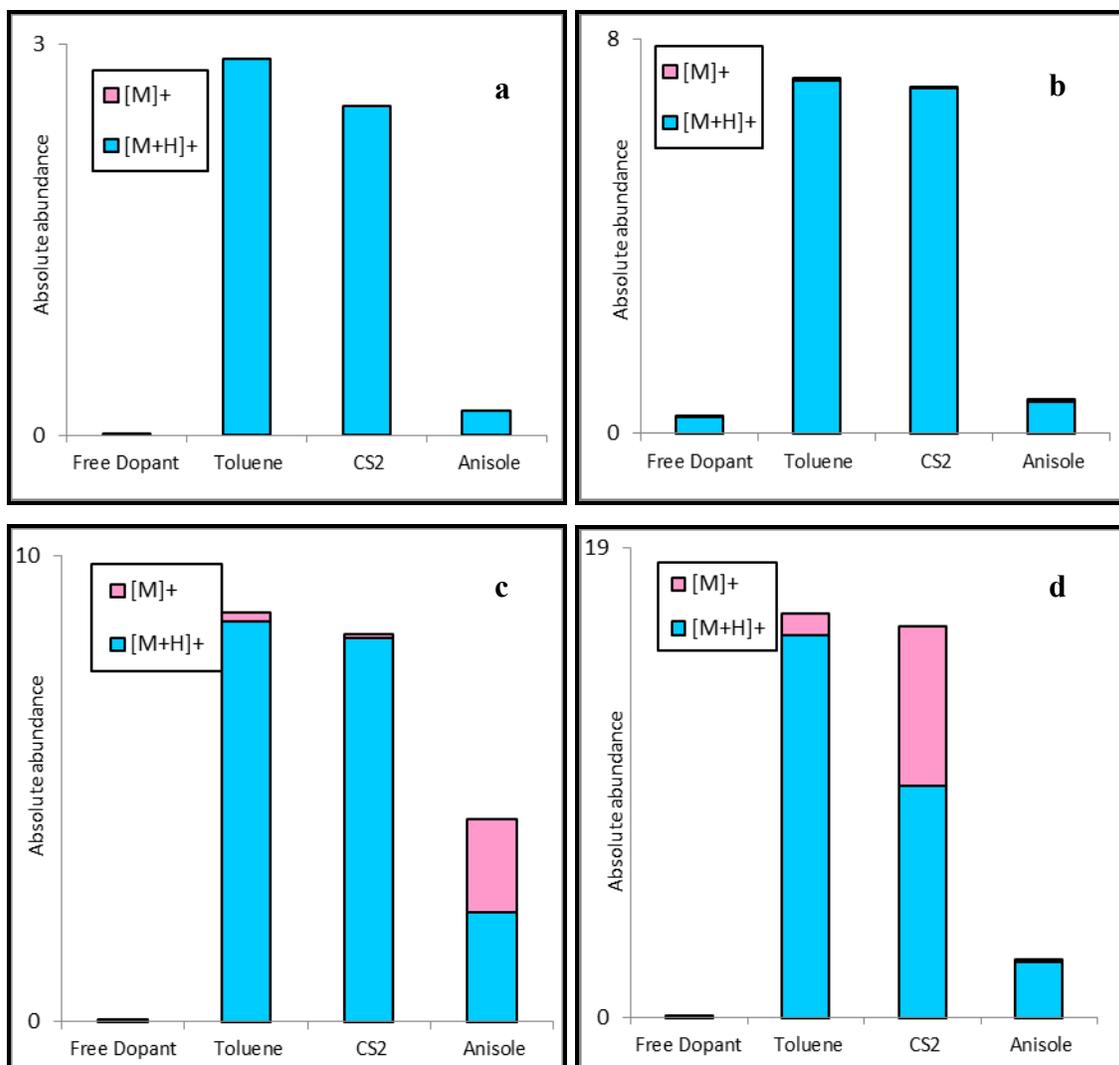
Acetonitrile and methanol were each used as a mobile phase in the isocratic mode. The flow rates of the solvents were 200  $\mu\text{L}/\text{min}$  for all experiments. Samples were injected by using a 5  $\mu\text{L}$  loop into a continuous solvent stream. The dopant flow rate was 20  $\mu\text{L}/\text{min}$  and was continuously introduced into the ion source through a fused-silica capillary by a syringe pump to be mixed with the mobile phase in a tee. Tandem mass spectrometric (MS/MS) measurements were carried out in the daughter scan mode. The scan range was from  $m/z$  50 to the  $m/z$  of the precursor ion + 50. Collision energy was varied between 10 and 60 V, depending on the stability of the precursor ion.

## 2.2 Results and discussion

### 2.2.1 Suitability of CS<sub>2</sub> as dopant

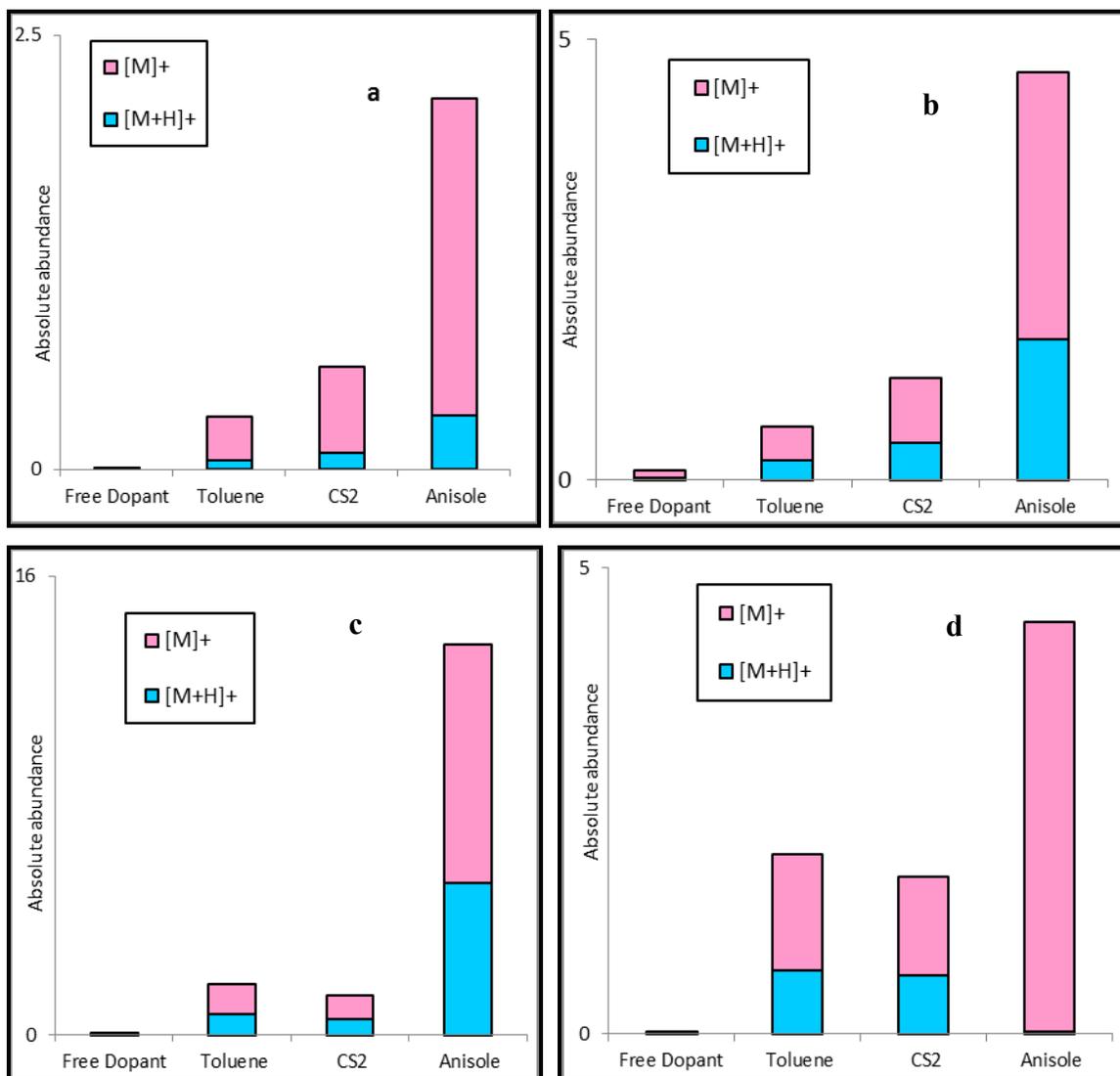
In order to investigate the suitability of carbon disulfide as a dopant for APPI, several analytes with various ionization energy and proton affinities (Figure 2.1) were analyzed in the positive mode and the results were compared to the results obtained with toluene and anisole as dopants.

The mobile phases used in this study were acetonitrile and methanol. All three of the dopants examined, toluene, anisole, and carbon disulfide, produced enhanced analyte signals in acetonitrile. Therefore, acetonitrile was used as a common eluent. Each compound was analyzed three times without dopant as a control, and three times with each of the dopants. Figure 2.2 shows the typical compounds that exhibited significant signal enhancements using toluene and carbon disulfide as dopants. As can be seen in Figure 2.2, these compounds have high PAs and the ion represented by  $[M+H]^+$  is dominant in their spectra.



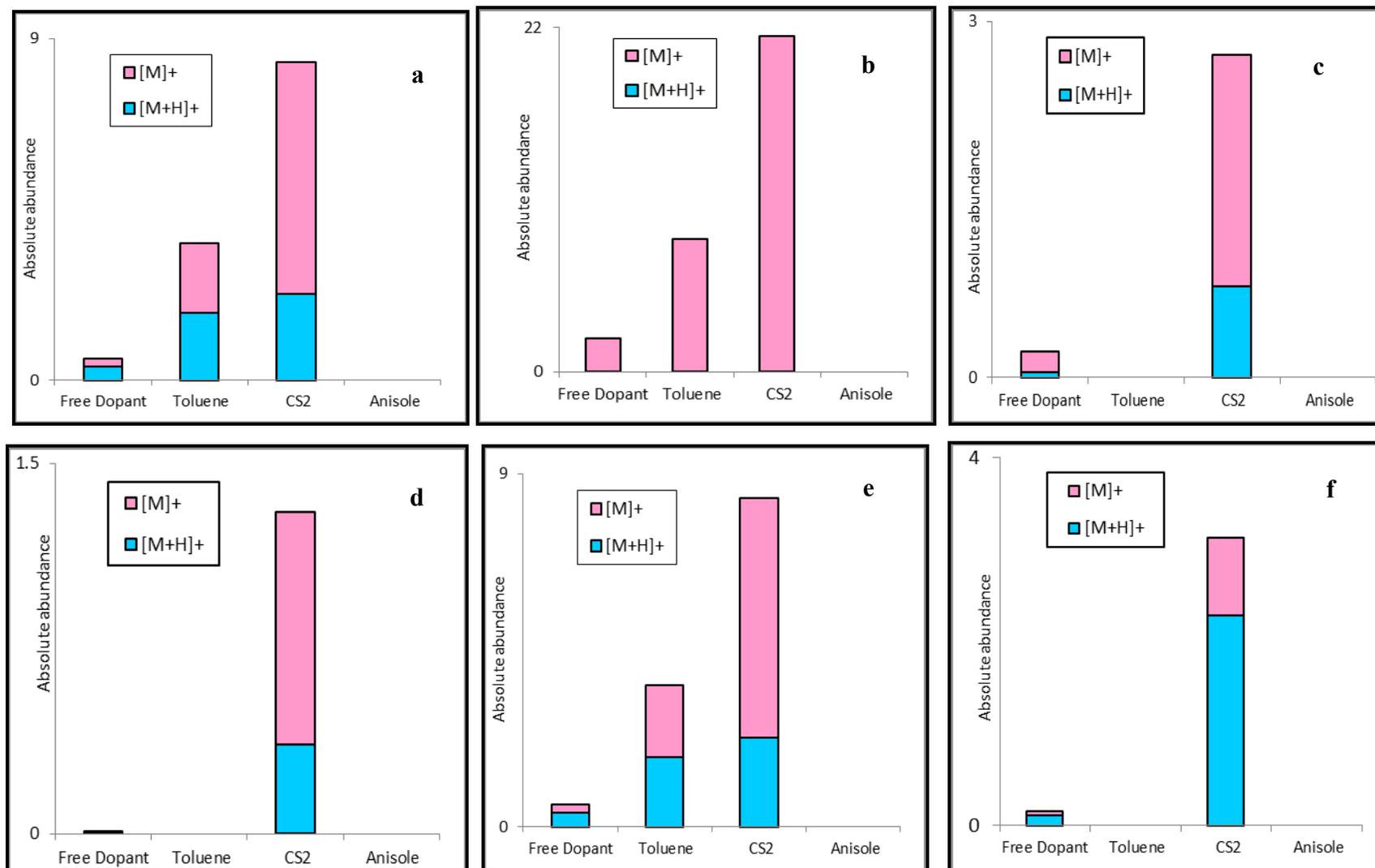
**Figure 2.2** Absolute abundance of the total ion currents and relative proportions of  $M^+$  and  $[M+H]^+$  for benzamide (a), melamine (b), caffeine (c), and vanillin (d) in ACN in the positive mode. Experiments were done in five replicates. Repeatability was assessed by calculating percent RSDs between areas of all five replicates, which was less than 5% for analytes.

Figure 2.3 shows a set of compounds that have the highest ionization rate with anisole as dopant compared to the results obtained with toluene and carbon disulfide. Each of these four compounds, 2-naphthol, phenanthrene, 9-methylanthracene, and butylated hydroxyanisole (BHA) all have low ionization energies, which are closely coupled to that of anisole. In the spectra of BHA and 2-naphthol, the  $M^{*+}$  ions are dominant. In the spectra of phenanthrene and 9-methylanthracene,  $[M+H]^+$  ions have the same intensity as  $M^{*+}$  which is explained by their comparably high PA.



**Figure 2.3** Absolute abundance of the total ion currents and relative proportions of  $M^{++}$  and  $[M+H]^+$  for 2-naphthol (a), phenanthrene (b), 9-methylanthracene (c), and BHA (d) in ACN in the positive mode. Experiments were done in five replicates. Repeatability was assessed by calculating percent RSDs between areas of all five replicates, which was less than 7% for analytes.

Figure 2.4 presents the analytes that have the best ionization efficiency with carbon disulfide as the dopant. The ionization energy (IE) of CS<sub>2</sub> is 10.01 eV, and analytes with IE of less than this value can be ionized via a simple charge transfer. Therefore, CS<sub>2</sub> can ionize analytes with a higher IE than anisole and toluene (8.83 < IE < 10.01). Examples of these compounds are biphenyl, diphenylmethane, 1,4-dibromobenzene, 1,2,4,5 tetrachlorobenzene, 3-methoxybenzoic acid, and 2,4,6- trichloroanisole all of which exhibit much higher intensities with CS<sub>2</sub> as dopant.

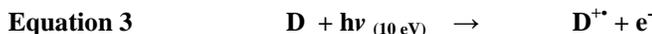


**Figure 2.4** Absolute abundance of the total ion currents and relative proportions of  $M^{++}$  and  $[M+H]^+$  for biphenyl (a), diphenylmethane (b), 1,4-dibromobenzene (c), 1,2,4,5-tetrachlorobenzene (d), 3-methoxybenzoic acid (e), and 2,4,6-trichloroanisole (f) in ACN in the positive mode.

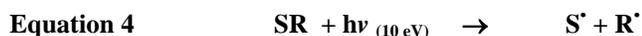
Experiments were done in five replicates. Repeatability was assessed by calculating percent RSDs between areas of all five replicates, which was less than 10% for analytes.

**In APPI, the photon energy is absorbed by the dopant molecules resulting in the formation of reactive radical cations as described in**

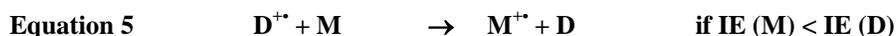
Equation 3.



It should be noted that other molecules in the gas phase matrix of the ion source can also absorb the available photon energy to undergo photoionization, photodissociation or photo-induced reactions to produce reactive species. Solvent molecules that are present in relatively high concentration are of particular concern to undergo photodissociation. However, in typical LCMS practice, the solvents selected commonly have ionization energies higher than 10 eV. Therefore, the extent of photoionization is minimized, although studies have shown that acetonitrile can be isomerized to the species with ionization energy lower than 10 eV which can take part in the ionization routes (Marotta et al., 2003). However, the photodissociation of solvents or other matrix species is described in Equation 4, where SR represents the solvent species. Reactions such as these lead to non-ionized reagents and therefore are more difficult to monitor but should not be ignored when considering the chemistry in the source as the radical species produced by these reactions could undergo radical chain reactions and produce a diverse range of chemical species.



The ionic reagents generated by the dopant can lead to analyte (M) ionization in a number of ways, for example if the IE of the analyte (or other molecules in the matrix) is lower than that of dopants, charge transfer between analyte molecules and dopant can occur by Equation 5.



**It is well established that gas phase ions, such as the dopant ions generated by**

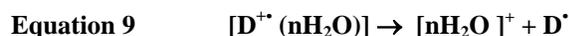
Equation 3, will be readily solvated in the gas phase to form solvent clusters or clusters with water that is ubiquitous under ambient conditions. These reactions are described in Equation 6 and Equation 7.



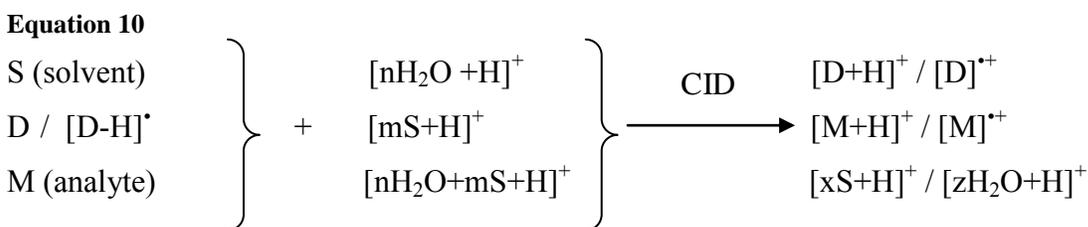
It is possible that if the dopant contains a proton, it can be deprotonated by the cluster as described in Equation 8;



It is also possible that the dopant could simply transfer its charge to the cluster as described in Equation 9 below. In the case of dopants without a proton, this is the only charge transfer reaction possible with clusters.



There are analogous Reactions to Equation 8 and Equation 9 that could occur with solvent ions if they are produced. The charged clusters formed by reactions such as Equation 8 and Equation 9 above can further undergo ligand switching with solvent, deprotonated dopant, non-photoionized dopant, and analyte molecules depending on the polarity of these compounds. The collision-induced dissociation (CID) created by the electrical field in the mass spectrometer's source dissociates these clusters and creates solvent/dopant/analyte protonated cations that are observed in the final spectra.

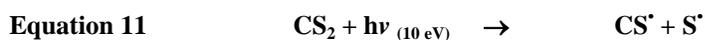


In the API sources, it is safe to assume that there is a thermodynamic equilibrium between dopant radical cations that are clustered with surrounded molecules and dopant radical cations which do not enter the clusters. Our data confirms that the latter plays the main role

in the promotion of formation of  $M^{++}$  via charge transfer mechanism. In this case, depending on the IE of dopant compared to analytes (Equation 5), each dopant can assist the ionization of different classes of analytes that have IE's that are less than that of the dopant.

### 2.2.2 Reagent chemistry generated by $CS_2$

As is demonstrated by the spectra of  $CS_2$  with ACN (Figure 2.5) the presence of  $CS_2^{++}$  does result in the formation of protonated solvent/water clusters. As a result, the observed  $[M+H]^+$  analyte signals could result from the interaction with these or isomerized protonated ACN molecules. It is also clear that  $CS_2$  can photodissociate via a mechanism described in Equation 11:



In this case, each of the radicals generated could enter into cluster complexes as well. I can safely assume that  $CS_2^{\bullet}$  radicals, neutral  $CS_2$  and solvent molecules are dominant in the source. In addition, it appears that there is a sufficient quantity of  $CS_2^{++}$  radical cations available to enable analyte ionization by the charge transfer reaction described by Equation 5. Given the higher IE of  $CS_2$  (10.01) compared to that of toluene (8.83) and anisole (8.20), the use of  $CS_2$  as a dopant can result in the ionization of analytes with a wider range of ionization energies ( $8.83 < IE < 10.01$ ). In Figure 2.4, a series of compounds were presented, which meet this criteria, i.e., biphenyl, diphenylmethane, 1,4-dibromobenzene, and 1,2,4,5-tetrachlorobenzene. In these cases,  $M^{++}$  ions are dominant which implies that the charge transfer reaction described by Equation 5 is the main ionization mechanism. In the spectra of 3-methoxybenzoic acid, and 2,4,6-trichloroanisole which have both high IE and higher PAs, significant  $[M+H]^+$  ions are also seen. This implies that their higher PAs favor the ligand switching with protonated solvent/water clusters and in turn leads to the formation of  $[M+H]^+$  ions.

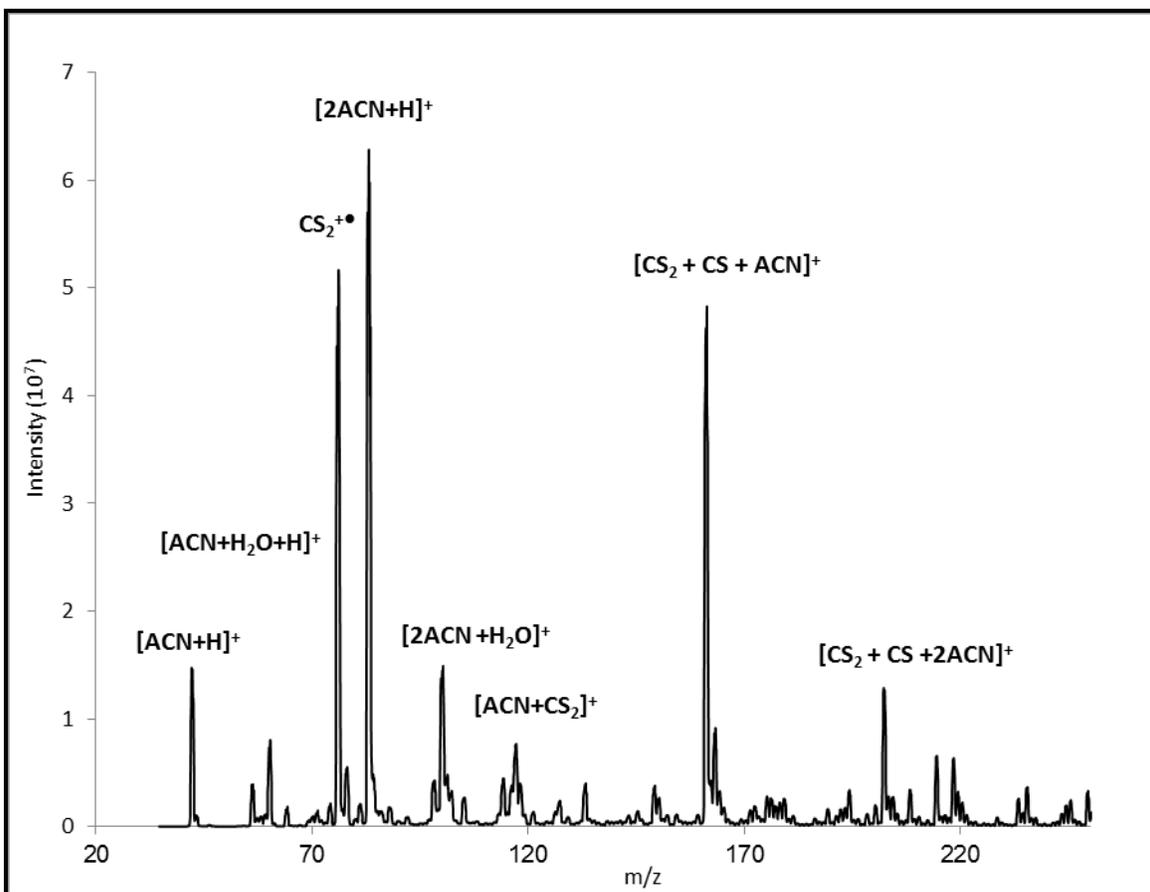


Figure 2.5 Mass Spectra of ACN with carbon disulfide as dopant.

## 2.3 Conclusion

The results presented in Chapter 2 demonstrate that CS<sub>2</sub> is an effective dopant in APPI sources. It was hypothesized that this compound would be effective based on its known photochemistry and absorption coefficient at the photon energies used in APPI sources. The matching of carbon disulfide's IE of 10.01 with the photon energy of the lamp enabled a wider range of analytes to be effectively ionized. Even though carbon disulfide does not contain a proton, significant [M+H]<sup>+</sup> ion formation was observed for some compounds. These results conclusively demonstrate that in this case these protons must originate from solvent, water, and other molecules existing in the source. It is highly probable that this is also the case with other dopants as well. For compounds with high IEs and low PAs, CS<sub>2</sub> is an especially valuable dopant as the other common dopants are ineffective with this subset of compounds.

### **3 Chapter: The Use of Isoprene as a Novel Dopant in Negative Ion Atmospheric Pressure Photo Ionization (NI-APPI) Mass Spectrometry Coupled to HPLC**

#### **3.1 Abstract**

As in the case with positive ion-atmospheric pressure photoionization (PI-APPI), the addition of dopants significantly improves the sensitivity of negative ion-APPI (NI-APPI). However, the research on dopant assisted-NI-APPI has been quite limited compared to the studies on dopant-assisted PI-APPI. This work presents the potential of isoprene as a novel dopant for NI-APPI. Thirteen compounds, possessing suitable gas-phase ion energetic properties in order to make stable negative ions, were selected. Dopants were continuously introduced into a tee junction prior to the ion source through a fused-silica capillary, while analytes were directly injected into the same tee. Then both were mixed with the continuous solvent from HPLC, nebulized, and entered the source. The nebulized stream was analyzed by an APPI-tandem quadrupole mass spectrometer in the negative ion mode. The results obtained with isoprene were compared with those obtained with toluene as a dopant and dopant-free NI-APPI. Isoprene enhanced the ionization intensities of the studied compounds comparable to and, in some cases, more effectively than toluene. The mechanisms, leading to the observed set of negative analyte ions, were also discussed. Because in NI-APPI, thermal electrons, which are produced during the photoionization of a dopant, are considered the main reagent ions. Thus, both isoprene and toluene promoted the ionization of analytes through the same mechanisms, as expected. Isoprene was shown to perform well as a novel dopant for NI-APPI. Isoprene has a high photoabsorption cross section in the VUV region; therefore, its photoionization leads to a highly effective production of thermal electrons, which further promotes the ionization of analytes.

## 3.2 Experimental

### 3.2.1 Materials and Methods

Figure 3.1 shows the structure of compounds studied in this work. Table 3.1 presents some relevant thermodynamic data for the solvent, dopants, and compounds utilized in this study. The ionization energies (IEs), electron affinities (EAs), and gas phase acidities were obtained from NIST Chemistry WebBook (NIST Standard Reference Database Number 69). Since for some compounds full gas-phase energetic data were not available, some estimates had to be done using the available experimental data for their structural analogs. Acetonitrile (HPLC grade), isoprene, and toluene (HPLC grade) were purchased from Fisher Scientific Canada. Isoprene, with a purity of 99+% (w/w), contains less than 1000 ppm 4-tert butyl catechol as an inhibitor and impurities can include isoprene dimer (limonene) at 0.5% (w/w) maximum. All other chemicals were of the highest purity available and were used as received.

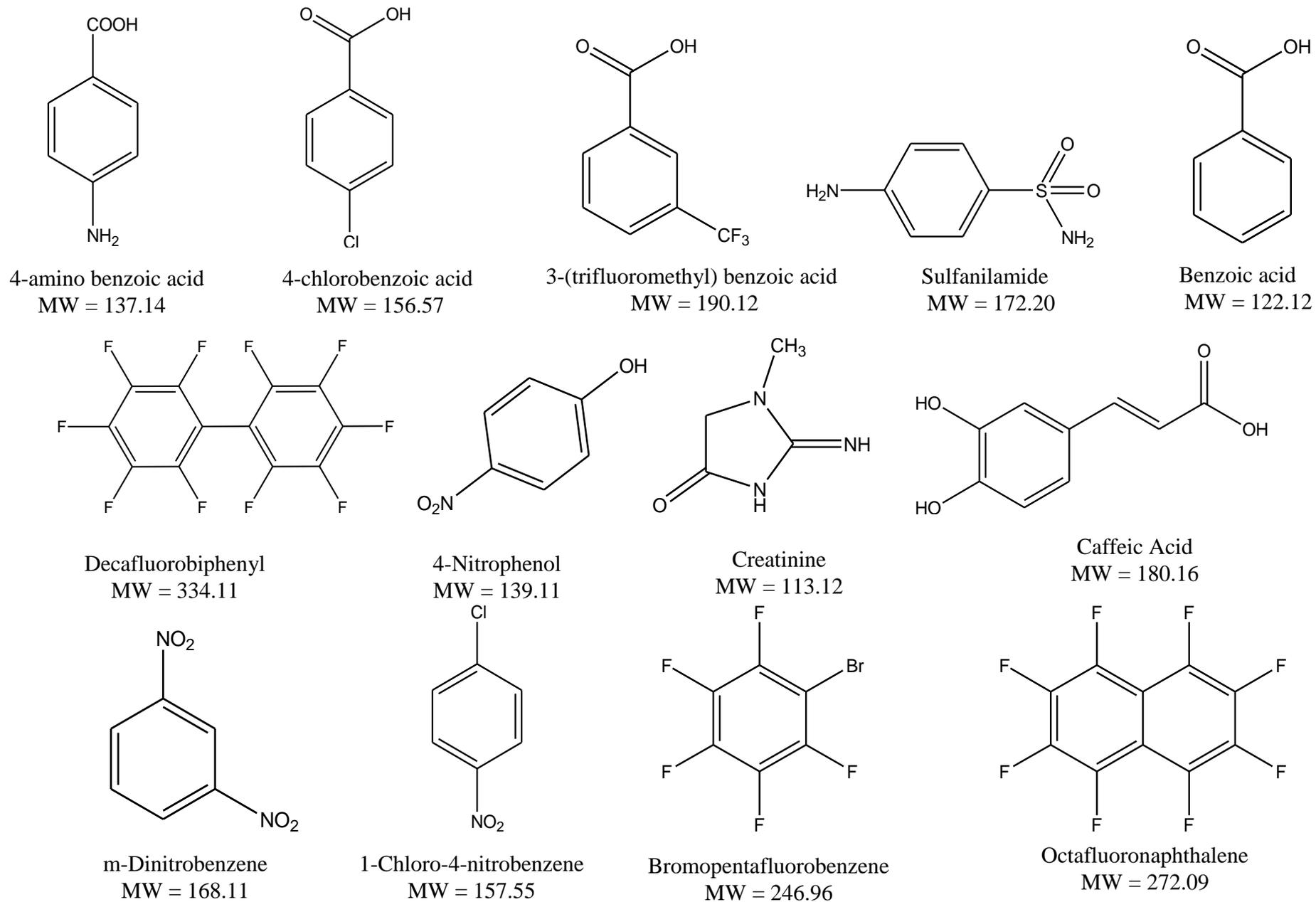


Figure 3.1 Structures of the studied compound

**Table 3.1 Ion energetics of the studied solvent, dopants, and compounds.**

compound	acronym	IE (eV)	PA (kJ/mol)	EA (eV)	$\Delta_{\text{acid}}G$ (kJ/mol)	$\Delta_{\text{acid}}H$ (kJ/mol)
Toluene		8.83	784.0		1564	1599
Isoprene		8.86	826.4		1586	1613
4-tert-butyl catechol	4-tBC	~8.1 <sup>a</sup>			~1392 <sup>a</sup>	~1420 <sup>a</sup>
tert-Butyl-ortho-benzoquinone	tBoBQ	~9.1 <sup>a</sup>		> 1.5		
Acetonitrile		12.20	779.2	0.01	1528	1560
Oxygen		12.07	421.0	0.45	1450	1477
Superoxide anion					1450	1477
Hydroperoxy radical		11.35	660.0	1.08	1450	1633
4-aminobenzoic acid	4ABZA	8.3	864.7		1407	1437
4-chlorobenzoic acid	4CBZA				1374	1404
3-(trifluoromethyl) benzoic	3-triFMBZA				1361	1390
Sulfanilamide	SA				< 1394 <sup>a</sup>	
Benzoic acid	BZA	9.3	821.1		1393	1423
Decafluorobiphenyl	decaFB	9.40		0.82		
4-nitrophenol	4NPHL	9.1		0.9 <sup>a</sup>	1343	1371
Creatinine		~8.7 <sup>a</sup>			~ 1434 <sup>a</sup>	
Caffeic acid	CA				<1392 <sup>a</sup>	
1,4-Dinitrobenzene	14DNBZ	10.4		1.66		
1-Chloro-4-nitrobenzene	1C4NBZ	10.0		1.26		
Bromopentafluorobenzene	BpentaFB	9.62		1.15		
Octafluoronaphthalene	octaFNPT	8.87		>0.5		

IE, ionization energy; PA, proton affinity; EA, electron affinity;  $\Delta_{\text{acid}}G$ , gas-phase acidity;  $\Delta_{\text{acid}}H$  is the enthalpy change of the deprotonation reaction of  $AH \rightarrow A^- + H^+$ , which is equal to the minus PA of the formed anion; while  $\Delta_{\text{acid}}G$  is the Gibbs energy change of the mentioned deprotonation reaction, with the higher value corresponding to the lower acidity.

The Gibbs energy change associated with the protonation reaction,  $A + H^+ \rightarrow AH^+$ , is called gas-phase basicity of the molecule A, with the higher value corresponding to the lower basicity.

<sup>a</sup> The gas-phase ion energetic data of compounds, for which NIST data are not available, were estimated using available experimental data for their structural analogs. For example,  $\Delta_{\text{acid}}G$  of caffeic acid is expected to be less than 1392 kJ/mol, considering the available gas-phase acidities values for catechol, benzoic acid, styrene, and benzene. Similarly, considering the  $\Delta_{\text{acid}}G$  of benzenesulfamide, aniline and benzene, the  $\Delta_{\text{acid}}G$  of sulfanilamide is expected to be less than 1394 kJ/mol.

### 3.2.2 Instrumentation

All experiments were performed with a Waters Quattro Premier LCT MS/MS (Waters, Milford, MA, USA) and controlled with the Masslynx V4.0 data analysis system (Micromass, Cary, USA). Compounds were detected within the MS/MS using optimized parameters for each compound. Photons were provided to the system via a Photomate™ Photoionization source. The Photomate ion source utilizes a low-pressure krypton VUV lamp which emits photons at 10.0 and 10.6 eV. Nitrogen was provided to the nebulizer, cone and desolvation systems by a nitrogen generator. Desolvation and cone gas flow rates were kept constant for all experiments at 50 and 250 L/h respectively. Argon was used as the collision gas. Experimental parameters for mass spectrometry including repeller, cone, extractor, and RF lens voltages were optimized based on the relative abundances of the peaks for each compound.

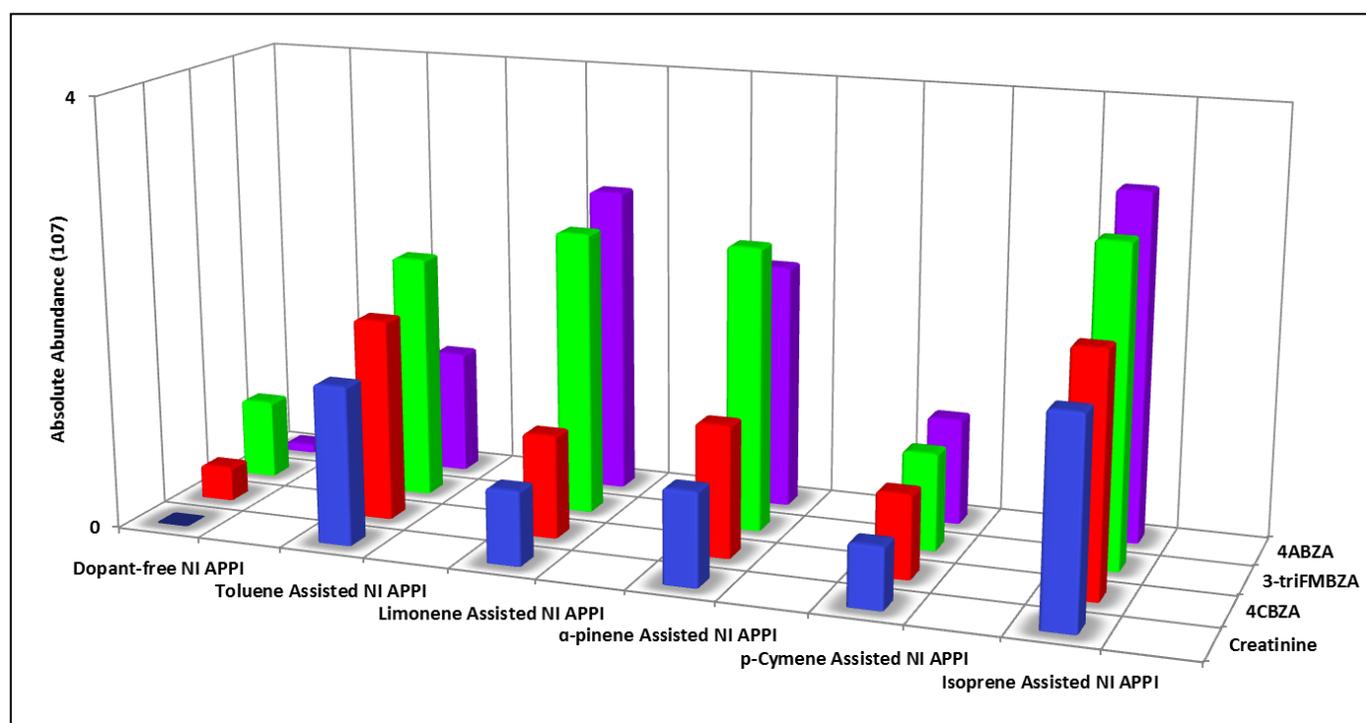
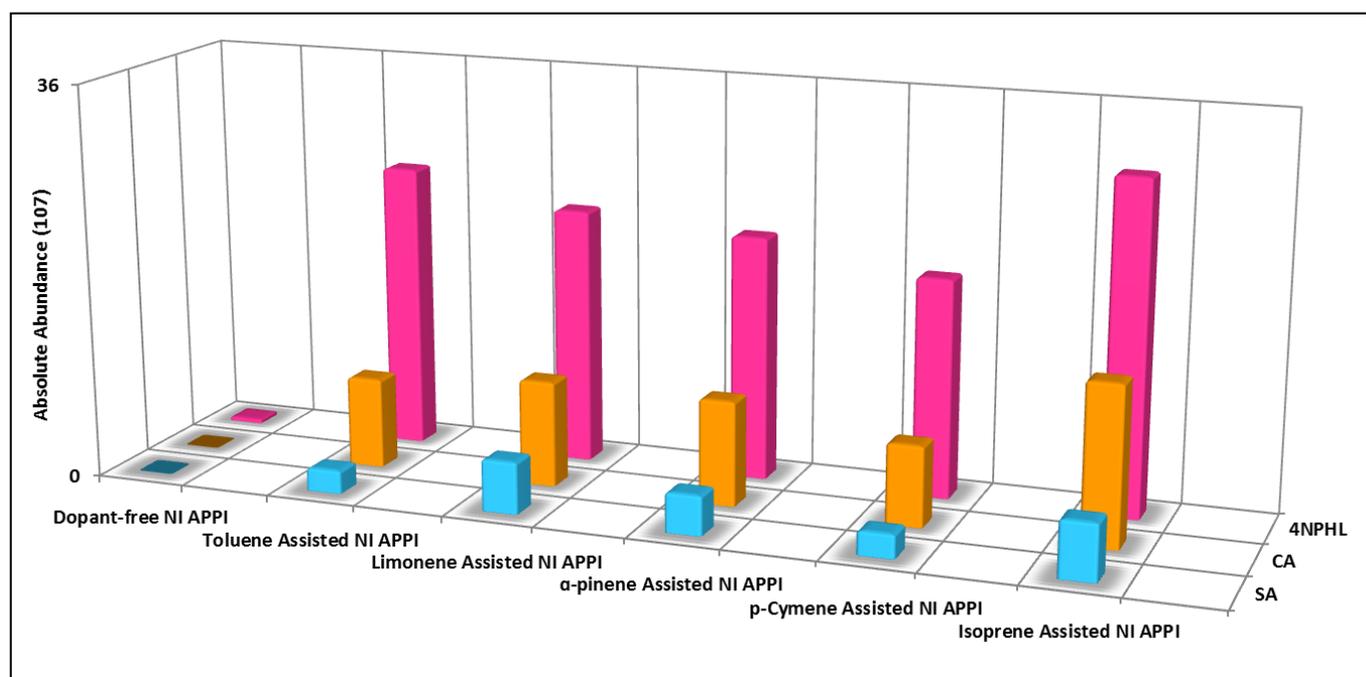
Acetonitrile, as the mobile phase from an Alliance HPLC system (Waters), enabled flow injection analysis in a constant flow rate of 200  $\mu\text{L}/\text{min}$  for all experiments. Samples in the concentration range of 5-10 mg/L were injected by using a 5  $\mu\text{L}$  loop into a continuous flow stream from HPLC. Dopants were continuously introduced into the APPI source through a fused-silica capillary with a syringe pump at a flow rate of 20  $\mu\text{L}/\text{min}$  and were mixed with HPLC stream at a tee junction just prior to the ion source. Tandem mass spectrometric (MS/MS) measurements were carried out in full scan mode with a mass range of  $m/z$  2–1000 and the product ion mode had a range of the precursor ion  $m/z$ , plus/minus 50  $m/z$ . Collision energy was varied between 10 and 60 V, depending on the stability of the precursor ion.

### 3.3 Results and discussion

Preliminary experiments were performed in this study to evaluate the potential of monoterpenes including limonene, alpha-pinene, and p-cymene and their structural unit, isoprene, as dopants for APPI. According to data available on photoabsorption cross sections (Gallagher, Brion, Samson, & Langhoff, 1988; Kubalaa et al., 2009; X. Liu et al., 2009; Martins et al., 2009; “MPI-Mainz-UV-VIS Spectral Atlas of Gaseous Molecules, A database

of atmospherically relevant species, including numerical data and graphical representations"; Shaw et al., 1998; Śmiałek et al., 2012), these species absorb the VUV photons from the Krypton lamp as effectively as toluene and do not have positive electron affinities (NIST Standard Reference Database Number 69). Moreover, these compounds are natural compounds ubiquitous in nature and are considered less hazardous (Degenhardt, Köllner, & Gershenzon, 2009). Available inhalation LD50 values for isoprene and toluene suggest that isoprene is less toxic and poses fewer occupational hazards and less environmental impact ("Isoprene; MSDS No. 464953" , "Toluene; MSDS No. 244511").

In the positive mode, their capability as dopants was compared with toluene and anisole and they gave comparable results (data not shown). Due to high background ions in the low mass range of the spectrum, which interfered with the detection of small organic molecules, further studies in the positive mode were discontinued, since toluene and anisole gave cleaner spectra with approximately the same ionization efficiencies. However, for the negative mode, in which the spectra inherently have lower background signals compared to the positive mode, monoterpenes and specifically limonene and isoprene showed preliminary results compared to those obtained by toluene as a dopant (Figure 3.2). Since isoprene gave the best results, further studies were limited to this chemical.



**Figure 3.2** A preliminary investigation of the ability of monoterpenes (isoprene, limonene,  $\alpha$ -pinene, and p-cymene) to enhance the ionization efficiency in the NI-APPI by comparing the results obtained for seven analytes with those obtained by APPI with no dopant and toluene assisted NI-APPI. The analytical responses are in terms of the total ion currents of the dominant negative ion for each analyte. List of abbreviations for analytes can be found in Table 3.1.

### 3.3.1 Reagent ions produced by isoprene in the NI-APPI with ACN as eluent

Figure 3.3 shows the reagent ions produced from isoprene when it was added at the flow rate of 1/10 of the solvent, ACN. These ions were tentatively identified by MS/MS experiments. As shown in Table 3.1, superoxide ions are the main reagent ions that control the deprotonation ionization route and only compounds with  $\Delta_{\text{acid}}G$  less than that of the  $\text{HO}_2^\bullet$  can be deprotonated by superoxide ions (reaction 5 and 6 in Table 1.3). Acetonitrile and toluene both do not have sufficient gas-phase acidities to form deprotonated ions in the spectra. Therefore, in the background spectra of toluene with acetonitrile, deprotonated solvent and dopant molecular ions or their clusters were not of a considerable intensity (data not shown).

The same applied for isoprene whose gas phase acidity is not strong enough to be deprotonated by superoxide ions (Table 3.1). However, the isoprene used in this study contains less than 1000 ppm 4-tert-butylcatechol (4-tBC), as an inhibitor of isoprene polymerization. The value of  $\Delta_{\text{acid}}G$  for 4-tBC is not available; however, the  $\Delta_{\text{acid}}G$  for catechol is 1392 kJ/mol and the tert-butyl- substituent does not reduce the acidity of the compound significantly. As reported, compounds with gas-phase acidities of only a few kJ/mol lower than  $\text{HO}_2^\bullet$  could be deprotonated by peroxide reagent ions (L. Song, Wellman, Yao, & Bartmess, 2007). Therefore, the deprotonation of 4-tBC by superoxide ions is thermodynamically feasible. As seen in Figure 3.3, in the spectra of isoprene with acetonitrile, the main peak was identified as the deprotonated 4-tert-butylcatechol molecular ion ( $[4\text{-tBC} - \text{H}]^-$ ).

On the one hand, this deprotonated molecular ion could act as a reagent ion and deprotonate the analyte molecules, if gas-phase acidity of the analyte is stronger than that of 4-tBC. On the other hand, 4-tBC could compete for electron/superoxide ions with analytes, reduce the accessible thermal electrons, and subsequently suppress the deprotonation of analytes with weaker gas-phase acidities. Moreover, the  $m/z$  164 could be tert-Butyl-ortho-benzoquinone negative molecular ion ( $[\text{tBoBQ}]^-$ ), the oxidized form of the inhibitor. The estimated EA of tert-Butyl-ortho-benzoquinone, using available experimental data for its structural analogs, was calculated to be at least 1.5 eV (Table 3.1), which is strong enough to result in an

effective electron capture, forming high intensities of its negative molecular ion. However, most analytes do not possess high enough EAs to seize its charge through a charge transfer reaction (Table 1.3, Reaction 4); therefore, it can act as a competent sink for thermal electrons, and adversely affect the performance of isoprene as a dopant. The efficiency of isoprene as a dopant for NI could thus strongly benefit from the removal of 4-tBC. This removal could be done by several washings of isoprene with dilute NaOH and water, followed by drying it over CaH<sub>2</sub> and distilling it under nitrogen at atmospheric pressure. The fraction distilled at 32°C is inhibitor-free isoprene; however, it has to be kept under nitrogen at -15°C in order to avoid polymerization (Armarego & Chai, 2013).

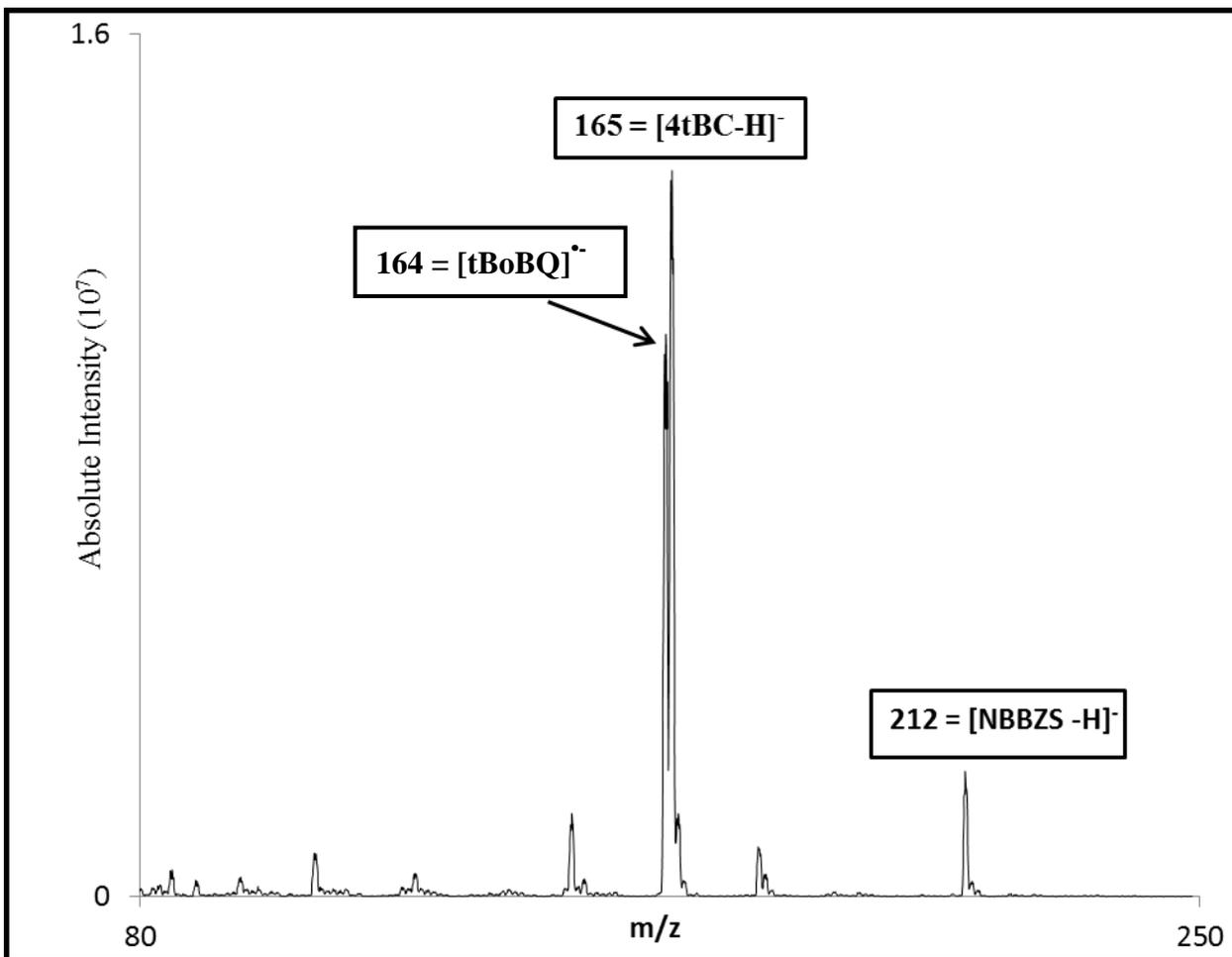


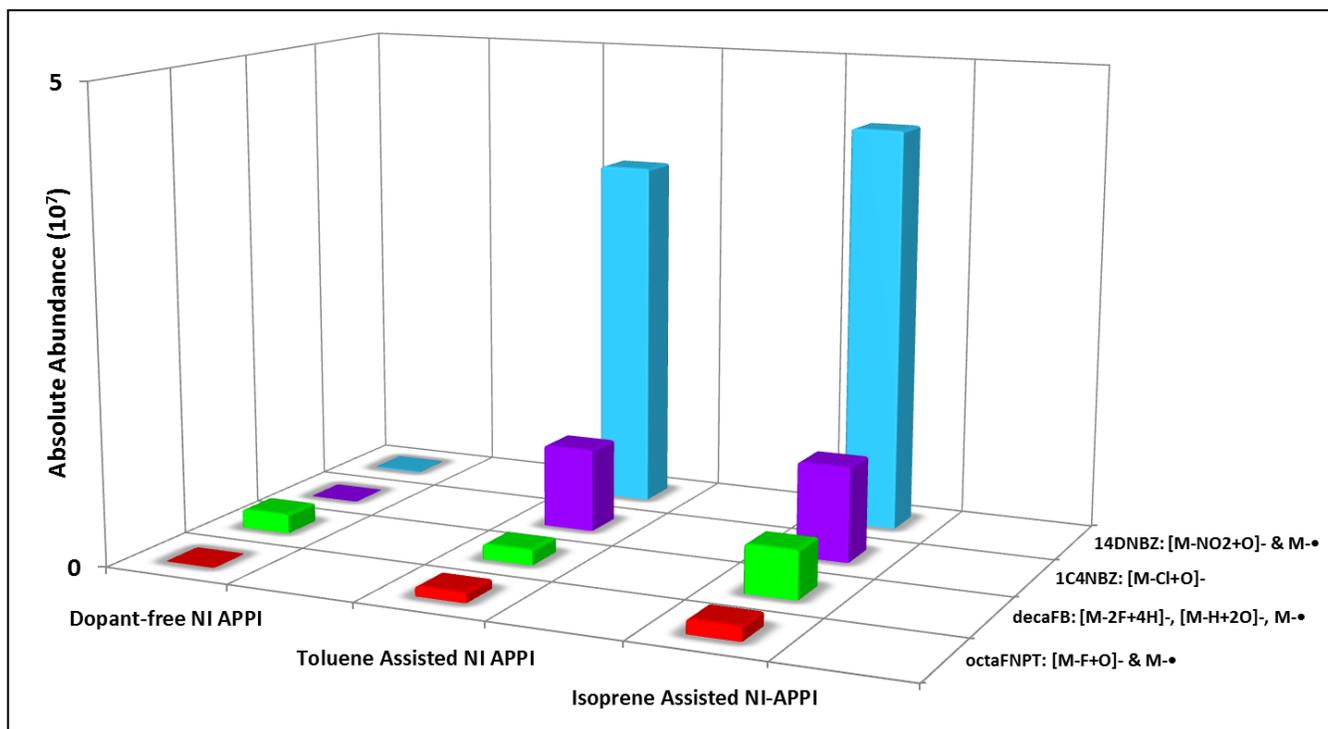
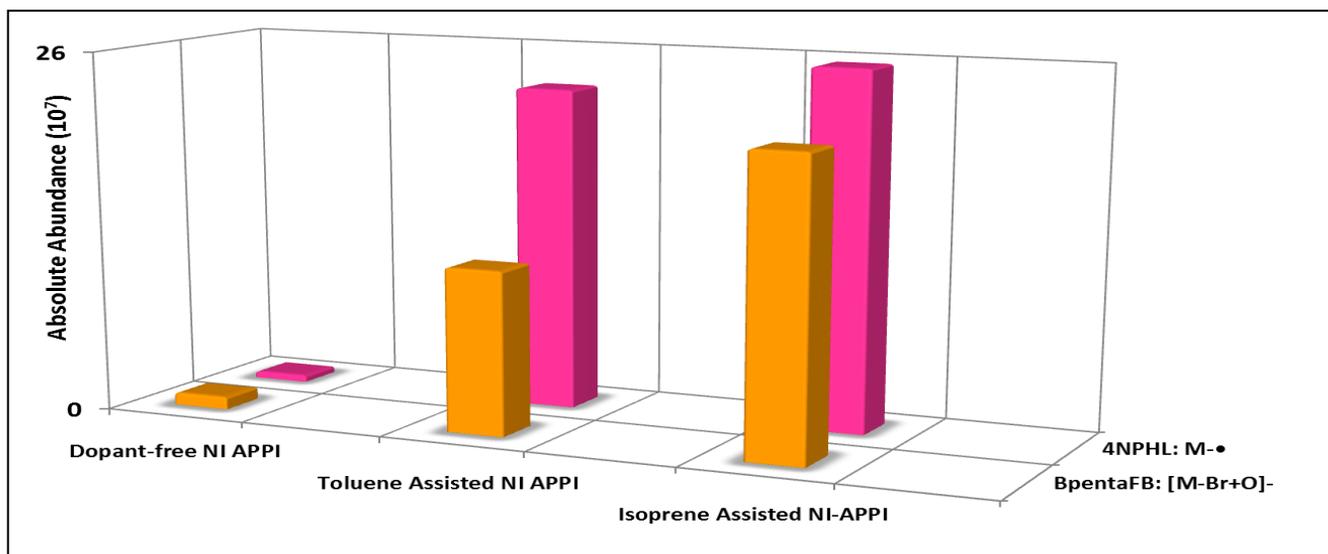
Figure 3.3 Tentatively identified reagent ions formed from isoprene (flow rate =20  $\mu\text{l}/\text{min}$ ) in acetonitrile (flow rate = 200  $\mu\text{l}/\text{min}$ ) in the NI-APPI. tBoBQ, 4-tBC, and NBBZS stand for tert-Butyl-o-benzoquinone, 4-tert-butylcatechol, and n-butyl benzene sulfonamide, respectively. The ion at  $m/z$  212 is a background ion existing in the spectra of acetonitrile and toluene as well and is likely the deprotonated molecular ion of n-butyl benzene sulfonamide (NBBZS), which could enter the source as a water contaminant (Grøn & Dybdahl, 1996; Kolakowski, Grossert, & Ramaley, 2004). The corresponding protonated molecular radical cation of this compound was seen in the positive ion mode.

### 3.3.2 Capability of isoprene as a dopant for NI-APPI

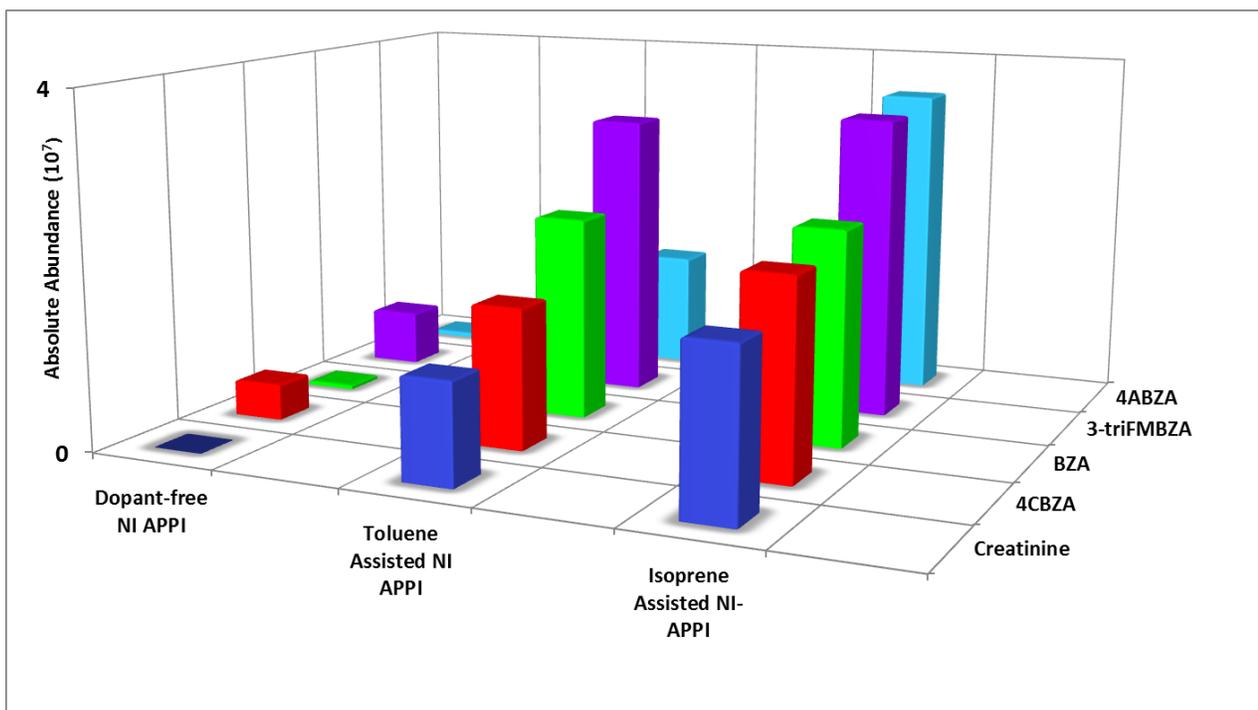
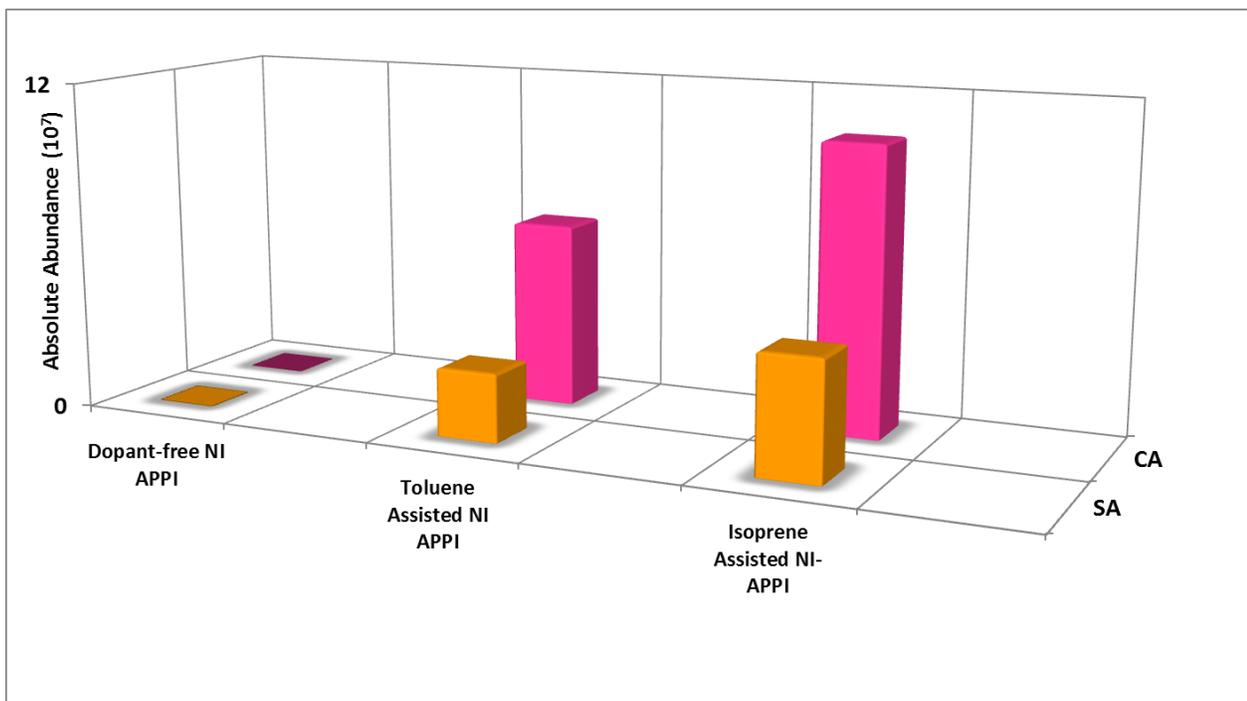
Figure 3.4 and Figure 3.5 compare the absolute abundances of the total ion currents of all observed negative ions including substitution products in direct APPI without using any dopant and DA-APPI using toluene and isoprene as dopants.

As shown in Table 3.1, 4-nitrophenol, decafluorobiphenyl, 1,4-dinitrobenzene, 1-chloro-4-nitrobenzene, bromopentafluorobenzene, and octafluoronaphthalene all possess high positive electron affinities, well above that of O<sub>2</sub>. Therefore, for these compounds both electron capture and charge transfer are thermodynamically possible (Reactions 2 and 4 in Table 1.3, respectively), which resulted in the formation of analyte molecular radical anions as the ionization product (Figure 3.4). However, as already stated, for compounds with positive EAs, substitution reactions compete with electron capture and charge transfer routes. Substituted products can effectively dominate the ionization products and sometimes be the only ionization product, as occurred with 1-chloro-4-nitrobenzene and Bromopentafluorobenzene (Figure 3.4).

On the other hand, 4-aminobenzoic acid, 4-chlorobenzoic acid, 3-(trifluoromethyl) benzoic acid, sulfanilamide, benzoic acid, creatinine, and caffeic acid all possess sufficient gas-phase acidities to be deprotonated by superoxide ions or in case of isoprene by 4-tBC deprotonated ions (Table 1.3, Reactions 5 and 7). As shown in Figure 3.5, the data confirmed this hypothesis and for these compounds deprotonated molecules formed the main ionization products.



**Figure 3.4** Comparison of the absolute abundances of the total ion currents of  $M^{\bullet-}$  and substitution products in APPI without a dopant and with toluene and isoprene as dopants. These compounds all possess positive electron affinities; therefore, they were ionized by electron capture, charge transfer, and substitution reactions. List of abbreviations for analytes can be found in Table 3.1.



**Figure 3.5** Comparison of the absolute abundances of the total ion currents of  $[M-H]^-$  in APPI without a dopant and with toluene and isoprene as dopants. These compounds all possess high gas-phase acidities; therefore, they were mainly ionized by proton transfer, forming deprotonated analyte molecular ions. List of abbreviations for analytes can be found in Table 3.1.

The enhancement factor of analytical responses obtained by using toluene and isoprene compared to dopant-free NI-APPI is shown in Table 3.2. In addition, repeatability of the analysis was examined by calculating the percent RSDs of the peak areas (Table 3.2), which were below 11% and indicates acceptable precision and repeatability of the analysis.

**Table 3.2 The enhancement factor of analytical responses obtained with using toluene and isoprene compared to dopant free NI-APPI. In addition, repeatability of the analysis in terms of percent RSDs of the peak areas is shown.**

		octaFNPT	decaFB	1C4NBZ	14DNBZ	BpentaFB	4NPHL	Creatinine	4CBZA	BZA	3-triFMBZA	4ABZA	SA	CA
Isoprene-Assisted NI APPI	Enhancement factor / dopant-free NI APPI	27	3	10,000	1200	24	54	16000	5	51	6	62	448	100000
	Reproducibility (%RSD)	11	6	5	7	5	9	10	6	5	7	9	8	5
Toluene-Assisted NI APPI	Enhancement factor / dopant-free NI APPI	19	1	9,000	1000	13	49	10000	4	49	5	23	260	67000
	Reproducibility (%RSD)	11	9	8	9	7	6	9	6	3	10	11	10	6
Dopant-free NI APPI	Reproducibility (%RSD)	5	6	10	4	8	7	8	4	4	5	7	7	10

### 3.4 Conclusion

Similar to PI-APPI, dopants significantly enhance the ionization efficiency in the negative mode as well; however, the research dedicated to discovering novel dopants and investigating the role of dopants in NI-APPI has been very limited compared to PI-APPI. This work demonstrated the potential of isoprene as a novel dopant for NI-APPI, by comparing the results using isoprene with those obtained by toluene as a dopant and dopant-free NI-APPI. Moreover, the mechanisms leading to the observed set of negative analyte ions were investigated, which proved to be identical for both dopants. For the studied compounds, isoprene gave comparable, and in some cases better results, than those obtained with toluene. The good ionization efficiency obtained with isoprene can be ascribed to its high photoabsorption cross section in VUV range, which leads to the generation of high quantities of thermal electrons. However, 4-tert butyl catechol, the inhibitor present in the isoprene reagent, produced a high intensity of stable negative ions, with the high probability of adversely affecting the performance of isoprene as a dopant due to the depletion of accessible thermal electrons and/or superoxide ions. Therefore, using an inhibitor-free isoprene could lead to a far more sensitive method. Removing the inhibitor offers an attractive prospective for further research in this area.

## **4 Chapter: Performance of carbon disulfide as a charge transfer dopant in GC-PI-APPI-MS**

Recently, a custom-built design of an atmospheric pressure interface (API) for coupling a GC to a MS was introduced (H Kersten, Haberer, Kroll, & Benter, 2014). A custom-made lamp holder places a commercial Kr lamp above the MgF<sub>2</sub> window of the ionization source to irradiate the entire ionization chamber. The design offers some advantages over commercially available APPI ionization chambers: (a) the source size is minimized compared to the commercial APPI sources; therefore, analyte stream and photons meet in a much closer proximity which enhances the probability of direct ionization. (b) The interface is tightly sealed. The carrier stream from GC (helium) is mixed with highly purified nitrogen make-up stream inside the source and enters a mass spectrometer after ionization. Sweeping the source with highly pure, VUV transparent N<sub>2</sub> limits the suppression of VUV light by oxygen and water. This suppression is caused by the fact that background water and oxygen, similar to LC solvents, significantly absorb the VUV photons, photo-dissociate, then advance radical-induced charge transfers, which are unwanted reactions that lead to a decrease in the targeted photoionization of analyte (Hendrik Kersten et al., 2009; Ladenburg & Van Voorhis, 1933; WATANABE & ZELIKOFF, 1953). (c) The source shape (conical) and the flow pattern have been designed in a way to maximize the chance of photoionization and transmitting ions to the mass spectrometer. Purified N<sub>2</sub> enters the cone as the make-up gas and produces a vortex flow. The GC column is positioned around the center of the cone from which the GC eluent mixes with the vortex flow. The residence time of ions is about 20 ms with no dead volume. The initial results with the API interface irradiated by a commercial Kr lamp showed high sensitivity in direct mode and without using any dopant (Peterson et al., 2014).

In this chapter, I report on the developments in adapting this custom-built API source for use as a DA-APPI. The hypothesis is to enhance the charge transfer route by producing an abundant stream of reactant ions, which further ionize the less and non-polar compounds. The advantage is that in the absence of LC solvents, reactant ions are not neutralized and are reserved in the source for a longer time and are able to effectively transfer their charge to analytes. Pronouncing the charge transfer route and minimizing the proton transfer route,

eradicates the discrimination against polar compounds. By using different dopants with different ionization energies, the range of ionizable compounds can be controlled. In addition, due to a chemically simplified ionization matrix, the examination of ionization mechanisms can be performed more simply and the data can be interpreted more reliably.

#### 4.1 Abstract

The performance of carbon disulfide as a charge transfer dopant in a custom-built Atmospheric Pressure Interface (API) for coupling a gas chromatograph (GC) to an orbitrap mass spectrometer (MS) with a photoionization Kr lamp was demonstrated in the positive mode by analyzing an EPA mixture containing 78 diverse compounds. The unique and carefully designed aspects of the source, in combination with the pure and simple matrix from the GC, enabled an inclusive sensitive ionization of all compounds detectable in the positive mode. Effective interaction between photons and analytes, leading to the formation of molecular analyte cations ( $M^{+}$ ), controlled the ionization in the direct mode, without using any dopant. Toluene and carbon disulfide were utilized as dopants and produced a high abundance of dopant radical cations as reactant ions, which further ionized the analytes through charge transfer. Therefore, ionization energies (IEs) of dopants controlled the range of ionizable compounds in the dopant assisted ionization mode because only compounds with IEs less than that of the dopant can be ionized. Carbon disulfide ( $CS_2$ ) showed a better performance as a dopant compared to toluene.  $CS_2$  not only was more effective in enhancing the ionization intensities of almost all compounds, but also its high IE (10 eV), enabled the ionization of compounds possessing high IEs. In contrast, toluene suppressed the ionization of all compounds having higher IEs than its own, which is 8.8 eV. Additionally, the potential of the source for quantitative analysis was examined with respect to repeatability, linearity, linear range, and limit of detection (LOD) for the three approaches: Chromatographic performance was good with peak half-widths of 1.0–3.0 s. Linearity with the coefficient of determination ( $R^2$ ) higher than 0.990 was acceptable. The spectral LODs (based on S/N of 3) were calculated. Excellent sensitivities with LODs in the range of fg/ $\mu$ L were observed in the direct APPI mode. Although the addition of dopants mostly decreased the LODs to some extent; in some cases, elevated background noise led to slightly higher LODs. Repeatability

of the injection with GC-APPI MS was tested by calculating the percent RSDs of the peak areas, which were below 10% indicating reliable reproducible GC injections. Overall, unlike previous inlet ionization methods, which discriminate against non-polar compounds, the novel API interface with photoionization offers a great potential as a universal ionization source, capable of simultaneously ionizing polar and non-polar compounds.

## **4.2 Experimental**

### **4.2.1 Chemicals and sample preparations**

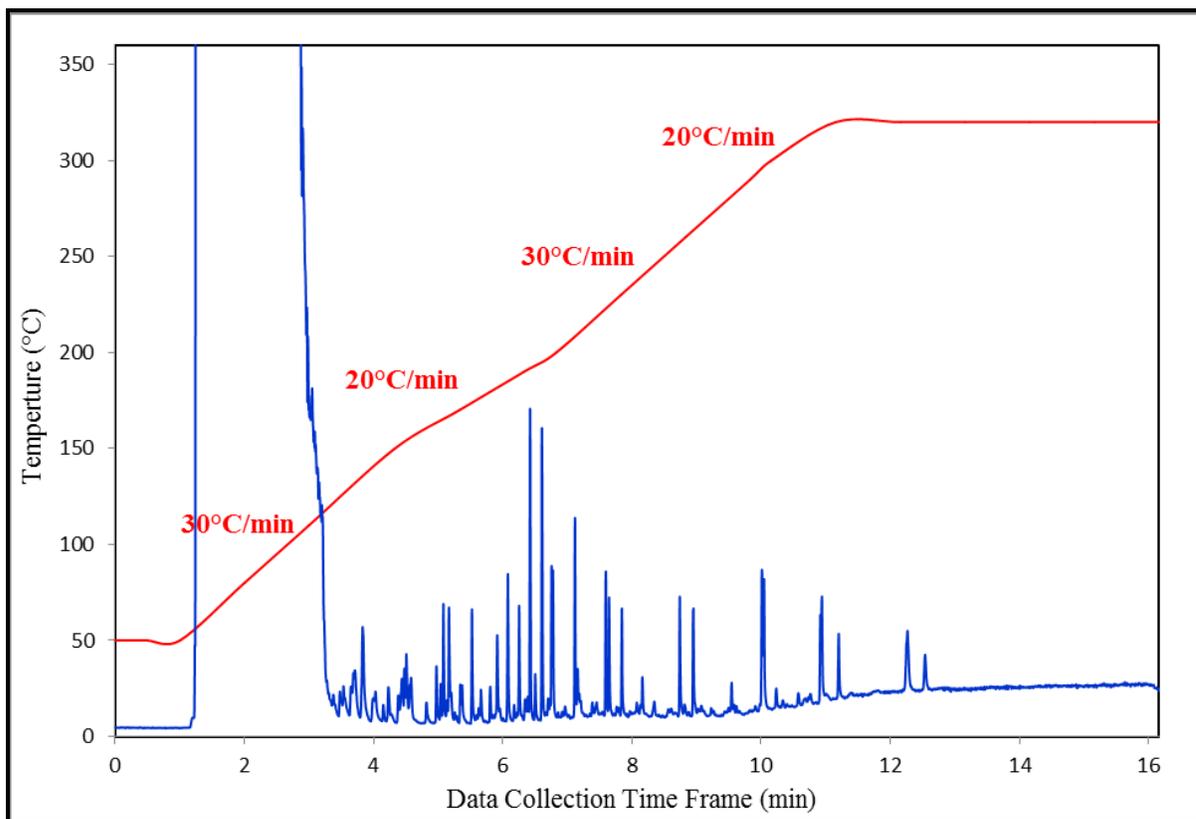
EPA 8270 LCX Mix 1 (100 µg/mL in acetone: methylene chloride (90:10)) was purchased from SUPELCO (Bellefonte, PA, USA) (Appendix A). A series of working solutions in the range of 1 fg/ µL to 800 pg/ µL were made in dichloromethane by serial dilution. Stock and working solutions were kept in freezer at -20°C. Toluene (99.9 %), anisole (99.7 %), and Carbon disulfide (≥99 %) were purchased from Sigma-Aldrich (Steinheim, Germany) and used unaltered. For dopant assisted experiments, headspace of dopants was continuously introduced into a tee junction prior to the source with a gas-tight syringe and a syringe pump through a fused-silica capillary at a flow rate of 300 µL/min. Dopants were mixed with the make-up gas inside the tee and then entered the ionization chamber.

### **4.2.2 Instrumentation**

#### **4.2.2.1 Gas chromatograph**

A Thermo Scientific 450 Series gas chromatograph equipped with a TR-Dioxin 5MS column (30 m×0.25 mm i.d. × 0.1 µ; Milan, Italy) was used for chemical separation. The temperature program used in this work, along with a total ion chromatograph (TIC) obtained by injection of 1 µL of EPA mixture at a concentration of 100 pg/µL and ionized by direct APPI, are shown in Figure 4.1. The GC transfer line and injector temperature were 325°C and 250 °C

respectively. Helium with 99.999% purity (Messer Industriegase GmbH, Bad Soden, Germany) at a constant flow rate of 1.50 mL/min was used as the carrier gas. All experiments were done by splitless injection of 1  $\mu$ L of EPA mix.



**Figure 4.1** Ramp chart of the GC oven temperature program. Total Ion Chromatogram (TIC) was obtained by 1  $\mu$ L injection of 100 pg/ $\mu$ L of EPA mix. The TIC was accordingly scaled to be shown in parallel to the oven program.

#### 4.2.2.2 Ionization source

The cone shaped ionization chamber was made of invar 36 alloy (Enpar Sonderwerkstoffe GmbH, Gummersbach, Germany) in order to adapt to the thermal expansion coefficient of the  $\text{MgF}_2$  window. A PVD double layer of Al and  $\text{MgF}_2$  refined the surface of the cone. The  $\text{MgF}_2$  window (Edmund Optics Inc., Barrington, NJ, USA) was sealed tightly on the base of the cone with cement (OMEGA CC High Temperature Cement from OMEGA Engineering Inc., Stamford, CT, USA) and a protective inorganic layer of IpsealKhaki (Indestructible Paint, Ltd.) Two fine holes allow the entry of the GC stream and make-up gas (with or without dopant) into the source, and a third hole conducts the ionized stream toward the mass spectrometer. The schematic of the ion source and the positioning of the three holes are shown in Figure 4.2 (a). As shown, the GC column enters the ionization source right below the window and is located in its optimum position, which is 1 mm before the tip of the cone (Appendix B). Perpendicular to this entry, though not toward the tip but instead with an angle, make-up gas (with or without dopant) enters and creates a vortex flow inside the source. GC eluent enters the middle of this vortex and is effectively irradiated by a Kr lamp. The ionized stream enters the MS through the third hole, parallel to the GC entry and positioned in the middle of the cone wall.

Figure 4.2 (b) shows the entire GC-API interface. The photon source used was a low-pressure Kr discharge lamp with a RF driver from Syagen (Santa Ana, CA, USA) emitting two lines of photons at 10.0 and 10.6 eV. A custom-made lamp holder positioned the lamp above the  $\text{MgF}_2$  window in order to effectively irradiate the entire source. The entire source can be heated up to 350°C with two heater cartridges (HORST GmbH, Lorsch, Germany), with the total heat output of 400 W. For this work, the interface was kept at a constant temperature of 300°C. Flat gasket sealing (Sigraflex®, A.W. Schultze, Geesthacht, Germany) were used where electrical isolation were needed. The GC column was directed to the source by a commercial transfer line (Thermo Scientific, Dreieich, Germany). A GC ferrule was used to provide a leak free secure connection between the GC column and the source.  $\text{N}_2$  from a compressed gas cylinder (Messer Industriegase GmbH, Bad Soden, Germany) was used as the make-up gas at an optimum flow rate of 700 mL/min (Appendix C). A Vici

Metronics (Poulsbo, WA, USA) N<sub>2</sub> purifier was placed in the line to reduce all impurities to ppb levels. The N<sub>2</sub> flow rate was controlled by a multichannel mass flow controller 647C from MKS Instruments Deutschland (München, Germany).

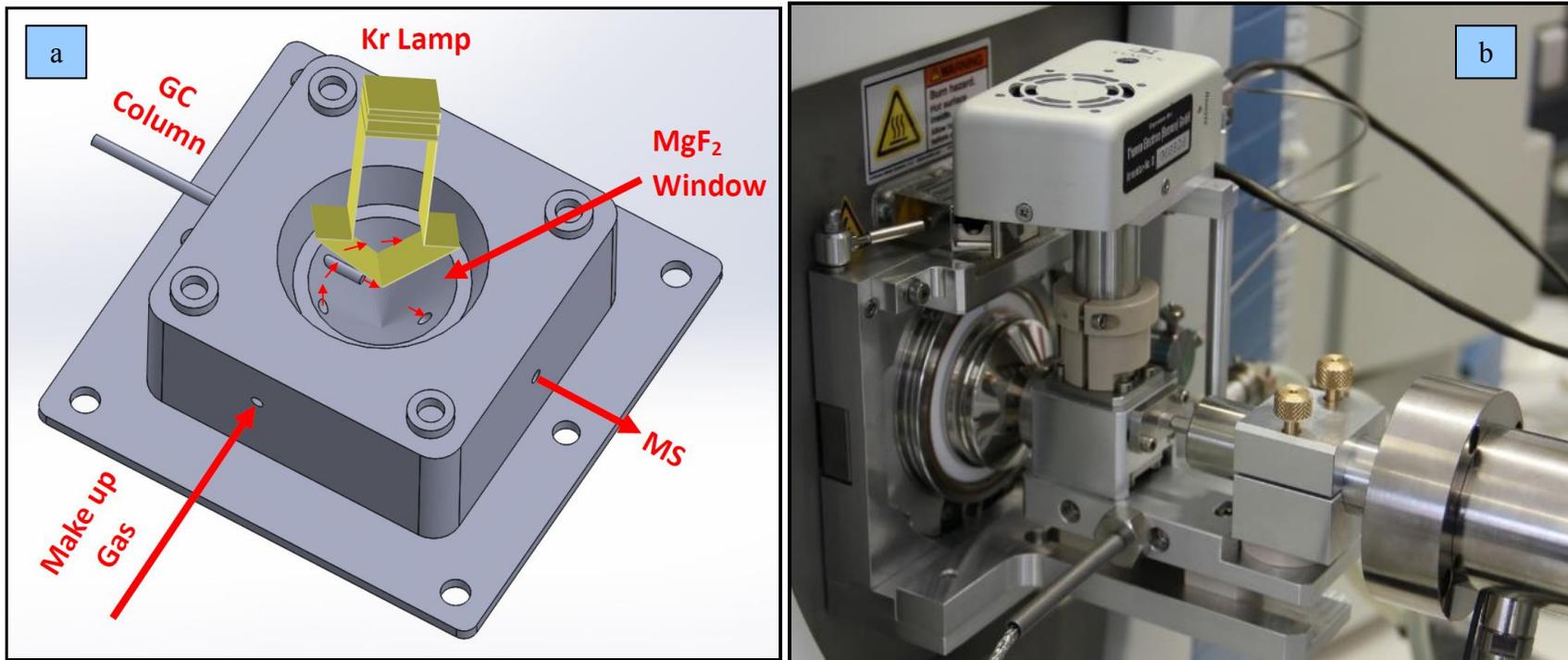


Figure 4.2 (a) Schematic of API interface showing GC and make up gas entries, and the exit hole to the MS. (b) the entire GC-API interface and the custom-made lamp holder that positions the lamp on the MgF<sub>2</sub> window of the interface. Because of the matched size of lamp and cone bases, the entire source volume is irradiated by photons. The small size of the cone assures the closest distance of photons and analyte stream.

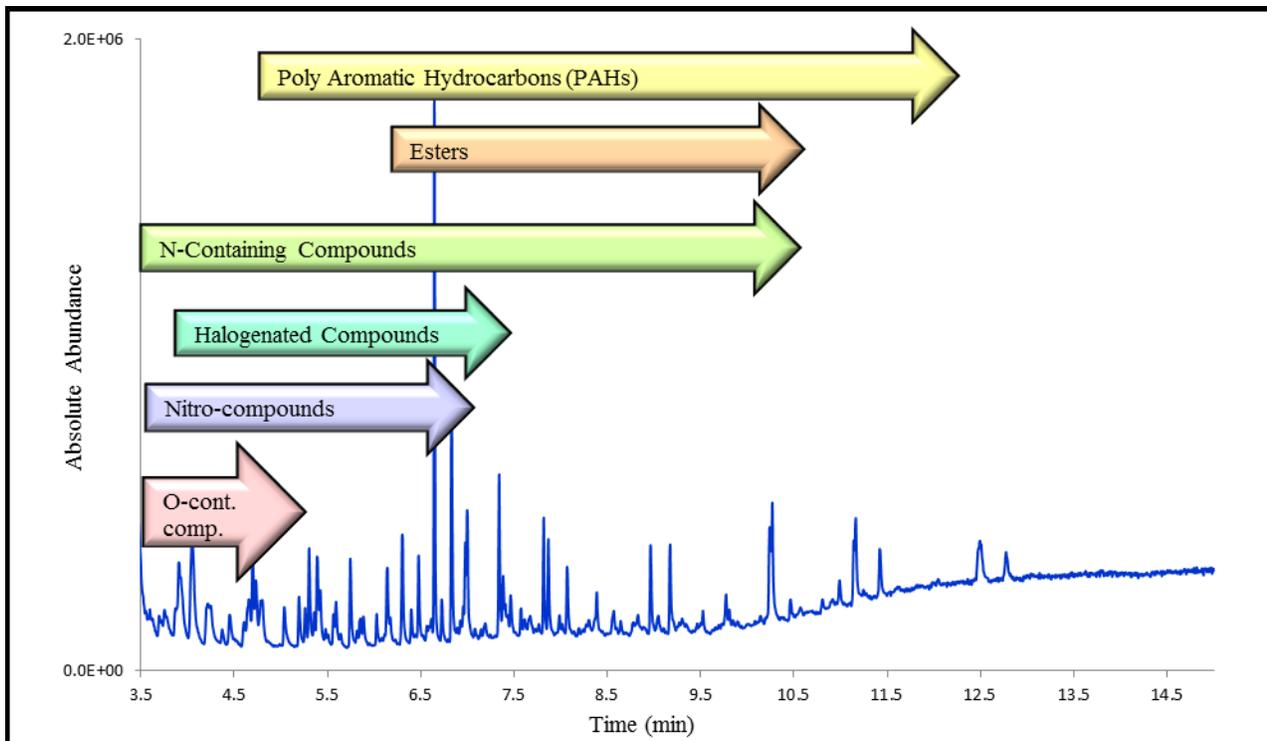
### 4.2.2.3 Mass spectrometer

A Thermo Scientific (Bremen, Germany) Exactive Orbitrap with a resolution of 10,000 collected mass spectra. To adapt the custom-built API interface, minor modification of the mass spectrometer is needed: A stainless steel capillary (Klaus Ziemer GmbH, Langerwehe, Germany) was modified with a gas-tight adapter and replaced the original MS transfer capillary. For this work, all the experiments were performed in the positive mode. The flow injection of carbon disulfide vapor by a syringe pump was used to optimize the mass spectrometer parameters in terms of obtaining the maximum intensity of its radical cation. Both auto tuning and manual tuning were performed for optimization. The optimized parameters were used in this work are as follows: Capillary temperature: 300°C, capillary, tube lens, and skimmer voltages were 25, 39.00, and 15.80 V respectively. The mass range was  $m/z$  50-1000 for direct ionization and it was limited to  $m/z$  80-1000 for CS<sub>2</sub> Assisted APPI and  $m/z$  95-1000 for toluene assisted APPI.

## 4.3 Results and discussion

### 4.3.1 GC Direct APPI-MS

The EPA mixture used in this work contains 78 chemicals representing different classes of compounds (nineteen poly aromatic hydrocarbons (PAHs), eight oxygen-containing, thirteen nitro-containing, twenty halogenated, eight nitrogen containing, seven esters, and three ethoxy ethers compounds). More details on this list of compounds, their percent purities and concentrations can be found in the MSDS sheet of the EPA mix. Using the EPA mixture provided a diverse range of gas-phase ion energetic properties and functional groups; this vast range of analytes enabled a comprehensive study on the evaluation of direct and dopant assisted APPI in GC-MS applications. Figure 4.3, which is a TIC obtained by the injection of 1  $\mu$ L of the EPA mixture at a concentration of 100 pg/ $\mu$ L, shows the retention time windows of different classes of compounds.

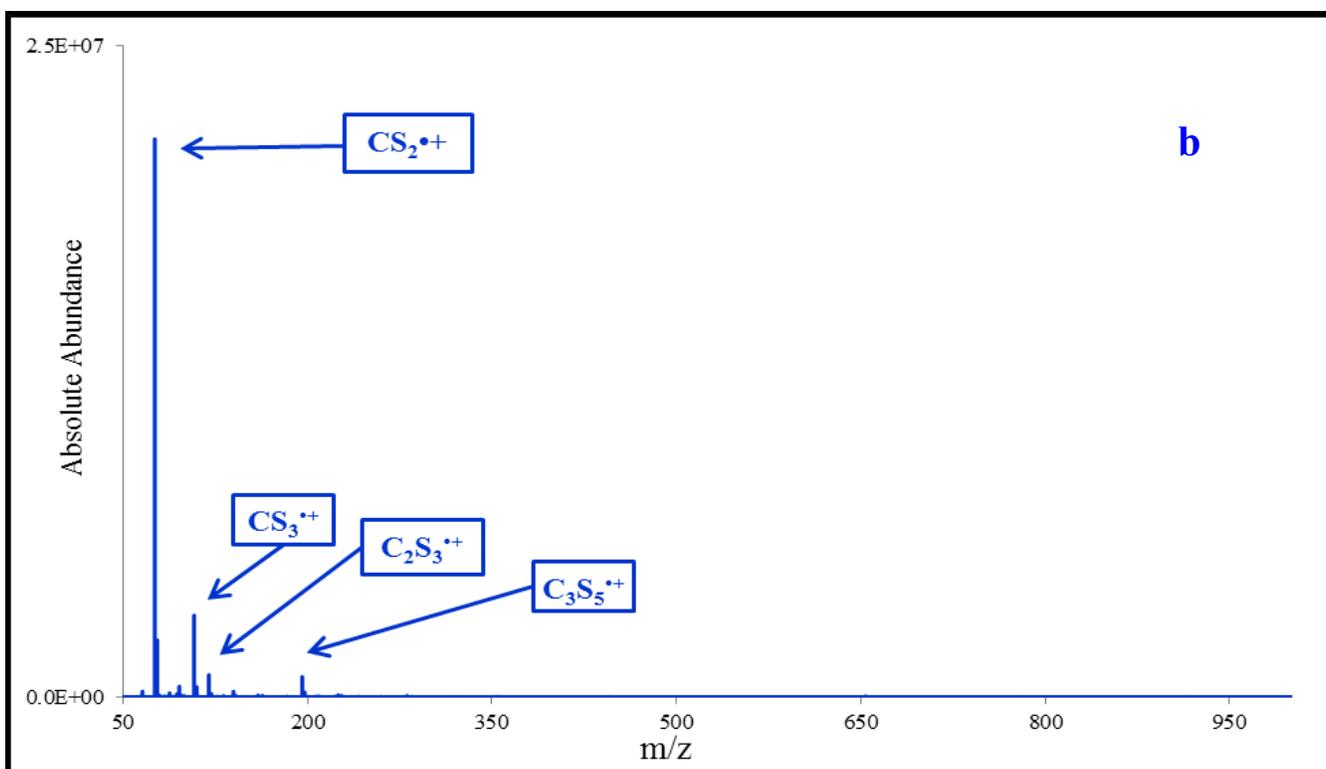
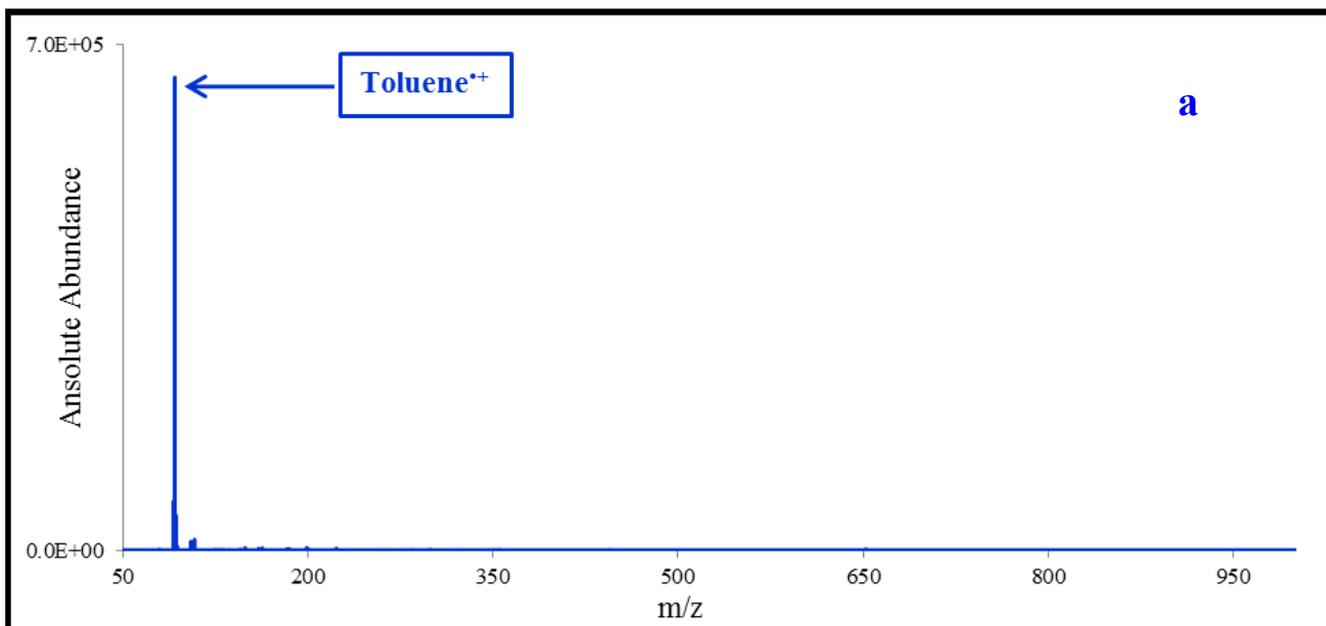


**Figure 4.3** The retention time windows of different classes of compounds during a GC run.

## 4.3.2 GC Dopant Assisted APPI MS

### 4.3.2.1 Reactant ion profiles with toluene and carbon disulfide as dopants.

Figure 4.4 shows the reactant ion profiles obtained by flow injection of dopants' vapor at a flow rate of 300  $\mu\text{L}/\text{min}$  inside the source, while the make-up gas flow rate was kept at 700 mL/min. As seen in Figure 4.4 (a), toluene produced a high intensity of radical cations, which can further promote the charge transfer route. Although carbon disulfide's dominant reactant ion is also its radical cation, photo-dissociation of carbon disulfide ( $\text{CS}_2 \rightarrow \text{CS}^{\bullet} + \text{S}^{\bullet}$ ) and subsequent ion molecule chemistry produced other radical cations i.e.  $\text{S}_2^{+\bullet}$ ,  $\text{CS}_3^{+\bullet}$ ,  $\text{C}_2\text{S}_3^{+\bullet}$ ,  $\text{CS}_4^{+\bullet}$ ,  $\text{C}_2\text{S}_3^{+\bullet}$  (Figure 4.4 (b)). The same photoionization pattern was observed in our previous study, where carbon disulfide was used a dopant for an APPI coupled to a HPLC (Dousty, O'Brien, Gahler, Kersten, & Benter, 2013).



**Figure 4.4** The reactant ion profiles obtained by using toluene (a) and carbon disulfide (b) as dopants. The spectra were obtained by flow injection of dopants' vapor into the ionization source at a flow rate of 300  $\mu\text{L}/\text{min}$ . Prior to the ionization source the dopant vapour was mixed with the make-up gas ( $\text{N}_2$  at a flow rate of 700  $\text{mL}/\text{min}$ ) in a tee junction.

### 4.3.3 Comparison of direct APPI, toluene, and carbon disulfide assisted APPI

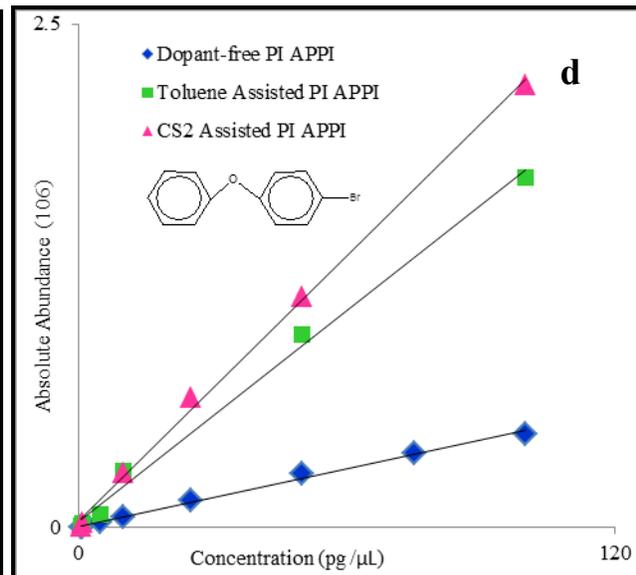
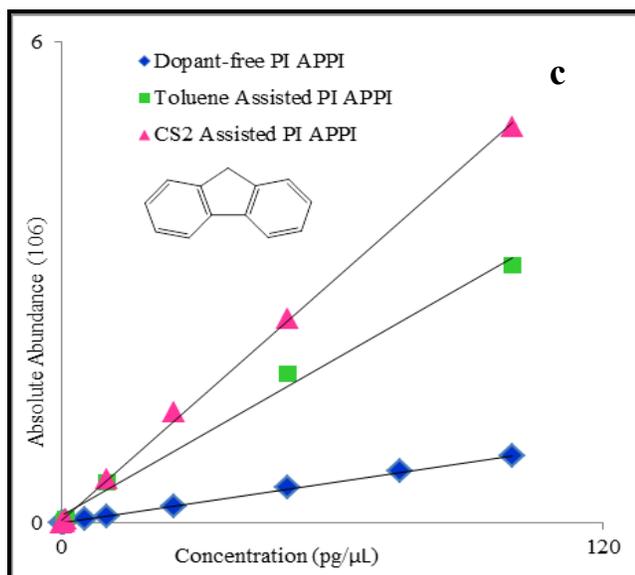
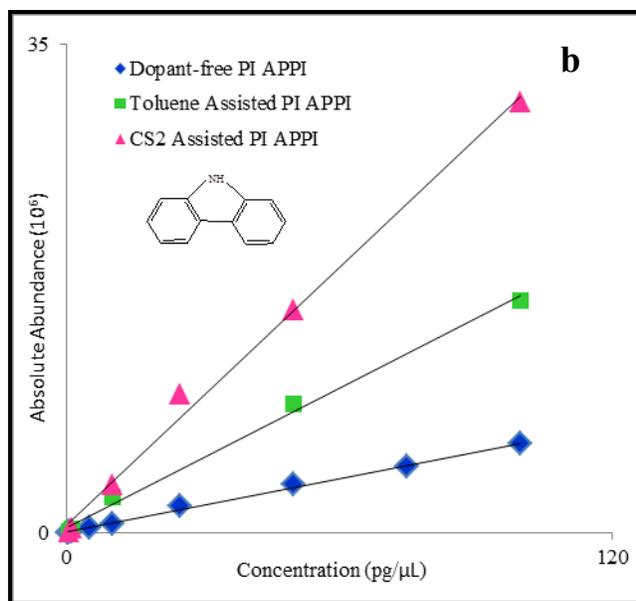
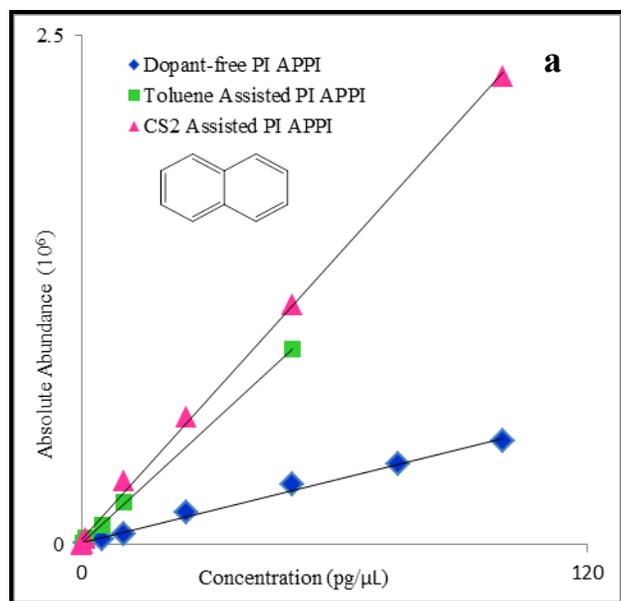
#### 4.3.3.1 Linear dynamic range and detection limits.

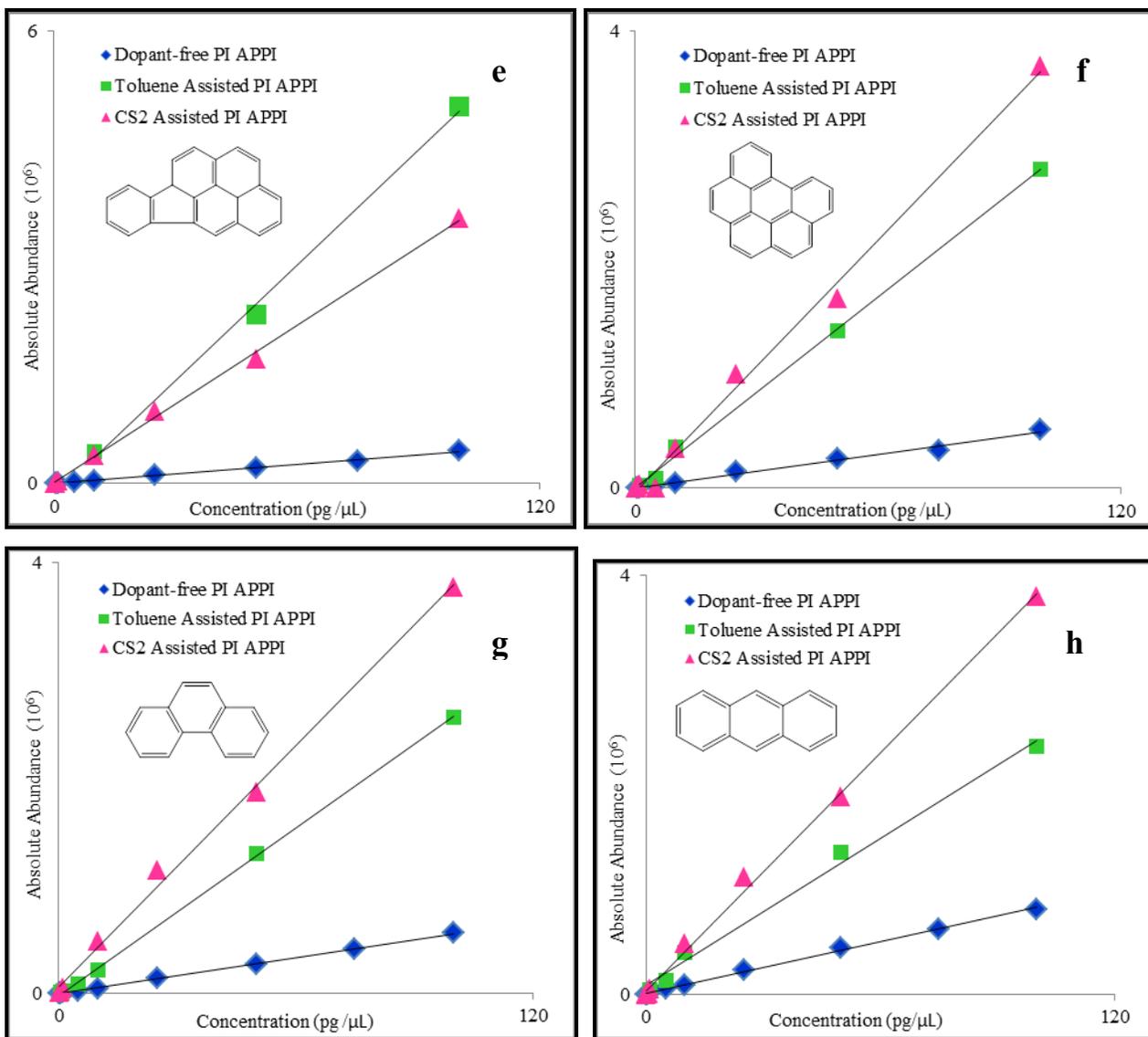
Addition of dopants to a commercial photoionization source coupled to a LC significantly increases the ionization efficiency. Increases of up to two orders of magnitude compared to direct APPI have been reported by different research groups (Dousty et al., 2013; Robb et al., 2000). In the commercial APPI sources coupled to a LC, the presence of LC solvents and a considerable amount of oxygen and background water, which significantly absorb VUV photons and deviate the photoionization toward other unwanted photo-induced reactions and drastically diminishes the likelihood of photoionization of analytes.

Therefore, addition of high quantity of dopants effectively converts the available photon density to reactant ions and decreases the chance of radiation suppression caused by other constituents inside the source.

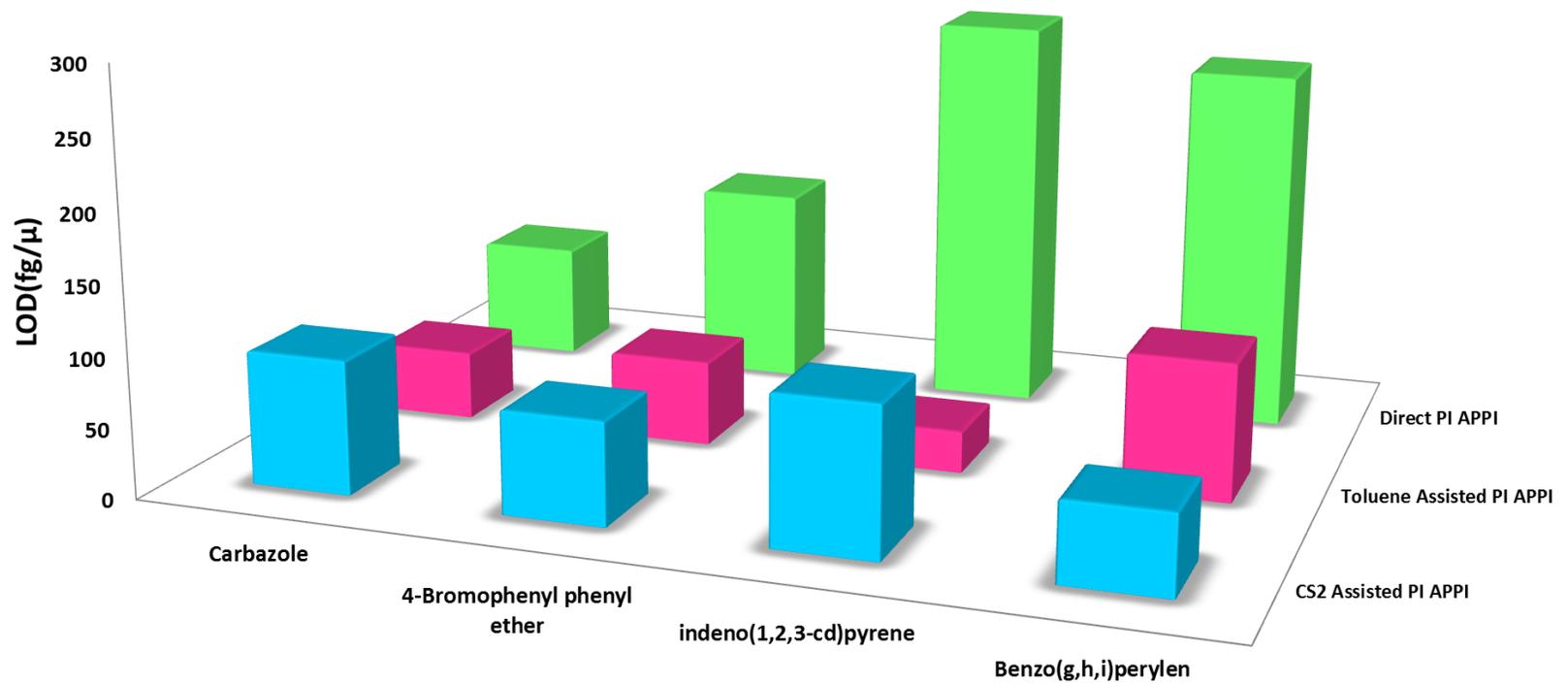
Using a GC as a separation technique eliminates the radiation loss and interferences caused by LC eluents and therefore, a significant increase in the ionization efficiency is expected without the necessity to use dopants. The custom-built design of API source in this work benefits from an extremely simple ionization matrix from GC, but also, the physical design of the source, the analyte-photon distance, and the flow pattern inside the source have been planned in a way to maximize the likelihood of direct ionization. The LODs as low as femto-gram per microliter has been already reported using this design to couple a GC to a quadrupole-Orbitrap MS (Peterson et al., 2014).

In this work, a comprehensive research on direct and dopant-assisted APPI performance of the interface was performed by using the EPA mix containing 78 compounds. Linear dynamic ranges and limit of detections (LODs) were shown for eight compounds with direct APPI, toluene, and carbon disulfide assisted APPI (Figure 4.5 and Figure 4.6), respectively. The analytical response was determined from the peak area of the single ion chromatography (SIM) for molecular analyte ion ( $M^{\bullet+}$ ). LODs were obtained from the spectra for the molecular analyte ion ( $M^{\bullet+}$ ) based on S/N of three.





**Figure 4.5** Calibration curves for (a) naphthalene, (b) carbazole, (c) fluorene, (d) 4-bromophenyl phenyl ether, (e) indeno(1,2,3-cd)pyrene, (f) benzo(g,h,i)perylene, (g) Phenanthrene, and (h) anthracene. Analytical response was obtained from absolute abundance of peak area for single ion chromatography (SIM) of molecular radical cation of each analyte.



	Carbazole	4-Bromophenyl phenyl ether	indeno(1,2,3-cd)pyrene	Benzo(g,h,i)perylene
CS2 Assisted PI APPI	95.5	73.5	105.6	56.6
Toluene Assisted PI APPI	49.2	61.0	29.9	98.4
Direct PI APPI	83.3	141.5	283.0	258.6

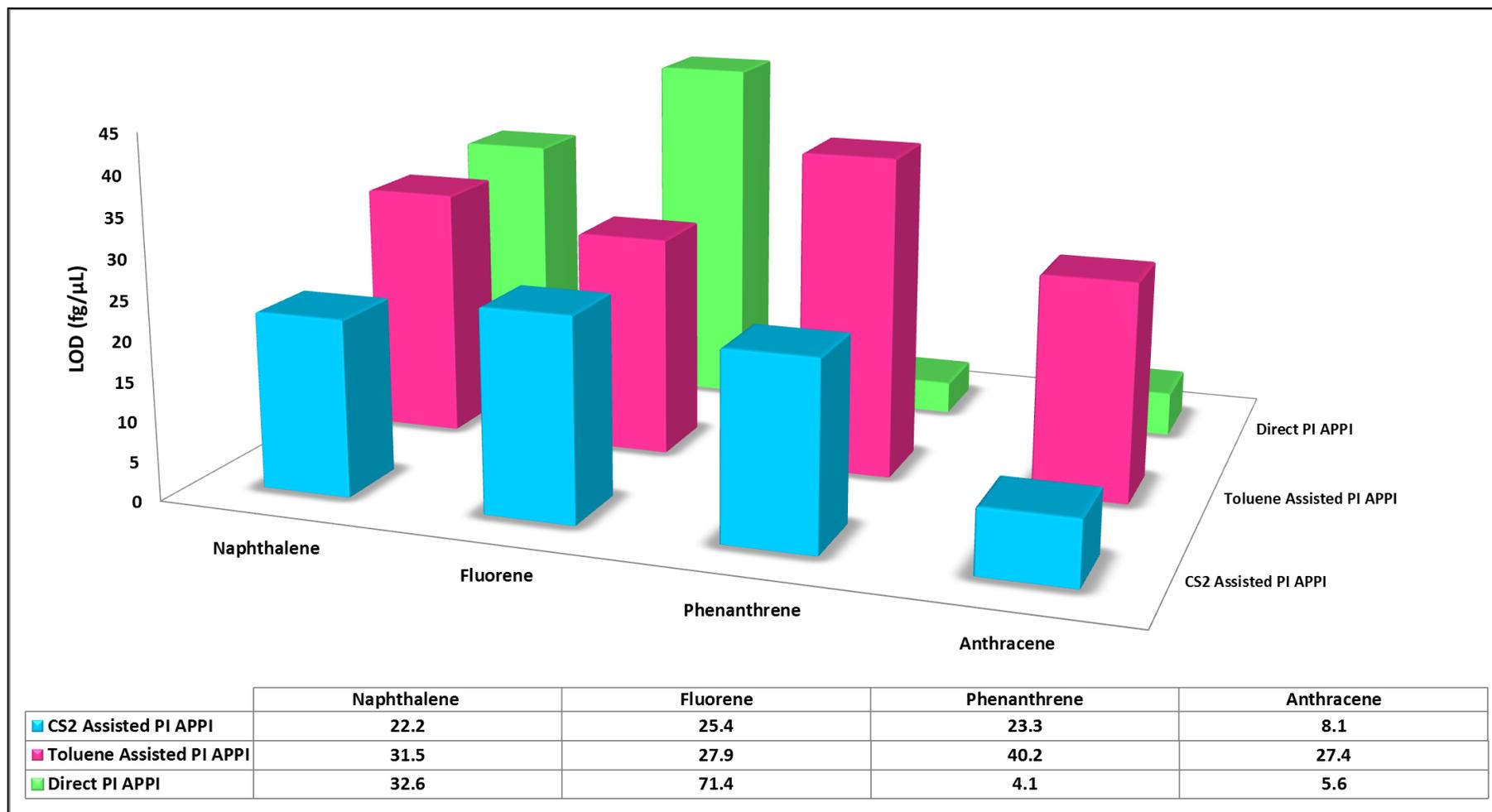


Figure 4.6 Comparison of LODs obtained by direct APPI, Toluene and Carbon disulfide Assisted APPI for eight compounds. Spectral LODs were calculated for the molecular radical cation of analytes based on S/N of 3.

On the whole, higher analytical responses were obtained with CS<sub>2</sub> assisted APPI compared to toluene assisted APPI. Carbon disulfide increased the ion intensity for the mixture in the range of 5 to 10 times, while with toluene this was 1.5 to 7 times when results were compared with direct APPI. In terms of LODs (Figure 4.6), utilizing both dopants led to lower LODs; however, for some compounds like isomers phenanthrene and anthracene, the addition of dopants slightly increased the LODs. These compounds have sensitive direct photoionization responses in low concentrations and the addition of dopants, which leads to higher background ions and noise, is not necessary to obtain better results. Therefore, these compounds can be sensitively analyzed by direct APPI. Generally, with toluene and CS<sub>2</sub> assisted APPI, LODs were as low as fg/μL and direct APPI gave slightly higher LODs. The reason of not gaining significant signal increases by utilizing dopants in the positive mode is that this custom-built design of API gives excellent sensitivities with LODs in the fg/μL levels already with direct photoionization. This does not leave much room for dopants to improve the sensitivity. To the best of our knowledge, this is the only API interface that enables such a sensitive quantitative analysis in the direct photoionization mode and other reported designs still greatly benefit from the addition of dopants in the positive mode (Hintikka et al., 2010; Revelsky & Yashin, 2012).

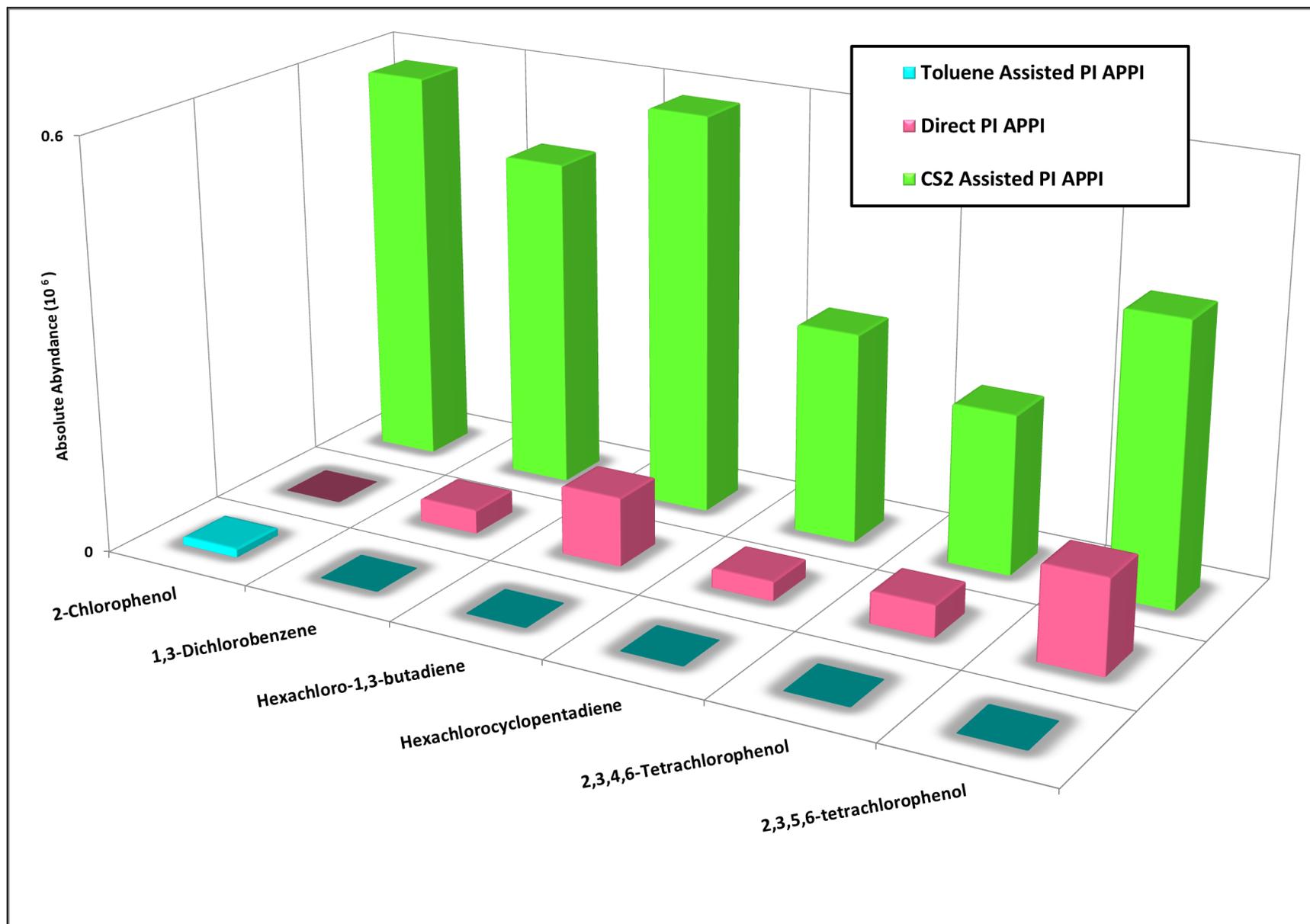
#### **4.3.3.2 Range of ionizable compounds by each method**

In GC/APPI MS, ionization mechanisms are much simpler than LC/APPI MS; direct photoionization, which directly occurs between analytes and photons (in the direct mode) and direct charge transfer, which occurs between analytes and reactant ions (in dopant assisted mode). Therefore, the photoionization is a selective process, which is controlled by the energy of photons and/or the ionization energies of reactant ions.

In direct APPI, all compounds with ionization energies less than 10 eV can be directly ionized with photons. When dopants are added, due to the very efficient ionization of the API interface, photons could efficiently be absorbed by dopant molecules, generating abundant quantities of reactant radical cations which further control the ionization of analytes: All compounds with ionization energies less than that of dopants are ionized by charge transfer.

Therefore, the ionization energies of dopants control the range of ionizable compounds: A dopant with higher IE covers the wider range of analytes, since it reserves higher energies for charge transfer.

The IE of toluene and carbon disulfide are 8.82 and 10.07 eV (NIST Standard Reference Database Number 69); therefore the radical cation of carbon disulfide carries more energy compared to toluene and it can ionize compounds having higher IE. The present data confirm the hypothesis, when using toluene as a dopant, the ionization of compounds having higher IE than 8.82 were suppressed while with direct APPI and CS<sub>2</sub> assisted APPI, more compounds were successfully ionized. Figure 4.7 compares the ionization of compounds with high IE ionized by direct APPI, toluene, and CS<sub>2</sub> assisted APPI. To further confirm this result, anisole with IE of 8.20 eV was utilized as a dopant. Since anisole radical cation carries the least IE among three utilized dopants, IE of all compounds possessing higher IE than that of anisole were suppressed (Figure 4.8). The same trend was observed in my previous study when CS<sub>2</sub> was used as a dopant for an APPI coupled to a LC (Dousty et al., 2013). In that study, low PA compounds with high IE were only ionized by CS<sub>2</sub> and their ionization were suppressed by toluene assisted APPI, since toluene radical cation does not possess enough energy to ionize compounds with high IEs.



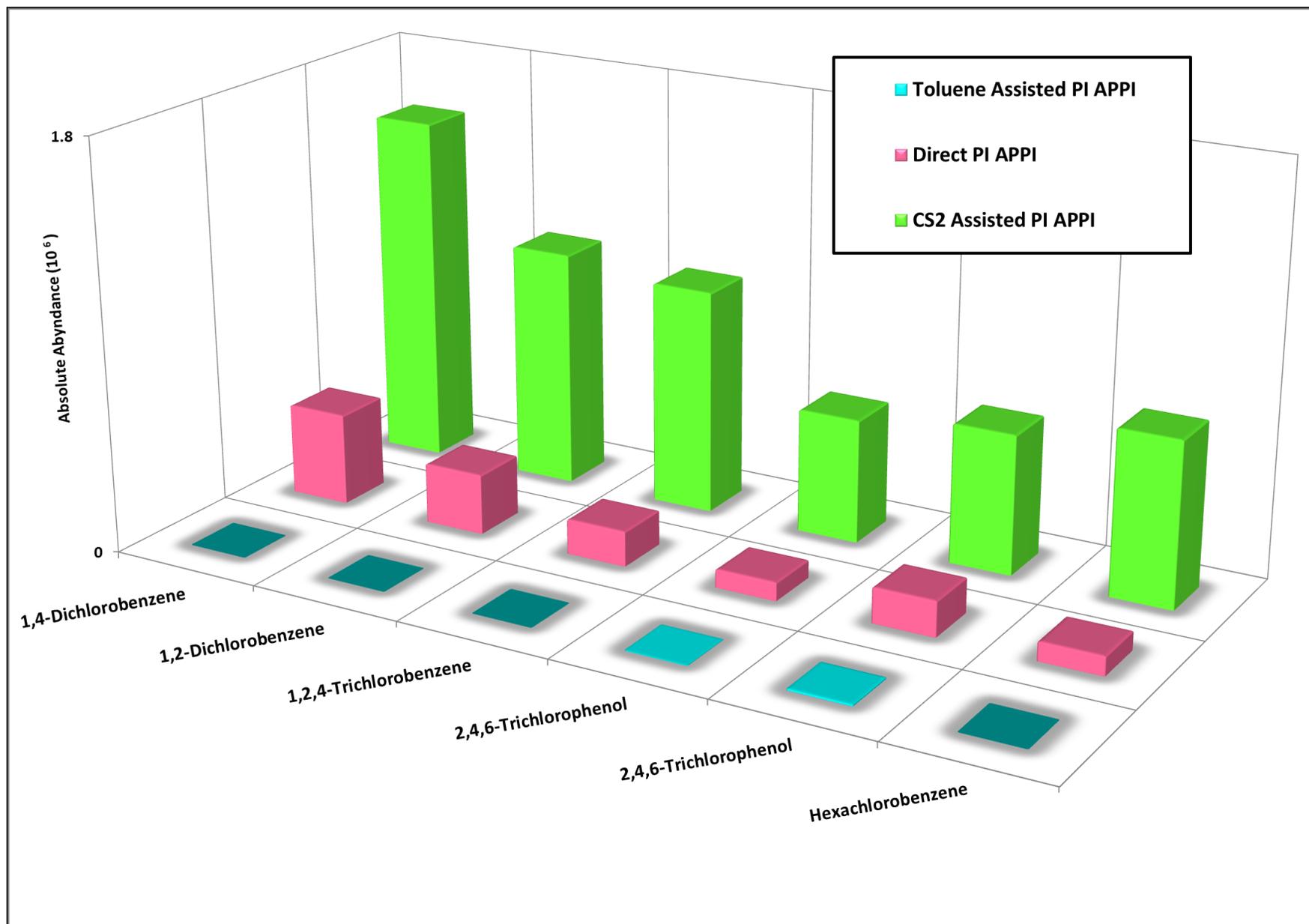
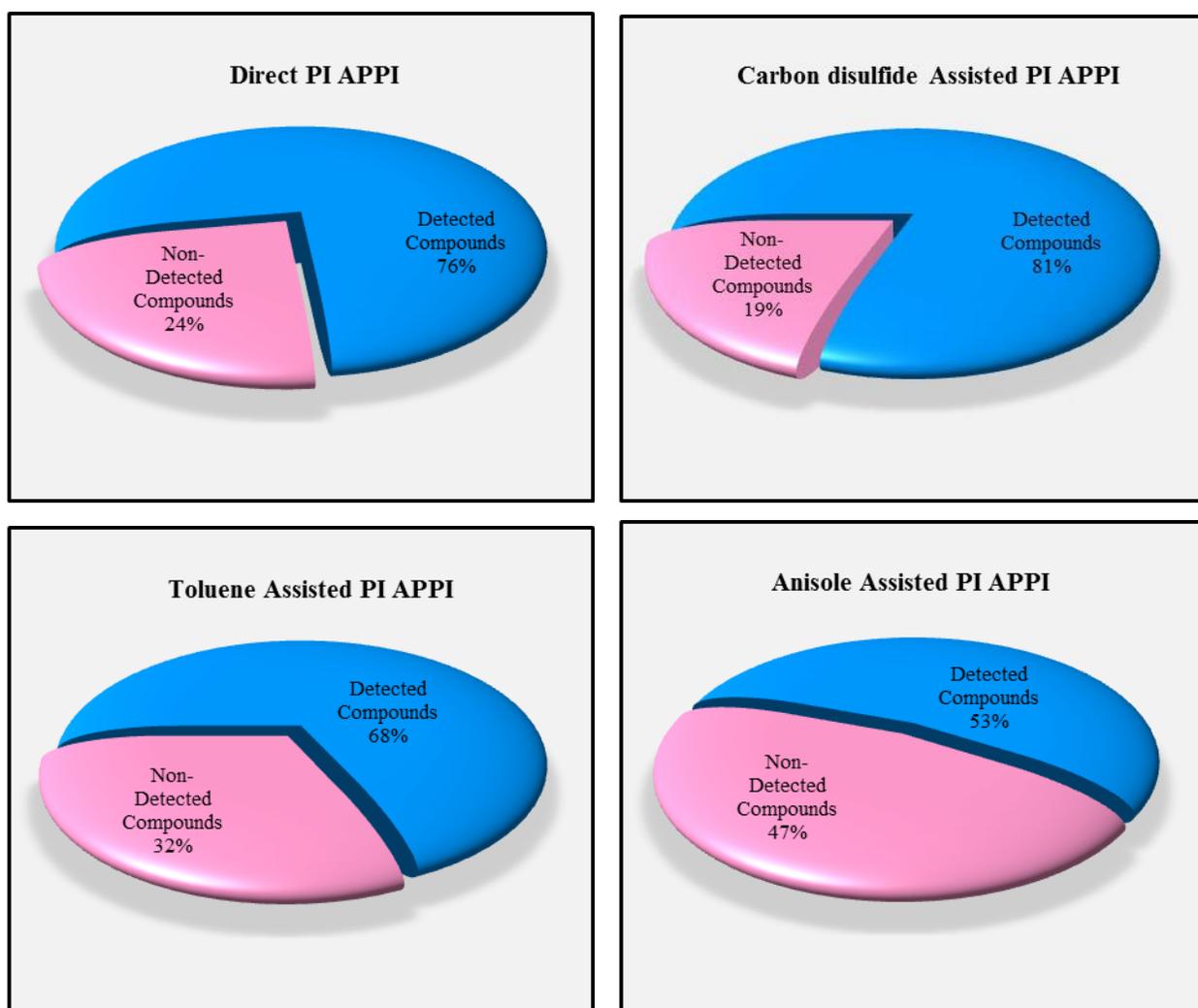


Figure 4.7 Comparison of absolute abundances of SIM of molecular radical cations for compounds with high IE. In toluene assisted APPI, ionization of compounds having higher IE than that of the toluene were completely suppressed or decreased drastically.

Since with GC-APPI, the main ionization route is charge transfer, the choice of dopant becomes critically even more important, as the dopants with low ionization energies suppress the ionization of compounds with high IEs (Figure 4.8), which can be a disadvantage when wide ranges of analytes are being studied, for example in metabolomics. CS<sub>2</sub>, which possesses the highest IE among all dopants, can be utilized as a reliable efficient charge transfer reagent, which enables the ionization of all compounds with a wide range of IEs, and excludes the discrimination against non-polar compounds that usually have high IEs.



**Figure 4.8** Comparison of range of ionizable compounds with direct APPI, toluene, CS<sub>2</sub>, anisole assisted APPI. As seen, toluene and anisole with the IEs of 8.8 and 8.2 eV, reduces the range of ionizable compounds, since their radical cations do not have sufficient energies to ionize compounds with high IE. On the contrary, CS<sub>2</sub> with IE of 10 eV, provides with the broadest range of ionizable compounds

The tightly sealed source, which is swept with highly pure nitrogen, minimizes the presence of background water, which can promote proton transfer route. Although the delivery dopant system is not completely sealed, addition of dopants still does not increase the water level to an extent to enable the formation of protonated dopant cations. This can be seen in Figure 4.4 that shows the exclusive formation of radical dopant cations with no protonated cation. Consequently, lack of LC solvents and background water eliminates the chance of proton transfer in both direct and dopant assisted modes. My data confirms this hypothesis, as all studied compounds formed  $M^{*+}$ , and  $[M+H]^+$  was only observed for four compounds (isophrone, N-nitrosodimethylamine, N-nitroso-di-n-propylamine, and pyridine). This can be ascribed to the possible dopant-effect of some analytes radical cations that may contribute in the formation of  $[M+H]^+$  ions for some analytes.

The comparative selectivity level of each ionization approach (direct APPI, toluene and  $CS_2$  assisted APPI) to different classes of compounds is shown in

. Due to the united ionization mechanism (charge transfer) in three approaches, a similar pattern was observed: while the studied ethoxy ethers were not ionized with any of these approaches, PAHs and nitro-compounds showed the highest and lowest degree of ionization, respectively.

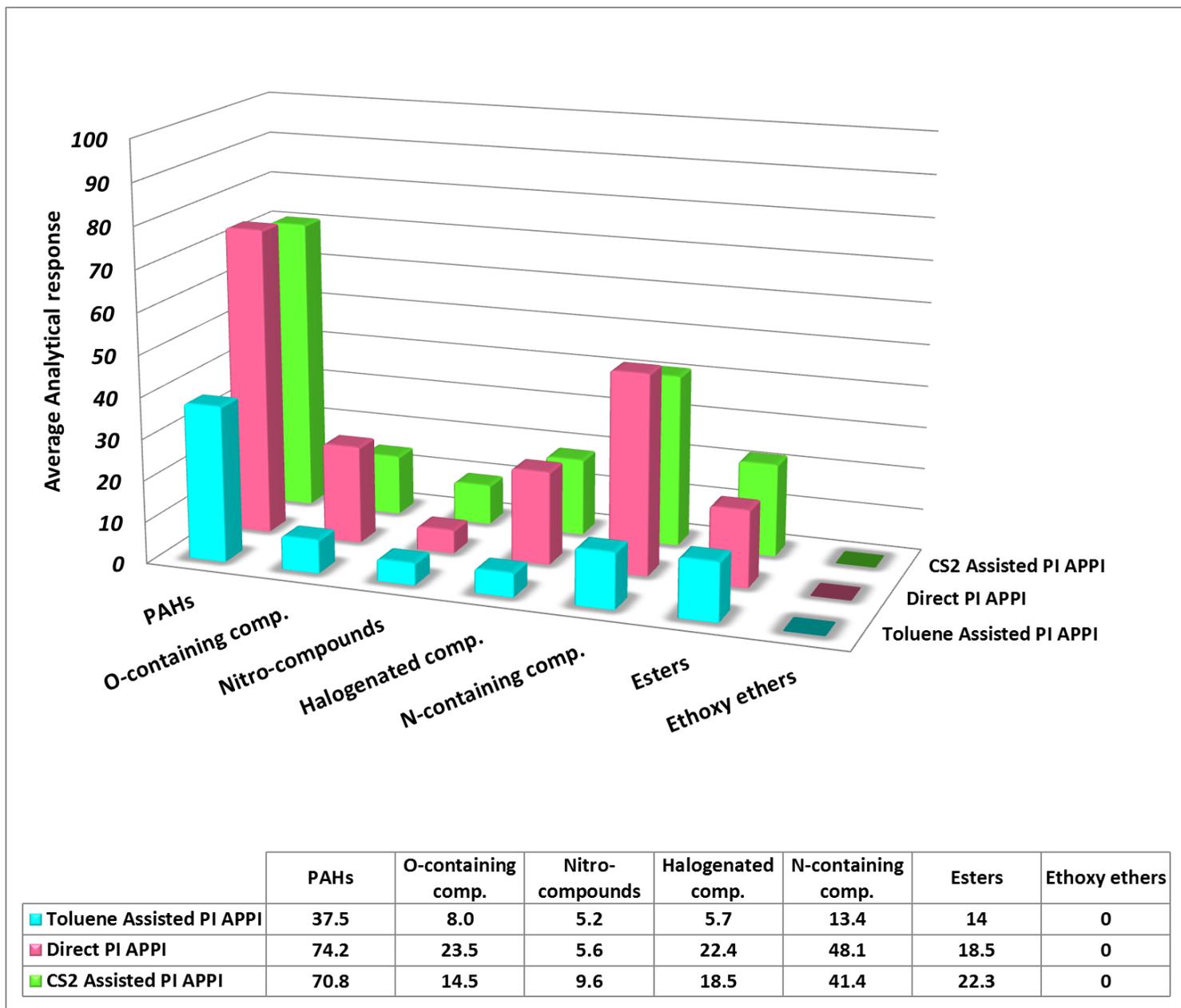


Figure 4.9 The sensitivity of the three ionization approaches to different classes of compounds. The analytical responses for all compounds were normalized against the maximum response in each method and then for each class of compounds the average of responses was calculated for comparison. This shows a comparative general degree of ionization obtained with each method for each class of compounds.

#### 4.4 Conclusion

The custom-built API interface can be easily employed to couple a GC to any mass spectrometer with only minor modification(s). Using GC as a separation technique not only adds all of its superior chromatographic advantages, including its higher resolution and peak capacities compared to LC, but also eliminates the presence of LC eluents. This elimination eradicates all interference caused by LC solvents, including radiation loss, suppression of reactant ions, and unwanted charge transfer reactions, which all complicate the analysis. On the other hand, the main aspects of the custom-built API interface design (e.g. its sealed conical shape, flow pattern of analytes inside the source and toward the MS, minimum distance between the photon and analyte stream with maximum overlap) have all led to a maximization of the ionization efficiency. Due to the minimized background water, only charge transfer controls the ionization, leading to the formation of analyte molecular ions in the final spectra. Eradicating proton transfer event eliminates the possible discrimination against low proton affinities compounds, which is a significant drawback in API coupled to LCs. The GC-APPI MS showed excellent sensitivities with LODs as low as  $\text{fg}/\mu\text{L}$  in direct ionization approach with no dopant. When toluene and carbon disulfide were used as dopants, a high abundance of dopant radical cations were formed, which further ionized the analytes through charge transfer.  $\text{CS}_2$  showed greater performance in enhancing the ionization efficiencies of almost all compounds, and promoted the ionization of compounds having high IEs, due to the fact that its IE is as high as photons' energy (10 eV). On the other hand, toluene suppressed the ionization of compounds possessing higher IE than itself which is 8.8 eV.

GC-APPI MS offers a promising tool for complex and thorough analysis, for example, of biological samples and metabolome screening in metabolomics. Due to the substantial diversity and dynamism of metabolome, the main challenge in metabolomics is to obtain an unbiased fingerprint of metabolome. Effective ionization through charge transfer, which is controlled exclusively by IEs occurring in a chemically simple ionization matrix, eliminates matrix effects and the bias against polarities, which are the main concerns with the currently used ionization methods in metabolomics. The limited energy of photons and reactant ions minimize possible fragmentations and maximize the abundance of the unique characteristic

molecular ions, both of which facilitate a reliable identification of compounds on the basis of their molecular weight and isotopic patterns. In addition, utilizing different dopants with different ionization energies provides a further management tool to control the range of ionizable compounds and ionization force. For example, using dopants with lower IEs can be important when even less ionization power than 10 eV is needed to avoid fragmentation of sensitive and fragile compounds. This factor can be useful in lipidomics. On the other hand, using CS<sub>2</sub> with the highest IE, provides us with the widest range of ionizable compounds.

## 5 Chapter: Performance of Isoprene as a dopant in GC-NI-APPI-MS

### 5.1 Abstract

The performance of isoprene as a dopant for the negative ion mode in a custom-built atmospheric pressure interface (API) — with a Kr lamp as a photoionization source—, which couples a gas chromatograph (GC) to an Orbitrap mass spectrometer (MS), is presented. The tightly sealed, conically shaped interface has two inlets for GC eluent and make-up gas (with or without dopant) and one outlet toward the MS. The sample eluted from the GC is mixed with the make-up gas, forming a vortex pattern, in which ionization takes place. The shape, size, and flow patterns inside the source and toward the MS have all been carefully designed to maximize the likelihood of direct photoionization. The GC-custom-built APPI/MS was shown to achieve excellent sensitivities without the necessity to use dopants in the positive mode (Chapter 4). In this work, the analytical performance of the source in the negative mode was evaluated in both direct- and dopant-assisted APPI with an EPA mixture containing 78 compounds with vast gas-phase properties. Toluene and isoprene were utilized as dopants. Unlike the positive mode, dopants were proved to drastically increase the ionization responses of analytes, in some cases up to several thousand times compared to direct APPI. Isoprene was proven to be a more effective dopant compared to toluene leading to higher signal intensities. The quantitative performance of the GC-APPI/MS was evaluated in terms of linearity, limit of detection (LOD) with a signal-to-noise ratio (S/N) of 3, and repeatability (%RSD): Direct APPI provided a dynamic range of 100-800 pg/ $\mu$ L with good linearity ( $R^2 > 0.996$ ), LODs down to 50 fg/ $\mu$ L - 10 pg/ $\mu$ L, and % RSD of less than 10%. Toluene Assisted APPI offered a dynamic range of 1-100 pg/ $\mu$ L with good linearity ( $R^2 > 0.995$ ), and decreased the LODs to 1-200 fg/ $\mu$ L, and %RSDs were less than 14%. Last but not least, the best results were obtained by Isoprene Assisted APPI: A dynamic range of 0.1-100 pg/ $\mu$ L with good linearity ( $R^2 > 0.995$ ), LODs down to 1-50 fg/ $\mu$ L, and % RSDs of less than 10%.

## **5.2 Experimental**

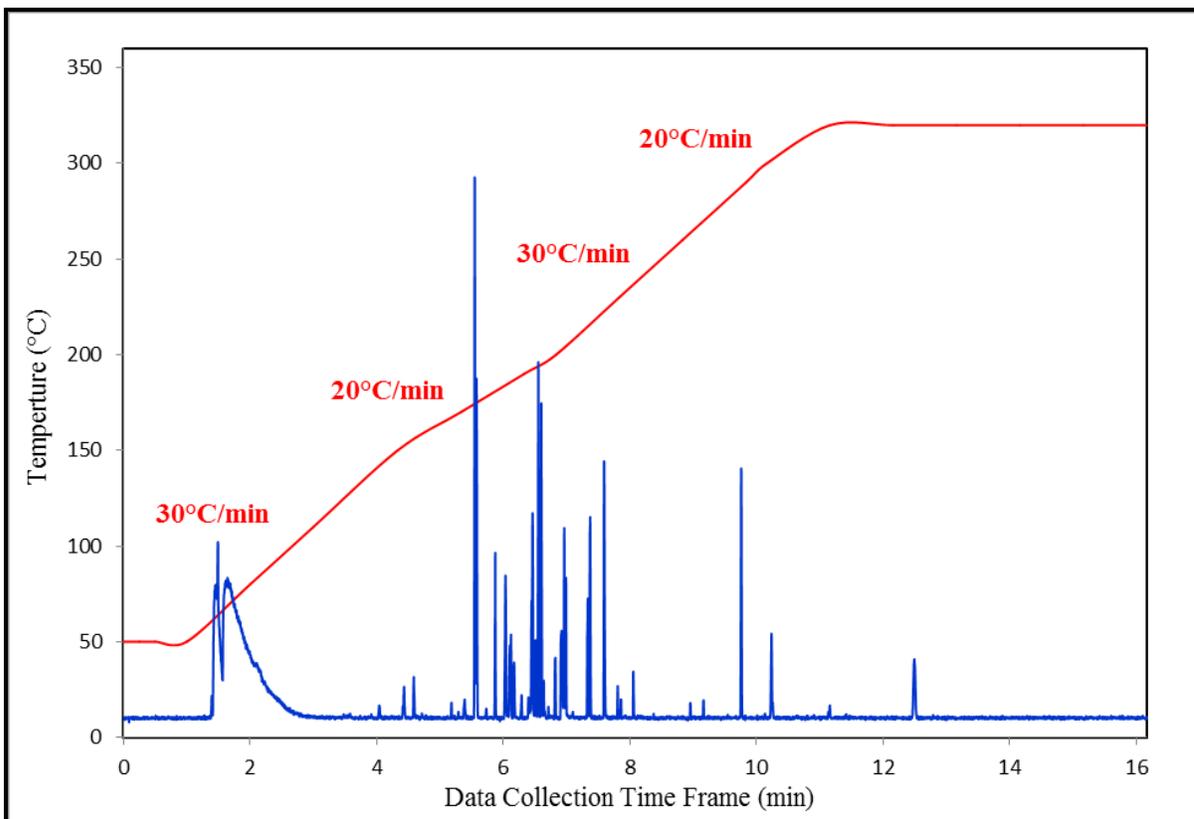
### **5.2.1 Chemicals and sample preparations**

EPA 8270 LCX Mix 1 (100  $\mu\text{g}/\text{mL}$  in acetone: methylene chloride (90:10)) was purchased from SUPELCO (Bellefonte, PA, USA). Working solutions in the range of 1  $\text{fg}/\mu\text{L}$  to 800  $\text{pg}/\mu\text{L}$  were made in dichloromethane by serial dilution. Stock and working solutions were kept in a freezer at  $-20^{\circ}\text{C}$ . All solvents were of analytical or HPLC grade. Toluene (99.9 %) and isoprene ( $\geq 99\%$ ) were purchased from Sigma-Aldrich (Steinheim, Germany). Isoprene contains less than 1000 ppm 4-tert butyl catechol as a stabilizer and impurities can include isoprene dimer (limonene) at less or equal to 0.5% (w/w). For dopant assisted APPI, a syringe pump with a gas-tight syringe was used to continuously introduce the headspace of dopants into a tee junction prior to the source. The dopant vapor (with a flow rate of 0.3  $\text{mL}/\text{min}$ ) joined the make-up gas stream (with a flow rate of 700  $\text{mL}/\text{min}$ ) inside the tee and together they entered the source.

### **5.2.2 Instrumentation**

#### **5.2.2.1 Gas chromatograph**

A Thermo Scientific 450 Series gas chromatograph equipped with a TR-Dioxin 5MS column (30  $\text{m} \times 0.25 \text{ mm i.d.} \times 0.1 \mu$ ; Milan, Italy) was used for chromatographic separation. The temperature program used in this work, along with a total ion chromatograph (TIC) obtained by injection of 500  $\text{pg}$  of mixture on the column and ionized by direct APPI in the negative mode, are shown in Figure 5.1. The GC transfer line and injector temperature were  $325^{\circ}\text{C}$  and  $250^{\circ}\text{C}$  respectively. Helium with 99.999% purity (Messer Industriegase GmbH, Bad Soden, Germany) at a constant flow rate of 1.50  $\text{mL}/\text{min}$  was used as the carrier gas. All experiments were done by splitless injection of 1  $\mu\text{L}$  of EPA mix.



**Figure 5.1** Ramp chart of the oven temperature program of GC. Total Ion Chromatograph (TIC) was obtained by 1  $\mu\text{L}$  injection of 500  $\text{pg}/\mu\text{L}$  of the EPA mix. The TIC was accordingly scaled to be shown in parallel to the oven program.

### 5.2.2.2 Ionization source

A detailed description of the design of the custom-built API source and the way it couples the GC to the orbitrap mass spectrometer is found in Chapter 4. Briefly described here, the tightly sealed interface has a conical shape and its base is covered by a MgF<sub>2</sub> window through which irradiation of the GC eluent by the Kr VUV lamp occurs. Two fine holes right below the MgF<sub>2</sub> window conduct the GC eluent and the make-up gas into the source. Make-up gas enters the interface at an angle toward the center of the cone and creates a vortex flow inside the source while mixing with the GC eluent. The whole gas stream is irradiated by the lamp as it exits the source toward the MS through a third opening. Figure 4.2(a) in Chapter 4 shows a schematic view of the ion source, inlet, and outlet paths. A low-pressure Kr discharge lamp with a RF driver from Syagen (Santa Ana, CA, USA) emitting two lines of photons at 10.0 and 10.6 eV was used as the photoionization source. The lamp is positioned right above the MgF<sub>2</sub> window of the source by a custom-made lamp holder and irradiates the entire source volume. Figure 4.2(b) in Chapter 4 shows the entire GC-API interface.

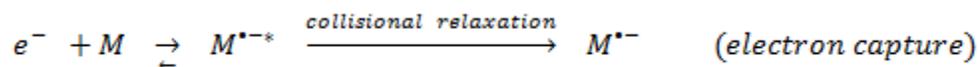
### 5.2.2.3 Mass spectrometer

A Thermo Scientific (Bremen, Germany) Exactive Orbitrap at a resolution of 10,000 was used. A minor modification with the mass spectrometer is needed to adapt the custom-built API interface: A stainless steel capillary (Klaus Ziemer GmbH, Langerwehe, Germany) modified with a gas-tight adapter replaced the original MS transfer capillary. All the experiments were run in the negative mode. The flow injection of isoprene vapor by a syringe pump was used to optimize the mass spectrometer parameters in terms of obtaining the maximum intensity for the *m/z* of 126.94 with both auto tuning and manual tuning. The optimized parameters used in this work are as follows: Capillary temperature: 300°C, capillary, tube lens, and skimmer voltages were -37.50, -55.00, and -13.00 volts respectively. The mass range was 50-1000 for all measurements.

### 5.3 Results and discussion

In DA-APPI, dopant molecules are effectively photo-ionized producing a dopant radical cation and an electron. What is important in the negative ion APPI (NI-APPI) is the formation of electrons. Under atmospheric pressure, the electrons are collisionally cooled resulting in thermal-energy electrons which can attach to the compounds having high electron affinities (EAs):

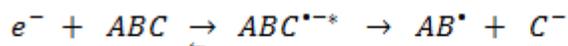
**Equation 12:**



This electron capture by a compound leads to the formation of a radical anion that carries excess energy equal to its EA. With the high collisional rate under atmospheric pressure, the excited state may get stabilized losing its excess energy and forming a stable thermal-energy radical anion. For compounds with high EAs, the rate constant of this electron capture is large enough to effectively produce stable anions, while compounds with low EAs undergo the reverse reaction leading to auto detachment of the electron (E. Chen, Wentworth, Desai, & Batten, 1987; Williamson, Knighton, & Grimsrud, 2000). High electron affinities are related to the electronegativity of compounds. Therefore, compounds having strong electronegative moieties such as fluorine, aromatic nitro groups, and highly conjugated compounds show high EAs.

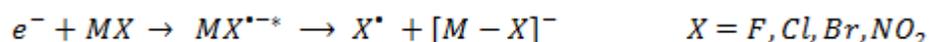
This formation of stable molecular radical anions is not the only ionization process accounting for the high sensitivities in the negative ion MS applications. The second main process is the dissociative electron capture process yielding highly valuable negative ions as fragmentation products which provide great sensitivities for some analytes:

**Equation 13:**



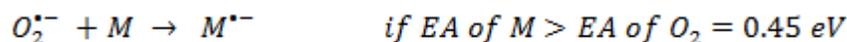
This process largely depends on the ability of the leaving group AB to absorb the excess internal energy: The larger the size of AB, the greater the possibility of Equation 13 occurring. Halogens and nitro groups usually leave the analyte carrying the excess internal energies and producing quantitative fragments (H. Leis, Fauler, Rechberger, & Windischhofer, 2004):

**Equation 14:**



In addition to the above ionization routes, the oxygen molecule, which is a principal constituent in API sources, performs an important function in the negative mode; O<sub>2</sub> with a positive electron affinity of 0.45 eV can easily capture an electron forming a superoxide ion. The formed superoxide ion induces different ionization paths as follows: Compounds, with higher EAs than that of oxygen, seize oxygen's negative charge and form molecular radical anions:

**Equation 15:**



Gas-phase superoxide is a relatively strong base ( $\Delta_{acid} G (HO_2^{\bullet}) = 1451 \text{ kJ/mol}$ ). Therefore, compounds with higher gas-phase acidities than that of HO<sub>2</sub><sup>•</sup> can be deprotonated by superoxide anions, forming deprotonated molecular anions:

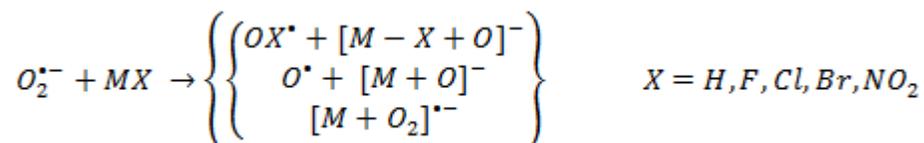
**Equation 16:**



When photoionization occurs within LC eluent matrices, superoxide can deprotonate solvent molecules and these solvent molecules can further deprotonate analyte molecules, provided thermodynamical conditions make these proton transfers feasible.

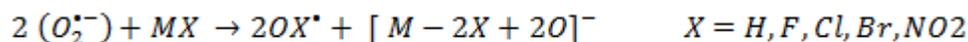
Another major contribution of oxygen and superoxide molecules in the formation of anions in the negative mode is through formation of oxidation products. A variety of reactions are possible, which may be accompanied by abstraction of halogens, hydrogen, and nitro groups. Below are some major pathways (Basso et al., 2003; Derpmann et al., 2012; L. Song, Wellman, Yao, & Adcock, 2007; L. Song, Wellman, Yao, & Bartmess, 2007):

**Equation 17:**



A further oxidation may result in:

**Equation 18:**



Although the negative mode has been largely overlooked compared to the positive mode, its unique dependence on electron capture event adds another dimension of selectivity to the mass spectrometry-based analysis, which can be considered as a valuable aspect in inclusive screening of complex mixtures, for example in metabolomics.

Conventional gas chromatography/ mass spectrometry (GC/MS) use electron ionization (EI) and chemical ionization (CI) under vacuum condition. In terms of the performance of these ionization sources in the negative mode, while EI lacks the selectivity in the negative mode, negative ion-chemical ionization (NI-CI) provides good sensitivities and selectivities (Chung, Lin, Yang, & Lee, 2009; Fitzgerald, Rixin, & Herold, 1993; N Karlonas,

Padarauskas, Ramanavicius, Minkuviene, & Ramanaviciene, 2012; Nerijus Karlonas, Ramanavicius, & Ramanaviciene, 2014; H. J. Leis & Windischhofer, 2010; Maurer, Kraemer, Kratzsch, Peters, & Weber, 2002; Wille, Van Hee, Neels, Van Peteghem, & Lambert, 2007).

Like modern generations of liquid chromatography/ mass spectrometry (LC/MS) instruments that utilize API interfaces to perform ionizations under atmospheric pressure (Carroll et al., 1975; Robb et al., 2000; Syage et al., 2000; Whitehouse et al., 1985) innovative generations of GC/MS are currently being developed to facilitate the API also in conjunction with GC (Bristow et al., 2010; Carrasco-Pancorbo et al., 2009; Haapala et al., 2013; Hintikka et al., 2013; Luosujärvi et al., 2008; McEwen & McKay, 2005; Suominen et al., 2013). This groundbreaking generation of GC/MS-based analytical approaches combines all three outstanding analytical strategies together in the vision of achieving a comprehensive analysis. First, the new generation of atmospheric pressure ionizations methods, which are primarily intended for LC/MS applications, can now be exploited with any GC. Second, using GC as the chemical separation method, adds all of its superior advantages over LC to the analysis, including its better chromatographic resolution, higher peak capacities, versatility, comparative low cost of purchase and maintenance, ease of routine operation and maintenance, absence of matrix-dependent ion suppression, and green nature (Fritz, 1981). Third, with the API interfaces, any high resolution mass spectrometer can be coupled to GCs, and in turn, all of its impressive capabilities such as,  $MS^n$ , high mass resolution, and accurate mass measurements can benefit an analysis.

In this thesis, the performance of isoprene as a dopant for GC/NI-APPI applications is presented in the negative mode. The same custom-built API stage with a commercial Kr lamp, which was used in Chapter 4 was utilized for this work as well. The custom-built interface combined a Thermo GC with an orbitrap mass spectrometer. The design aspects of the custom-built interface have been fully discussed in Chapter 4. In that work, the performance of the custom-built APPI source in the positive mode was presented in both direct APPI mode (with no dopant) and dopant assisted-APPI (with toluene and carbon disulfide as dopants). An EPA mixture containing 78 compounds with vast varieties of gas-phase ion energetic properties was investigated. The source was shown to enable excellent sensitivities in the direct mode, without the need to use dopants in order to enhance the

ionization intensities and LODs; however, using dopants exclusively produced a high quantity of dopant radical cations, in which their ionization energies controlled the range of ionizable compounds in DA-APPI.

Isoprene was already introduced in Chapter 3 as an efficient dopant to enhance the ionization efficiency of an APPI coupled to a HPLC in the negative mode. Results proved the potential of isoprene as an effective dopant for NI-APPI, since better signal intensities were obtained by isoprene compared to those obtained by using toluene as a dopant (Dousty & O'Brien, 2015).

Isoprene with high photoabsorption and photoionization cross sections in the VUV range, effectively is photo-ionized and produces a range of products including its molecular radical cation, its dimer and trimer cations, etc. and each ionization event produces one electron as well. The complex background of isoprene in the positive mode disqualifies its application as a dopant in the positive mode. On the other hand, its efficient electron production under photoionization along with its clean background spectrum in the negative ion mode, makes it a good dopant candidate for NI-APPI. Additionally, the high vapor pressure of isoprene at room temperature facilitates its usage as a dopant for GC/MS-based applications.

In this work, the utilization of isoprene as a NI-dopant for the custom-built GC-APPI source is presented. The results are compared with those obtained in the direct APPI mode (with no dopant present) and with toluene-assisted APPI. The same EPA mixture that was used in the previous study in the positive mode (Chapter 4) was utilized here in the negative mode. The vast range of analytes, including those possessing the required gas-phase ion energetic properties in the negative mode, enabled a comprehensive investigation of the performance of the instrument in NI direct APPI. Additionally using dopant, isoprene, in comparison to the most commonly used dopant, toluene, enabled a comprehensive investigation of dopant utilization in GC-NI -APPI MS analysis.

### **5.3.1 GC Direct NI-APPI-MS**

Among 78 compounds of the EPA mixture, only compounds with positive electron affinities and relatively high gas-phase acidities are capable of producing detectable stable anions in the negative mode. Compounds possessing halogens, aromatic nitro groups, and highly

conjugated segments have generally positive electron affinities and compounds with hydroxyl groups and nitrogen-containing functional groups typically have enough gas-phase acidities to generate deprotonated anions. Table 5.1 presents the compounds identified in the NI-direct APPI with no dopant by injecting 1  $\mu\text{L}$  of 100  $\text{pg}/\mu\text{L}$  of the EPA mixture. As shown in Table 5.1, compounds having positive EAs undergo electron capture or dissociative electron capture, while compounds having high gas phase acidities become deprotonated. Butyl benzyl phthalate, which is a fragile ester, underwent fragmentation by ionization and/or collisionally induced dissociation during molecular anion transfer.

**Table 5.1 Compounds detected in NI-Direct APPI with no dopant**

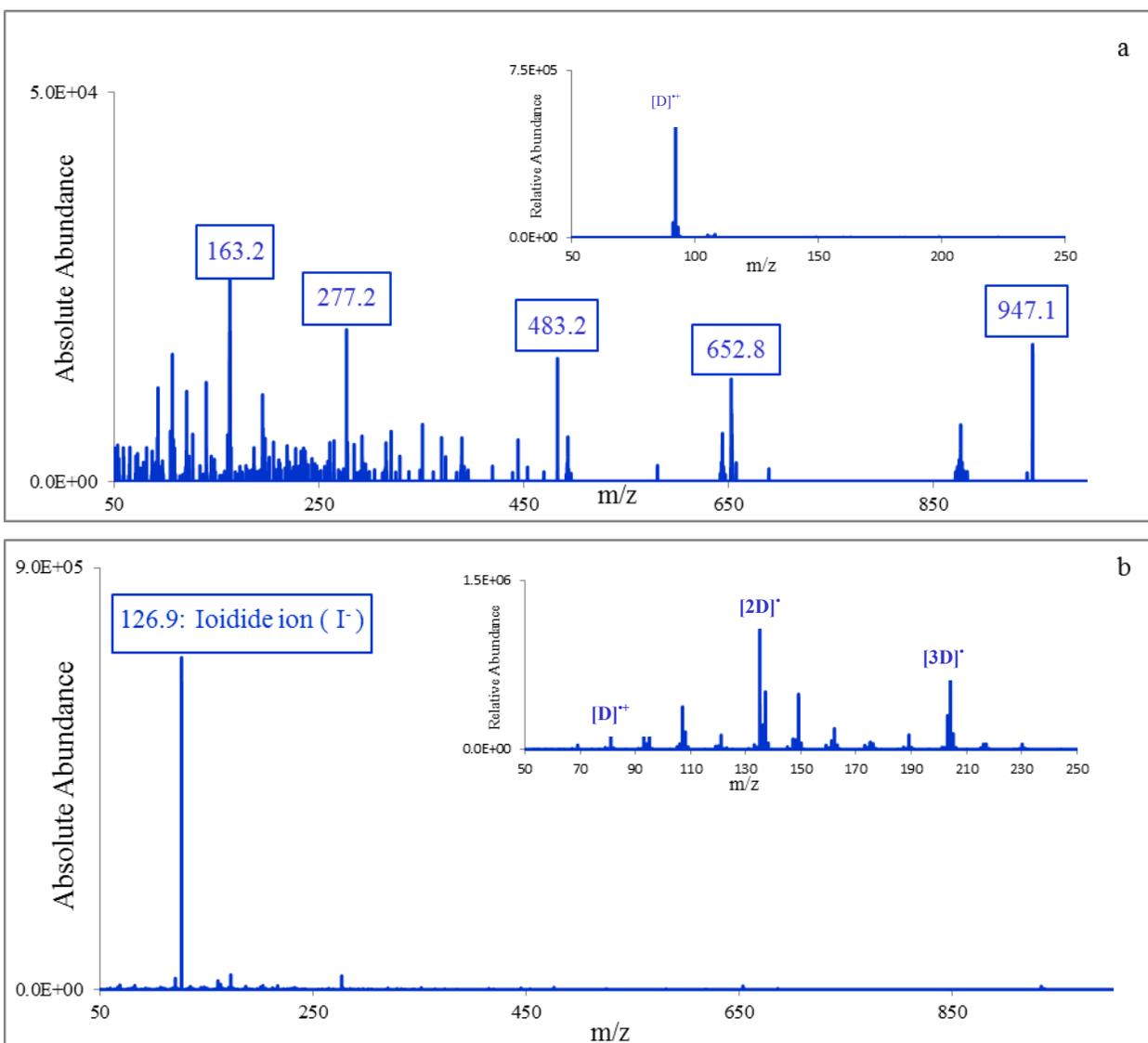
<b>Compound</b>	<b>EA (eV)</b>	<b><math>\Delta_{\text{acid}} G</math> (kJ/mol)</b>	<b>Main ion (base peak)</b>	<b>Other ions (% relative to the base)</b>
<b>Poly Aromatic Hydrocarbons (PAHs)</b>				
Acenaphthylene	<0.403		$M^{\cdot-}$	$[M+O]^-$ (50%)
indeno(1,2,3-cd)pyrene	$\sim 0.42^b$		$M^{\cdot-}$	$[M-H+O]^-$ (20%)
<b>Nitro compounds</b>				
2-Nitrophenol		1379	$[M-H]^-$	-----
4-Nitrophenol		1343	$[M-H]^-$	-----
2-Nitroaniline	0.95	1443	$[M-H]^-$	-----
4-nitroaniline	0.92	1407	$[M-H]^-$	-----
1,4-dinitrobenzene	2.00		$[M-NO_2+O]^-$	$M^{\cdot-}$ (30%)
1,3-Dinitrobenzene	1.66		$[M-NO_2+O]^-$	$M^{\cdot-}$ (30%)
1,2-Dinitrobenzene	1.65		$[M-NO_2+O]^-$	$M^{\cdot-}$ (10%)
2,6-Dinitrotoluene	1.47		$[M-NO_2+O]^-$	$[M-H]^-$ (30%)
2,6-Dinitrotoluene	<1.60		$[M-H]^-$	$[M-NO_2+O]^-$ (30%)
2-methyl-4,6-dinitrophenol	>1 <sup>b</sup>		$[M-OH]^-$	$[M-H]^-$ (10%)
<b>Halogenated Compounds</b>				
2,4-Dichlorophenol		$\sim 1360^b$	$[M-H]^-$	-----
Hexachlorocyclopentadiene	>0.7 <sup>b</sup>		$[M-Cl]^-$	-----
2,4,6-Trichlorophenol		$\sim 1375^b$	$[M-H]^-$	-----
2,4,5-Trichlorophenol		$\sim 1367^b$	$[M-H]^-$	-----
2,3,4,6-Tetrachlorophenol		$\sim 1348^b$	$[M-H]^-$	$[M-H-Cl+O]^-$ (10%)
2,3,5,6-tetrachlorophenol		$\sim 1346^b$	$[M-H]^-$	$[M-H-Cl+O]^-$ (10%)
4-Bromophenyl phenyl ether	>0 <sup>b</sup>	>1407 <sup>b</sup>	$[M-Br+O_2]^-$	$[Br]^-$
Hexachlorobenzene	0.92		$[M-Cl+O]^-$	-----
Pentachlorophenol		$\sim 1327^b$	$[M-H]^-$	-----
3,3-dichlorobenzidine	>1.16 <sup>b</sup>		$[M-Cl-2H]^-$	$[M-H]^-$ (40%)
<b>Esters</b>				
Butyl benzyl phthalate	>0 & <0.8 <sup>b</sup>		Fragment: $[M-C_6H_5CH_3-H]^-$	Fragment: $[M-C_6H_5CH_3-C_3H_7]^-$ (20%)

<sup>b</sup> Due to the lack of full gas-phase ion energetic data for some compounds in NIST data base, some estimates had to be done using the available experimental data for their structural analogs. For example,  $\Delta_{\text{acid}} G$  values for trichlorophenols, tetrachlorophenols, and pentachlorophenol have been estimated using available  $\Delta_{\text{acid}} G$  values for phenol, 2-chlorophenol, 3-chlorophenol, and 4-chlorophenol.

## 5.3.2 GC Dopant Assisted NI-APPI MS

### 5.3.2.1 Reactant ion profiles for toluene and isoprene as dopants.

Mass spectra showing the reactant ion profiles of dopants, toluene and isoprene, are shown in Figure 5.2. To obtain these profiles, the dopants' vapors at a liquid flow rate of a 300  $\mu\text{L}/\text{min}$  were combined with the make-up gas stream with a gas flow rate of 700  $\text{mL}/\text{min}$  in a tee junction prior to photoionization. Figure 5.2(a) depicts the reactant ion profile for toluene. As shown, only background ions at the noise level were observed in the toluene spectrum indicating that toluene does not produce any reactant anion in the negative mode. The same trend was observed in the reactant ion profile of isoprene (Figure 5.2(b)). The only difference was the presence of the mass of 126.9 with the intensity of about three orders of magnitude higher than background ions. Collision induced dissociation (CID) experiments were performed in order to obtain structural information for this mass, but the anion did not undergo any fragmentation. Moreover, the high resolution of the orbitrap mass spectrometry can illustrate isotopic patterns, which can lead to useful insights on the elemental composition of a compound. Yet, no isotopic distribution was observed for this mass, which excludes the possibility of the presence of poly isotopic elements (such as carbon, oxygen, nitrogen, chlorine and bromine) in its formula. Therefore, this mass is associated with only mono-isotopic elements. These facts and the result of CID experiments has led to the conclusion that the mass of 126.9 is related to iodide ion ( $\text{I}^-$ ). Iodide can be a contaminant in the isoprene reagent.



**Figure 5.2** The reactant ion profiles obtained by using toluene (a) and isoprene (b) as dopants in the NI APPI mode. The insets display the reactant ion profile of each dopant in the positive mode. The letter D stands for dopant.

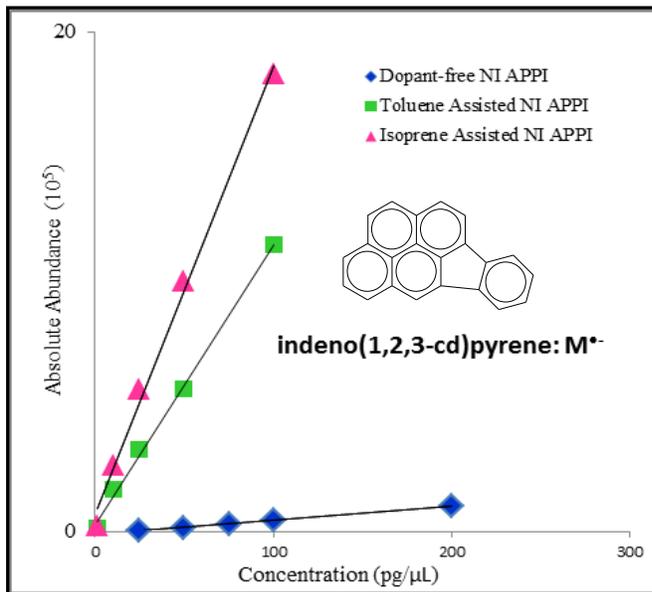
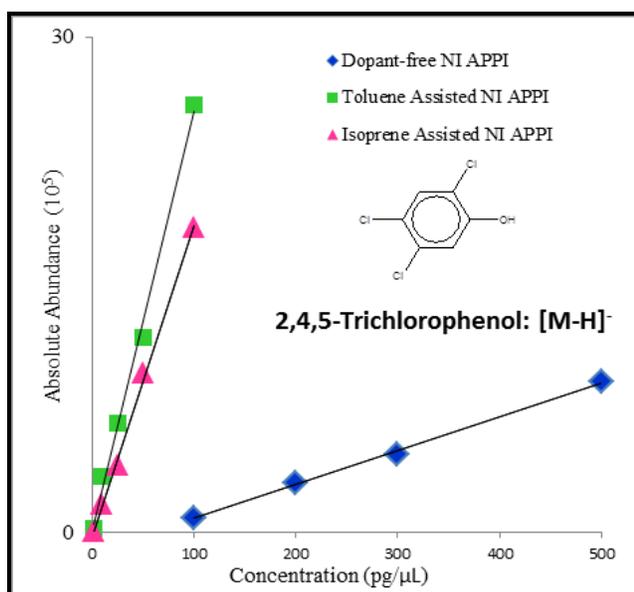
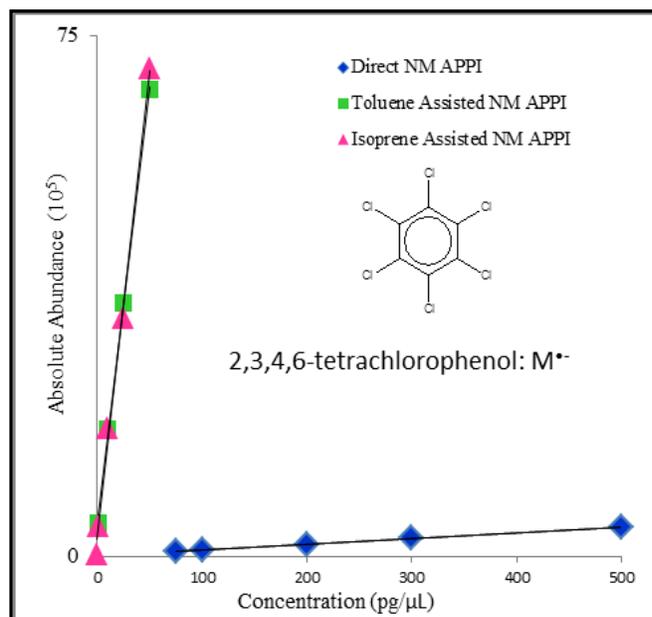
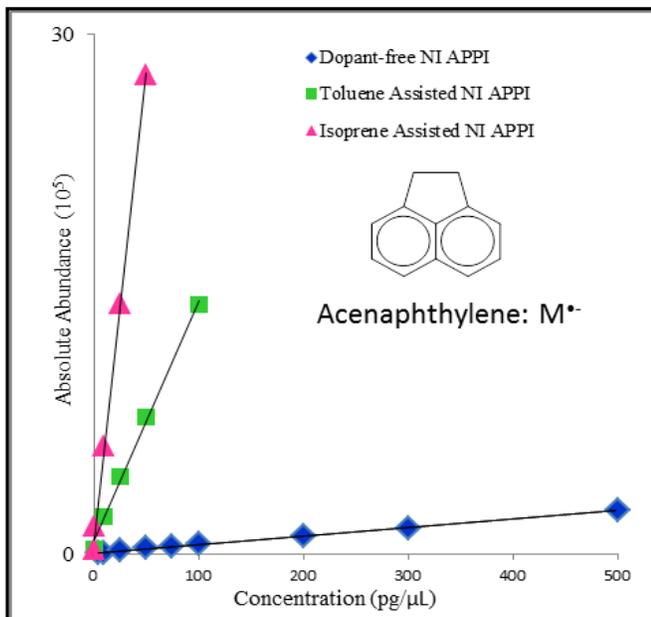
Since both dopants do not produce any reactant anions for the charge transfer mechanism in the negative mode, they can only enhance the ionization by increasing the number of accessible photo-electrons. Therefore, the dopant that becomes more effectively photoionized produces more thermal electrons, which further can be captured by analytes and oxygen molecules. Inserted smaller graphs in Figure 5.2, shows the reactant profile ions of dopants in the positive mode. As shown, while toluene only produces a radical cation, isoprene produces a range of radical cations with high quantities including its dimer and trimer. Since each photo ionization event is accompanied by production of an electron, it is safe to assume that photoionization of isoprene produces higher quantities of thermal electrons compared to toluene. The present results, which show that isoprene performs better as a dopant, confirm this hypothesis.

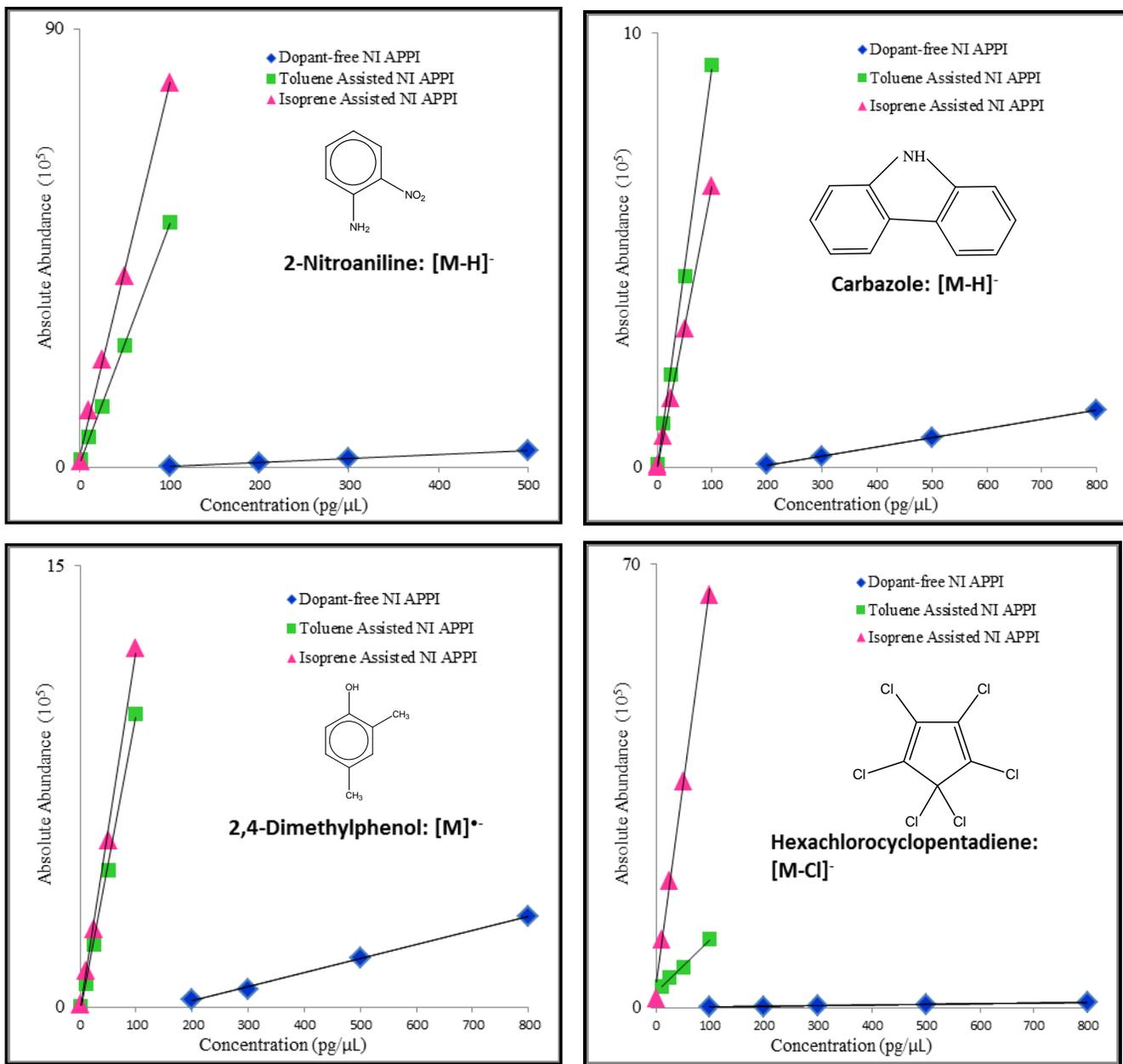
### **5.3.2.2 Comparison of direct APPI, toluene, and carbon disulfide assisted APPI**

#### **5.3.2.2.1 Linear dynamic range and detection limits.**

In Chapter 4, the performance of the custom-built GC-APPI source was examined in the positive mode, and dopants were shown to increase the sensitivity to some extent; however, excellent sensitivities were as well obtained in the direct APPI mode with no dopant present. The clean and simple matrix from GC, which effectively excluded the matrix absorption of photons, and the fundamental design features of the custom-built source facilitate an effective direct ionization of analytes in the positive mode. Although all of these aspects benefit the ionization in the negative mode as well, due to the nature of ionization mechanism in the negative mode, which are almost exclusively controlled by thermal electrons, dopants contribute in another way: Efficient photoionization of dopants greatly increases the number of available thermal electrons compared to direct photoionization in which only limited ionization of trace analytes and the photo electric effect produces a restricted number of electrons. The present data confirmed this hypothesis, as the addition of dopants increased the ionization intensities up to three orders of magnitude compared to direct NI-APPI. Figure 5.3 compares the linear dynamic range obtained for eight analytes with direct NI-APPI,

Toluene-Assisted NI-APPI, and isoprene Assisted APPI and Table 5.2 correspondingly presents the correlation coefficient ( $R^2$ ) of linearity, LODs and reproducibility of the method in terms of %RSDs of analytical responses. The analytical responses were determined from the peak area of the single ion chromatography (SIM) for the dominant anion for each analyte. LODs were calculated from the corresponding spectra based on S/N of three.





**Figure 5.3** Calibration curves for eight compounds in the negative mode with direct APPI- Toluene Assisted APPI and Isoprene Assisted APPI. For each analyte, the absolute abundance of the peak area of single ion chromatography (SIM) for its dominant anion, as shown in each graph, has been considered.

**Table 5.2 The R<sup>2</sup> values of linearity, LODs, and %RSDs obtained with Direct NI-APPI, Toluene Assisted NI-APPI, and Isoprene-Assisted NI-APPI.**

Compound	NI-Direct APPI			Toluene Assisted NI-APPI			Isoprene Assisted NI-APPI		
	R <sup>2</sup>	LOD (fg/μL)	%RSD	R <sup>2</sup>	LOD (fg/μL)	%RSD	R <sup>2</sup>	LOD (fg/μL)	%RSD
Acenaphthylene	0.996	1500	<6	0.996	2	<8	0.999	2	<8
Hexachlorobenzene	0.997	1600	<5	0.998	1.5	<5	0.995	1	<6
2,4,5-Trichlorophenol	0.997	3600	<8	0.995	5	<12	0.998	10	<5
indeno(1,2,3-cd)pyrene	0.996	12,300	<7	0.997	12	<12	0.995	9	<8
2-Nitroaniline	0.998	15	<9	0.999	2.5	<14	0.997	4	<5
Carbazole	0.999	110	<9	0.998	12	<6	0.999	1.5	<7
2,4-Dimethylphenol	0.998	45	<10	0.999	160	<8	0.998	17	<10
Hexachlorocyclopentadiene	0.996	5500	<10	0.998	90	<5	0.996	46	<7

Toluene and isoprene both greatly increased the analytical responses. Overall, higher analytical responses were obtained within one order of magnitude with isoprene assisted APPI compared to toluene assisted APPI for most analytes. The iodide anion present in the spectra of isoprene did not take part in any charge transfer reaction, as its SIM was constant throughout the whole run. Iodine, with the high EA of 2.5 eV (NIST Standard Reference Database Number 69), can effectively capture thermal electrons and thus reduces the accessible number of electrons for analytes. The high intensity of iodide in the spectra of isoprene assisted APPI demonstrates and verifies the efficiency of the electron capture by iodine. Therefore, an iodine-free isoprene reagent could result in the enhanced performance of isoprene as a dopant, because then all the produced photo-electrons can be captured by analytes without any competition from iodine.

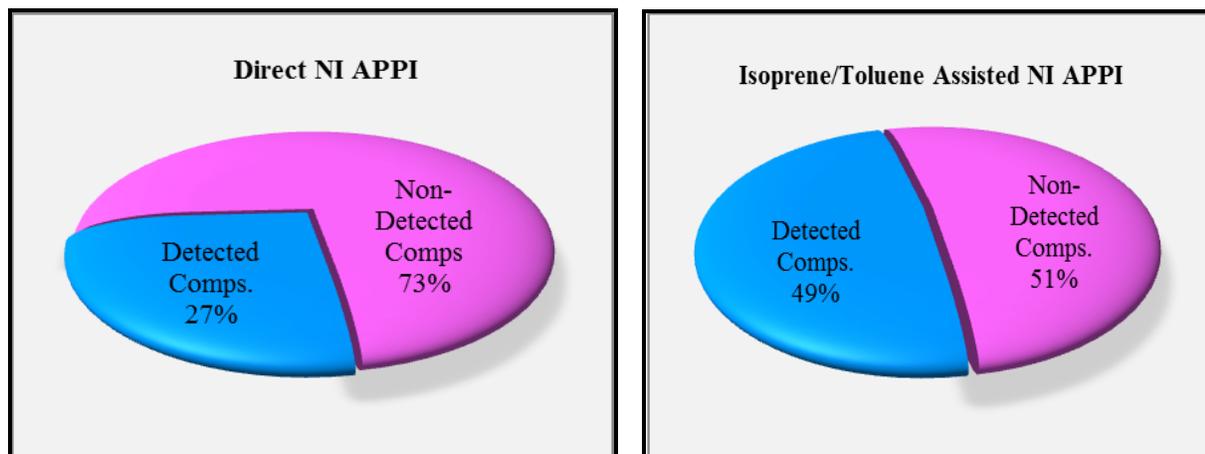
#### **5.3.2.2.2 Range of ionizable compounds obtained by each method.**

The addition of dopants significantly increases the ionization intensity of analytes, and also broadens the range of ionizable compounds compared to direct NI APPI. Table 5.3 represents the analytes that were only detected in dopant assisted modes (with both toluene and isoprene) but not by direct APPI, and Figure 5.4 compares the range of ionizable compounds with direct APPI, dopant Assisted APPI with both isoprene and toluene as dopants. Note that all the comparisons were made with the results obtained by injecting 1  $\mu\text{L}$  of the EPA mixture with a concentration of 100  $\text{pg}/\mu\text{L}$  into the GC.

**Table 5.3 Compounds detected only with dopant assisted NI-APPI and not with Direct NI-APPI.**

<b>Compound</b>	<b>EA (eV)</b>	<b><math>\Delta_{\text{acid}} G</math> (kJ/mol)</b>	<b>Main ion (base peak)</b>	<b>Other ions (% relative to the base)</b>
<b>Poly Aromatic Hydrocarbons (PAHs)</b>				
Dibenzofurane	>0 <sup>b</sup>		[M-H] <sup>-</sup>	[M-H+O] <sup>-</sup> (20%)
Fluorene	<0.278	1434	[M-H] <sup>-</sup>	
Benzo(b)fluoranthene	>0.6 <sup>b</sup>		[M-H +O] <sup>-</sup>	[M-H ] <sup>-</sup> (10%)
Benzo(k)fluoranthene	>0.6 <sup>b</sup>		[M-H +O] <sup>-</sup>	[M-H ] <sup>-</sup> (10%)
Benzo(a)pyrene	0.82		[M-H +O] <sup>-</sup>	[M-H ] <sup>-</sup> (20%)
Benzo(g,h,i)perylene	0.60		[M-H +O] <sup>-</sup>	-----
<b>Oxygen-containing compounds</b>				
Benzyl alcohol		1520	[M-H] <sup>-</sup>	-----
2-methylphenol		1431	[M-H] <sup>-</sup>	-----
3-methylphenol		1434	[M-H] <sup>-</sup>	-----
4-methylphenol		1437	[M-H] <sup>-</sup>	-----
Isophorone		~1300 <sup>b</sup>	[M-H] <sup>-</sup>	-----
2,4-Dimethylphenol		~1430 <sup>b</sup>	[M+O] <sup>-</sup>	M <sup>-</sup> (80%)
Benzoic acid		1394	[M+O] <sup>-</sup>	M <sup>-</sup> (80%)
<b>Nitro compounds</b>				
Nitrobenzene	1.00	1545	[M-H+O] <sup>-</sup>	M <sup>-</sup> (10%)
2,4-Dinitrophenol		1291	[M-H] <sup>-</sup>	[M-NO <sub>2</sub> +O] <sup>-</sup> (50%)
<b>Halogenated Compounds</b>				
4-chloro-3-methylphenol		~1409 <sup>b</sup>	[M-H] <sup>-</sup>	-----
<b>Nitrogen-containing</b>				
N-nitrosodiphenylamine	>0 & <2 <sup>b</sup>		[M-NO] <sup>-</sup>	-----
Carbazole		1412	[M-H] <sup>-</sup>	

<sup>b</sup> Due to the lack of full gas-phase ion energetic data for some compounds in NIST data base, some estimates had to be done using the available experimental data for their structural analogs.



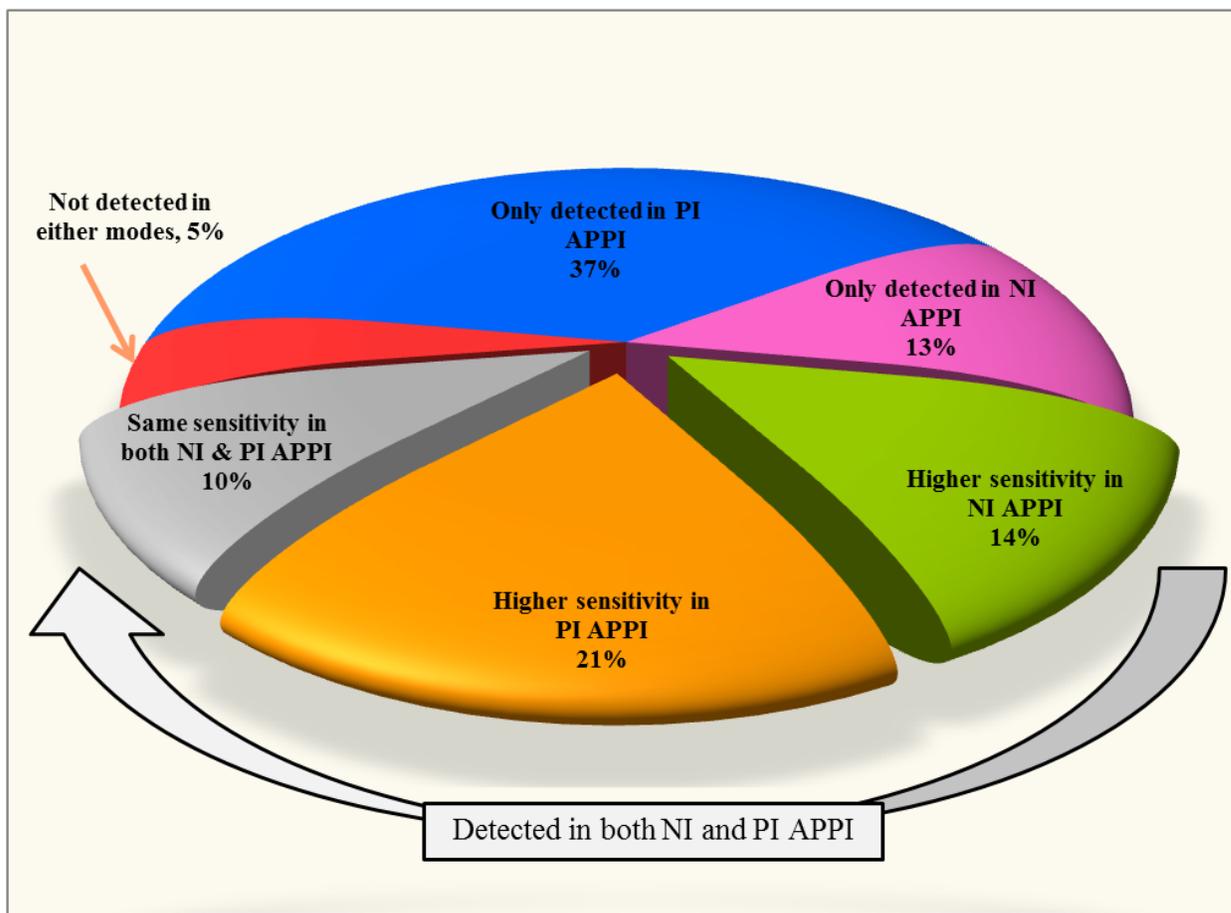
**Figure 5.4** A comparison of the range of ionizable compounds with direct APPI, toluene Assisted APPI, and isoprene assisted APPI. The comparison was made with the results obtained by injecting 1  $\mu\text{L}$  of the EPA mixture with a concentration of 100  $\text{pg}/\mu\text{L}$  into the GC.

### 5.3.2.2.3 Comparison of sensitivity and selectivity of Dopant Assisted APPI in NI and PI approaches.

The exact same instrumentation and EPA mixture were used in previous work (cf. Chapter 4) to examine the performance of the custom-built APPI in the PI. Therefore, a reliable comparison can be made with the result of this work in the NI, which indicates the general selectivity and sensitivity of the Dopant Assisted APPI technique in both NI and PI approaches. Figure 5.5 illustrates the selectivity of Dopant Assisted APPI in NI and PI by comparing the range of ionizable compounds obtained by isoprene assisted NI APPI and carbon disulfide assisted PI APPI. For both methods, 1  $\mu\text{L}$  of the EPA mixture at a concentration of 100  $\text{Pg}/\mu\text{L}$  was injected into the GC and the headspace of dopants at a flow rate of 300  $\mu\text{L}/\text{min}$  was introduced into the source with make-up gas with a flow rate of 700  $\text{ml}/\text{min}$ .

In Figure 5.5, the pink section contains compounds that were only ionized in the NI, while the blue section shows the ionizable compounds only in PI. The protruding sections in the graph, which contain compounds ionized in both modes, have been sub-divided in three sections: the

green section indicates the compounds that were strongly ionized in NM compared to PM, while the orange section displays otherwise, i.e. compounds which were ionized in NM to some extent but were effectively ionized in PM. The gray area displays compounds ionized with the same sensitivity in both dopant assisted NI and PI approaches. In addition to the above comparison, Figure 5.5 clearly demonstrates the comprehensive ionization coverage that can be achieved by applying APPI in both modes, as only four compounds out of 78 were not detected with either approach (red section of the graph). The ability of PI/NI APPI to cover a broad range of analytes is fundamental, especially with mass spectrometers capable of positive/negative polarity switching (Yuan, Breitkopf, Yang, & Asara, 2012) or with newly developed dual-polarity mass spectrometers (H.-K. Chen, Chang, Wu, Huang, & Wang, 2009; Hsiao et al., 2011; Tsai et al., 2006) that allow simultaneous measurements of positive and negative ions in a single analysis. By using APPI for the ionization source of these mass spectrometers, a comprehensive mass analysis can be obtained in a single analysis. This is very advantageous for metabolomics platforms, as a larger number of metabolites can be profiled in one experiment.



**Figure 5.5** The general selectivity of CS<sub>2</sub> Assisted PI APPI and Isoprene Assisted NI APPI obtained with the EPA mixture (which can be regarded as a relatively comprehensive selection of various analytes). The comparison was made when 1 μL of the EPA mixture at a concentration of 100 pg/μL was injected into the GC.

As mentioned earlier, using GC as the chromatographic separation brings some unique advantages to the analysis in general. When GC is used with NI, it can specifically benefit the ionization in an additional aspect, i.e. chemical derivatisation. In GC-MS analysis, chemical derivatisation of analytes is commonly used prior to analysis in order to modify the analytes for GC process, since many analytes are not volatile enough or thermolabile for GC (Pang, Lewis, & Hamilton, 2011; Poli et al., 2010). Sometimes, even thermolabile compounds may undergo a chemical derivatisation step in order to improve their chromatographic properties. Although the main purpose of chemical derivatisation is to affect the volatility, thermostability, and/or chromatographic behaviors of analytes, this derivatisation can be regarded as a tactic to enhance the ionization response of analytes as well. Various chemical derivatisation reagents for selective functional-groups modifications are available; fortunately many derivatisation reagents have highly electronegative sites, which benefit the negative ionization, as they impart EAs to analytes that may not be inherently capable of forming stable negative ions. Examples of these derivatisation reagents that can change the characteristic of analytes toward negative ion acceptors include perfluoroacyl, pentafluorobenzyl, and o-pentafluorobenzoyloxime derivatives (H. Leis et al., 2004).

#### **5.4 Conclusion**

The custom-built API with a photoionization source combined with a GC offered sensitive and selective performances in the negative mode. Isoprene was utilized as a dopant for GC-NI APPI/MS for the first time and the results were compared with those obtained by toluene. While isoprene was shown to offer better sensitivities compared to toluene, both dopants significantly increased the sensitivity and selectivity of the method by lowering the LODs to fg/ $\mu$ L degree and broaden the range of ionizable compounds compared to direct NI APPI in which no dopant was added. The excellent performance of the dopants is related to the highly increased quantity of thermal electrons produced from dopant photoionization. In direct NI-APPI, electrons are only produced from photoionization of trace analytes, which are very limited, compared to the quantity of dopants. The dependence of the negative

ionization on the EAs of analytes, and thus on their electronegativities, is both limiting and advantageous. On one hand, it greatly reduces the range of ionizable compounds; on the other hand, it adds to the analysis an additional dimension of selectivity with high sensitivity for this class of compounds that cannot be achieved by PI. Compounds having high EAs, suitable for NI, usually are non-polar compounds with high IEs for which PI lacks sufficient sensitivity. The chemical derivatisation with GC-MS analysis benefits the NI, since many derivatisation reagents have electronegative moieties, which facilitate forming negative ions. Therefore, the GC/dopant assisted NI-APPI/MS bears a huge potential for trace level measurements where PI fails, and although in a small scale compared to PI, NI adds an additional indispensable part to the profiles obtained by MS analysis.

## 6 Chapter: Evaluation of direct and dopant-assisted APLI in GC-MS applications

### 6.1 Abstract

The analytical potential of direct and dopant-assisted APLI for GC/MS applications was evaluated using an EPA mixture as analyte solution that contains 78 chemicals representing different classes of compounds. A gas-tight custom-built ionization interface coupled a GC to an orbitrap mass spectrometer. An excimer laser delivered laser radiation at a 248 nm (5 eV) wavelength, which provided a total of 10 eV for two-step ionization. Toluene, anisole, carbon disulfide, and isoprene were examined as dopants. The pure and well-controlled matrix from both GC and make-up gas (highly purified N<sub>2</sub>) along with the gas-tight layout of the ionization interface provided a simplified matrix, which facilitates the investigation of ionization mechanisms by minimizing interferences and parallel ionization/ion transformation events. Therefore, ionization mechanisms underlying direct and dopant-assisted APLI in both PI and NI modes were also investigated. Direct-PI APLI almost exclusively led to the formation of molecular radical cations and offered a unique selectivity toward polycyclic aromatic compounds. The short lifetime of the excited virtual intermediate states of carbon disulfide suppressed its laser ionization. Therefore, carbon disulfide radical cations were not produced to assist the ionization. Positive electron affinity of carbon disulfide disqualifies its applications for NI APLI. Toluene provided the best results for both PI and NI APLI compared to anisole and isoprene. Laser ionization of toluene generated abundant dopant radical cations, which determined the subsequent chemical system with charge transfer as the predominating mechanism and covered the ionization of analytes whose spectroscopic features do not favor laser ionization. In PI APLI, addition of toluene led to a 2- to 10-fold increase in ion intensities and broadened the range of ionizable compounds from 20 to 51 analytes. The ionization mechanisms in toluene-assisted NI APLI were controlled by thermal electrons, similar to NI APPI. Laser ionization of toluene increased the number of thermal electrons and led to a 2- to 50-fold increase in ion intensities and expanded the range of ionizable compounds from 15 to 44 analytes. Although the geometry of the custom-built API is not at optimum to achieve the maximum sensitivity for

APLI, quantitative performance of the current GC APLI MS system was evaluated in terms of linearity, limit of detection (LOD) with a signal-to-noise ratio (S/N) of 3, and repeatability (%RSD) for both direct and toluene-assisted APLI in PI and NI modes. Both approaches offered acceptable linearity and repeatability and the addition of toluene improved the LODs to some extent in NI mode and more significantly in PI approach.

## **6.2 Experimental**

### **6.2.1 Chemicals and sample preparations**

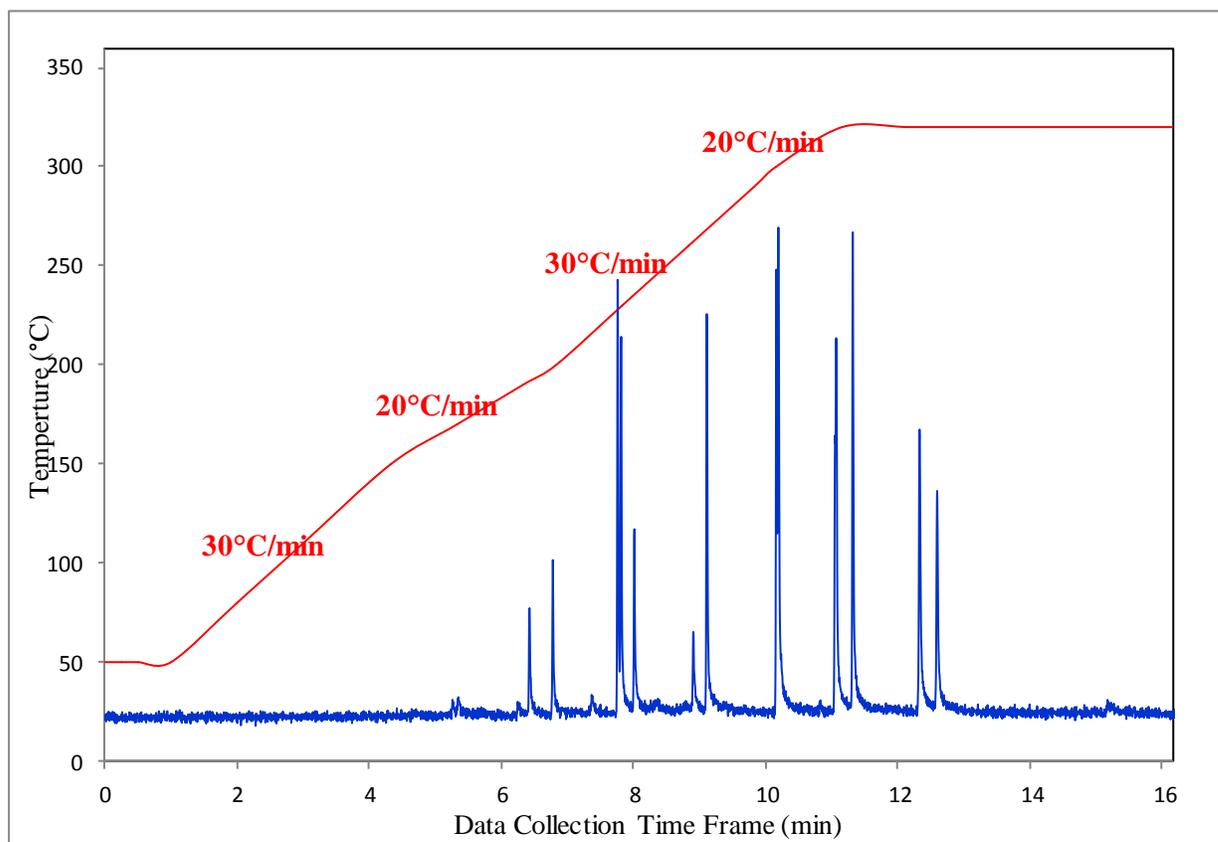
EPA 8270 LCX Mix 1 (100 µg/mL in acetone: methylene chloride (90:10)) was purchased from SUPELCO (Bellefonte, PA, USA). A series of working solutions in the range of 1 fg/µL to 800 pg/µL were made in dichloromethane by serial dilution. Stock and working solutions were kept in a freezer at -20°C. Toluene (99.9 %), anisole (99.7 %), Carbon disulfide (≥99 %), and isoprene (≥ 99%) were purchased from Sigma-Aldrich (Steinheim, Germany). Isoprene contains less than 1000 ppm 4-tert butyl catechol as a stabilizer and impurities can include isoprene dimer (limonene) at less or equal to 0.5% (w/w). For dopant assisted experiments, the headspace of dopants was continuously introduced into a tee junction prior to the source with a gas-tight syringe and a syringe pump through a fused-silica capillary at a flow rate of 300 µL/min. Dopants were mixed with the make-up gas inside the tee and then entered the ionization chamber.

### **6.2.2 Instrumentation**

#### **6.2.2.1 Gas chromatograph**

A Thermo Scientific 450 Series gas chromatograph equipped with a TR-Dioxin 5MS column (30 m×0.25 mm i.d. × 0.1 µ; Milan, Italy) was used for chemical separation. The temperature program used in this work, along with a total ion chromatograph (TIC) obtained by injection

of 1  $\mu\text{L}$  of EPA mixture at a concentration of 50  $\text{pg}/\mu\text{L}$  and ionized by direct PI APLI, are shown in Figure 6.1. The GC transfer line and injector temperature were 325°C and 250 °C respectively. Helium with 99.999% purity (Messer Industriegase GmbH, Bad Soden, Germany) at a constant flow rate of 1.50 mL/min was used as the carrier gas. All experiments were done by splitless injection of 1  $\mu\text{L}$  of EPA mix.



**Figure 6.1** Ramp chart of the GC oven temperature program. Total Ion Chromatograph (TIC) was obtained by 1  $\mu\text{L}$  injection of 50  $\text{pg}/\mu\text{L}$  of EPA mix, which was then ionized by direct PI APLI. The TIC was accordingly scaled to be shown in parallel to the oven program.

### 6.2.2.2 Ionization source

A detailed description of the design of the custom-built API interface and the way it couples the GC to the orbitrap mass spectrometer can be found in chapter 4. Briefly described here, the base of the gas-tight conical shaped interface is covered by a MgF<sub>2</sub> window through which laser radiations meet the GC eluent. Two fine holes right below the MgF<sub>2</sub> window conduct the GC eluent and the make-up gas into the source. Make-up gas enters the interface with an angle toward the center of the cone and creates a vortex flow inside the source while mixing with the GC eluent. The stream is irradiated by the laser as it exits the source toward the MS through a third opening. An ATLEX 300 excimer laser (ATL Lasertechnik, Wermelskirchen, Germany) was used to provide laser radiation and its operational parameters are as follows: the 248 nm (5 eV) radiation was obtained by a Kr/F<sub>2</sub> gas mixture, a repetition rate of 50 Hz, a pulse energy of 5mJ, a pulse width of 5 ns, a rectangular profile beam with 0.5 cm<sup>2</sup> beam area and a power density of 2E6 W/cm<sup>2</sup>.

Figure 6.2 (a) shows the custom-built radiation probe, which was used to direct the laser radiation to the MgF<sub>2</sub> window of the ionization chamber. Figure 6.2(b) shows the entire GC-APLI-MS set-up.

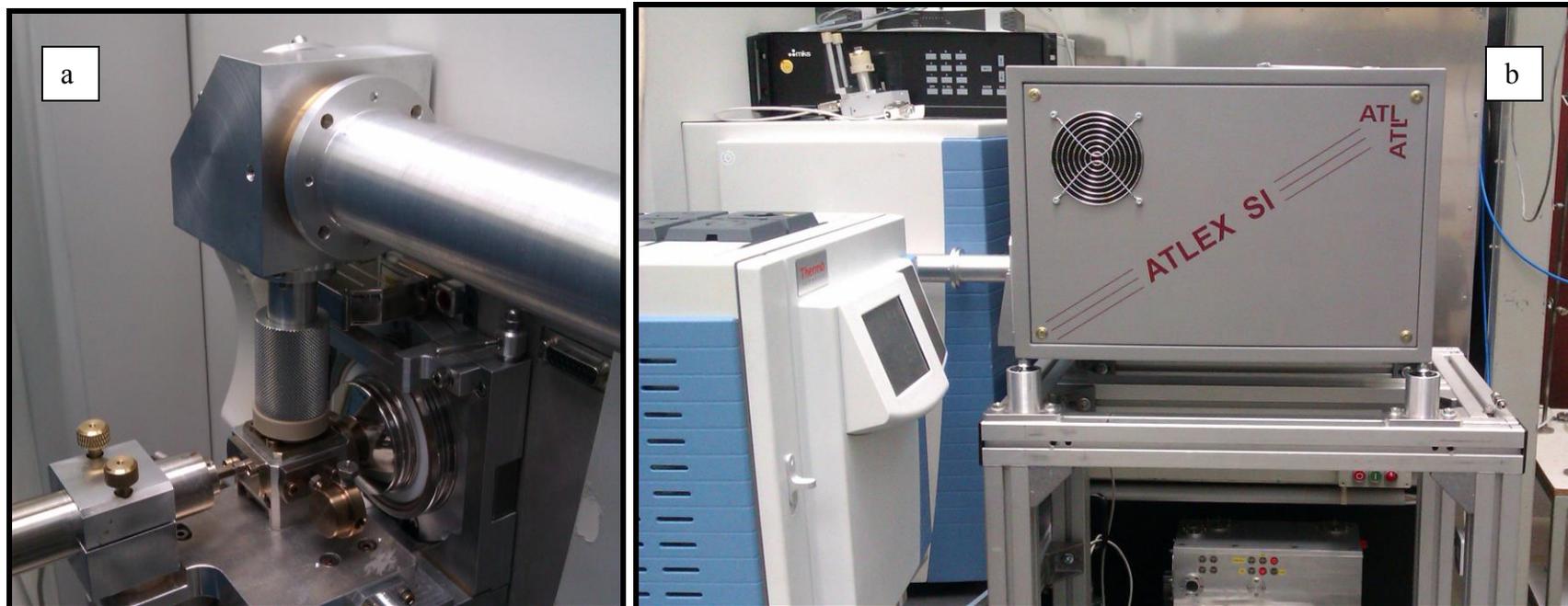


Figure 6.2 (a) The APLI probe, which directs laser radiation to the  $\text{MgF}_2$  window of the API interface. (b) The entire GC APLI MS system.

### 6.2.2.3 Mass spectrometer

A Thermo Scientific (Bremen, Germany) Exactive Orbitrap at a resolution of 10,000 was used. A minor modification with the mass spectrometry is needed to adapt the custom-built API interface: A stainless steel capillary (Klaus Ziemer GmbH, Langerwehe, Germany) modified with a gas-tight adapter replaced the original MS transfer capillary. The flow injection of isoprene vapor by a syringe pump was used to optimize the mass spectrometry parameters in the NI mode, in terms of obtaining the maximum intensity for the  $m/z$  of 126.94 by applying both auto tuning and manual tuning. For optimizing PI APLI parameters, the same approach was followed for the  $m/z$  92.06 of toluene. The optimized parameters that were used are as follows: In NI-APLI, capillary temperature was 300°C and capillary, tube lens, and skimmer voltages were -37.50, -60.06, and -16.00 volts respectively. In PI-APLI, capillary temperature was also 300°C and capillary, tube lens, and skimmer voltages were 26.50, 36.30, and 14.00 volts respectively. The mass range was  $m/z$  50-1000 for all measurements.

## 6.3 Results and discussion

The EPA mixture contains 78 chemicals representing different classes of compounds (nineteen Poly Aromatic Hydrocarbons (PAHs), eight oxygen-containing, thirteen nitro-containing, twenty halogenated, eight nitrogen containing, seven esters, and three ethoxy ethers compounds). Detailed information on the compounds, their percent purities and concentrations can be found in the MSDS sheet of the EPA mix. The diverse range of gas-phase ion energetic properties and functional groups of chemicals in the EPA mixture was used to evaluate the capability of direct and dopant-assisted PI/NI APLI for GC/MS application in detail. In addition, the pure and well-controlled matrix from both GC and make-up gas (highly purified N<sub>2</sub>) along with the gas-tight layout of the ionization interface provided a simplified matrix, which facilitates the investigation of ionization mechanisms by minimizing interferences and parallel ionization/ion transformation events. Therefore, ionization mechanisms underlying Dopant-free/ Dopant-assisted APLI in both PI and NI

were also investigated and are discussed along with the results. Furthermore, since the same EPA mixture, GC/API/MS configuration, and a total photon energy of 10 eV used for the GC-PI/NI APPI/MS studies discussed in chapters 4 and 5, were also used for the APLI studies discussed here, the selectivity (the range of ionizable compounds) of the current APLI system for GC/MS applications can be reliably interpreted in the context of selectivity with APPI. The quantitative performance of the GC-APLI/MS was also evaluated in terms of linearity, limit of detection (LOD) with a signal-to-noise ratio (S/N) of 3, and repeatability (%RSD) for some compounds in both direct and dopant-assisted APLI approaches. It is very important to note that the custom-built API has been optimized to achieve the most sensitive APPI performance; the wide base of the conical shaped interface matches the base of the krypton lamp, which maximizes photons and analyte stream overlap, and in turn maximizes the likelihood of direct ionization. In addition, the height of the cone is relatively short, which results in the largest possible volume of the analyte stream right below the MgF<sub>2</sub> window, where the photons' intensity is at its highest. This feature also helps in maximizing the intersection of photons and the largest volume of analyte stream, which in turn, maximizes the ionization responses. However, to obtain the most sensitivity for APLI, a different geometry of the API source is needed; for the pointed small-sized spot of laser radiation, a much smaller optical window and a longer beam travel path are required to maximize the likelihood of laser and analyte overlap and ionization. Therefore, by using the current interface for APLI, the maximum ionization efficiencies of analytes cannot be achieved, and this fact has to be considered when quantitative results are presented. Thus, a comparison of ionization efficiencies (sensitivities) obtained by APPI and APLI is irrelevant, since the source has been tailored toward obtaining the maximum intensity for APPI, not APLI. However, a comparison of ionization products (selectivities) in a relatively high concentration of 100 pg/μL between APPI and APLI is pertinent, which is reported in the results section.

For all methods, analytical responses were determined from the peak area of the single ion chromatography (SIM) for the dominant ion. The reported limits of detections (LODs) were obtained from the corresponding spectra for the dominant ion based on a signal-to-noise ratio (S/N) of three.

### 6.3.1 Dopant-free APLI

The laser used in this work delivers 248 nm (5 eV) photons, which provide a total of 10 eV energy for a two-photon ionization event. This energy is equal to that of a commercial krypton discharge lamp in an APPI source (VUV radiation with energies at 10.0 and 10.6 eV). Therefore, it is expected that all analytes with IEs less than 10 eV and favorable two-photon absorption cross-sections are ionized in this system. Table 6.1 presents GC/MS results obtained by the injection of 1  $\mu\text{L}$  of the mixture at a concentration of 100  $\text{pg}/\mu\text{L}$ , which was then ionized by direct APLI in both PI and NI modes.

**Table 6.1 Analytes ionized by dopant free APLI.**

<b>Dopant-Free PI APLI</b>		<b>Dopant-Free NI APLI</b>	
<b>Compound</b>	<b>Observed ions: m/z (% intensity) ion type</b>	<b>Compound</b>	<b>Observed ions: m/z (% intensity) ion type</b>
Naphthalene	128 (100) M <sup>+</sup>	Phenanthrene	208(100) [M-2H+2O] <sup>-</sup> ; 193(90) [M-H+O] <sup>-</sup> ; 177(40) [M-H] <sup>-</sup>
2-methylnaphthalene	142 (100) M <sup>+</sup>	Anthracene	208(100) [M-2H+2O] <sup>-</sup> ; 193(40) [M-H+O] <sup>-</sup> ; 177(40) [M-H] <sup>-</sup>
1-methylnaphthalene	142 (100) M <sup>+</sup>	Fluoranthene	233(100) [M-H+O <sub>2</sub> ] <sup>-</sup> ; 217(40) [M-H+O] <sup>-</sup>
Acenaphthene	154 (100) M <sup>+</sup>	Pyrene	232(100) [M-2H+2O] <sup>-</sup> ; 201(30) [M-H] <sup>-</sup> ; 217(20) [M-H+O] <sup>-</sup>
Dibenzofurane	168 (100) M <sup>+</sup>	Benzo(a)anthracene	242(100) [M-2H+O] <sup>-</sup> ; 227(10) [M-H] <sup>-</sup>
Fluorene	166 (100) M <sup>+</sup>	Chrysene	242(100) [M-2H+O] <sup>-</sup> ; 227(70) [M-H] <sup>-</sup>
Phenanthrene	178 (100) M <sup>+</sup>	Benzo(b)fluoranthene	282(100) [M-2H+2O] <sup>-</sup> ; 251(10) [M-H] <sup>-</sup>
Anthracene	178 (100) M <sup>+</sup>	Benzo(k)fluoranthene	282(100) [M-2H+2O] <sup>-</sup> ; 251(10) [M-H] <sup>-</sup>
Fluoranthene	202 (100) M <sup>+</sup>	Benzo(a)pyrene	282(100) [M-2H+2O] <sup>-</sup> ; 251(50) [M-H] <sup>-</sup>
Pyrene	202 (100) M <sup>+</sup>	indeno(1,2,3-cd)pyrene	276(100) [M-H] <sup>-</sup> ; 291(10) [M-H+O] <sup>-</sup>
Benzo(a)anthracene	228 (100) M <sup>+</sup>	Dibenzo(a,h)anthracene	277(100) [M-H] <sup>-</sup> ; 308(30) [M-2H+2O] <sup>-</sup>
Chrysene	228 (100) M <sup>+</sup>	Nitrobenzene	123(100) [M] <sup>+</sup> ; 138(30) [M-H+O] <sup>-</sup>
Benzo(b)fluoranthene	252 (100) M <sup>+</sup>	4- Nitrophenol	38(100) [M-H] <sup>-</sup> ; 108(30) [M-H-NO <sub>2</sub> +O] <sup>-</sup>
Benzo(k)fluoranthene	252 (100) M <sup>+</sup>	Carbazole	166(100) [M-H] <sup>-</sup> ; 181(10) [M-2H+O] <sup>-</sup>
Benzo(a)pyrene	252 (100) M <sup>+</sup>	3,3-dichlorobenzidine	215(100) [M-2H-Cl] <sup>-</sup> ; 251(30) [M-H] <sup>-</sup>
indeno(1,2,3-cd)pyrene	276 (100) M <sup>+</sup>		
Benzo(g,h,i)perylene	276 (100) M <sup>+</sup>		
Dibenzo(a,h)anthracene	278 (100) M <sup>+</sup>		
2-Chloronaphthalene	162 (100) M <sup>+</sup>		
Carbazole	167 (100) M <sup>+</sup>		

In typical analytical applications of APLI, the laser power intensities are limited in a range to restrict the REMPI process to only two-photon ionization. The first photon excites the molecule into a resonant state (Equation 19). If the lifetime of the resonant state is long enough (i.e. in the range of  $> \text{ns}$  to  $\mu\text{s}$ ) to have the opportunity of absorbing a second photon and also if the second photon provides the remaining energy needed for the ionization of the desired analyte, ionization occurs from the intermediate state (Equation 20). Therefore, for a two-step ionization, the sum of two photons' energy must exceed the IEs of analyte. The laser in this study delivers a 248 nm (5 eV) wavelength, which provides a total maximum energy of 10 eV. Since the IEs of most analytes are less than 10 eV, the ionization energy of the laser is not a limiting factor in this study. However, as already mentioned, this condition is not the only requirement; the lifetime of the excited state has to be long enough to undergo ionization with the absorption of a second photon, but not undergo the competing deactivation process, which can lead to an electronically lower-lying state by collisional deactivation (Equation 21a), simple light scattering (Equation 21b), fluorescence (Equation 21c), or intersystem crossing (Equation 21d).

**Equation 19**



**Equation 20**



**Equation 21**

(a)



(b)



(c)



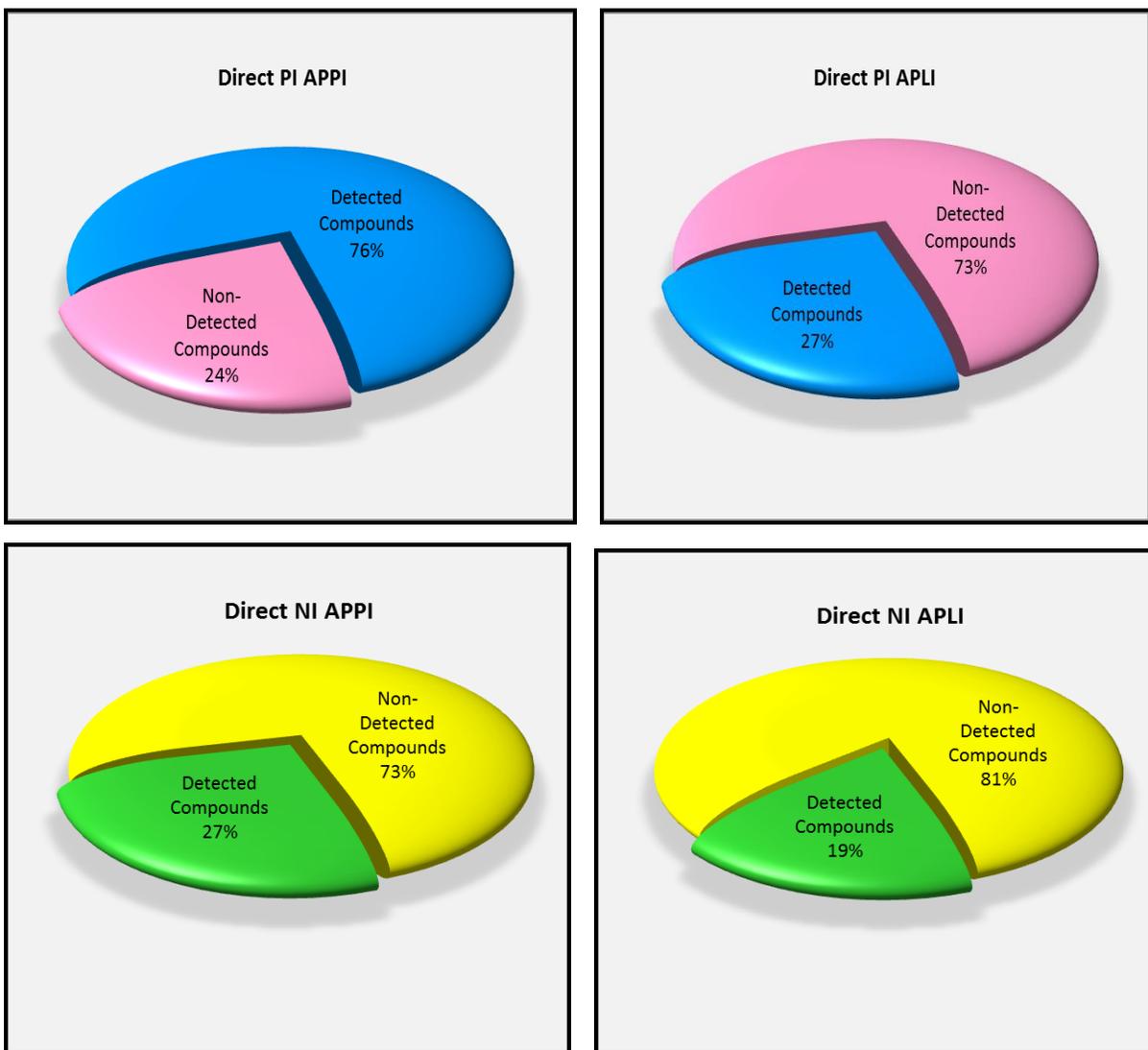
(d)



Despite different ionization mechanisms in dopant-free APPI and dopant-free APLI, the products of ionization are the same in such a clean matrix as used here; each ionization event leads to the production of a radical cation and an electron. Radical cations are detected in the PI mode and thermal electrons initiate ion formation in the NI mode.

As shown in Table 6.1, PI-APLI exclusively led to the formation of molecular radical cations and NI-APLI led to the production of a range of negative ions including molecular radical anions, deprotonated molecular ions, and oxidation products, which is similar to what occurs in NI-APPI.

The requirement of having relatively long-lived excited transition states for laser ionization highly restricts the selectivity of APLI toward aromatic compounds. APPI, on the other hand, offers a universal selectivity compared to APLI. Figure 6.3 compares the range of ionizable compounds obtained by dopant-free APLI and APPI.



**Figure 6.3** A comparison of the range of ionizable compounds by dopant-free (direct) APLI and APPI in both PI and NI modes obtained by injection of 1  $\mu\text{L}$  of the mixture at a concentration of 100  $\text{pg}/\mu\text{L}$ . APLI in positive mode offers exceptionally high selectivity toward polycyclic aromatic compounds, while PI APPI provides a universal selectivity, ionizing all compounds that were able to make stable positive ions. The *ratio* of ionized compounds to non-ionized compounds in NI APPI and NI APLI are comparable; however, the *types* of ionized compounds are not similar.

In PI APLI, out of 78 compounds of the EPA mixture all the nineteen studied PAHs, plus 2-chloronaphthalene and carbazole, were ionized, while in NI-APLI, eleven of the nineteen studied PAHs, plus nitrobenzene, 4-nitrophenol, carbazole, and 3,3-dichlorobenzidine, were ionized (Table 6.2).

Although this selectivity of APLI ionization mechanism toward analytes having long-lived excited transition states considerably limits its range of ionizable compounds compared to APPI, this high selectivity and high tolerance of APLI toward other compounds can be applied in targeted analyses of these compounds in complex mixtures, in the presence of interfering matrix components. All other ionization methods are subject to matrix effects in varying levels. APLI, in which all aliphatic compounds do not generate any signals and do not even take part in the so-called competition over ionization, could offer a matrix interference-free method for polycyclic aromatic compounds and could address the serious analytical issue of ion suppression in complex mixtures. Therefore, APLI could be directly utilized to analyze these compounds from their complex matrixes and eliminate the need for sample clean-up and chromatography separations. This advantage can highly improve the key analytical aspects of high-throughput analyses including reproducibility, matrix effects, precision, and accuracy for APLI favorable compounds. Compared to direct PI APLI, direct PI APPI provided a universal selectivity and ionized all the compounds that were able to make stable positive ions (Figure 6.3).

Negative ionization inherently provides much higher selectivity compared to positive ionization, simply due the fact that not many compounds are able to generate stable negative ions. As shown in Figure 6.3, the *percentage* of ionized compounds by NI APLI and NI APPI are comparable; however, the *type* of ionizable compounds by the two techniques are not similar. As already mentioned, In NI APLI, eleven of the nineteen studied PAHs, plus nitrobenzene, 4-nitrophenol, carbazole, and 3,3-dichlorobenzidine, were ionized, which all contain aromatic rings. In NI APPI, out of the nineteen studied PAHs analytes in the EPA mixture, only acenaphthylene and indeno(1,2,3-cd)pyrene were ionized, forming  $M^+$  as the dominant ion. These compounds both possess positive electron affinities (Table 5.1, Chapter 5). Other detectable compounds in the NI APPI include nitro-compounds (2-nitrophenol, 4-nitrophenol, 2-nitroaniline, 4-nitroaniline, 1,4-dinitrobenzene, 1,3-dinitrobenzene, 1,2-

dinitrobenzene, 2,6-dinitrotoluene, 2,6-dinitrotoluene, 2-methyl-4,6-dinitrophenol), halogenated compounds (2,4-dichlorophenol, hexachlorocyclopentadiene, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol, 2,3,4,6-tetrachlorophenol, 2,3,5,6-tetrachlorophenol, 4-Bromophenyl phenyl ether, hexachlorobenzene, pentachlorophenol, 3,3-dichlorobenzidine), and one ester (butyl benzyl phthalate) (Table 5.1, Chapter 5). These compounds all possess positive electron affinities or relatively high gas phase acidities, which facilitate the formation of stable negative ions. Nitrobenzene with a relatively high positive electron affinity of 1.0 eV (NIST Standard Reference Database Number 69) was not detected in NI APPI at a concentration of 100 pg/ $\mu$ L; however, it was detected at higher concentrations with NI APPI. These types of detected analytes are very different than the compounds ionized in NI APLI. All compounds ionized in NI APLI (Table 6.1) contain an aromatic ring. This difference suggests that a different ionization mechanism may be involved in NI APLI.

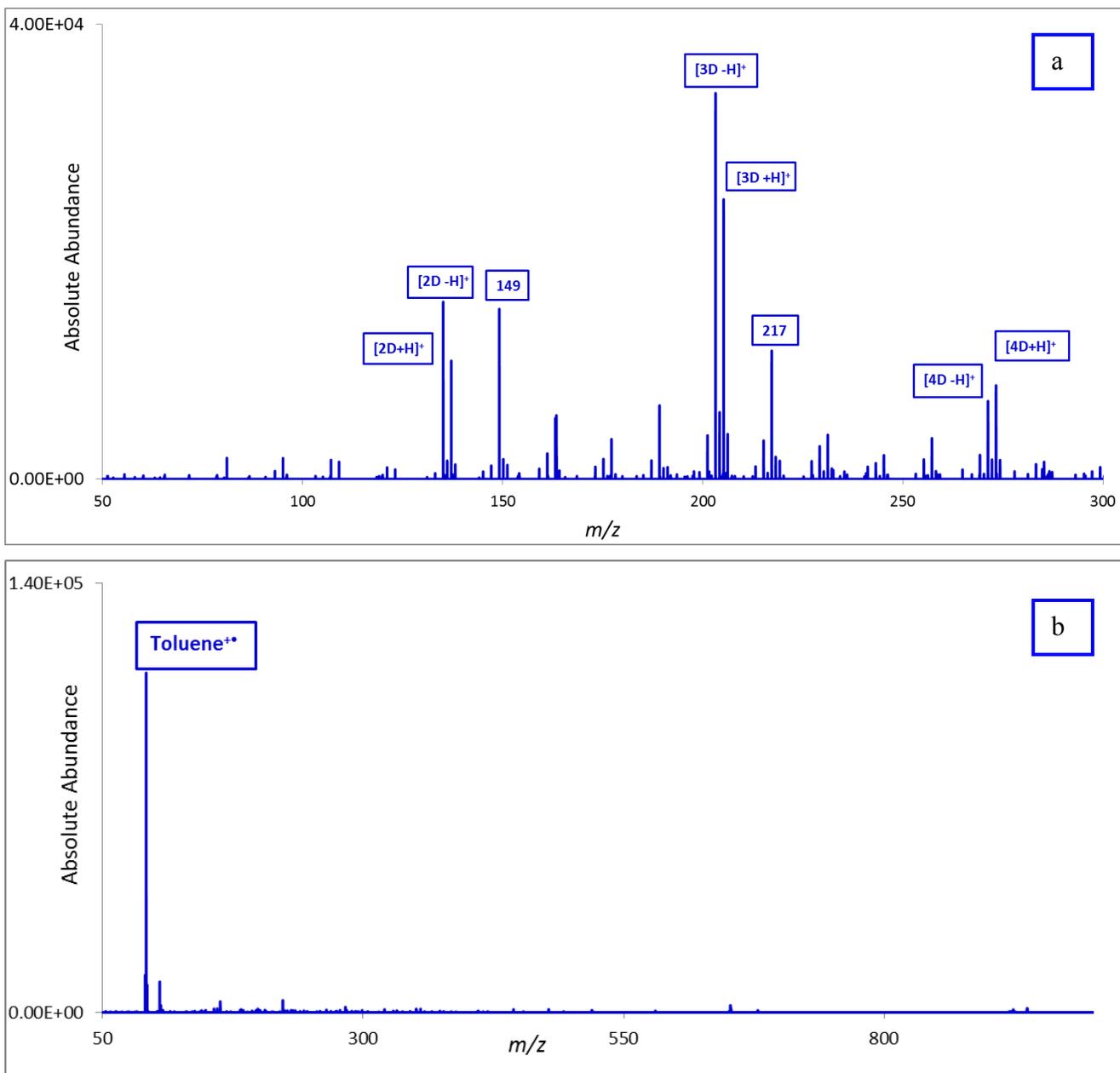
### 6.3.2 Dopant-assisted APLI

The unfavorable two-photon absorption cross sections of most analytes in the EPA mixture led to a very limited range of ionized compounds by APLI compared to APPI. The purpose of adding dopants to APLI is to drive the ionization mechanism toward gas-phase ion-molecule chemical reactions in order to ionize a wider range of analytes including those that cannot be ionized by two-step photon ionization. Similar to other dopant-assisted ionization techniques, in DA-APLI, the laser radiation only initiates the ionization by producing a high quantity of reagent ions, which further ionize analytes.

For PI-APLI, toluene, anisole, and carbon disulfide were examined as dopants. Carbon disulfide did not produce any radical cation under laser ionization. This is due to the comparably low linear absorption cross section at 248 nm along with the extremely short lifetime of its higher excited states, which were reported to be in the range of 500 fs to 3.0 ps, depending on the excitation conditions and targeted Rydberg states (Greening & King, 1976; Knappenberger, Kenneth L Lerch, Wen, & Leone, 2006). Therefore, in spite of the good performance of CS<sub>2</sub> as a dopant for PI-APPI, CS<sub>2</sub> cannot assist the ionization in APLI.

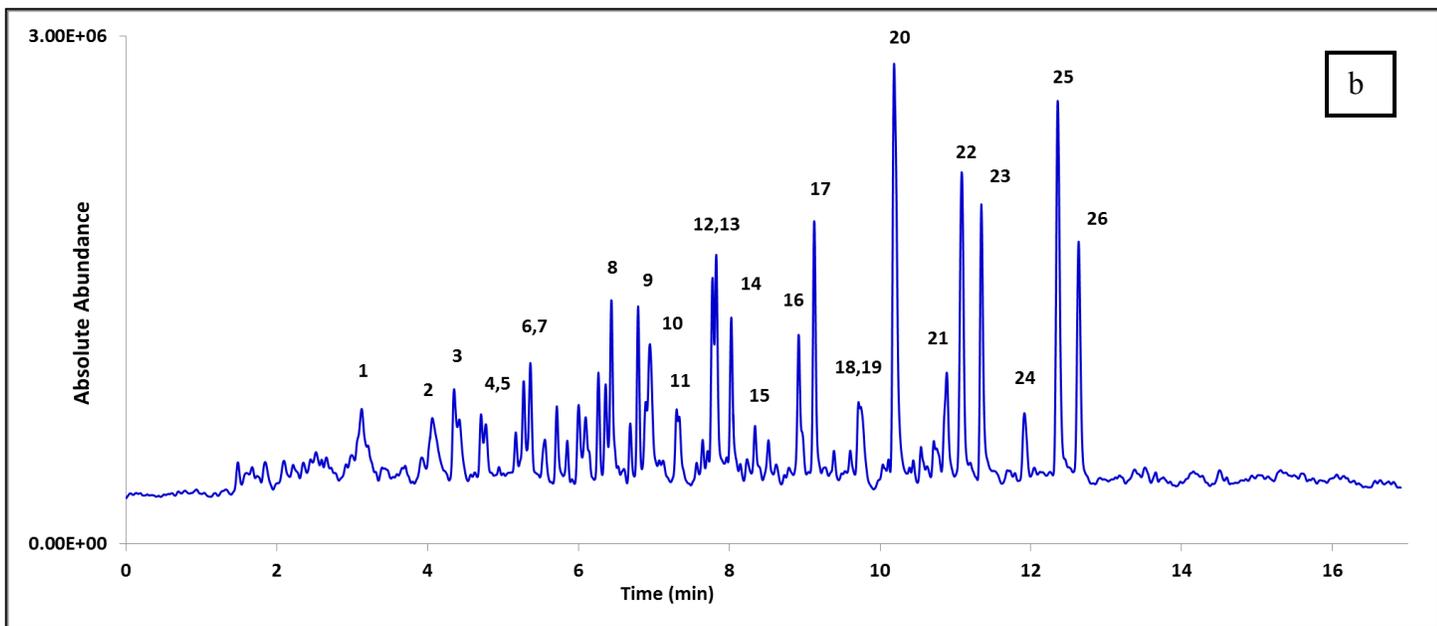
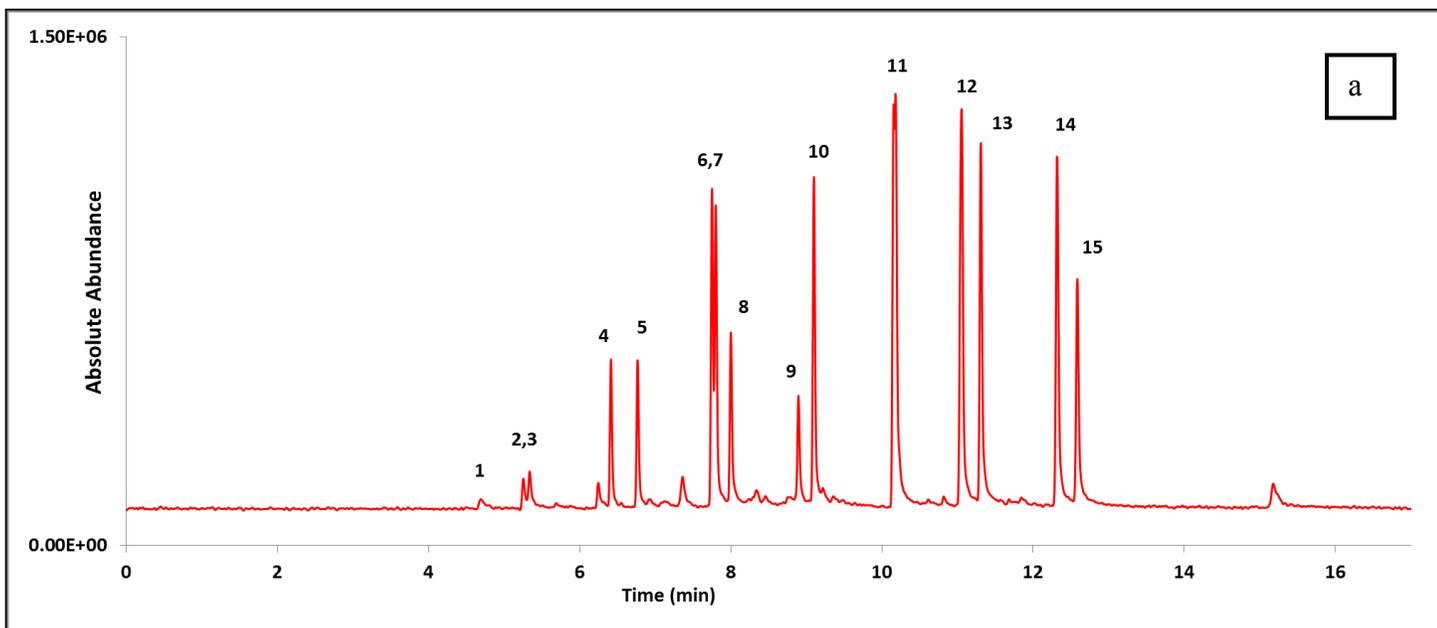
Laser ionization of isoprene produced a range of radical cations including its dimer and trimer (Figure 6.4(a)), similar to what was observed with PI APPI. Thus, the elevated chemical noise and the presence of many ions at the low  $m/z$  range of the isoprene spectrum can interfere with ion products of small molecules, which render its application as a dopant for PI APLI challenging and problematic, similar to PI APPI.

Toluene and anisole both generated a high quantity of dopant radical cations under laser ionization. The preliminary results showed that toluene provides better sensitivities than anisole. Therefore, quantification experiments were only limited to toluene-assisted APLI. Figure 6.4 (b) represents the profile of toluene reagent ions under laser ionization.



**Figure 6.4** The reagent ion profiles of isoprene (a) and toluene (b) as dopants for PI-APLI. The spectra were obtained by flow injection of dopant' headspace into the ionization source at a flow rate of 300  $\mu\text{L}/\text{min}$ , which was mixed with the make-up gas ( $\text{N}_2$ ) at a flow rate of 700  $\text{mL}/\text{min}$ .

The positive electron affinity of carbon disulfide (NIST Standard Reference Database Number 69) disqualifies its applications for the NI APLI, similar to NI-APPI. Isoprene, toluene, and anisole were examined as dopants for NI-APLI. The preliminary results showed that toluene offered a much more effective function as a dopant in NI APLI in terms of enhancing the ionization efficiencies compared to anisole and isoprene. Therefore, quantitative studies in NI APLI were also only performed using toluene as the dopant. Figure 6.5 shows TICs of toluene-assisted APLI (blue, b) compared to TICs obtained by dopant-free APLI (red, a) in PI mode and Figure 6.6 shows the same trend but in NI APLI. As the Figure clearly illustrates, the addition of toluene, highly increased the number and the intensities of peaks in the TICs obtained with both PI and NI APLI. The addition of toluene derived the ionization mechanisms toward gas-phase ion-molecule chemical reactions and covered the ionization of compounds whose two-photon absorption cross sections were not appropriate for laser ionization. The main peaks in TICs of Figure 6.5 and Figure 6.6 were labeled and listed in Table 6.2 and Table 6.3, respectively.



**Figure 6.5** Total Ion Chromatographs (TICs) that were obtained by (a) dopant-free APLI (red) and (b) toluene-assisted APLI (blue) in PI mode. TICs were obtained by 1  $\mu\text{L}$  injection of 100  $\text{pg}/\mu\text{L}$  of EPA mix. The labeled peaks were listed in Table 6.2.

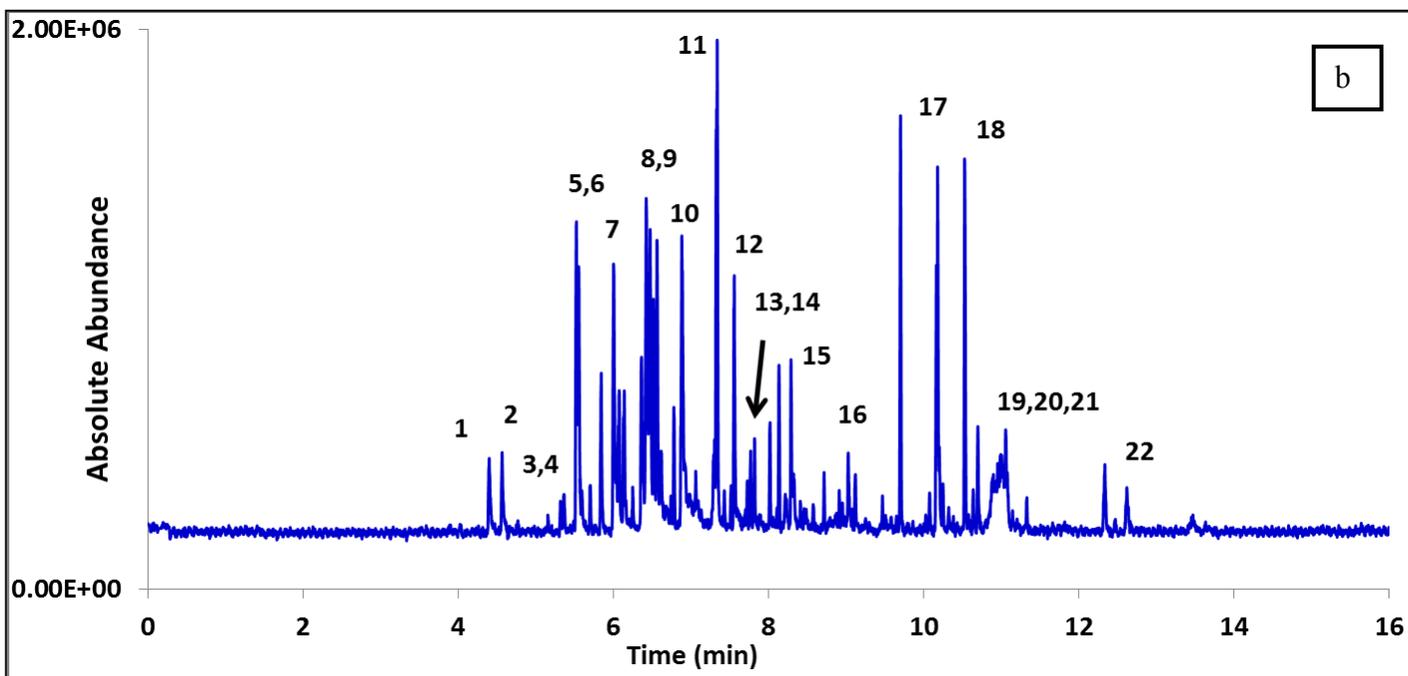
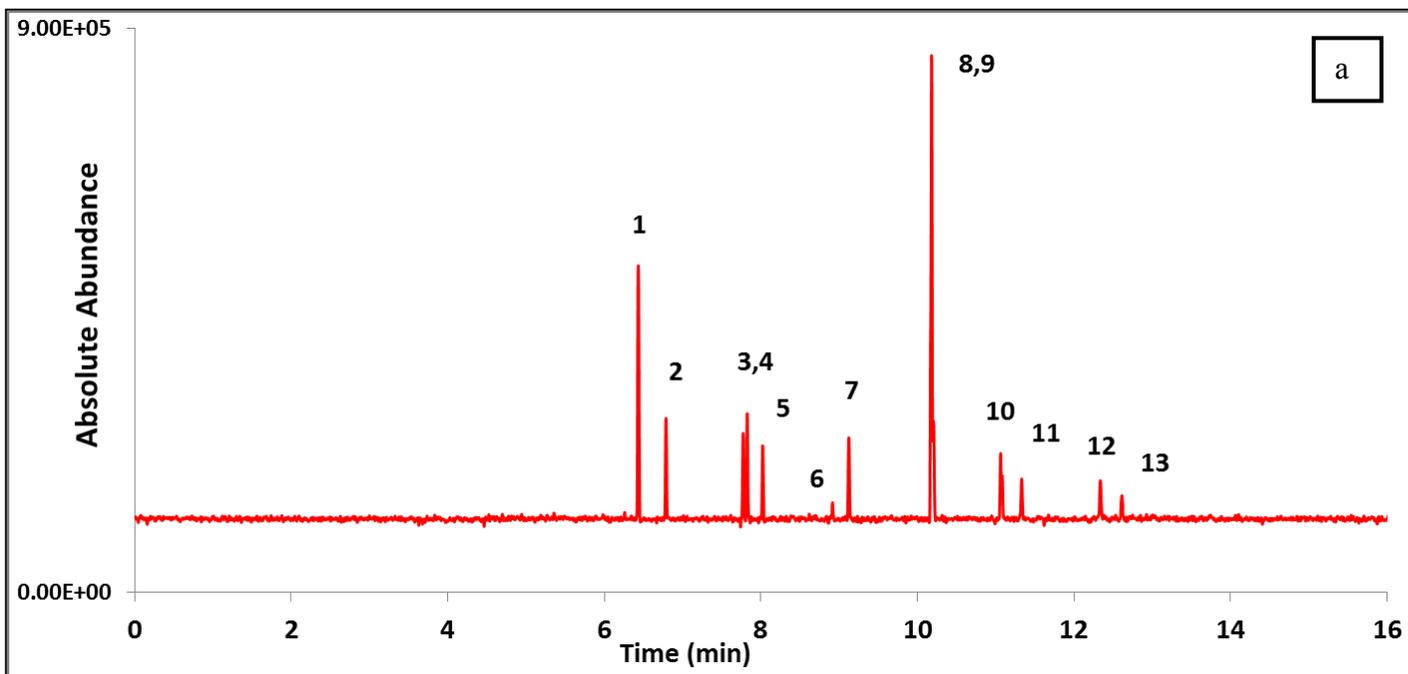


Figure 6.6- Total Ion Chromatographs (TICs) that were obtained by (a) dopant-free APLI (red) and (b) toluene-assisted APLI (blue) in NI mode. TICs were obtained by 1  $\mu\text{L}$  injection of 100  $\text{pg}/\mu\text{L}$  of EPA mix. The labeled peaks were listed in Table 6.3.

**Table 6.2** The list of labeled peaks shown in TICs of dopant-free PI APLI and toluene-assisted PI APLI.

<b>Dopant-free PI APLI</b>		<b>Toluene-assisted PI APLI</b>	
<b>Label #</b>	<b>Compound</b>	<b>Label #</b>	<b>Compound</b>
1	Naphthalene	1	Nitrobenzene
2	2-Methylnaphthalene	2	2-methylphenol
3	1-Methylnaphthalene	3	Isophorone
4	Dibenzofurane	4	Naphthalene
5	Fluorene	5	4-chloroaniline
6	Phenanthrene	6	2-Methylnaphthalene
7	Anthracene	7	1-Methylnaphthalene
8	Carbazole	8	Dibenzofurane
9	Fluoranthene	9	Fluorene
10	Pyrene	10	2,4-Dinitrotoluene
11	Benzo(a)anthracene	11	4-Bromophenyl phenyl ether
12	Benzo(b)fluoranthene	12	Phenanthrene
13	Benzo(a)pyrene	13	Anthracene
14	Dibenzo(a,h)anthracene	14	Carbazole
15	Benzo(g,h,i)perylene	15	di-n-butyl phthalate
		16	Fluoranthene
		17	Pyrene
		18	Butyl benzyl phthalate
		19	bis-2-ethylhexyl adipate
		20	Benzo(a)anthracene
		21	Di-n-octylphthalate
		22	Benzo(b)fluoranthene
		23	Benzo(k)fluoranthene
		24	Benzo(a)pyrene
		25	Dibenzo(a,h)anthracene
		26	Benzo(g,h,i)perylene

**Table 6.3 The list of labeled peaks shown in TICs of dopant-free NI APLI and toluene-assisted NI APLI.**

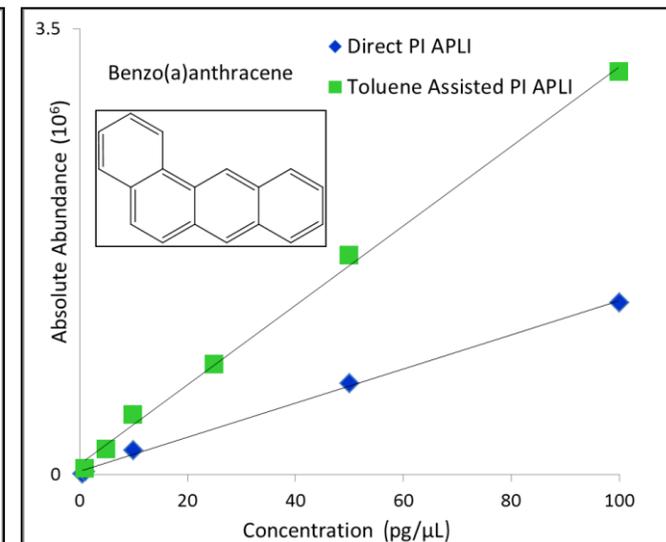
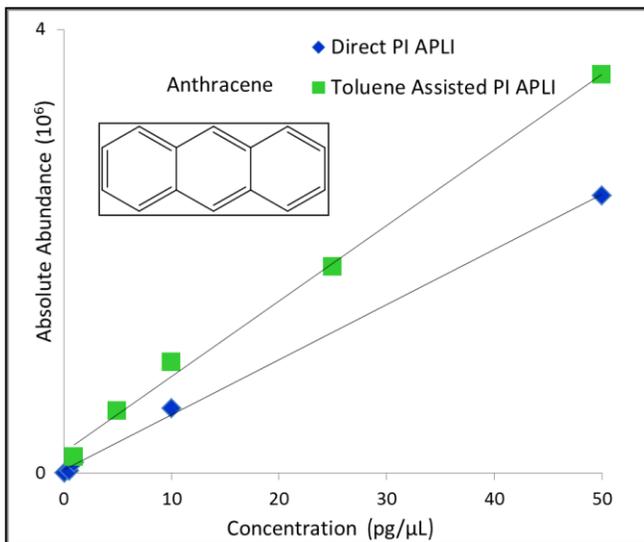
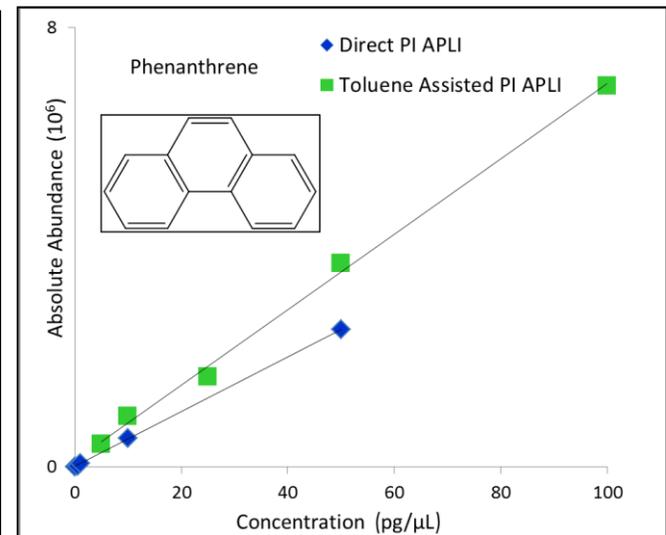
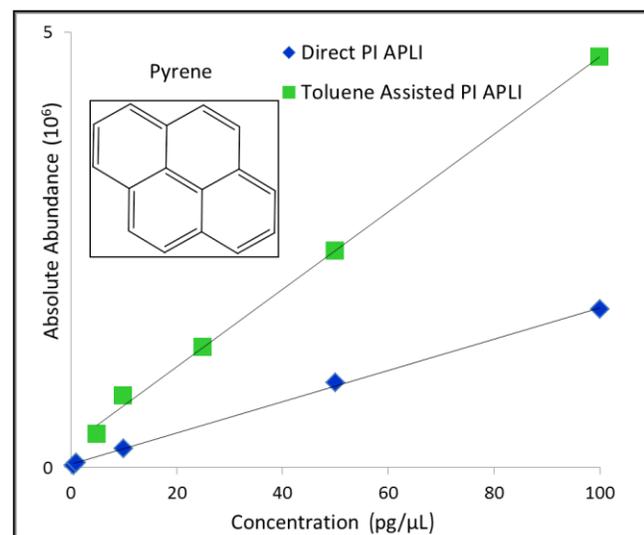
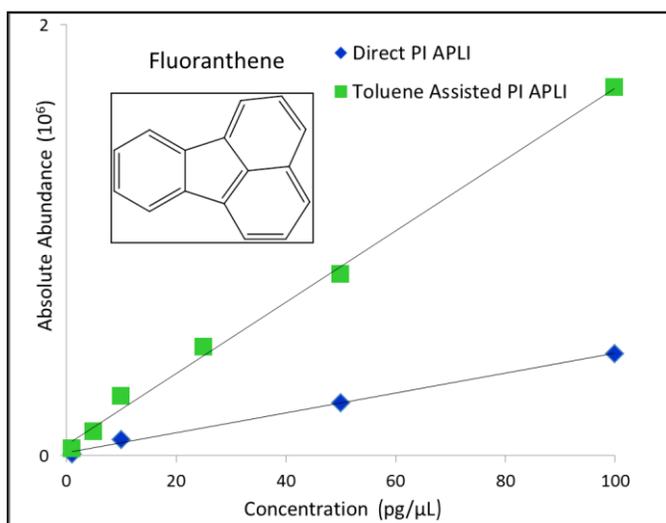
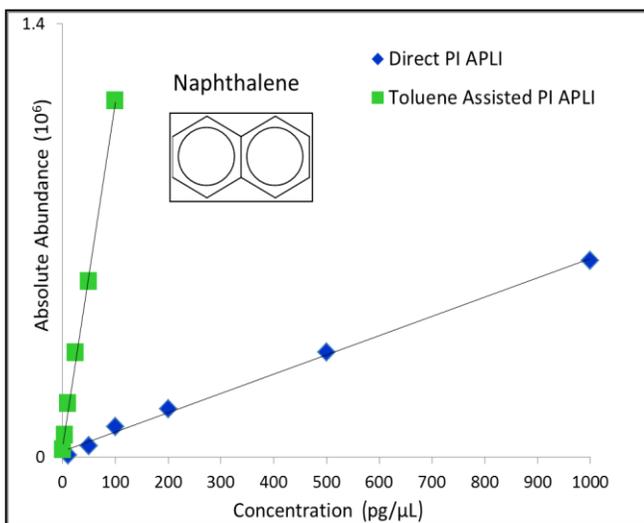
<b>Dopant-free NI APLI</b>		<b>Toluene-assisted NI APLI</b>	
<b>Label #</b>	<b>Compound</b>	<b>Label #</b>	<b>Compound</b>
1	4- Nitrophenol	1	2,4-Dimethylphenol
2	Nitrobenzene	2	2,4-Dichlorophenol
3	Phenanthrene	3	4-chloro-3-methylphenol
4	Anthracene	4	Hexachlorocyclopentadiene
5	Carbazole	5	2,4,6-Trichlorophenol
6	Fluoranthene	6	2,4,5-Trichlorophenol
7	Pyrene	7	1,4-dinitrobenzene
8	Benzo(a)anthracene	8	2-Nitrophenol
9	Chrysene	9	4-Nitrophenol
10	Benzo(b)fluoranthene	10	2,4-Dinitrotoluene
11	Benzo(a)pyrene	11	Hexachlorobenzene
12	Dibenzo(a,h)anthracene	12	Pentachlorophenol
13	Benzo(g,h,i)perylene	13	Phenanthrene
14		14	Anthracene
15		15	Carbazole
		16	Fluoranthene
		17	3,3-dichlorobenzidine
		18	Chrysene
		19	Benzo(b)fluoranthene
		20	Benzo(k)fluoranthene
		21	Benzo(a)pyrene
		22	indeno(1,2,3-cd)pyrene

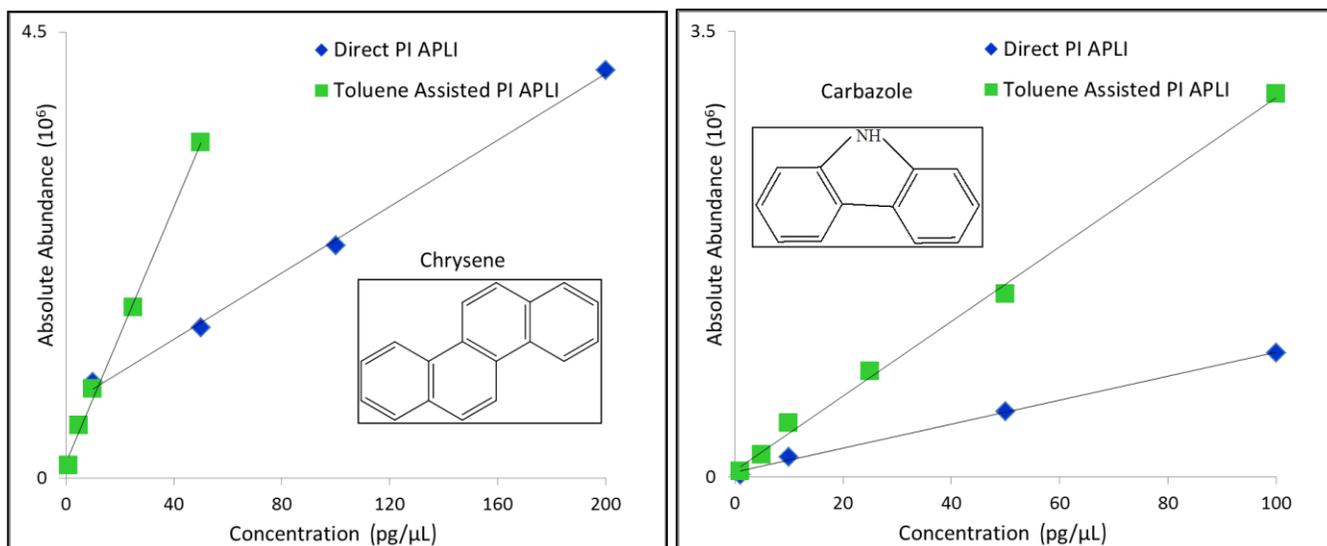
### **6.3.3 Comparison of direct APLI and toluene-assisted APLI**

#### **6.3.3.1 Linear dynamic range and detection limits.**

As mentioned earlier, the API interface does not possess the optimal geometry to achieve the maximum sensitivity for laser ionization, and using a different geometry customized specifically for APLI could lead to much higher sensitivities. Considering that fact, the results of the quantitative studies are presented here as a base to compare the ionization efficiencies obtained by dopant-free and dopant-assisted APLI.

Figure 6.7 compares the linear dynamic range obtained for eight analytes with direct PI-APLI and toluene-assisted PI-APLI and Table 6.4 correspondingly presents the correlation coefficient ( $R^2$ ) of linearity, LODs and reproducibility of the method in terms of %RSDs of analytical responses. The analytical responses were determined from the peak area of the single ion chromatography (SIM) of the molecular radical cation for each analyte. LODs were calculated from the corresponding spectra based on an S/N of three.





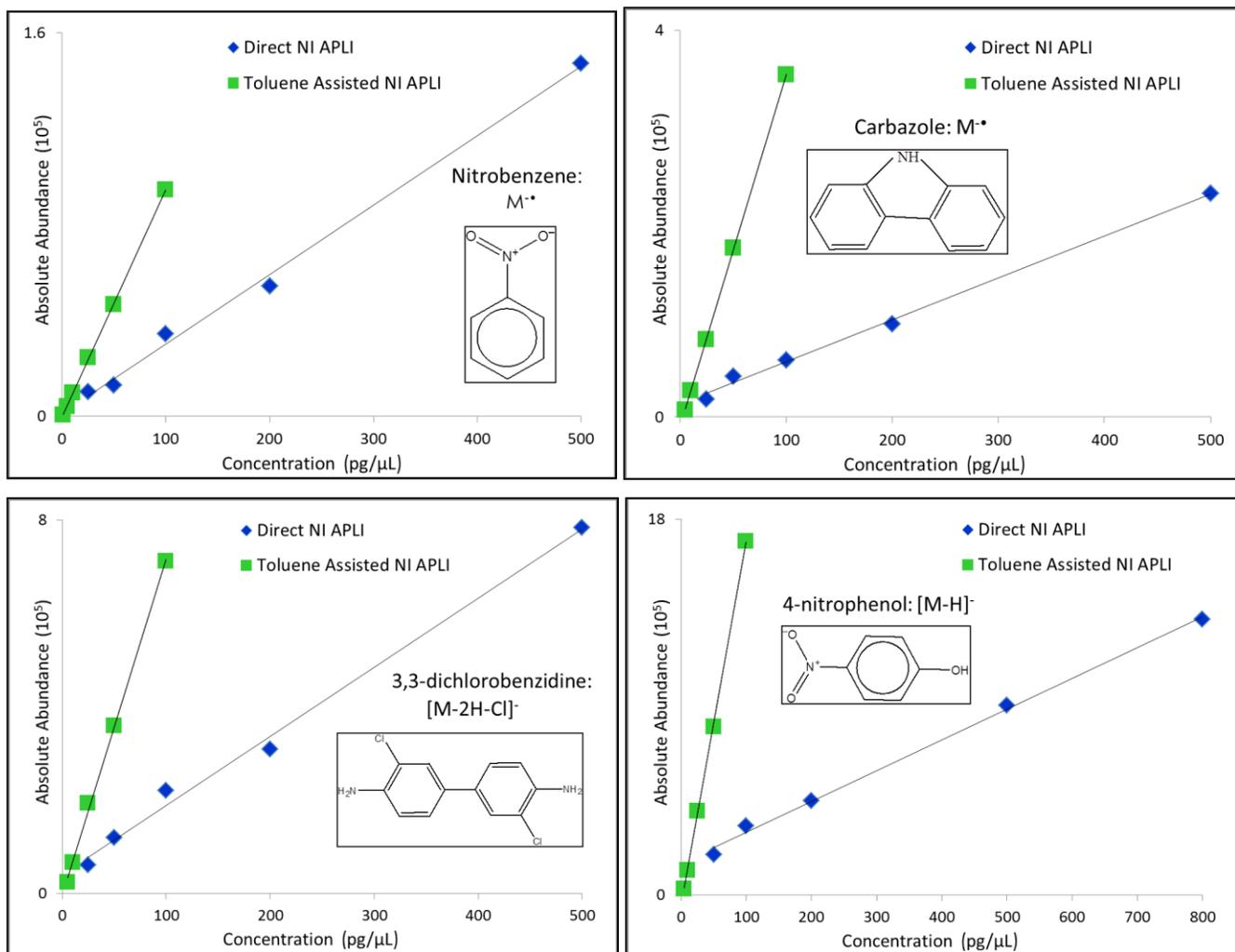
**Figure 6.7** Calibration curves for eight compounds in PI mode with direct APLI and toluene-assisted APLI. For each analyte, the absolute abundance of the peak area of single ion chromatography (SIM) for the molecular radical cation has been considered.

**Table 6.4** The  $R^2$  values of linearity, LODs, and %RSDs obtained with Direct PI-APLI and toluene assisted PI-APPI.

Compound	Direct PI APLI			Toluene-assisted PI APLI		
	$R^2$	LOD (fg/ $\mu$ L)	%RSD	$R^2$	LOD (fg/ $\mu$ L)	%RSD
Naphthalene	0.996	1250	<8	0.997	4	<8
Fluoranthene	0.997	265	<5	0.996	3	<5
Pyrene	0.999	30	<5	0.998	0.4	<10
Phenanthrene	0.999	10	<6	0.997	0.3	<9
Anthracene	0.998	20	<7	0.996	0.5	<11
Benzo(a)anthracene	0.998	40	<8	0.997	1	<12
Chrysene	0.998	22	<6	0.997	0.5	<9
Carbazole	0.997	205	<7	0.997	1.5	<5

As shown in Table 6.4, for the studied compounds, toluene-assisted APLI increased ionization responses and in turn, led to improved LODs up to a few orders of magnitude. In addition both approaches provided acceptable linearity and repeatability.

Figure 6.8 compares the linear dynamic range obtained for four analytes with direct NI-APLI and toluene-assisted NI-APLI and Table 6.5 correspondingly presents the correlation coefficient ( $R^2$ ) of linearity, LODs and reproducibility of the methods in terms of %RSDs of analytical responses. The analytical responses were determined from the peak area of the single ion chromatography (SIM) of the dominant negative ion for each analyte. LODs were calculated from the corresponding spectra based on an S/N of three.



**Figure 6.8** Calibration curves for four compounds in NI mode with direct APLI and toluene-assisted NI APLI. For each analyte, the absolute abundance of the peak area of single ion chromatography (SIM) of the dominant anion, as shown in each graph, has been considered.

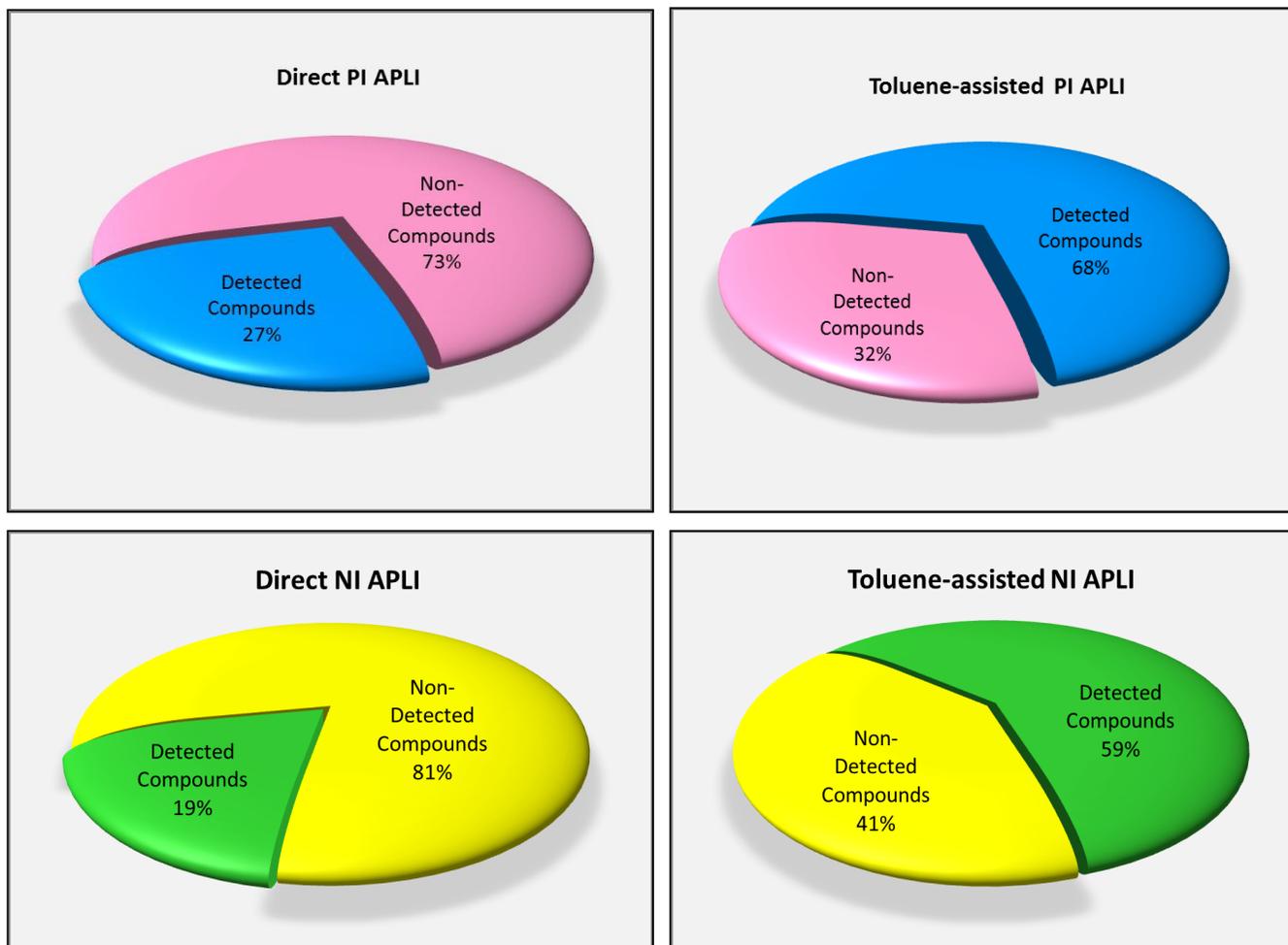
**Table 6.5** The  $R^2$  values of linearity, LODs, and %RSDs obtained with direct NI APLI and toluene-assisted NI APLI.

Compound	Direct NI APLI			Toluene-assisted NI APLI		
	$R^2$	LOD (fg/ $\mu$ L)	%RSD	$R^2$	LOD (fg/ $\mu$ L)	%RSD
Nitrobenzene	0.996	4800	<6	0.999	1500	<7
Carbazole	0.996	4400	<5	0.999	400	<10
3,3-dichlorobenzidine	0.997	1200	<7	0.999	200	<11
4-nitrophenol	0.997	1200	<5	0.999	30	<9

As shown, in Table 6.5, toluene assisted NI APLI, improved the LODs compared to direct NI APLI and both methods provided acceptable linearity and repeatability.

### 6.3.3.2 Range of ionizable compounds obtained by each method.

Figure 6.9 illustrates the broader range of ionized compounds achieved by the addition of toluene as a dopant to APLI in both PI and NI modes. Direct APLI, which offers unique selectivity toward aromatic compounds with absolutely no interference from other aliphatic compounds, can be employed in the targeted analysis of these compounds in complex mixtures, without the necessity of removing matrix components. However, if a universal selectivity by APLI is desired, the addition of a suitable dopant can simply evolve the ionization mechanisms toward ion-molecule gas-phase chemical reactions and cover the ionization of analytes, whose spectroscopic features are not appropriate for laser ionization mechanism.



**Figure 6.9** The range of ionizable compounds obtained by direct APLI and toluene-assisted APLI in both PI and NI modes.

## 6.4 Conclusion

The custom-built API with an excimer laser as the ionization source combined with a GC offered highly selective performance toward polycyclic aromatic compounds in the direct mode with no dopant. These compounds, whose spectroscopic features favor the two-step laser ionization mechanism, can be directly analyzed in complex mixtures with no interference from other matrix components. To achieve a more universal applicability for APLI, suitable dopants can be added. Similar to other dopant-assisted ionization techniques, laser ionization of dopants generates primary reagent ions and electrons, which further ionize analytes through gas-phase ion-molecule reactions and cover the ionization of aliphatic analytes that cannot be ionized through the two-step ionization mechanism in APLI.

Therefore, direct APLI or dopant-assisted APLI may be preferred according to the selectivity needed for an analysis. Toluene was proven to give the best efficiencies in both NI and PI APLI compared to anisole and isoprene. Although the geometry of the custom-built API was tailored to achieve the maximum sensitivity for APPI and some major changes are needed to customize the API source in order to achieve the best APLI performance, quantitative studies showed acceptable results in terms of sensitivity in both direct APLI and dopant-assisted APLI. These results are promising for a more sensitive performance from a specifically customized API design for laser ionization.

This study demonstrated the unique role of GC/APLI MS technique for highly selective and sensitive analysis of polymeric and polycyclic aromatic in complex mixtures, with almost no interference from other matrix components, and also the role of broadening the applicability by the addition of suitable dopants.

## 7 Chapter: Conclusion

### 7.1 Overall conclusions and contributions of the dissertation

Dopants are chemicals that are added to ionization sources in order to assist the ionization process. Although they have been applied in different ionization techniques, they found general applicability in the field of Atmospheric Pressure Photoionization (APPI). APPI is based on a krypton discharge lamp that generates vacuum-ultraviolet (VUV) photons of 10.0 and 10.6 eV energy. The principal ionization mechanism in APPI is photoabsorption by an analyte molecule, which leads to a photoionization event that forms an analyte radical cation by ejection of an electron. Although atmospheric gases and chromatographic mobile phases do not become ionized upon VUV excitation at 10 eV due to their high ionization energies, these species highly absorb the photons and decrease the accessible energy for analytes ionization. To increase the total ion production, dopants are added in a high quantity. These chemicals that have high photoabsorption and photoionization cross sections easily get photoionized in large quantity and the photo-dopant ions ionize analyte molecules through ion-molecule chemical reactions.

The research described in this thesis can be divided into four parts. In the first chapter, the history and areas of application of dopants as “ionization assisting” chemicals were reviewed. For each dopant, the underlying ionization mechanisms and their pros and cons in the atmospheric matrix, as well as in the presence of mobile phases of liquid chromatograph and in a much simpler matrix from a gas chromatograph, were discussed. With the understanding obtained from this in depth analysis on ion formation mechanisms, two novel dopants for positive ion and negative ion modes were introduced, i.e., carbon disulfide and isoprene, respectively.

In the second part, which includes chapters 2 and 3, the potential of these chemicals as dopants for APPI in the presence of mobile phases from a HPLC, alongside their underlying ionization mechanisms, were examined. Carbon disulfide was proven to be an efficient dopant for positive ion APPI (PI-APPI). The advantage of carbon disulfide over commonly

used dopants is its high ionization energy (10 eV), which enables the ionization of analytes with high IEs through charge transfer routes. Other commonly used dopants, i.e. toluene (IE of 8.8 eV) and anisole (IE of 8.2 eV) suppress the ionization of analytes with higher IEs than that of toluene and anisole. Isoprene gave effective results in the negative ion APPI (NI-APPI).

In the third part, which includes chapters 4 and 5, the potential of carbon disulfide and isoprene as dopants were examined for an APPI that coupled a gas chromatograph (GC) to a mass spectrometer. GC provides a very clean matrix for photoionization and does not lead to the interferences and ion suppression issues caused by LC mobile phases. Our results in these chapters were in agreement with the results obtained in chapters 2 and 3. In addition, the pure and well-defined matrix from GC enabled me to more reliably investigate the corresponding ionization mechanisms.

In the last Chapter, the utilization of dopants in an Atmospheric Pressure Laser Ionization (APLI) setup was explored. Laser ionization is also a photoionization method, but instead of one-step VUV photoionization as in APPI, two-step UV photoionization is applied here. The first photon excites an analyte to an excited state. If the lifetime of this state is long enough to absorb a second photon and if the energy of the second photon provides the remaining energy needed for the ionization of the analyte, the ionization occurs from the long-lived state and leads to the formation of a radical cation and an electron. APLI has been only recently introduced; thus, the utilization of dopants in this technique has not been explored as extensively as for APPI. The potential of carbon disulfide and isoprene as dopants were also examined for this technique. The low linear absorption cross section and the short lifetime of highly excited electronic states of carbon disulfide suppressed its laser ionization. Therefore, laser ionization of carbon disulfide did not increase the total ion production in order to assist the ionization. Laser ionization of isoprene produced a range of radical cations. Thus, the elevated chemical noise and the presence of many ions at the low  $m/z$  range of the isoprene spectrum can interfere with ion products of small molecules, which renders its application as a dopant for PI-APLI challenging and problematic. In NI APLI, toluene offered more effective results than isoprene. Therefore, toluene was chosen to investigate the role of

dopants in enhancing the ionization responses and to study the corresponding ionization mechanisms in the two-step photoionization of APLI.

Although electrospray is considered the popular ionization technique in analytical and bioanalytical fields, its bias against the polarity of analytes and its vulnerability to matrix effects results in a largely incomprehensive image of the key components of a complex sample. Thus, the complementary profile that can be exclusively obtained by a photoionization technique, which inherently provides a more universal ionization, is becoming a greater area of interest for the thorough analysis of complex mixtures, such as biological samples and metabolome screening in metabolomics in order to obtain an unbiased fingerprint of components.

The crucial step towards successful application of a photoionization technique is to fully understand underlying ion formation mechanisms in each particular matrix. Charge transfer and proton transfer are considered the two main ionization mechanisms, which assist the ionization of non-polar and polar analytes, respectively. Depending on the matrix components under study (atmospheric gases, GC/LC or other separation techniques' mobile phases) different dopants may promote one of the main ionization mechanisms over another. Therefore, a deep understanding of ionization mechanisms can help to select the best dopant/dopant mixture in order to achieve a maximum ionization efficiency for a particular analysis, depending on the polarity and nature of the targeted analytes and the matrix components under study. My research contributes to providing a profound understanding on underlying ion formation mechanisms with/without dopants in APPI for both GC/MS and LC/MS applications and in APLI for GC/MS applications. These findings can be reliably expanded for other ionization techniques because it has been well established that despite the different mechanisms for generating primary reactant ions in any ionization technique, the subsequent ion-molecule chemical reactions are similar. Therefore, in all ionization techniques, dopants can be added in a systematic way to manipulate the analysis in order to enhance and/or prefer charge transfer over proton transfer and vice versa. Therefore, the results presented in this thesis are of general interest for researchers that wish to utilize

dopants in order to assist the ionization responses and provide them with a guide to systematically manipulate their analysis in order to achieve the maximum desired ionization.

## 7.2 Future research directions

There are several possible research directions for future work. On the whole, the results of this research contributes to the current knowledge on the ion formation mechanisms in API techniques and can be applied to further expand APPI /APLI MS applications in vital areas, such as metabolomics, biomarker cancer identifications, pharmaceutical research, environmental analyses and many more.

Other more specific possible research directions are listed as follows:

- Carbon disulfide radical cations were shown to be very effective charge transfer reagents, which carry the highest possible energy, equal to 10 eV, among all commonly used dopants. This compound is the best option for ionization of non-polar compounds with high IEs, for which the proton transfer route is not possible and other dopants with less IEs suppress their ionization. Examples of these chemicals are polybrominated diphenyl ethers and halogenated pesticides.
- Isoprene was shown to be an effective dopant for the negative ionization mode because its photoionization generates a range of ionized products with accompanying electron ejection. Therefore, its photoionization highly increases the number of thermal electrons, which initiate the ion formation events in the negative ion mode. However, isoprene reagents typically contain less than 1000 ppm 4-tert-butylcatechol (4-tBC), as an inhibitor of isoprene polymerization. As shown in chapter 3, 4-tBC competes for electron/superoxide ions with analytes, reduces the number of thermal electrons, and subsequently suppresses the deprotonation of analytes with weaker gas-phase acidities. Moreover, the oxidized form of the inhibitor (tert-Butyl-ortho-benzoquinone negative molecular ion ( $[tBoBQ]^-$ )), is also present in the mass spectra of isoprene. Because of the relatively high estimated EA of tert-Butyl-ortho-

benzoquinone, this species can act as an effective sink for thermal electrons and adversely affect the performance of isoprene as a dopant. Therefore, the efficiency of isoprene as a dopant for NI could strongly benefit from the removal of 4-tBC. This removal could be done by several washings of isoprene with dilute NaOH and water, followed by drying over CaH<sub>2</sub> and distilling under nitrogen at atmospheric pressure. The fraction distilled at 32°C is inhibitor-free isoprene; however, it has to be kept under nitrogen at -15°C in order to avoid polymerization. Due to time restrictions, I was not able to conduct this research. Moreover, in chapter 5, where isoprene was examined as a dopant for GC/MS application, the inhibitor did not interfere due to the absence of LC solvents. However, an intense iodide peak was observed in the background spectrum of isoprene, thus implying the presence of iodine as impurity. Here, also removing iodide could significantly increase the efficiency of function of isoprene as dopant. Therefore, effectively purifying the isoprene reagent could lead to much more enhanced dopant efficiency and this path can be further explored.

- In the studies with negative ionization for GC/MS application, purified nitrogen was used as the make-up gas in the ionization source. Another possible research direction is to use synthetic air as an alternative make-up gas and explore the dopant function of oxygen in the negative ionization. The role of oxygen in ion formation mechanisms in the negative mode, through the formation of superoxide anions, has been well documented. Therefore, alongside its role as a make-up gas, the oxygen content of synthetic air could promote the ionization efficiencies by enhancing the concentration of superoxide anions. I have conducted a series of quantitative and qualitative experiments and my primary results supported this hypothesis (Appendix D). Using synthetic air as the make-up gas increased both the number and the intensity of detected analytes in dopant-free NI APPI. However, in the background spectra, the carbonate anion ( $\text{CO}_3^{2-}$ ,  $m/z$  60) was observed with a considerable intensity, which originates from CO<sub>2</sub> impurity in the bottle. CO<sub>2</sub> acts as a sink for thermal electrons and reduces the accessible negative charges for analytes. Therefore, removing CO<sub>2</sub> from the synthetic air can further increase the efficiency of the negative ionization. In the data sheet of the synthetic air cylinder used, the impurity of CO<sub>2</sub> was stated to

be below 1 ppm. The content of CO<sub>2</sub> in the synthetic air bottle was measured by an LI-7000 CO<sub>2</sub>/ H<sub>2</sub>O analyzer. With the exact measurement mode of the analyzer, the content of CO<sub>2</sub> was determined to be 500 +/- 200 ppb. The next step included attempts to remove the CO<sub>2</sub> content of the synthetic air using a Ca(OH)<sub>2</sub> saturated solution as a CO<sub>2</sub> trap. This was done by placing a wash-bottle with a Ca(OH)<sub>2</sub> saturated solution in the line between the synthetic air bottle and the CO<sub>2</sub> analyzer in order to measure the possible reduction in CO<sub>2</sub> concentration. In addition, the same trap was placed in the line between the synthetic air bottle and the ionization source to see if the intensity of signal at *m/z* 60 was reduced. Although, due to time restrictions, I could not finish these series of experiments, this path can be further explored and could lead to much more effective ionization in the negative mode.

- As explained in Chapter 6, the custom-built API has been customized to achieve the most sensitive APPI performance; the wide base of the conical-shaped interface and its relatively short height maximize VUV photons and analyte stream overlap, which in turn, maximizes ionization responses. However, these features are not appropriate to achieve the best efficiency in laser ionization. For the pointed small-sized spot of laser radiation, a much smaller optical window and a longer beam travel path are required to maximize the likelihood of laser radiation and analyte stream overlap and in turn, ionization. It would therefore be an interesting area of research to construct an API interface with an appropriate geometry for laser ionization, which could drastically increase the sensitivity of the technique.

## Bibliography

- Ackloo, S. Z., Smith, R. W., Terlouw, J. K., & McCarry, B. E. (2000). Characterization of ginseng saponins using electrospray mass spectrometry and collision-induced dissociation experiments of metal-attachment ions. *The Analyst*, *125*(4), 591–597.
- Adamiya, T., Budovich, V., & Shlyakhov, A. (1994). Relative sensitivity of a photoionization detector to volatile organic compounds. *Industrial Laboratory*, *60*(7), 387–389.
- Allegrand, J., Touboul, D., Giuliani, A., Brunelle, A., & Laprévote, O. (2012). Atmospheric pressure photoionization mass spectrometry of guanine using tunable synchrotron VUV radiation. *International Journal of Mass Spectrometry*, *321-322*, 14–18.
- Anacleto, J. F., Ramaley, L., Benoit, F. M., Boyd, R. K., & Quilliam, M. A. (1995). Comparison of liquid-chromatography mass-spectrometry interfaces for the analysis of polycyclic aromatic compounds. *Analytical Chemistry*, *67*(22), 4145–4154.
- Andersson, J. T., Hegazi, A. H., & Roberz, B. (2006). Polycyclic aromatic sulfur heterocycles as information carriers in environmental studies. *Analytical and Bioanalytical Chemistry*, *386*(4), 891–905.
- Andrés, V., Villanueva, M. J., & Tenorio, M. D. (2014). Simultaneous determination of tocopherols, retinol, ester derivatives and  $\beta$ -carotene in milk- and soy-juice based beverages by HPLC with diode-array detection. *LWT - Food Science and Technology*, *58*(2), 557–562.
- Armarego, W. L. F., & Chai, C. L. L. (2013). *Purification of laboratory chemicals* (7th ed.). Oxford, UK ; Waltham, MA : Butterworth-Heinemann/Elsevier.
- Arthen-Engeland, T., & Dunsbach, R. (2008). New Generation of GC/High Resolution TOF-MS: APCI/APLI Source for Increased Flexibility. *LCGC Europe*, *21*(7), 34–35.
- Axén, J., Malmström, D., Axelsson, B.-O., Petersson, P., & Sjöberg, P. J. R. (2010). Efforts to improve detection sensitivity for capillary electrophoresis coupled to atmospheric pressure photoionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, *24*(9), 1260 – 1264.
- Bacaloni, A., Callipo, L., Corradini, E., Giansanti, P., Gubbiotti, R., Samperi, R., & Laganà, A. (2009). Liquid chromatography–negative ion atmospheric pressure photoionization tandem mass spectrometry for the determination of brominated flame retardants in environmental water and industrial effluents. *Journal of Chromatography. A*, *1216*(36), 6400 – 6409.

- Bae, E., Na, J., Chung, S., Kim, H., & Kim, S. (2010). Identification of about 30 000 Chemical Components in Shale Oils by Electrospray Ionization (ESI) and Atmospheric Pressure Photoionization (APPI) Coupled with 15 T Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) and a Comparison to. *Energy & Fuels*, *24*, 2563–2569.
- Bagag, A., Giuliani, A., & Laprévotte, O. (2007). Atmospheric pressure photoionization mass spectrometry of nucleic bases, ribonucleosides and ribonucleotides. *International Journal of Mass Spectrometry*, *264*(1), 1 – 9.
- Bagag, A., Giuliani, A., & Laprévotte, O. (2008). Atmospheric pressure photoionization mass spectrometry of oligodeoxyribonucleotides. *European Journal of Mass Spectrometry*, *14*(2), 71 – 80.
- Bagag, A., Laprévotte, O., Hirsch, J., & Kováčik, V. (2008). Atmospheric pressure photoionization mass spectrometry of per-O-methylated oligosaccharides related to D-xylans. *Carbohydrate Research*, *343*(16), 2813 – 2818.
- Baim, M. A., Eatherton, R. L., & Hill, H. H. (1983). Ion mobility detector for gas chromatography with a direct photoionization source. *Analytical Chemistry*, *55*(11), 1761–1766.
- Banoub, J. H., Benjelloun-Mlayah, B., Ziarelli, F., Joly, N., & Delmas, M. (2007). Elucidation of the complex molecular structure of wheat straw lignin polymer by atmospheric pressure photoionization quadrupole time-of-flight tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, *21*(17), 2867 – 2888.
- Barbounis, E., Moukas, A., Xiskia, A., Tzatzarakis, M., Stivaktakis, P., Kokkinaki, A., & Tsatsakis, A. (2012). Liquid chromatography-mass spectrometry of polychlorinated biphenyls (PCBs) using dopant assisted atmospheric pressure photoionization. *Toxicology Letters*, *211*, S52–S53.
- Basso, E., Marotta, E., Seraglia, R., Tubaro, M., & Traldi, P. (2003). On the formation of negative ions in atmospheric pressure photoionization conditions. *Journal of Mass Spectrometry*, *38*(10), 1113 – 1115.
- Becker, G., Nilsson, U., Colmsjö, A., & Östman, C. (1998). Determination of polycyclic aromatic sulfur heterocyclic compounds in airborne particulate by gas chromatography with atomic emission and mass spectrometric detection. *Journal of Chromatography A*, *836*(1), 57 – 66.
- Black, B. A., Sun, C., Zhao, Y. Y., Gänzle, M. G., & Curtis, J. M. (2013). Antifungal Lipids Produced by Lactobacilli and Their Structural Identification by Normal Phase LC/Atmospheric Pressure Photoionization-MS/MS. *Journal of Agricultural and Food Chemistry*, *61*(22), 5338 – 5346.

- Boesl, U. (2000). Laser mass spectrometry for environmental and industrial chemical trace analysis. *Journal of Mass Spectrometry*, 35(3), 289 – 304.
- Bonfanti, L., Careri, M., Mangia, A., Manini, P., & Maspero, M. (1996). Simultaneous identification of different classes of hydrocarbons and determination of nitro-polycyclic aromatic hydrocarbons by means of particle beam liquid chromatography-mass spectrometry. *Journal of Chromatography A*, 728(1), 359–369.
- Bouyssiére, B., Leonhard, P., Pr frock, D., Baco, F., Lopez Garcia, C., Wilbur, S., & Prange, A. (2004). Investigation of the sulfur speciation in petroleum products by capillary gas chromatography with ICP-collision cell-MS detection. *Journal of Analytical Atomic Spectrometry*, 19(5), 700–702.
- Bristow, T., Harrison, M., & Sims, M. (2010). The application of gas chromatography/atmospheric pressure chemical ionisation time-of-flight mass spectrometry to impurity identification in Pharmaceutical Development. *Rapid Communications in Mass Spectrometry*, 24(11), 1673–1681.
- Bruins, A. P., Covey, T. R., & Henion, J. D. (1987). Ion spray interface for combined liquid chromatography/atmospheric pressure ionization mass spectrometry. *Analytical Chemistry*, 59(22), 2642–2646.
- Cai, S.-S., Hanold, K. A., & Syage, J. A. (2007). Comparison of atmospheric pressure photoionization and atmospheric pressure chemical ionization for normal-phase LC/MS chiral analysis of pharmaceuticals. *Analytical Chemistry*, 79(6), 2491 – 2498.
- Cai, S.-S., Short, L. C., Syage, J. A., Potvin, M., & Curtis, J. M. (2007). Liquid chromatography-atmospheric pressure photoionization-mass spectrometry analysis of triacylglycerol lipids--effects of mobile phases on sensitivity. *Journal of Chromatography A*, 1173(1), 88 – 97.
- Cai, S.-S., Stevens, J., & Syage, J. A. (2012). Ultra high performance liquid chromatography–atmospheric pressure photoionization-mass spectrometry for high-sensitivity analysis of US Environmental Protection Agency sixteen priority pollutant polynuclear aromatic hydrocarbons in oysters. *Journal of Chromatography. A*, 1227, 138 – 144.
- Cai, S.-S., & Syage, J. A. (2006a). Atmospheric pressure photoionization mass spectrometry for analysis of fatty acid and acylglycerol lipids. *Journal of Chromatography A*, 1110(1), 15 – 26.
- Cai, S.-S., & Syage, J. A. (2006b). Comparison of atmospheric pressure photoionization, atmospheric pressure chemical ionization, and electrospray ionization mass spectrometry for analysis of lipids. *Analytical Chemistry*, 78(4), 1191 – 1199.

- Cai, S.-S., Syage, J. A., Hanold, K. A., & Balogh, M. P. (2009). Ultra Performance Liquid Chromatography–Atmospheric Pressure Photoionization–Tandem Mass Spectrometry for High-Sensitivity and High-Throughput Analysis of U.S. Environmental Protection Agency 16 Priority Pollutants Polynuclear Aromatic Hydrocarbons. *Analytical Chemistry*, *81*(6), 2123 – 2128.
- Cai, Y., Herold, D., McConnell, O., & Bach, A. (2005). THF as a new dopant for atmospheric pressure photoionization mass spectrometry and comparison to other existing dopants. In *Proceedings of the 229th National Meeting of the American-Chemical-Society* (pp. U126–U126). San Diego, CA.
- Cai, Y., Kingery, D., McConnell, O., & Bach, 2nd, A. C. (2005). Advantages of atmospheric pressure photoionization mass spectrometry in support of drug discovery. *Rapid Communications in Mass Spectrometry*, *19*(12), 1717 – 1724.
- Cai, Y., McConnell, O., & Bach, 2nd, A. C. (2009). Suitability of tetrahydrofuran as a dopant and the comparison to other existing dopants in dopant-assisted atmospheric pressure photoionization mass spectrometry in support of drug discovery. *Rapid Communications in Mass Spectrometry*, *23*(15), 2283 – 2291.
- Cariou, R., Antignac, J.-P., Debrauwer, L., Maume, D., Monteau, F., Zalko, D., ... Andre, F. (2006). Comparison of analytical strategies for the chromatographic and mass spectrometric measurement of brominated flame retardants: 1. Polybrominated diphenylethers. *Journal of Chromatographic Science*, *44*(8), 489 – 497.
- Carrasco-Pancorbo, A., Nevedomskaya, E., Arthen-Engeland, T., Zey, T., Zurek, G., Baessmann, C., ... Mayboroda, O. A. (2009). Gas chromatography/atmospheric pressure chemical ionization-time of flight mass spectrometry: analytical validation and applicability to metabolic profiling. *Analytical Chemistry*, *81*(24), 10071–10079.
- Carroll, D. I., Dzidic, I., Stillwell, R. N., Haegele, K. D., & Horning, E. C. (1975). Atmospheric pressure ionization mass spectrometry. Corona discharge ion source for use in a liquid chromatograph-mass spectrometer-computer analytical system. *Analytical Chemistry*, *47*(14), 2369–2373.
- Carroll, D. I., Dzidic, I., Stillwell, R. N., Horning, M. G., & Horning, E. C. (1974). Subpicogram detection system for gas phase analysis based upon atmospheric pressure ionization (API) mass spectrometry. *Analytical Chemistry*, *46*(6), 706–710.
- Casetta, B., & Garofolo, F. (1994). Characterization of explosives by liquid chromatography mass spectrometry and liquid chromatography tandem mass spectrometry using electrospray ionization and parent-ion scanning techniques. *ORGANIC MASS SPECTROMETRY*, *29*(10), 517–525.
- Cassada, D. ., Monson, S. ., Snow, D. ., & Spalding, R. . (1999). Sensitive determination of RDX, nitroso-RDX metabolites, and other munitions in ground water by solid-phase

extraction and isotope dilution liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *Journal of Chromatography A*, 844(1), 87–95.

Castello, G., Benzo, M., & Gerbino, T. C. (1995). Automated gas chromatographic analysis of volatile organic compounds in air. *Journal of Chromatography A*, 710(1), 61 – 70.

Cavalcante, R. M., de Andrade, Márcia V.F Marins, R. V., & Oliveira, L. D. . (2010). Development of a headspace-gas chromatography (HS-GC-PID-FID) method for the determination of VOCs in environmental aqueous matrices: Optimization, verification and elimination of matrix effect and VOC distribution on the Fortaleza Coast. *Microchemical Journal*, 96(2), 337 – 343.

Cavaliere, C., Foglia, P., Pastorini, E., Samperi, R., & Laganà, A. (2006). Liquid chromatography/tandem mass spectrometric confirmatory method for determining aflatoxin M1 in cow milk: comparison between electrospray and atmospheric pressure photoionization sources. *Journal of Chromatography. A*, 1101(1-2), 69 – 78.

Chen, E., Wentworth, W., Desai, E., & Batten, C. (1987). Importance of kinetics and thermodynamics to the electron-capture detector. *Journal of Chromatography*, 399, 121–137.

Chen, H.-C., Kuo, H.-W., & Ding, W.-H. (2009). Determination of estrogenic compounds in wastewater using liquid chromatography–tandem mass spectrometry with electrospray and atmospheric pressure photoionization following desalting extraction. *Chemosphere*, 74(4), 508 – 514.

Chen, H.-K., Chang, C.-K., Wu, C.-C., Huang, M.-C., & Wang, Y.-S. (2009). Synchronized Dual-Polarity Electrospray Ionization Mass Spectrometry. *Journal of the American Society for Mass Spectrometry*, 20(12), 2254–2257.

Chen, J., Korfmacher, W. A., & Hsieh, Y. (2005). Chiral liquid chromatography-tandem mass spectrometric methods for stereoisomeric pharmaceutical determinations. *Journal of Chromatography B*, 820(1), 1–8.

Chiaberge, S., Fiorani, T., Savoini, A., Bionda, A., Ramello, S., Pastori, M., & Cesti, P. (2013). Classification of crude oil samples through statistical analysis of APPI FTICR mass spectra. *Fuel Processing Technology*, 106, 181–185.

Chiaia-Hernandez, A. C., Krauss, M., & Hollender, J. (2013). Screening of Lake Sediments for Emerging Contaminants by Liquid Chromatography Atmospheric Pressure Photoionization and Electrospray Ionization Coupled to High Resolution Mass Spectrometry. *Environmental Science & Technology*, 47(2), 976 – 986.

Cho, Y., Jin, J., Witt, M., Birdwell, J., Na, J., Roh, N., & Kim, S. (2013). Comparing Laser Desorption Ionization and Atmospheric Pressure Photoionization Coupled to Fourier

Transform Ion Cyclotron Resonance Mass Spectrometry To Characterize Shale Oils at the Molecular Level. *Energy & Fuels*, 27(4), 1830 – 1837.

Cho, Y., Na, J., Nho, N., & Kim, S. (2012). Application of Saturates, Aromatics, Resins, and Asphaltenes Crude Oil Fractionation for Detailed Chemical Characterization of Heavy Crude Oils by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Equipped with Atmospheric Pressure Photoionization. *Energy & Fuels*, 26(5), 2558–2565.

Chung, L.-W., Lin, K.-L., Yang, T. C.-C., & Lee, M.-R. (2009). Orthogonal array optimization of microwave-assisted derivatization for determination of trace amphetamine and methamphetamine using negative chemical ionization gas chromatography–mass spectrometry. *Journal of Chromatography A*, 1216(18), 4083–4089.

Clark II, C. J., & Delfino, J. J. (2003). Correlations between PID and FID field analytical instruments in the analysis of soil contaminated with diesel fuel. *Soil and Sediment Contamination: An International Journal*, 12(2), 151 – 164.

Cogliano, V. J., Baan, R., & Straif, K. (2011). Updating IARC's carcinogenicity assessment of benzene. *American Journal of Industrial Medicine*, 54(2), 193–197.

Cole, R. B., & Zhu, J. (1999). Chloride anion attachment in negative ion electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, 13(7), 607 – 611.

Comisarow, M. B., & Marshall, A. G. (1974). Frequency-sweep fourier transform ion cyclotron resonance spectroscopy. *Chemical Physics Letters*, 26(4), 489 – 490.

Constapel, M., Schellenträger, M., Schmitz, O. J., Gäb, S., Brockmann, K. J., Giese, R., & Benter, T. (2005). Atmospheric-pressure laser ionization: a novel ionization method for liquid chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry*, 19(3), 326 – 336.

Cotte-Rodríguez, I., Hernandez-Soto, H., Chen, H., & Cooks, R. . (2008). In situ trace detection of peroxide explosives by desorption electrospray ionization and desorption atmospheric pressure chemical ionization. *Analytical Chemistry*, 80(5), 1512 – 1519.

Cutter, L. S., Cutter, G. A., & San Diego-McGlone, M. L. C. (1991). Simultaneous determination of inorganic arsenic and antimony species in natural waters using selective hydride generation with gas chromatography/photoionization detection. *Analytical Chemistry*, 63(11), 1138–1142.

D'Andrilli, J., Dittmar, T., Koch, B. P., Purcell, J. M., Marshall, A. G., & Cooper, W. T. (2010). Comprehensive characterization of marine dissolved organic matter by Fourier transform ion cyclotron resonance mass spectrometry with electrospray and atmospheric

- pressure photoionization. *Rapid Communications in Mass Spectrometry*, 24(5), 643 – 650.
- De Wit, J. S. ., & Jorgenson, J. . (1987). Photoionization detector for open-tubular liquid chromatography. *Journal of Chromatography A*, 411, 201–212.
- Deal, C. H., Otvos, J. W., Smith, V. N., & Zucco, P. S. (1956). Radiological Detector for Gas Chromatography. *Anal.Chem.*, 28(12), 1958–1964.
- Debois, D., Giuliani, A., & Laprévotte, O. (2006). Fragmentation induced in atmospheric pressure photoionization of peptides. *JOURNAL OF MASS SPECTROMETRY*, 41(12), 1554 – 1560.
- Debrauwer, L., Riu, A., Jouahri, M., Rathahao, E., Jouanin, I., Antignac, J.-P., ... Zalko, D. (2005). Probing new approaches using atmospheric pressure photo ionization for the analysis of brominated flame retardants and their related degradation products by liquid chromatography–mass spectrometry. *Journal of Chromatography A*, 1082(1), 98 – 109.
- Deery, M. J., Jennings, K. R., Jasieczek, C. B., Haddleton, D. M., Jackson, A. T., Yates, H. T., & Scrivens, J. H. (1997). A Study of Cation Attachment to Polystyrene by Means of Matrix-assisted Laser Desorption/Ionization and Electrospray Ionization-Mass Spectrometry. *Rapid Communications in Mass Spectrometry*, 11(1), 57–62.
- Degenhardt, J., Köllner, T. G., & Gershenzon, J. (2009). Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry*, 70(15), 1621–1637.
- Delobel, A., Halgand, F., Laffranchise-Gosse, B., Snijders, H., & Laprévotte, O. (2003). Characterization of Hydrophobic Peptides by Atmospheric Pressure Photoionization-Mass Spectrometry and Tandem Mass Spectrometry. *Analytical Chemistry*, 75(21), 5961 – 5968.
- Delobel, A., Roy, S., Touboul, D., Gaudin, K., Germain, D. P., Baillet, A., ... Laprévotte, O. (2006). Atmospheric pressure photoionization coupled to porous graphitic carbon liquid chromatography for the analysis of globotriaosylceramides. Application to Fabry disease. *Journal of Mass Spectrometry*, 41(1), 50–58.
- Derpmann, V., Albrecht, S., & Benter, T. (2012). The role of ion-bound cluster formation in negative ion mass spectrometry. *Rapid Communications in Mass Spectrometry*, 26(17), 1923 – 1933.
- Desmazières, B., Legros, V., Giuliani, A., & Buchmann, W. (2014). Synthetic oligomer analysis using atmospheric pressure photoionization mass spectrometry at different photon energies. *Analytica Chimica Acta*, 808(220–230).

- Diachenko, G. W., Perfetti, G. A., & de Jager, L. S. (2004). LC-UV and LC-MS analysis of food and drink products containing kava. *Food Additives and Contaminants*, 21(10), 921 – 934.
- Doering, H., Arnold, G., Adler, J., Roebel, T., & Riemenschneider, J. (1996). Detecting gas traces in air using photoionisation ion mobility spectrometer. *Geramny*.
- Dougherty, R. C., & Roberts, J. D. (1974). Ammonia-isobutane chemical ionization mass spectra of oligosaccharide peracetates. *The Journal of Organic Chemistry*, 39(4), 451 – 455.
- Dousty, F., & O'Brien, R. (2015). The use of isoprene as a novel dopant in negative ion atmospheric pressure photoionization mass spectrometry coupled to high-performance liquid chromatography. *Rapid Communications in Mass Spectrometry*, 29, 1–8.
- Dousty, F., O'Brien, R. T., Gahler, R., Kersten, H., & Benter, T. (2013). Carbon disulfide as a dopant in photon-induced chemical ionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, 27(17), 1969 – 1976.
- Driscoll, J. (1982). Identification of hydrocarbons in complex mixtures using a variable energy PID and capillary column gas chromatography. *Journal of Chromatographic Science*, 20(2), 91 – 94.
- Driscoll, J. ., Conron, D. ., Ferioli, P., Krull, I. ., & Xie, K.-H. (1984). Trace analysis of organic compounds by high-performance liquid chromatography with photoionization detection. *Journal of Chromatography A*, 302, 43–50.
- Driscoll, J. N. (1976). Application of a photoionization detector in gas chromatography. *Am Lab*, 8(10), 71–75.
- Driscoll, J. N. (1977). Evaluation of a new photoionization detector for organic compounds. *Journal of Chromatography A*, 134(1), 49–55.
- Driscoll, J. N., & Clarici, J. B. (1976). Ein neuer Photoionisationsdetektor für die Gas-Chromatographie. *Chromatographia*, 9(11), 567 – 570.
- Driscoll, J. N., Ford, J., Jaramillo, L. F., & Gruber, E. T. (1978). Gas chromatographic detection and identification of aromatic and aliphatic hydrocarbons in complex mixtures by coupling photoionization and flame-ionization detectors. *Journal of Chromatography A*, 158, 171–180.
- Driscoll, J., & Spaziani, F. (1976). PID development gives new performance levels. *Research-Development*, 27(5), 50–&.
- Droste, S., Schellenträger, M., Constapel, M., Gäb, S., Lorenz, M., Brockmann, K. J., Schmitz, O. J. (2005). A silica-based monolithic column in capillary HPLC and CEC

coupled with ESI-MS or electrospray-atmospheric-pressure laser ionization-MS. *Electrophoresis*, 26(21), 4098 – 4103.

- Dzidic, I., Carroll, D. I., Stillwell, R. N., & Horning, E. C. (1974). Gas phase reactions. Ionization by proton transfer to superoxide anions. *Journal of the American Chemical Society*, 96(16), 5258–5259.
- Dzidic, I., Carroll, D. I., Stillwell, R. N., & Horning, E. C. (1975). Atmospheric pressure ionization (API) mass spectrometry: formation of phenoxide ions from chlorinated aromatic compounds. *Analytical Chemistry*, 47(8), 1308 – 1312.
- Dzidic, I., Carroll, D. I., Stillwell, R. N., & Horning, E. C. (1976). Comparison of Positive Ions Formed in Nickel-63 and Corona Discharge Ion Sources Using Nitrogen, Argon, Isobutane, Ammonia and Nitric Oxide as Reagents in Atmospheric Pressure Ionization Mass Spectrometry. *Analytical Chemistry*, 48(12), 1763–1768.
- Ehrenhauser, F. S., Wornat, M. J., Valsaraj, K. T., & Rodriguez, P. (2010). Design and evaluation of a dopant-delivery system for an orthogonal atmospheric-pressure photoionization source and its performance in the analysis of polycyclic aromatic hydrocarbons. *Rapid Communications in Mass Spectrometry*, 24(9), 1351 – 1357.
- Eiceman, G. A., & Vandiver, V. J. (1986). Charge-exchange in binary mixtures of polycyclic aromatic hydrocarbons using photoionization-ion mobility spectrometry. *Analytical Chemistry*, 58(11), 2331–2335.
- Eiceman, G., Fleischer, M., & Leasure, C. (1987). Sensing of Petrochemical Fuels in Soils Using Headspace Analysis with Photoionization-Ion Mobility Spectrometry. *International Journal of Environmental Analytical Chemistry*, 28(4), 279–296.
- Evans, C. S., Sleeman, R., Luke, J., & Keely, B. J. (2002). A rapid and efficient mass spectrometric method for the analysis of explosives. *Rapid Communications in Mass Spectrometry*, 16(19), 1883–1891.
- Field, F. (1972). Chemical Ionization Mass Spectrometry. In *Ion-Molecule Reactions Volume 1* (pp. 261–313). Springer US.
- Fitzgerald, R. L., Rexin, D. A., & Herold, D. A. (1993). Benzodiazepine Analysis by Negative Chemical Ionization Gas Chromatography/Mass Spectrometry. *Journal of Analytical Toxicology*, 17(6), 342–347.
- Fredenhagen, A., & Kühnöl, J. (2014). Evaluation of the optimization space for atmospheric pressure photoionization (APPI) in comparison with APCI. *Journal of Mass spectrometry*, 49(8), 727–736.

- Fritz, D. (1981). Introduction to analytical gas chromatography: History, principles and practice ( Chromatographic Science Series, Vol. 14). *Journal of Chromatography A*, 219(1), 193–193.
- Gallagher, J. W., Brion, C. E., Samson, J. A. R., & Langhoff, P. W. (1988). Absolute Cross Sections for Molecular Photoabsorption, Partial Photoionization, and Ionic Photofragmentation Processes. *Journal of Physical and Chemical Reference Data*, 17(1), 9–153.
- Gapeev, A., Sigman, M., & Yinon, J. (2003). Liquid chromatography/mass spectrometric analysis of explosives: RDX adduct ions. *Rapid Communications in Mass Spectrometry*, 17(9), 943–948.
- Gaspar, A., Zellermann, E., Lababidi, S., Reece, J., & Schrader, W. (2012). Characterization of Saturates, Aromatics, Resins, and Asphaltenes Heavy Crude Oil Fractions by Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy & Fuels*, 26(6), 3481–3487.
- Gaspar, A., Zellermann, E., Lababidi, S., Reece, J., & Schrader, W. (2012). Impact of different ionization methods on the molecular assignments of asphaltenes by FT-ICR mass spectrometry. *Analytical Chemistry*, 84(12), 5257–5267.
- Geyer, R., Peacock, A. D., White, D. C., Lytle, C., & Van Berkel, G. J. (2004). Atmospheric pressure chemical ionization and atmospheric pressure photoionization for simultaneous mass spectrometric analysis of microbial respiratory ubiquinones and menaquinones. *Journal of Mass Spectrometry*, 39(8), 922 – 929.
- Gimeno, R. ., Altelaar, A. F. ., Marcé, R. ., & Borrull, F. (2002). Determination of polycyclic aromatic hydrocarbons and polycyclic aromatic sulfur heterocycles by high-performance liquid chromatography with fluorescence and atmospheric pressure chemical ionization mass spectrometry detection in seawater and sediment samp. *Journal of Chromatography A*, 958(1), 141–148.
- Gómez-Ariza, J. ., García-Barrera, T., & Lorenzo, F. (2006). Anthocyanins profile as fingerprint of wines using atmospheric pressure photoionisation coupled to quadrupole time-of-flight mass spectrometry. *Analytica Chimica Acta*, 570(1), 101 – 108.
- González-Domínguez, R., García-Barrera, T., & Gómez-Ariza, J. L. (2015). Application of a novel metabolomic approach based on atmospheric pressure photoionization mass spectrometry using flow injection analysis for the study of Alzheimer’s disease. *Talanta*, 131, 480 – 489.
- González-Domínguez, R., García-Barrera, T., & Gómez-Ariza, J.-L. (2012). Iberian ham typification by direct infusion electrospray and photospray ionization mass spectrometry fingerprinting. *Rapid Communications in Mass Spectrometry*, 26(7), 835 – 844.

- Greening, F. ., & King, G. . (1976). Rydberg states of carbon dioxide and carbon disulfide. *Journal of Molecular Spectroscopy*, 59(2), 312–325.
- Greig, M. J., Bolaños, B., Quenzer, T., & Bylund, J. M. R. (2003). Fourier transform ion cyclotron resonance mass spectrometry using atmospheric pressure photoionization for high-resolution analyses of corticosteroids. *Rapid Communications in Mass Spectrometry*, 17(24), 2763 – 2768.
- Grøn, C., & Dybdahl, H. . (1996). Determination of total organic halogens (TOX); bias from a non-halogenated organic compound. *Environment International*, 22(3), 325 – 329.
- Guevremont, R. (2004). High-field asymmetric waveform ion mobility spectrometry: A new tool for mass spectrometry. *Journal of Chromatography A*, 1058(1), 3 – 19.
- Guo, T., Chan, M., & Soldin, S. J. (2004). Steroid profiles using liquid chromatography-tandem mass spectrometry with atmospheric pressure photoionization source. *Archives of Pathology & Laboratory Medicine*, 128(4), 469–475.
- Haapala, M., Luosujärvi, L., Saarela, V., Kotiaho, T., Ketola, R. A., Franssila, S., & Kostianen, R. (2007). Microchip for combining gas chromatography or capillary liquid chromatography with atmospheric pressure photoionization-mass spectrometry. *Analytical Chemistry*, 79(13), 4994 – 4999.
- Haapala, M., Suominen, T., & Kostianen, R. (2013). Capillary photoionization: a high sensitivity ionization method for mass spectrometry. *Analytical Chemistry*, 85(12), 5715–5719.
- Hakala, K. S., Laitinen, L., Kaukonen, A. M., Hirvonen, J., Kostianen, R., & Kotiaho, T. (2003). Development of LC/MS/MS Methods for Cocktail Dosed Caco-2 Samples Using Atmospheric Pressure Photoionization and Electrospray Ionization. *Analytical Chemistry*, 75(21), 5969–5977.
- Hanold, K. A., Fischer, S. M., Cormia, P. H., Miller, C. E., & Syage, J. A. (2004). Atmospheric Pressure Photoionization. 1. General Properties for LC/MS. *Analytical Chemistry*, 76(10), 2842 – 2851.
- Headley, J., Peru, K., Mohamed, M., Wilson, L., McMartin, D., Mapolelo, M., ... Marshall, A. (2014). Atmospheric Pressure Photoionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Characterization of Tunable Carbohydrate-Based Materials for Sorption of Oil Sands Naphthenic Acids. *Energy & Fuels*, 28(3), 1611 – 1616.
- Henion, J. D., Thomson, B. A., & Dawson, P. H. (1982). Determination of Sulfa Drugs in Biological-Fluids by Liquid-Chromatography Mass-Spectrometry Mass-Spectrometry. *Analytical Chemistry*, 54(3), 451–456.

- Herrera, L. C., Ramaley, L., & Grossert, J. S. (2009). Fragmentation pathways of some benzothiophene radical cations formed by atmospheric pressure chemical ionisation. *Rapid Communications in Mass Spectrometry*, 23(5), 571 – 579.
- Hester, N. E., & Meyer, R. A. (1979). A sensitive technique for measurement of benzene and alkylbenzenes in air. *Environmental Science & Technology*, 13(1), 107–109.
- Hill, H. H. (1975). *A hydrogen-atmosphere flame ionization detector for gas chromatography*. Dalhousie University (Canada). Retrieved from <http://search.proquest.com.ezproxy.library.ubc.ca/docview/302804168?accountid=14656>
- Himmelsbach, M., Buchberger, W., & Reingruber, E. (2009). Determination of polymer additives by liquid chromatography coupled with mass spectrometry. A comparison of atmospheric pressure photoionization (APPI), atmospheric pressure chemical ionization (APCI), and electrospray ionization (ESI). *Polymer Degradation and Stability*, 94(8), 1213–1219.
- Hintikka, L., Haapala, M., Franssila, S., Kuuranne, T., Leinonen, A., & Kostainen, R. (2010). Feasibility of gas chromatography–microchip atmospheric pressure photoionization-mass spectrometry in analysis of anabolic steroids. *Journal of Chromatography A*, 1217(52), 8290–8297.
- Hintikka, L., Haapala, M., Kuuranne, T., Leinonen, A., & Kostainen, R. (2013). Analysis of anabolic steroids in urine by gas chromatography–microchip atmospheric pressure photoionization-mass spectrometry with chlorobenzene as dopant. *Journal of Chromatography A*, 1312, 111–117.
- Hollosi, L., & Wenzl, T. (2011). Development and optimisation of a dopant assisted liquid chromatographic-atmospheric pressure photo ionisation-tandem mass spectrometric method for the determination of 15 + 1 EU priority PAHs in edible oils. *Journal of Chromatography A*, 1218(1), 23 – 31.
- Hommerson, P., Khan, A. M., & Bristow, T. (2007). Photon-Independent Gas-Phase-Ion Formation in Capillary Electrophoresis-Mass Spectrometry Using Atmospheric Pressure Photoionization. *Analytical Chemistry*, 79(14), 5351–5357.
- Horning, E. ., Horning, M. ., Carroll, D. ., Dzidic, I., Haegele, K. ., & Stillwell, R. . (1974). Liquid chromatograph—mass spectrometer—computer analytical systems: A continuous-flow system based on atmospheric pressure ionization mass spectrometry. *Journal of Chromatography A*, 99, 13–21.
- Horning, E. C., Carroll, D. I., Dzidic, I., Haegele, K. D., Horning, M. G., & Stillwell, R. N. (1974). Atmospheric pressure ionization (API) mass spectrometry. Solvent-mediated ionization of samples introduced in solution and in a liquid chromatograph effluent stream. *Journal of Chromatographic Science*, 12(11), 725 – 729.

- Horning, E. C., Horning, M. G., Carroll, D. I., Dzidic, I., & Stillwell, R. N. (1973). New picogram detection system based on a mass spectrometer with an external ionization source at atmospheric pressure. *Analytical Chemistry*, 45(6), 936–943.
- Horváth, P., Gergely, A., Mazák, K., Kökösi, J., & Szász, G. (2013). Novel Data on the Effect of Tetrahydrofuran as an Organic Co-Modifier in RP-HPLC. *Chromatographia*, 76(9), 441–448.
- Hourani, N., Andersson, J. T., Möller, I., Amad, M., Witt, M., & Sarathy, S. M. (2013). Atmospheric pressure chemical ionization Fourier transform ion cyclotron resonance mass spectrometry for complex thiophenic mixture analysis. *Rapid Communications in Mass Spectrometry*, 27(21), 2432 – 2438.
- Hourani, N., & Kuhnert, N. (2012a). Development of a novel direct-infusion atmospheric pressure chemical ionization mass spectrometry method for the analysis of heavy hydrocarbons in light shredder waste. *Analytical Methods*, 4(3), 730–735.
- Hourani, N., & Kuhnert, N. (2012b). High molecular weight non-polar hydrocarbons as pure model substances and in motor oil samples can be ionized without fragmentation by atmospheric pressure chemical ionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, 26(19), 1–8.
- Hsiao, C.-H., Hong, C.-W., Liu, B.-H., Chen, C. W., Wu, C.-C., & Wang, Y.-S. (2011). Comprehensive molecular imaging of photolabile surface samples with synchronized dual-polarity time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, 25(7), 834–842.
- Hsieh, Y., Merkle, K., & Wang, G. (2003). Zirconia-based column high-performance liquid chromatography/atmospheric pressure photoionization tandem mass spectrometric analyses of drug molecules in rat plasma. *Rapid Communications in Mass Spectrometry*, 17(15), 1775 – 1780.
- Hsieh, Y., Merkle, K., Wang, G., Brisson, J.-M., & Korfmacher, W. A. (2003). High-performance liquid chromatography-atmospheric pressure photoionization/tandem mass spectrometric analysis for small molecules in plasma. *Analytical Chemistry*, 75(13), 3122 – 3127.
- Hunt, D. F., & Ryan, J. F. (1972). Chemical ionization mass spectrometry studies. Nitric oxide as a reagent gas. *Journal of the Chemical Society, Chemical Communications*, (10), 620–621.
- Hunter, W. J., & Kuykendall, L. D. (2004). Determination of dimethylselenide and dimethyldiselenide by gas chromatography–photoionization detection. *Journal of Chromatography A*, 2004, Volume 1038, Issue 1, 1038(1), 295 – 297.

- I.G., W., R.A., D., & Desty, D. H. (1958). *Gas Chromatography*. London. Retrieved from William I.G.; Dewar R.A.; D.H. Desty (Ed.), Proc. 2nd Symp. Gas Chromatography. Discussion Group, Butterworth, London, 1958.
- Imbert, L., Gaudin, M., Libong, D., Touboul, D., Abreu, S., Loiseau, P. M., ... Chaminade, P. (2012). Comparison of electrospray ionization, atmospheric pressure chemical ionization and atmospheric pressure photoionization for a lipidomic analysis of *Leishmania donovani*. *Journal of Chromatography A*, 1242, 75–83.
- Iribarne, J. V, & Thomson, B. A. (1976). On the evaporation of small ions from charged droplets. *The Journal of Chemical Physics*, 64(6), 2287–2294.
- Isoprene; MSDS No. 464953. (n.d.).
- Itoh, N., Aoyagi, Y., & Yarita, T. (2006). Optimization of the dopant for the trace determination of polycyclic aromatic hydrocarbons by liquid chromatography/dopant-assisted atmospheric-pressure photoionization/mass spectrometry. *Journal of Chromatography A*, 1131(1), 285 – 288.
- Itoh, N., Otake, T., Aoyagi, Y., Matsuo, M., & Yarita, T. (2009). Application of Pesticide Quantification in Unpolished Rice by LC-Dopant-Assisted Atmospheric Pressure Photoionization-MS. *Chromatographia*, 70(7-8), 1073–1078.
- Itoh, N., Yarita, T., Narukawa, T., Aoyagi, Y., Numata, M., & Takatsu, A. (2009). Mechanism of ionization of polycyclic aromatic hydrocarbons by a toluene/anisole mixture as a dopant in liquid chromatography/dopant-assisted atmospheric-pressure photoionization/mass spectrometry. *Polycyclic Aromatic Compounds*, 29(1), 41 – 55.
- Jelus, B. L., Munson, B., Babiak, K. A., & Murray, R. K. (1974). Mass spectrometry. Comparison of the electron impact and chemical ionization fragmentations of 8,9-dehydro-2-adamantanol and 2-exo-protoadamantenol. *The Journal of Organic Chemistry*, 39(22), 3250–3254.
- Jelus, B. L., Munson, B., & Fenselau, C. (1974). Reagent gases for G.C.-M.S. analyses. *Biomedical Mass Spectrometry*, 1(2), 96 – 102.
- Jesse, W., & Sadauskis, J. (1955). IONIZATION BY ALPHA-PARTICLES IN MIXTURES OF GASES. *Phys. Rev.*, 100(6), 1755–1762.
- Kamel, A., Jeanville, P., Colizza, K., & J-Rivera, L. E. (2008). Mechanism of [M + H] + Formation in Atmospheric Pressure Photoionization Mass Spectrometry: Identification of Propionitrile in Acetonitrile with High Mass Accuracy Measurement and Tandem Mass Spectrometry and Evidence for Its Involvement in the Protonatio. *Journal of the American Society for Mass Spectrometry*, 19(11), 1579 – 1589.

- Kapila, S., Bornhop, D. J., Manahan, S. E., & Nickell, G. L. (1983). Design of a combined photoionization detector and photoionization-based electron-capture detector. *Journal of Chromatography A*, 259, 205–210.
- Karlonas, N., Padarauskas, A., Ramanavicius, A., Minkuviene, Z., & Ramanaviciene, A. (2012). Rapid and highly sensitive determination of clonazepam and 7-aminoclonazepam in whole blood using gas chromatography with negative-ion chemical ionization mass spectrometry. *Chemija*, 23(2), 91 – 99.
- Karlonas, N., Ramanavicius, A., & Ramanaviciene, A. (2014). Development of an SPE method for the determination of zaleplon and zopiclone in hemolyzed blood using fast GC with negative-ion chemical ionization MS. *Journal of Separation Science*, 37(5), 551 – 557.
- Karmen, A., & Giuffrida, L. (1964). ENHANCEMENT OF THE RESPONSE OF THE HYDROGEN FLAME IONIZATION DETECTOR TO COMPOUNDS CONTAINING HALOGENS AND PHOSPHORUS. *Nature*, 201(4925), 1204–1205.
- Karst, U., Leeuwen, van, S. M., & Bos, S. J. (2006). From fundamentals to applications: recent developments in atmospheric pressure photoionization mass spectrometry: review. *Analytical and Bioanalytical Chemistry*, 384(1), 85–99.
- Karuna, R., von Eckardstein, A., & Rentsch, K. M. (2009). Dopant assisted-atmospheric pressure photoionization (DA-APPI) liquid chromatography–mass spectrometry for the quantification of 27-hydroxycholesterol in plasma. *Journal of Chromatography B*, 877(3), 261 – 268.
- Kauppila, T. J., Bruins, A. P., & Kostianen, R. (2005). Effect of the Solvent Flow Rate on the Ionization Efficiency in Atmospheric Pressure Photoionization-Mass Spectrometry. *Journal of the American Society for Mass Spectrometry*, 16(8), 1399–1407.
- Kauppila, T. J., Kostianen, R., & Bruins, A. P. (2004). Anisole, a new dopant for atmospheric pressure photoionization mass spectrometry of low proton affinity, low ionization energy compounds. *Rapid Communications in Mass Spectrometry*, 18(7), 808 – 815.
- Kauppila, T. J., Kotiaho, T., Kostianen, R., & Bruins, A. P. (2004). Negative ion-atmospheric pressure photoionization-mass spectrometry. *Journal of the American Society for Mass Spectrometry*, 15(2), 203–211.
- Kauppila, T. J., Kuuranne, T., Meurer, E. C., Eberlin, M. N., Kotiaho, T., & Kostianen, R. (2002). Atmospheric pressure photoionization mass spectrometry. Ionization mechanism and the effect of solvent on the ionization of naphthalenes. *Analytical Chemistry*, 74(21), 5470 – 5479.

- Kauppila, T. J., Nikkola, T., Ketola, R. A., & Kostianen, R. (2006). Atmospheric pressure photoionization-mass spectrometry and atmospheric pressure chemical ionization-mass spectrometry of neurotransmitters. *Journal of Mass Spectrometry*, 41(6), 781 – 789.
- Kauppila, T. J., Ostman, P., Marttila, S., Ketola, R. A., Kotiaho, T., Franssila, S., & Kostianen, R. (2004). Atmospheric pressure photoionization-mass spectrometry with a microchip heated nebulizer. *Analytical Chemistry*, 76(22), 6797 – 6801.
- Kéki, S., Nagy, L., Kuki, A., & Zsuga, M. (2008). A New Method for Mass Spectrometry of Polyethylene Waxes: The Chloride Ion Attachment Technique by Atmospheric Pressure Photoionization. *Macromolecules*, 41(11), 3772 – 3774.
- Kéki, S., Török, J., Nagy, L., & Zsuga, M. (2008). Atmospheric Pressure Photoionization Mass Spectrometry of Polyisobutylene Derivatives. *Journal of the American Society for Mass Spectrometry*, 19(5), 656 – 665.
- Kersten, H., Funcke, V., Lorenz, M., Brockmann, K. J., Benter, T., & O'Brien, R. (2009). Evidence of Neutral Radical Induced Analyte Ion Transformations in APPI and Near-VUV APLI. *Journal of the American Society for Mass Spectrometry*, 20(10), 1868–1880.
- Kersten, H., Haberer, K., Kroll, K., & Benter, T. (2014). Progress in the development of a GC-APPI source with femtogram sensitivity. In *Proceedings of the 62nd ASMS Conference on Mass Spectrometry and Allied Topics*. Baltimore, MD, USA.
- Keski-Hynnälä, H., Kurkela, M., Elovaara, E., Antonio, L., Magdalou, J., Luukkanen, L., Kostianen, R. (2002). Comparison of electrospray, atmospheric pressure chemical ionization, and atmospheric pressure photoionization in the identification of apomorphine, dobutamine, and entacapone phase II metabolites in biological samples. *Analytical Chemistry*, 74(14), 3449 – 3457.
- Keski-Rahkonen, P., Huhtinen, K., Desai, R., Harwood, D. T., Handelsman, D. J., Poutanen, M., & Auriola, S. (2013). LC-MS analysis of estradiol in human serum and endometrial tissue: Comparison of electrospray ionization, atmospheric pressure chemical ionization and atmospheric pressure photoionization. *Journal of Mass Spectrometry*, 48(9), 1050 – 1058.
- Ketkar, S. ., Dulak, J. ., Dheandhanoo, S., & Fite, W. . (1991). Benzene charge exchange at atmospheric pressure for low-level detection of pollutants in ambient air. *Analytica Chimica Acta*, 245, 267 – 270.
- King, R., Bonfiglio, R., Fernandez-Metzler, C., Miller-Stein, C., & Olah, T. (2000). Mechanistic Investigation of Ionization Suppression in Electrospray Ionization. *Journal of the American Society for Mass Spectrometry*, 11(11), 942–950.

- Klee, S., Dierpmann, V., Wißdorf, W., Klopotoski, S., Kersten, H., Brockmann, K. J., Syage, J. A. (2014). Are clusters important in understanding the mechanisms in atmospheric pressure ionization? Part 1: Reagent ion generation and chemical control of ion populations. *Journal of the American Society for Mass Spectrometry*, 25(8), 1310–1321.
- Knappenberger, Kenneth L Lerch, E.-B. W., Wen, P., & Leone, S. R. (2006). Coherent polyatomic dynamics studied by femtosecond time-resolved photoelectron spectroscopy: dissociation of vibrationally excited CS<sub>2</sub> in the 6s and 4d Rydberg states. *The Journal of Chemical Physics*, 125(17), 174314–174314–12.
- Koch, W., Forcisi, S., Lehmann, R., & Schmitt-Kopplin, P. (2014). Sensitivity improvement in hydrophilic interaction chromatography negative mode electrospray ionization mass spectrometry using 2-(2-methoxyethoxy)ethanol as a post-column modifier for non-targeted metabolomics. *Journal of Chromatography A*, 1361, 209–216.
- Kok, P. W., & Ong, C. N. (1994). Blood and urinary benzene determined by headspace gas chromatography with photoionization detection: application in biological monitoring of low-level nonoccupational exposure. *International Archives of Occupational and Environmental Health*, 66(3), 195–201.
- Kolakowski, B. M., Grossert, J. S., & Ramaley, L. (2004). Studies on the positive-ion mass spectra from atmospheric pressure chemical ionization of gases and solvents used in liquid chromatography and direct liquid injection. *Journal of the American Society for Mass Spectrometry*, 15(3), 311–324.
- Korfmacher, W. A., Cox, K. A., Bryant, M. S., Veals, J., Ng, K., Lin, C., & Watkins, R. (1997). HPLC-API/MS/MS: a powerful tool for integrating drug metabolism into the drug discovery process. *Drug Discovery Today*, 2(12), 532 – 537.
- Kubalaa, D., Drageb, E. A., Al-Faydhic, A. M. E., Kočíšeka, J., Pappa, P., Matejčíkd, V., Masonb, N. J. (2009). Electron impact ionisation and UV absorption study of alpha- and beta-pinene. *International Journal of Mass Spectrometry*, 280(1-3), 169–173.
- Kühlewind, H., Boesl, U., Weinkauff, R., Neusser, H. J., & Schlag, E. W. (1983). Multiphoton Mass Spectrometry: Metastables. *Laser Chemistry*, 3(1-6), 3 – 17.
- Kushnir, M. M., Neilson, R., Roberts, W. L., & Rockwood, A. L. (2004). Cortisol and cortisone analysis in serum and plasma by atmospheric pressure photoionization tandem mass spectrometry. *Clinical Biochemistry*, 37(5), 357 – 362.
- Lababidi, S., Panda, S. K., Andersson, J. T., & Schrader, W. (2013). Direct Coupling of Normal-Phase High-Performance Liquid Chromatography to Atmospheric Pressure Laser Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry for the Characterization of Crude Oil. *Analytical Chemistry*, 85(20), 9478–9485.

- Ladenburg, R., & Van Voorhis, C. C. (1933). The Continuous Absorption of Oxygen Between 1750 and 1300A and Its Bearing Upon the Dispersion. *Physical Review*, 43(5), 315–321.
- Lafleur, A. L., Taghizadeh, K., Howard, J. B., Anacleto, J. F., & Quilliam, M. A. (1996). Characterization of flame-generated C10 to C 160 polycyclic aromatic hydrocarbons by atmospheric-pressure chemical ionization mass spectrometry with liquid introduction via heated nebulizer interface. *Journal of the American Society for Mass Spectrometry*, 7(3), 276 – 286.
- Lagalante, A. F., & Oswald, T. D. (2008). Analysis of polybrominated diphenyl ethers (PBDEs) by liquid chromatography with negative-ion atmospheric pressure photoionization tandem mass spectrometry (LC/NI-APPI/MS/MS): application to house dust. *Analytical and Bioanalytical Chemistry*, 391(6), 2249 – 2256.
- Langhorst, M. (1981). Photo-ionization detector sensitivity of organic-compounds. *Journal of Chromatographic Science*, 19(2), 98–103.
- Langhorst, M. L., & Nestruck, T. J. (1979). Determination of chlorobenzenes in air and biological samples by gas chromatography with photoionization detection. *Analytical Chemistry*, 51(12), 2018–2025.
- Leasure, C. S., Fleischer, M. E., Anderson, G. K., & Eiceman, G. A. (1986). Photoionization in air with ion mobility spectrometry using a hydrogen discharge lamp. *Analytical Chemistry*, 58(11), 2142–2147.
- Leinonen, A., Kuuranne, T., & Kostianen, R. (2002). Liquid chromatography/mass spectrometry in anabolic steroid analysis—optimization and comparison of three ionization techniques: electrospray ionization, atmospheric pressure chemical ionization and atmospheric pressure photoionization. *Journal of Mass Spectrometry*, 37(7), 693 – 698.
- Leis, H., Fauler, G., Rechberger, G., & Windischhofer, W. (2004). Electron-Capture Mass Spectrometry: A Powerful Tool in Biomedical Trace Level Analysis. *Current Medicinal Chemistry*, 11(12), 1585–1594.
- Leis, H. J., & Windischhofer, W. (2010). o-(Pentafluorobenzyloxycarbonyl)benzoyl chloride: a novel electrophoric derivatisation reagent for amino compounds designed for negative ion chemical ionisation mass spectrometry. *Rapid Communications in Mass Spectrometry*, 24(22), 3320 – 3324.
- Lembcke, J., Ceglarek, U., Fiedler, G. M., Baumann, S., Leichtle, A., & Thiery, J. (2005). Rapid quantification of free and esterified phytosterols in human serum using APPI-LC-MS/MS. *Journal of Lipid Research*, 46(1), 21 – 26.

- Letcher, R. J., & Chu, S. (2010). High-sensitivity method for determination of tetrabromobisphenol-S and tetrabromobisphenol-A derivative flame retardants in great lakes herring gull eggs by liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry. *Environmental Science & Technology*, *44*(22), 8615–8621.
- Lewis, A. C., Hamilton, Jacqueline F Rhodes, Christopher N Halliday, J., Bartle, Keith D Homewood, P., Grenfell, R. J., Goody, B., Harling, A. M., Milton, M. J. (2010). Microfabricated planar glass gas chromatography with photoionization detection. *Journal of Chromatography A*, *1217*(5), 768 – 774. doi:10.1016/j.chroma.2009.12.009
- Liu, Q., Liu, Y., & Wang, X. (2011). Determination of dimethylsulfide and dimethylselenide in human urine by portable gas chromatography–photoionization detection with headspace sampling. *Microchemical Journal*, *2011*, Volume 99, Issue 2, *99*(2), 352 – 355.
- Liu, X., Zhang, W., Wang, Z., Huang, M., Yang, X., Tao, L., Sheng, L. (2009). Dissociative photoionization of isoprene: experiments and calculations. *Journal of Mass Spectrometry*, *44*(3), 404 – 409.
- Liu, Y., Chen, C., Zhang, L., Zhang, X., Wang, H., & Kong, D. (2014). Detection of Chemical Warfare Agent Simulants by UV Photoionization High-Field Asymmetric Ion Mobility Spectrometry. *Chinese Journal of Analytical Chemistry*, *42*(9), 1259–1263.
- Locke, D. C., Dhingra, B. S., & Baker, A. D. (1982). Liquid-phase photoionization detector for liquid chromatography. *Analytical Chemistry*, *54*(3), 447–450.
- Locke, D. C., & Meloan, C. E. (1965). Study of the Photoionization Detector for Gas Chromatography. *Analytical Chemistry*, *37*(3), 389–395.
- Louw, S., Njoroge, M., Chigorimbo-Murefu, N., & Chibale, K. (2012). Comparison of electrospray ionisation, atmospheric pressure chemical ionisation and atmospheric pressure photoionisation for the identification of metabolites from labile artemisinin-based anti-malarial drugs using a QTRAP (R) mass spectrometer. *Rapid Communications in Mass Spectrometry*, *26*(20), 2431 – 2442.
- Lovelock, J. (1958). A SENSITIVE DETECTOR FOR GAS CHROMATOGRAPHY. *JOURNAL OF CHROMATOGRAPHY*, *1*(1), 35–46.
- LOVELOCK, J. E. (1960). A Photoionization Detector for Gases and Vapours. *Nature*, *188*(4748), 401–401.
- Lovelock, J. E. (1961). Ionization Methods for the Analysis of Gases and Vapors. *Analytical Chemistry*, *33*(2), 162–178.
- LOVELOCK, J. E. (1962). Free Electrons with Thermal Energy: their Generation for Chemical Use. *Nature*, *195*(4840), 488–489.

- Lovelock, J. E. (1963). Electron Absorption Detectors and Technique for Use in Quantitative and Qualitative Analysis by Gas Chromatography. *Analytical Chemistry*, 35(4), 474–481.
- Lubman, D. M. (1990). *Lasers and Mass Spectrometry*. Oxford university Press.
- Lung, S.-C. C., & Liu, C.-H. (2011). High-Sensitivity Analysis of Six Synthetic Musks by Ultra-Performance Liquid Chromatography–Atmospheric Pressure Photoionization–Tandem Mass Spectrometry. *Analytical Chemistry*, 83(12), 4955–4961.
- Luosujärvi, L., Karikko, M.-M., Haapala, M., Saarela, V., Huhtala, S., Franssila, S., Kauppila, T. J. (2008). Gas chromatography/mass spectrometry of polychlorinated biphenyls using atmospheric pressure chemical ionization and atmospheric pressure photoionization MICROCHIPS. *Rapid Communications in Mass Spectrometry*, 22(4), 425 – 431.
- Marchi, I., Rudaz, S., & Veuthey, J.-L. (2009). Atmospheric pressure photoionization for coupling liquid-chromatography to mass spectrometry: a review. *Talanta*, 78(1), 1 – 18.
- Marotta, E., Seraglia, R., Fabris, F., & Traldi, P. (2003). Atmospheric pressure photoionization mechanisms - 1. The case of acetonitrile. *International Journal of Mass Spectrometry*, 228(2-3), 841 – 849.
- Marshall, A. G., Hendrickson, C. L., & Jackson, G. S. (1998). Fourier transform ion cyclotron resonance mass spectrometry: a primer. *Mass Spectrometry Reviews*, 17(1), 1 – 35.
- Marteau, C., Chevolleau, S., Jouanin, I., Perdu, E., De Sousa, G., Rahmani, R., Debrauwer, L. (2012). Development of a liquid chromatography/atmospheric pressure photo-ionization high-resolution mass spectrometry analytical method for the simultaneous determination of polybrominated diphenyl ethers and their metabolites: application to BDE--47 metabolism . *Rapid Communications in Mass Spectrometry*, 26(6), 599 – 610.
- Martins, G., Ferreira-Rodrigues, A. M., Rodrigues, F. N., de Souza, G. G. B., Mason, N. J., Eden, S., ... Limão-Vieira, P. (2009). Valence shell electronic spectroscopy of isoprene studied by theoretical calculations and by electron scattering, photoelectron, and absolute photoabsorption measurements. *Physical Chemistry Chemical Physics*, 11(17), 11219 – 11231.
- Mascher, D. G., Mascher, H. J., & Zech, K. (2008). Sensitive simultaneous determination of ciclesonide, ciclesonide-M1-metabolite and fluticasone propionate in human serum by HPLC-MS/MS with APPI. *Journal of Chromatography B*, 869(1), 84 – 92.
- Mascolo, G., Locaputo, V., & Mininni, G. (2010). New perspective on the determination of flame retardants in sewage sludge by using ultrahigh pressure liquid chromatography-

- tandem mass spectrometry with different ion sources. *Journal of Chromatography A*, 1217(27), 4601–4611.
- Maurer, H. H., Kraemer, T., Kratzsch, C., Peters, F. T., & Weber, A. A. (2002). Negative ion chemical ionization gas chromatography-mass spectrometry and atmospheric pressure chemical ionization liquid chromatography-mass spectrometry of low-dosed and/or polar drugs in plasma. *Therapeutic Drug Monitoring*, 24(1), 117–124.
- McClaine, J. W., Zhang, X., & Wornat, M. J. (2006). First identification of benzo[ghi]naphtho[8,1,2-bcd]perylene as a product of fuel pyrolysis, using high-performance liquid chromatography with diode-array ultraviolet-visible absorbance detection and mass spectrometry. *Journal of Chromatography A*, 1127(1), 137 – 146.
- McCulloch, R. D., Robb, D. B., & Blades, M. W. (2008). A dopant introduction device for atmospheric pressure photoionization with liquid chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry*, 22(22), 3549 – 3554.
- McEwen, C. N., & McKay, R. G. (2005). A Combination Atmospheric Pressure LC/MS:GC/MS Ion Source: Advantages of Dual AP-LC/MS:GC/MS Instrumentation. *Journal of the American Society for Mass Spectrometry*, 16(11), 1730–1738.
- McWilliam, I. G., & Dewar, R. A. (1958). Flame Ionization Detector for Gas Chromatography. *Nature*, 181(4611), 760–760.
- Meier, F., Garrard, K. P., & Muddiman, D. C. (2014). Silver dopants for targeted and untargeted direct analysis of unsaturated lipids via infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI). *Rapid Communications in Mass Spectrometry*, 28(22), 2461–2470.
- Meurer, E. C., Sabino, A. A., & Eberlin, M. N. (2003). Ionic transacetalization with acylium ions: a class-selective and structurally diagnostic reaction for cyclic acetals performed under unique electrospray and atmospheric pressure chemical ionization in-source ion-molecule reaction conditions. *Analytical Chemistry*, 75(17), 4701–4709.
- Miller, C. A., Cormia, P. H., & Fischer, S. M. (2001). Atmospheric Pressure Photoionization Ion Trap Analysis of Fat-Soluble Vitamins. In *49th ASMS conference on Mass Spectrometry and Allied Topics*. Chicago, IL.
- Milne, G. W., Fales, H. M., & Axenrod, T. (1971). Identification of dangerous drugs by isobutane chemical ionization mass spectrometry. *Analytical Chemistry*, 43(13), 1815–1820.
- Miyabe, K., Sotoura, S., & Guiochon, G. (2001). Retention and mass transfer characteristics in reversed-phase liquid chromatography using a tetrahydrofuran–water solution as the mobile phase. *Journal of Chromatography A*, 919(2), 231–244.

- Mohamed, R., Hammel, Y.-A., LeBreton, M.-H., Tabet, J.-C., Jullien, L., & Guy, P. A. (2007). Evaluation of atmospheric pressure ionization interfaces for quantitative measurement of sulfonamides in honey using isotope dilution liquid chromatography coupled with tandem mass spectrometry techniques. *Journal of Chromatography A*, *1160*(1), 194 – 205.
- Mol, R., de Jong, G. J., & Somsen, G. W. (2005). On-line capillary electrophoresis-mass spectrometry using dopant-assisted atmospheric pressure photoionization: setup and system performance. *Electrophoresis*, *26*(1), 146 – 154.
- Mol, R., Jong, G. J. de, & Somsen, G. W. (2005). Atmospheric Pressure Photoionization for Enhanced Compatibility in On-Line Micellar Electrokinetic Chromatography-Mass Spectrometry. *Analytical Chemistry*, *77*(16), 5277–5282.
- Mopper, K., Stubbins, A., Ritchie, J. D., Bialk, H. M., & Hatcher, P. G. (2007). Advanced Instrumental Approaches for Characterization of Marine Dissolved Organic Matter: Extraction Techniques, Mass Spectrometry, and Nuclear Magnetic Resonance Spectroscopy. *Chemical Reviews*, *107*(2), 419 – 442.
- Moriwaki, H., Harino, H., Yoshikura, T., Ohe, T., Nukaya, H., Terao, Y., Alary, J.-F. (2004). Simultaneous determination of 2-phenylbenzotriazole-type mutagens, PBTA-1 through-8, in river water by liquid chromatography-tandem mass spectrometry. *Journal of Environmental Monitoring*, *6*(11), 897–902.
- Moriwaki, H., Ishitake, M., Yoshikawa, S., Miyakoda, H., & Alary, J.-F. (2004). Determination of polycyclic aromatic hydrocarbons in sediment by liquid chromatography-atmospheric pressure photoionization-mass spectrometry. *Analytical Sciences : The International Journal of the Japan Society for Analytical Chemistry*, *20*(2), 375–377.
- Moukas, A. I., Thomaidis, N. S., & Calokerinos, A. C. (2014). Determination of polychlorinated biphenyls by liquid chromatography-atmospheric pressure photoionization-mass spectrometry. *Journal of Mass Spectrometry*, *49*(11), 1096 – 1107.
- MPI-Mainz-UV-VIS Spectral Atlas of Gaseous Molecules, A database of atmospherically relevant species, including numerical data and graphical representations. (n.d.). Retrieved October 1, 2014, from <http://www.atmosphere.mpg.de/enid/2295/>
- Müller, H., & Andersson, J. (2004). Gel permeation chromatography of sulfur containing aromatics in vacuum residues. *Polycyclic Aromatic Compounds*, *24*(4), 299–308.
- Müller, H., Andersson, J. T., & Schrader, W. (2005). Characterization of High-Molecular-Weight Sulfur-Containing Aromatics in Vacuum Residues Using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Analytical Chemistry*, *77*(8), 2536 – 2543.

- Mullins, O. C., Cunico, R. L., & Sheu, E. Y. (2004). Molecular weight measurement of UG8 asphaltene using APCI mass spectroscopy. *Petroleum Science and Technology*, 22(7), 787 – 798.
- Nagy, L., Pálfi, V., Narmandakh, M., Kuki, Á., Nyíri, A., Iván, B., ... Kéki, S. (2009). Dopant-Assisted Atmospheric Pressure Photoionization Mass Spectrometry of Polyisobutylene Derivatives Initiated by Mono- and Bifunctional Initiators. *Journal of the American Society for Mass Spectrometry*, 20(12), 2342 – 2351.
- Nakahara, T., Yamaguchi, K., Takino, M., & Tanaka, T. (2004). Atmospheric pressure photo-ionization liquid chromatography/mass spectrometric determination of aflatoxins in food. *Food Additives and Contaminants*, 21(1), 76 – 84.
- Neukermans, A., Kruger, W., & McManigill, D. (1982). Non-radioactive electron-capture detector. *Journal of Chromatography A*, 235(1), 1–20.
- Niessen, W. M. A. (1999). *Liquid chromatography-mass spectrometry* (2nd ed.). New York : M. Dekker.
- Nilsson, S. L., Andersson, C., Sjöberg, P. J. R., Bylund, D., Petersson, P., Jörntén-Karlsson, M., & Markides, K. E. (2003). Phosphate buffers in capillary electrophoresis/mass spectrometry using atmospheric pressure photoionization and electrospray ionization. *Rapid Communications in Mass Spectrometry*, 17(20), 2267 – 2272.
- NIST Standard Reference Database Number 69. NIST Chemistry WebBook. Retrieved May 20, 2003, from <http://webbook.nist.gov/chemistry/> Date accessed: Feb 2015
- Norlander, B., Carlsson, B., & Bertler, A. (1986). Sensitive assay of methadone in plasma by using capillary gas chromatography with photoionization detection. *Journal of Chromatography*, 375(2), 313 – 319.
- Núñez, O., Gallart-Ayala, H., Martins, C. P. B., Moyano, E., & Galceran, M. T. (2012). Atmospheric Pressure Photoionization Mass Spectrometry of Fullerenes. *Analytical Chemistry*, 84(12), 5316–5326.
- Ogino, H., Aomura, Y., & Komuro, Masatugu Kobayashi, T. (1989). Determination of trace impurities in high-purity oxygen by gas chromatography with photoionization detection. *Analytical Chemistry*, 61(20), 2237–2240.
- Osborne, D. M., Podgorski, D. C., Bronk, D. A., Roberts, Q., Sipler, R. E., Austin, D., Cooper, W. T. (2013). Molecular level characterization of reactive and refractory dissolved natural organic nitrogen compounds by atmospheric pressure photoionization coupled to Fourier transform ion. *Rapid Communications in Mass Spectrometry*, 27(8), 851 – 858.

- Oyler, A. R., Bodenner, D. L., Welch, K. J., Liukkonen, R. J., Carlson, R. M., Kopperman, H. L., & Caple, R. (1978). Determination of aqueous chlorination reaction products of polynuclear aromatic hydrocarbons by reversed phase high performance liquid chromatography-gas chromatography. *Analytical Chemistry*, 50(7), 837–842.
- Panda, S. K., Andersson, J. T., & Schrader, W. (2009). Characterization of supercomplex crude oil mixtures: what is really in there? *Angewandte Chemie (International Ed. in English)*, 48(10), 1788 – 1791.
- Panda, S. K., Brockmann, K.-J., Benter, T., & Schrader, W. (2011). Atmospheric pressure laser ionization (APLI) coupled with Fourier transform ion cyclotron resonance mass spectrometry applied to petroleum samples analysis: comparison with electrospray ionization and atmospheric pressure photoionization methods. *Rapid Communications in Mass Spectrometry*, 25(16), 2317 – 2326.
- Pang, X., Lewis, A. C., & Hamilton, J. F. (2011). Determination of airborne carbonyls via pentafluorophenylhydrazine derivatisation by GC–MS and its comparison with HPLC method. *Talanta*, 85(1), 406–414.
- Papanastasiou, M., Allen, N. S., McMahon, A., Naegel, L. C., Edge, M., & Protopappas, S. (2012). Analysis of Indigo-type compounds in natural dyes by negative ion atmospheric pressure photoionization mass spectrometry. *Dyes and Pigments*, 92(3), 1192–1198.
- Papanastasiou, M., McMahon, A., Allen, N., Johnson, B., Keck-Antoine, K., Santos, L., & Neumann, M. (2008). Atmospheric pressure photoionization mass spectrometry as a tool for the investigation of the hydrolysis reaction mechanisms of phosphite antioxidants. *International Journal of Mass Spectrometry*, 275(1), 45 – 54.
- Peng, F., Luo, T., Yuan, Y., & Qiu, L. (2010). Performance of optimized TVOCs sensor. *Procedia Engineering*, 7, 392 – 398. doi:10.1016/j.proeng.2010.11.064
- Perazzolli, C., Mancini, I., & Guella, G. (2005). Benzene-assisted atmospheric-pressure chemical ionization: a new liquid chromatography/mass spectrometry approach to the analysis of selected hydrophobic compounds. *Rapid Communications in Mass Spectrometry*, 19(4), 461 – 469.
- Pereira, T. M., Vanini, G., Oliveira, E. C., Cardoso, F. M., Fleming, F. P., Neto, A. C., Romão, W. (2014). An evaluation of the aromaticity of asphaltenes using atmospheric pressure photoionization Fourier transform ion cyclotron resonance mass spectrometry – APPI(±)FT-ICR MS. *Fuel*, 118, 348–357.
- Peterson, A. C., Kersten, H., Krumwiede, D., Quarmby, S., D’Silva, K., Kroll, K., Benter, T. (2014). Analytical performance of a novel, dopant-free GC-APPI source with femtogram-level sensitivity for quadrupole-Orbitrap GC/MS. In *Proceedings of the 62nd ASMS Conference on Mass Spectrometry and Allied Topics*. Baltimore, MD, USA.

- Poli, D., Goldoni, M., Corradi, M., Acampa, O., Carbognani, P., Internullo, E., Mutti, A. (2010). Determination of aldehydes in exhaled breath of patients with lung cancer by means of on-fiber-derivatization SPME–GC/MS. *Journal of Chromatography B*, 878(27), 2643–2651.
- Price, J. G. W., Fenimore, David C Simmonds, P. G., & Zlatkis, A. (1968). Design and operation of a photoionization detector for gas chromatography. *Analytical Chemistry*, 40(3), 541–547.
- Puig, L., & Sacks, R. (1991). Low-Pressure Gas Chromatography with Split Injection and Photoionization Detection. *JOURNAL OF CHROMATOGRAPHIC SCIENCE*, 29(4), 158–164.
- Purcell, J. M., Hendrickson, C. L., Rodgers, R. P., & Marshall, A. G. (2006). Atmospheric pressure photoionization fourier transform ion cyclotron resonance mass spectrometry for complex mixture analysis. *Analytical Chemistry*, 78(16), 5906 – 5912.
- Purcell, J. M., Hendrickson, C. L., Rodgers, R. P., & Marshall, A. G. (2007). Atmospheric Pressure Photoionization Proton Transfer for Complex Organic Mixtures Investigated by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Journal of the American Society for Mass Spectrometry*, 18(9), 1682 – 1689.
- Purcell, J. M., Juyal, P., Kim, D.-G., Rodgers, R. P., Hendrickson, C. L., & Marshall, A. G. (2007). Sulfur Speciation in Petroleum: Atmospheric Pressure Photoionization or Chemical Derivatization and Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy & Fuels*, 21(5), 2869 – 2874.
- Purcell, J., Merdrignac, I., Rodgers, R., Marshall, A., Gauthier, T., & Guibard, I. (2010). Stepwise Structural Characterization of Asphaltenes during Deep Hydroconversion Processes Determined by Atmospheric Pressure Photoionization (APPI) Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Spectrometry. *Energy & Fuels*, 24, 2257–2265.
- Qian, S. Y., Tomer, K. B., Yue, G.-H., Guo, Q., Kadiiska, M. B., & Mason, R. P. (2002). Characterization of the initial carbon-centered pentadienyl radical and subsequent radicals in lipid peroxidation: identification via on-line high performance liquid chromatography/electron spin resonance and mass spectrometry. *Free Radical Biology and Medicine*, 33(7), 998–1009.
- Raffaelli, A., & Saba, A. (2003). Atmospheric pressure photoionization mass spectrometry. *Mass Spectrometry Reviews*, 22(5), 318 – 331.
- Ramirez, C. E., Wang, C., & Gardinali, P. R. (2014). Fully automated trace level determination of parent and alkylated PAHs in environmental waters by online SPE-LC-APPI-MS/MS. *Analytical and Bioanalytical Chemistry*, 406(1), 329–344.

- Rauha, J. P., Vuorela, H., & Kostianen, R. (2001). Effect of eluent on the ionization efficiency of flavonoids by ion spray, atmospheric pressure chemical ionization, and atmospheric pressure photoionization mass spectrometry. *JOURNAL OF MASS SPECTROMETRY*, 36(12), 1269 – 1280.
- Reid Asbury, G., Klasmeier, J., & Hill Jr, H. H. (2000). Analysis of explosives using electrospray ionization/ion mobility spectrometry (ESI/IMS). *Talanta*, 50(6), 1291–1298.
- Revelsky, I., & Yashin, Y. S. (2012). New approach to complex organic compounds mixtures analysis based on gas chromatography-atmospheric pressure photoionization-mass spectrometry. *Talanta*, 102, 110–113.
- Richeter, S., Jeandon, C., Sauber, C., Gisselbrecht, J., Ruppert, R., & Callot, H. (2002). Preparation, mass spectrometry and electrochemical studies of metal connected porphyrin oligomers. *JOURNAL OF PORPHYRINS AND PHTHALOCYANINES*, 6(6), 423–430.
- Riu, A., Zalko, D., & Debrauwer, L. (2006). Study of polybrominated diphenyl ethers using both positive and negative atmospheric pressure photoionization and tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, 20(14), 2133 – 2142.
- Rizvi, S., Shamsi, S., Zheng, J., & Hou, J. (2007). Photopolymerized Sol-Gel Monolithic Column for Capillary Electrochromatography (CEC) and CEC Coupled to Atmospheric Pressure Photoionization Mass Spectrometry. *Journal of Liquid Chromatography & Related Technologies*, 30(1), 43 – 57.
- Robb, D. B., & Blades, M. W. (2005). Effects of Solvent Flow, Dopant Flow, and Lamp Current on Dopant-Assisted Atmospheric Pressure Photoionization (DA-APPI) for LC-MS. Ionization via Proton Transfer. *Journal of the American Society for Mass Spectrometry*, 16(8), 1275 – 1290.
- Robb, D. B., & Blades, M. W. (2006a). Atmospheric pressure photoionization for ionization of both polar and nonpolar compounds in reversed-phase LC/MS. *Analytical Chemistry*, 78(23), 8162–8164.
- Robb, D. B., & Blades, M. W. (2006b). Factors affecting primary ionization in dopant-assisted atmospheric pressure photoionization (DA-APPI) for LC/MS. *Journal of the American Society for Mass Spectrometry*, 17(2), 130 – 138.
- Robb, D. B., & Blades, M. W. (2008). State-of-the-art in atmospheric pressure photoionization for LC/MS. *Analytica Chimica Acta*, 627(1), 34 – 49.
- Robb, D. B., Covey, T. R., & Bruins, A. P. (2000). Atmospheric pressure photoionization: An ionization method for liquid chromatography-mass spectrometry. *Analytical Chemistry*, 72(15), 3653 – 3659.

- Robb, D. B., Smith, D. R., & Blades, M. W. (2008). Investigation of Substituted-Benzene Dopants for Charge Exchange Ionization of Nonpolar Compounds by Atmospheric Pressure Photoionization. *Journal of the American Society for Mass Spectrometry*, 19(7), 955 – 963.
- Roberts, J. ., & Hill, H. . (1979). Parameter study of a hydrogen atmosphere flame ionization detector. *Journal of Chromatography A*, 176(1), 1–9.
- ROBIN, M. (1980). Multiphoton fragmentation and ionization. *APPLIED OPTICS*, 19(23), 3941–3947.
- Rodil, R., Schrader, S., & Moeder, M. (2009). Comparison of atmospheric pressure photoionization and electrospray ionization mass spectrometry for the analysis of UV filters. *Rapid Communications in Mass Spectrometry*, 23(5), 580–588.
- Roesler, J. F. (1964). Preliminary Study of Characteristics of Photoionization Detector for Gas Chromatography. *Analytical Chemistry*, 36(10), 1900–1903.
- Ronsein, G. E., Prado, F. M., Mansano, F. V, Oliveira, M. C. B., Medeiros, M. H. G., Miyamoto, S., & Di Mascio, P. (2010). Detection and Characterization of Cholesterol-Oxidized Products Using HPLC Coupled to Dopant Assisted Atmospheric Pressure Photoionization Tandem Mass Spectrometry. *Analytical Chemistry*, 82(17), 7293–7301.
- Ross, M. S., & Wong, C. S. (2010). Comparison of electrospray ionization, atmospheric pressure photoionization, and anion attachment atmospheric pressure photoionization for the analysis of hexabromocyclododecane enantiomers in environmental samples. *Journal of Chromatography A*, 1217(50), 7855 – 7863.
- Roy, S., Delobel, A., Gaudin, K., Touboul, D., Germain, D. ., Baillet, A., Chaminade, P. (2006). Liquid chromatography on porous graphitic carbon with atmospheric pressure photoionization mass spectrometry and tandem mass spectrometry for the analysis of glycosphingolipids. *Journal of Chromatography A*, 1117(2), 154–162.
- Rudzinski, W. E., & Rai, V. (2005). Detection of Polyaromatic Sulfur Heterocycles in Crude Oil Using Postcolumn Addition of Tropylium and Tandem Mass Spectrometry. *Energy & Fuels*, 19(4), 1611–1618.
- Rudzinski, W. E., Zhang, Y., & Luo, X. (2003). Mass spectrometry of polyaromatic sulfur compounds in the presence of palladium(II). *Journal of Mass Spectrometry*, 38(2), 167 – 173.
- Ruiz-Jiménez, J., Hautala, S., Parshintsev, J., Laitinen, T., Hartonen, K., Petäjä, T., Riekkola, M.-L. (2013). Liquid chromatography–dopant-assisted atmospheric pressure photoionization–mass spectrometry: Application to the analysis of aldehydes in atmospheric aerosol particles. *Journal of Separation Science*, 36(1), 164 – 172.

- Sacks, R. D. (2007). Performance characteristics of a new prototype for a portable GC using ambient air as carrier gas for on-site analysis. *Journal of Separation Science*, 30(7), 1052–1060.
- Saf, R., Mirtl, C., & Hummel, K. (1994). Electrospray mass spectrometry using potassium iodide in aprotic organic solvents for the ion formation by cation attachment. *Tetrahedron Letters*, 35(36), 6653 – 6656.
- Sanchís, J., Oliveira, L. F. S., de Leão, F. B., Farré, M., & Barceló, D. (2015). Liquid chromatography-atmospheric pressure photoionization-Orbitrap analysis of fullerene aggregates on surface soils and river sediments from Santa Catarina (Brazil). *The Science of the Total Environment*, 505, 172 – 179.
- Sanwald, P., Blankson, E. A., Duléry, B. D., Schoun, J., Huebert, N. D., & Dow, J. (1995). Isocratic high-performance liquid chromatographic method for the separation of testosterone metabolites. *Journal of Chromatography B: Biomedical Applications*, 672(2), 207–215.
- Schappler, J., Guillarme, D., Prat, J., Veuthey, J.-L., & Rudaz, S. (2007). Coupling CE with atmospheric pressure photoionization MS for pharmaceutical basic compounds: optimization of operating parameters. *Electrophoresis*, 28(17), 3078 – 3087.
- Schiewek, R., Mönnikes, R., Wulf, V., Gäb, S., Brockmann, K. J., Benter, T., & Schmitz, O. J. (2008). A Universal Ionization Label for the APLI-(TOF)MS Analysis of Small Molecules and Polymers. *Angewandte Chemie (International Ed. in English)*, 47(51), 9989 – 9992.
- Schiewek, R., Schellenträger, M., Mönnikes, R., Lorenz, M., Giese, R., Brockmann, K. J., Schmitz, O. J. (2007). Ultrasensitive determination of polycyclic aromatic compounds with atmospheric-pressure laser ionization as an interface for GC/MS. *Analytical Chemistry*, 79(11), 4135–4140.
- Schmermund, J., & Locke, D. (1975). Universal photoionization detector for liquid chromatography. *ANALYTICAL LETTERS*, 8(9), 611–625.
- Schmermund, J. T., & Locke, D. C. (1990). Design of a photoionization detector for high-performance liquid chromatography using an automated liquid-to-vapor phase interface and application to phenobarbital in an animal feed and to amantadine. *Journal of Chromatography A*, 505(2), 319–328. doi:10.1016/S0021-9673(01)93047-X
- Schmitt-Kopplin, P., Englmann, M., Rossello-Mora, R., Schiewek, R., Brockmann, K. J., Benter, T., & Schmitz, O. J. (2008). Combining chip-ESI with APLI (cESILI) as a multimode source for analysis of complex mixtures with ultrahigh-resolution mass spectrometry. *Analytical and Bioanalytical Chemistry*, 391(8), 2803–2809.

- Schrader, W., Panda, S. K., Brockmann, K. J., & Benter, T. (2008). Characterization of non-polar aromatic hydrocarbons in crude oil using atmospheric pressure laser ionization and Fourier transform ion cyclotron resonance mass spectrometry (APLI FT-ICR MS). *The Analyst*, 133(7), 867–869.
- Schreiber, A., Efer, J., & Engewald, W. (2000). Application of spectral libraries for high-performance liquid chromatography–atmospheric pressure ionisation mass spectrometry to the analysis of pesticide and explosive residues in environmental samples. *Journal of Chromatography A*, 869(1), 411–425.
- Shaw, D., Holland, D. M., MacDonald, M., Hayes, M., Shpinkova, L., Rennie, E., von Niessen, W. (1998). An experimental and theoretical study of the spectroscopic and thermodynamic properties of toluene. *Chemical Physics*, 230(1), 97 – 116.
- Shvartsburg, A. A. (2009). *Differential ion mobility spectrometry: nonlinear ion transport and fundamentals of FAIMS*. Boca Raton : CRC Press.
- Simmonds, P. (1987). Electron-capture detector with a photoemissive electron source. *Journal of Chromatography*, 399, 149–164.
- Sinha, V., Nölscher, A. C., Bockisch, S., Klüpfel, T., & Williams, J. (2012). Total OH reactivity measurements using a new fast Gas Chromatographic Photo-Ionization Detector (GC-PID). *Atmospheric Measurement Techniques*, 5(12), 2981–2992. doi:10.5194/amt-5-2981-2012
- Sioud, S., Amad, M., & Al-Talla, Z. A. (2012). Multicomponent mixed dopant optimization for rapid screening of polycyclic aromatic hydrocarbons using ultra high performance liquid chromatography coupled to atmospheric pressure photoionization high-resolution mass spectrometry. *Rapid Communications in Mass Spectrometry*, 26(12), 1488 – 1496.
- Siu, K. W. M., & Aue, W. A. (1980). Effect of dopants on the linearity of a d.c. electron-capture detector. *Journal of Chromatography A*, 192(2), 413–417.
- Siuzdak, G., Bothner, B., Yeager, M., Brugidou, C., Fauquet, C. M., Hoey, K., & Change, C.-M. (1996). Mass spectrometry and viral analysis. *Chemistry & Biology*, 3(1), 45–48.
- Śmiałek, M. A., Hubin-Franskin, M.-J., Delwiche, J., Duflot, D., Mason, N. J., Vronning-Hoffmann, S., Limão-Vieira, P. (2012). Limonene: electronic state spectroscopy by high-resolution vacuum ultraviolet photoabsorption, electron scattering, He(I) photoelectron spectroscopy and ab initio calculations. *Physical Chemistry Chemical Physics*, 14(6), 2056 – 2064.
- Smith, D. R., Robb, D. B., & Blades, M. W. (2009). Comparison of Dopants for Charge Exchange Ionization of Nonpolar Polycyclic Aromatic Hydrocarbons with Reversed-Phase LC-APPI-MS. *Journal of the American Society for Mass Spectrometry*, 20(1), 73 – 79.

- Snow, D. D., Damon-Powell, T., Onanong, S., & Cassada, D. A. (2013). Sensitive and simplified analysis of natural and synthetic steroids in water and solids using on-line solid-phase extraction and microwave-assisted solvent extraction coupled to liquid chromatography tandem mass spectrometry atmospheric pressure photoion. *Analytical and Bioanalytical Chemistry*, 405(5), 1759–1771.
- Song, K., Ahn, B., Jung, E., Lee, Y.-I., & Ko, S. (2007). Enhancement of the detection sensitivity for volatile organic compounds by using an annular type photoionization detector and a pre-concentration system. *Analytica Chimica Acta*, 583(1), 210 – 215.
- Song, L., & Bartmess, J. E. (2009). Liquid chromatography/negative ion atmospheric pressure photoionization mass spectrometry: a highly sensitive method for the analysis of organic explosives. *Rapid Communications in Mass Spectrometry*, 23(1), 77–84.
- Song, L., Cho, D. S., Bhandari, D., Gibson, S. C., McNally, M. E., Hoffman, R. M., & Cook, K. D. (2011). Liquid chromatography/dopant-assisted atmospheric pressure chemical ionization mass spectrometry for the analysis of non-polar compounds. *International Journal of Mass Spectrometry*, 303(2), 173 – 180.
- Song, L., Dykstra, A. B., Yao, H., & Bartmess, J. E. (2009). Ionization Mechanism of Negative Ion-Direct Analysis in Real Time: A Comparative Study with Negative Ion-Atmospheric Pressure Photoionization. *Journal of the American Society for Mass Spectrometry*, 1(42–50).
- Song, L., Wellman, A. D., Yao, H., & Adcock, J. (2007). Electron capture atmospheric pressure photoionization mass spectrometry: analysis of fullerenes, perfluorinated compounds, and pentafluorobenzyl derivatives. *Rapid Communications in Mass Spectrometry*, 21(8), 1343 – 1351.
- Song, L., Wellman, A. D., Yao, H., & Bartmess, J. E. (2007). Negative Ion-Atmospheric Pressure Photoionization: Electron Capture, Dissociative Electron Capture, Proton Transfer, and Anion Attachment. *Journal of the American Society for Mass Spectrometry*, 18(10), 1789–1798.
- Spangler, G. E., Roehl, J. E., Patel, G. B., & Dorman, A. (1992). Photoionization ion mobility spectrometer. Baltimore, MD.
- Stader, C., Beer, F. T., & Achten, C. (2013). Environmental PAH analysis by gas chromatography–atmospheric pressure laser ionization–time-of-flight–mass spectrometry (GC-APLI-MS). *Analytical and Bioanalytical Chemistry*, 405(22), 7041–7052.
- Starkey, J. A., Mechref, Y., Muzikar, J., McBride, W. J., & Novotny, M. V. (2006). Determination of salsolinol and related catecholamines through on-line preconcentration and liquid chromatography/atmospheric pressure photoionization mass spectrometry. *Analytical Chemistry*, 78(10), 3342 – 3347.

- Stearns, S. D., & E., W. W. (1997). Apparatus and methods for identifying and quantifying compounds using a plurality of pulsed rare gas photoionization detectors. Retrieved from <http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=/netahtml/PTO/srchnum.htm&r=1&f=G&l=50&s1=5594346.PN.&OS=PN/5594346&RS=PN/5594346>
- Straube, E. A., Dekant, W., & Völkel, W. (2004). Comparison of electrospray ionization, atmospheric pressure chemical ionization, and atmospheric pressure photoionization for the analysis of dinitropyrene and aminonitropyrene LC-MS/MS. *Journal of the American Society for Mass Spectrometry*, 15(12), 1853 – 1862.
- Straube, E., Dekant, W., & Völkel, W. (2005). Enhanced sensitivity for the determination of ambiphilic polyaromatic amines by LC–MS/MS after acetylation. *Journal of Chromatography A*, 1067(1), 181 – 190.
- Sudha, R., Panda, M., Chandrasekhar, J., & Balaram, P. (2002). Structural effects on the formation of proton and alkali metal ion adducts of apolar, neutral peptides: electrospray ionization mass spectrometry and Ab initio theoretical studies. *Chemistry (Weinheim an Der Bergstrasse, Germany)*, 8(21), 4980 – 4991.
- Suni, N. M., Lindfors, P., Laine, O., Östman, P., Ojanperä, I., Kotiaho, T., Kostiaainen, R. (2011). Matrix effect in the analysis of drugs of abuse from urine with desorption atmospheric pressure photoionization-mass spectrometry (DAPPI-MS) and desorption electrospray ionization-mass spectrometry (DESI-MS). *Analytica Chimica Acta*, 699(1), 73–80.
- Suominen, T., Haapala, M., Takala, A., Ketola, R. A., & Kostiaainen, R. (2013). Neurosteroid analysis by gas chromatography–atmospheric pressure photoionization–tandem mass spectrometry. *Analytica Chimica Acta*, 794, 76–81.
- Syage, J., Evans, M., & Hanold, K. (2000). Photoionization mass spectrometry. *AMERICAN LABORATORY*, 32(24), 24–29.
- Takada, Y., Sakairi, M., & Koizumi, H. (1995). Atmospheric Pressure Chemical Ionization Interface for Capillary Electrophoresis/Mass Spectrometry. *Analytical Chemistry*, 67(8), 1474 – 1476.
- Takeda, S., Tanaka, Y., Yamane, M., Siroma, Z., Wakida, S., Otsuka, K., & Terabe, S. (2001). Ionization of dichlorophenols for their analysis by capillary electrophoresis–mass spectrometry. *Journal of Chromatography A*, 924(1), 415 – 420.
- Takino, M., Daishima, S., & Nakahara, T. (2003a). Determination of chloramphenicol residues in fish meats by liquid chromatography–atmospheric pressure photoionization mass spectrometry. *Journal of Chromatography A*, 1011(1), 67 – 75.

- Takino, M., Daishima, S., & Nakahara, T. (2003b). Determination of perfluorooctane sulfonate in river water by liquid chromatography/atmospheric pressure photoionization mass spectrometry by automated on-line extraction using turbulent flow chromatography. *Rapid Communications in Mass Spectrometry*, 17(5), 383 – 390.
- Takino, M., Daishima, S., & Nakahara, T. (2003c). Liquid chromatography/mass spectrometric determination of patulin in apple juice using atmospheric pressure photoionization. *Rapid Communications in Mass Spectrometry*, 17(17), 1965–1972.
- Takino, M., Yamaguchi, K., & Nakahara, T. (2004). Determination of carbamate pesticide residues in vegetables and fruits by liquid chromatography-atmospheric pressure photoionization-mass spectrometry and atmospheric pressure chemical ionization-mass spectrometry. *Journal of Agricultural and Food Chemistry*, 52(4), 727–735.
- Tam, M., & Hill, Jr, H. H. (2004). Secondary electrospray ionization-ion mobility spectrometry for explosive vapor detection. *Analytical Chemistry*, 76(10), 2741–2747.
- Tanaka, Y., Otsuka, K., & Terabe, S. (2003). Evaluation of an atmospheric pressure chemical ionization interface for capillary electrophoresis-mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 30(6), 1889 – 1895.
- Tang, F., Li, H., Wang, X., Yang, J., Zhang, L., & Wang, F. (2011). Experimental and simulation research on the influence of voltage amplitude, frequency and carrier gas flow rate on the performance of FAIMS. *Science China Technological Sciences*, 54(6), 1407–1414.
- Terrier, P., Desmazières, B., Tortajada, J., & Buchmann, W. (2011). APCI/APPI for synthetic polymer analysis. *Mass Spectrometry Reviews*, 30(5), 854–874.
- Theron, H., Coetzee, C., Sutherland, F. C., Wiesner, J., & Swart, K. (2004). Selective and sensitive liquid chromatography–tandem mass spectrometry method for the determination of levonorgestrel in human plasma. *Journal of Chromatography B*, 813(1), 331 – 336.
- Theron, H. B., van der Merwe, M. J., Swart, K. J., & van der Westhuizen, J. H. (2007). Employing atmospheric pressure photoionization in liquid chromatography/tandem mass spectrometry to minimize ion suppression and matrix effects for the quantification of venlafaxine and O-desmethylvenlafaxine. *Rapid Communications in Mass Spectrometry*, 21(10), 1680–1686.
- Thomas, D., Crain, S. M., Sim, P. G., & Benoit, F. M. (1995). Application of reversed phase liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry to the determination of polycyclic aromatic sulfur heterocycles in environmental samples. *Journal of Mass Spectrometry*, 30(7), 1034 – 1040.

- Thomson, B. A., & Iribarne, J. V. (1979). Field induced ion evaporation from liquid surfaces at atmospheric pressure. *The Journal of Chemical Physics*, 71(11), 4451–4463.
- Toluene; MSDS No. 244511. (n.d.).
- Traldi, P., & Marotta, E. (2004). Some experiments and thoughts on atmospheric pressure photoionization. In A. Ashcroft, G. Brenton, & J. Monaghan (Eds.), *ADVANCES IN MASS SPECTROMETRY* (Vol. 16, pp. 275–293). AMSTERDAM: ELSEVIER SCIENCE BV.
- Trösken, E. R., Straube, E., Lutz, W. K., Völkel, W., & Patten, C. (2004). Quantitation of Lanosterol and Its Major Metabolite FF-MAS in an Inhibition Assay of CYP51 by Azoles with Atmospheric Pressure Photoionization Based LC-MS/MS. *Journal of the American Society for Mass Spectrometry*, 15(8), 1216 – 1221.
- Tsai, S.-T., Chen, C. W., Lora, L. C., Huang, M.-C., Chen, C.-H., & Wang, Y.-S. (2006). Simultaneous mass analysis of positive and negative ions using a dual-polarity time-of-flight mass spectrometer. *Analytical Chemistry*, 78(22), 7729–7734.
- Tubaro, M., Marotta, E., Seraglia, R., & Traldi, P. (2003). Atmospheric pressure photoionization mechanisms. 2. The case of benzene and toluene. *Rapid Communications in Mass Spectrometry*, 17(21), 2423 – 2429.
- Turnipseed, S. B., Roybal, J. E., Andersen, W. C., & Kuck, L. R. (2005). Analysis of avermectin and moxidectin residues in milk by liquid chromatography–tandem mass spectrometry using an atmospheric pressure chemical ionization/atmospheric pressure photoionization source. *Analytica Chimica Acta*, 529(1), 159 – 165.
- Van Leeuwen, S. M., Hendriksen, L., & Karst, U. (2004). Determination of aldehydes and ketones using derivatization with 2,4-dinitrophenylhydrazine and liquid chromatography–atmospheric pressure photoionization-mass spectrometry. *Journal of Chromatography A*, 1058(1), 107 – 112.
- Varga, M., Bartók, T., & Mesterházy, Á. (2006). Determination of ergosterol in Fusarium-infected wheat by liquid chromatography–atmospheric pressure photoionization mass spectrometry. *Journal of Chromatography A*, 1103(2), 278 – 283.
- Vien, S. H., & Fry, R. C. (1988). Ultrasensitive, simultaneous determination of arsenic, selenium, tin, and antimony in aqueous solution by hydride generation gas chromatography with photoionization detection. *Analytical Chemistry*, 60(5), 465 – 472.
- Wang, G., Hsieh, Y., & Korfmacher, W. A. (2005). Comparison of Atmospheric Pressure Chemical Ionization, Electrospray Ionization, and Atmospheric Pressure Photoionization for the Determination of Cyclosporin A in Rat Plasma. *Analytical Chemistry*, 77(2), 541 – 548.

- WATANABE, K., & ZELIKOFF, M. (1953). Absorption Coefficients of Water Vapor in the Vacuum Ultraviolet. *Journal of the Optical Society of America*, 43(9), 753–755.
- Wentworth, W. ., Huang, J., Sun, K., Zhang, Y., Rao, L., Cai, H., & Stearns, S. D. (1999). Non-radioactive electron-capture detector. *Journal of Chromatography A*, 842(1), 229–266.
- Wentworth, W., Limero, T., Batten, C. ., & Chen, E. C.(1988). Non-radioactive electron-capture detector operating in the pulsed mode. *Journal of Chromatography A*, 441(1), 45–62.
- Wentworth, W., Limero, T., Batten, C. ., & Chen, E. C. (1989). Non-radioactive electron-capture detector for gas chromatography. *Journal of Chromatography A*, 468, 215–224.
- Wentworth, W. ., Tishbee, A., Batten, C. ., & Zlatkis, A. (1975). Photo-electron capture detector. *Journal of Chromatography A*, 112, 229–246.
- Wentworth, W., Dsa, E., Cai, H., & Stearns, S. (1992). ENVIRONMENTAL APPLICATIONS OF THE PULSED-DISCHARGE ELECTRON-CAPTURE DETECTOR. *JOURNAL OF CHROMATOGRAPHIC SCIENCE*, 30(12), 478–485.
- Wentworth, W. E., & Stearns, S. D. (1992). High voltage spark excitation and ionization detector system. Retrieved from <http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=/netahtml/PTO/srchnum.htm&r=1&f=G&l=50&s1=5153519.PN.&OS=PN/5153519&RS=PN/5153519>
- Whitehouse, C. M., Dreyer, R. N., Yamashita, M., & Fenn, J. B. (1985). Electrospray interface for liquid chromatographs and mass spectrometers. *Analytical Chemistry*, 57(3), 675–679.
- Wille, S. M. ., Van Hee, P., Neels, H. M., Van Peteghem, C. H., & Lambert, W. E. (2007). Comparison of electron and chemical ionization modes by validation of a quantitative gas chromatographic–mass spectrometric assay of new generation antidepressants and their active metabolites in plasma. *Journal of Chromatography A*, 1176(1), 236 – 245.
- Williamson, D., Knighton, W., & Grimsrud, E. (2000). Effect of buffer gas alterations on the thermal electron attachment and detachment reactions of azulene by pulsed high pressure mass spectrometry. *INTERNATIONAL JOURNAL OF MASS SPECTROMETRY*, 195, 481 – 489.
- Wilm, M. S., & Mann, M. (1994). Electrospray and Taylor-Cone theory, Dole’s beam of macromolecules at last? *International Journal of Mass Spectrometry and Ion Processes*, 136(2), 167–180.
- Woodyard, J. R. (1944). Nonlinear circuit device utilizing germanium. Retrieved from <http://www.google.com/patents/US2530110>

- Wu, Z., Hendrickson, C. L., Rodgers, R. P., & Marshall, A. G. (2002). Composition of explosives by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Analytical Chemistry*, 74(8), 1879–1883.
- Yamamoto, A., Kakutani, N., Yamamoto, K., Kamiura, T., & Miyakoda, H. (2006). Steroid Hormone Profiles of Urban and Tidal Rivers Using LC/MS/MS Equipped with Electrospray Ionization and Atmospheric Pressure Photoionization Sources. *Environmental Science & Technology*, 40(13), 4132 – 4137.
- Yamamoto, A., Miyamoto, I., Kitagawa, M., Moriwaki, H., Miyakoda, H., Kawasaki, H., & Arakawa, R. (2009). Analysis of Chlorothalonil by Liquid Chromatography/Mass Spectrometry Using Negative-ion Atmospheric Pressure Photoionization. *Analytical Sciences : The International Journal of the Japan Society for Analytical Chemistry*, 25(5), 693–697.
- Yamamoto, A., Terao, T., Hisatomi, H., Kawasaki, H., & Arakawa, R. (2012). Evaluation of river pollution of neonicotinoids in Osaka City (Japan) by LC/MS with dopant-assisted photoionisation. *Journal of Environmental Monitoring*, 14(8), 2189 – 2194.
- Yamane, M. (1962). A new argon ionization detector for gas chromatography. *Journal of Chromatography A*, 9, 162–172.
- Yang, C., & Henion, J. (2002). Atmospheric pressure photoionization liquid chromatographic–mass spectrometric determination of idoxifene and its metabolites in human plasma. *Journal of Chromatography A*, 970(1), 155 – 165.
- Yoshioka, N., Akiyama, Y., & Teranishi, K. (2004). Rapid simultaneous determination of o-phenylphenol, diphenyl, thiabendazole, imazalil and its major metabolite in citrus fruits by liquid chromatography-mass spectrometry using atmospheric pressure photoionization. *Journal of Chromatography A*, 1022(1), 145 – 150.
- Yuan, M., Breitkopf, S. B., Yang, X., & Asara, J. M. (2012). A positive/negative ion-switching, targeted mass spectrometry-based metabolomics platform for bodily fluids, cells, and fresh and fixed tissue. *Nature Protocols*, 7(5), 872–881.
- Zaitseva, A., & Gliberman, A. (1962). Study of dopant distribution in the surface layer of photoelectric solar energy converters made from N-type silicon. *Soviet Physics-Solid State*, 3(8), 1724–1727. Retrieved from [http://apps.webofknowledge.com/full\\_record.do?product=WOS&search\\_mode=GeneralSearch&qid=2&SID=4DcU9cZ9tyellvssmBH&page=1&doc=1](http://apps.webofknowledge.com/full_record.do?product=WOS&search_mode=GeneralSearch&qid=2&SID=4DcU9cZ9tyellvssmBH&page=1&doc=1)
- Zavitsanos, P. (2003). *Using the LC-Atmospheric pressure ionization-ion trap for the analysis of steroids in water*. (I. Ferrer & E. Thurman, Eds.) *Liquid Chromatography/Mass Spectrometry, Ms/Ms and Time-of-Flight MS*.

- Zhang, K., Wong, J., Mai, H., & Trucksess, M. (2014). Dopant-Assisted Atmospheric Pressure Photoionization of Patulin in Apple Juice and Apple-Based Food with Liquid Chromatography-Tandem Mass Spectrometry. *Journal of Agricultural and Food Chemistry*, 62(18), 4112 – 4118.
- Zhao, X., & Yinon, J. (2002). Identification of nitrate ester explosives by liquid chromatography–electrospray ionization and atmospheric pressure chemical ionization mass spectrometry. *Journal of Chromatography A*, 977(1), 59–68.
- Zheng, A.-L., Fan, X., Liu, F.-J., Wei, X.-Y., Wang, S.-Z., Zhao, Y.-P., Zhao, W. (2014). Characterization of Zhundong subbituminous coal by time-of-flight mass spectrometry equipped with atmospheric pressure photoionization ion source. *Fuel Processing Technology*, 117, 60 – 65.
- Zheng, A.-L., Fan, X., Wang, S.-Z., Liu, F.-J., Wei, X.-Y., Zhao, Y.-P., Zhu, J.-L. (2014). Analysis of the Products from the Oxidation of Geting Bituminous Coal by Atmospheric Pressure Photoionization–Mass Spectrometry. *ANALYTICAL LETTERS*, 47(6), 958 – 969.
- Zheng, J., & Shamsi, S. A. (2006). Capillary electrochromatography coupled to atmospheric pressure photoionization mass spectrometry for methylated benzo[a]pyrene isomers. *Analytical Chemistry*, 78(19), 6921–6927.
- Zhou, S. N., Reiner, E. J., Marvin, C., Kolic, T., Riddell, N., Helm, P., Brindle, I. D. (2010). Liquid chromatography–atmospheric pressure photoionization tandem mass spectrometry for analysis of 36 halogenated flame retardants in fish. *Journal of Chromatography A*, 1217(5), 633 – 641.

## Appendices

**Appendix A : List of compounds in the EPA mixture with their available gas-phase ion energetic data from NIST Chemistry WebBook (NIST Standard Reference Database Number 69)**

### A.1 Poly Aromatic Hydrocarbons (PAHs)

**Table A.1 List of PAHs in the EPA mixture**

	Compound	MW	IE (eV)	PA (kJ/mol)	EA (eV)	$\Delta_{\text{acid}}G$ (kJ/mol)
1	Naphthalene	128.1705	8.1	802.9	-0.20	1613
2	2-Methylnaphthalene	142.1971	7.91	831.9	<0.143	1528
3	1-methylnaphthalene	142.1971	7.96	834.8	<1.16	1531
4	Acenaphthylene	152.1919	8.12	861.1	<0.403	
5	Acenaphthene	154.2078	7.75	851.7		1531
6	Dibenzofurane	168.1913	8.77			
7	Fluorene	166.2185	7.91	831.5	<0.278	1434
8	Phenanthrene	178.2292	7.90	825.7	-0.01	
9	Anthracene	178.2293	7.44	877.4	0.55	
10	Fluoranthene	202.2506	7.9	828.6	0.62	
11	Pyrene	202.2506	7.43	869.2	0.41	
12	Benzo(a)anthracene	228.2879	7.45		0.39	
13	Chrysene	228.2879	7.6	840.9	0.32	
14	Benzo(b)fluoranthene	252.3093				
15	Benzo(k)fluoranthene	252.3093				
16	Benzo(a)pyrene	252.3093	7.12		0.82	
17	indeno(1,2,3-cd)pyrene	276.3307				
18	Dibenzo(a,h)anthracene	278.3466	7.39		0.60	
19	Benzo(g,h,i)perylene	276.3307	7.17	876	0.42	

Note. The empty cells are due to unavailable data in the NIST Chemistry WebBook.

## A.2 Oxygen-containing compounds

Table A.2 List of oxygen-containing compounds in the EPA mixture

	Compound	MW	IE (eV)	PA (kJ/mol)	EA (eV)	$\Delta_{\text{acid}}G$ (kJ/mol)
20	Phenol	94.1112	8.49	817.3		1432
21	Benzyl alcohol	108.1378	8.26	778.3		1520
22	4-methylphenol	108.1378	8.34	814		1437
23	2-methylphenol	108.1378	8.14	832		1431
24	3-methylphenol	108.1378	8.29	841		1434
25	Isophorone	138.2069	9.07	893.5		
26	2,4-Dimethylphenol	122.1644	8			
27	benzoic acid	122.1213	9.3	821.1		1394

Note. The empty cells are due to unavailable data in the NIST Chemistry WebBook.

### A.3 Nitro-compounds

**Table A.3** List of nitro-compounds in the EPA mixture

	<b>Compound</b>	<b>MW</b>	<b>IE (eV)</b>	<b>PA (kJ/mol)</b>	<b>EA (eV)</b>	<b><math>\Delta_{\text{acid}}G</math> (kJ/mol)</b>
28	Nitrobenzene	123.1094	9.94	800.3	1.00	1545
29	2-Nitrophenol	139.1088	9.1			1379
30	2-Nitroaniline	138.1240	8.27			
31	1,4-dinitrobenzene	168.1070	10.3		2.00	
32	1,3-Dinitrobenzene	168.1070	10.4		1.66	
33	2,6-Dinitrotoluene	182.1335			1.47	
34	2,4-Dinitrophenol	184.1064	9.57			1291
35	1,2-Dinitrobenzene	168.1070	10.71		1.65	
36	3-Nitroaniline	138.1240	8.31		0.95	1443
37	2,4-Dinitrotoluene	182.1355			<1.600	
38	4-nitroaniline	138.1240	8.43	866	0.92	1407
39	2-methyl-4,6-dinitrophenol	198.1329				
40	4-nitrophenol	139.1088	9.1			1343

Note. The empty cells are due to unavailable data in the NIST Chemistry WebBook.

#### A.4 Halogenated compounds

**Table A.4** List of halogenated compounds in the EPA mixture

	Compound	MW	IE (eV)	PA (kJ/mol)	EA (eV)	$\Delta_{\text{acid}}G$ (kJ/mol)
41	2-Chlorophenol	128.5560	9.28			1410
42	1,3-Dichlorobenzene	147.0020	9.1		0.09	1543
43	1,4-Dichlorobenzene	147.0020	9.1			1543
44	1,2-Dichlorobenzene	147.0020	9.06			1533
45	2,4-Dichlorophenol	163.0010	8.65			
46	1,2,4-Trichlorobenzene	181.4470	9.04			1517
47	Hexachloro-1,3-butadiene	260.7610				
48	4-chloroaniline	127.5720	7.8	873.8		1477
49	Hexachloroethane	236.7390			1.48	
50	4-chloro-3-methylphenol	142.5830				
51	Hexachlorocyclopentadiene	272.7720				
52	2,4,6-Trichlorophenol	197.4460				
53	2,4,5-Trichlorophenol	197.4460				
54	2-Chloronaphthalene	162.6160	8.11			1607
55	2,3,4,6-Tetrachlorophenol	231.8910				
56	2,3,5,6-tetrachlorophenol	231.8910				
57	4-Chlorophenyl phenyl ether	204.6250				
58	4-Bromophenyl phenyl ether	248.1030			0.92	
59	Hexachlorobenzene	284.7820	9			
60	Pentachlorophenol	266.3370				

Note. The empty cells are due to unavailable data in the NIST Chemistry WebBook.

## A.5 Nitrogen-containing compounds

**Table A.5** List of nitrogen-containing compounds in the EPA mixture

	Compound	MW	IE (eV)	PA (kJ/mol)	EA (eV)	$\Delta_{\text{acid}}G$ (kJ/mol)
61	N-nitrosodimethylamine	74.0818	8.69			1564
62	Pyridine	79.0000	9.26	930.1		
63	Aniline	93.0000	7.72	882.5		
64	N-nitroso-di-n-propylamine	130.1882				
65	N-nitrosodiphenylamine	198.2206				
66	Azobenzene	182.2212	8.5			
67	Carbazole	167.2066	7.57	940		1412
68	3,3-dichlorobenzidine	252.1270				

Note. The empty cells are due to unavailable data in the NIST Chemistry WebBook.

## A.6 Esters

**Table A.6** List of esters in the EPA mixture

	Compound	MW	IE (eV)	PA (kJ/mol)	EA (eV)	$\Delta_{\text{acid}}G$ (kJ/mol)
69	Dimethyl phthalate	194.1840	9.64		0.55	
70	Diethyl phthalate	222.2372			0.54	
71	di-n-butyl phthalate	278.3435				
72	Butyl benzyl phthalate	312.3597				
73	bis-2-ethylhexyl adipate	370.5665				
74	Bis(2-ethylhexyl)phthalate	390.5561				
75	Di-n-octylphthalate	390.5561				

Note. The empty cells are due to unavailable data in the NIST Chemistry WebBook.

## A.7 Ethoxy Esters

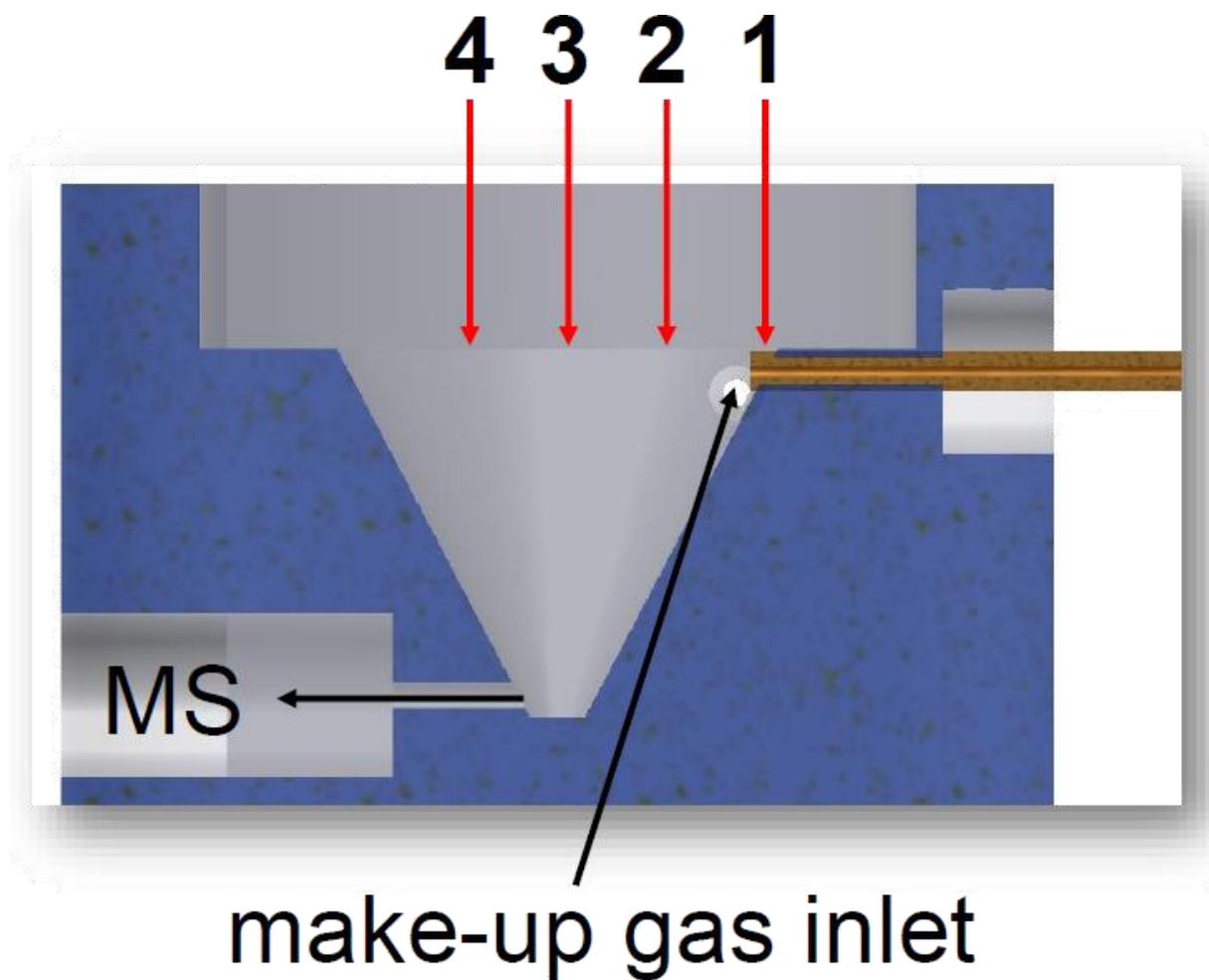
Table A.7 List of ethoxy esters in the EPA mixture

	Compound	MW	IE (eV)	PA (kJ/mol)	EA (eV)	$\Delta_{\text{acid}}G$ (kJ/mol)
76	Bis(2-chloroethyl)ether	143.0120				
77	Bis(2-chloroisopropyl)ether	171.0650				
78	Bis(2-chloroethoxy)methane	173.0380				

Note. The empty cells are due to unavailable data in the NIST Chemistry WebBook.

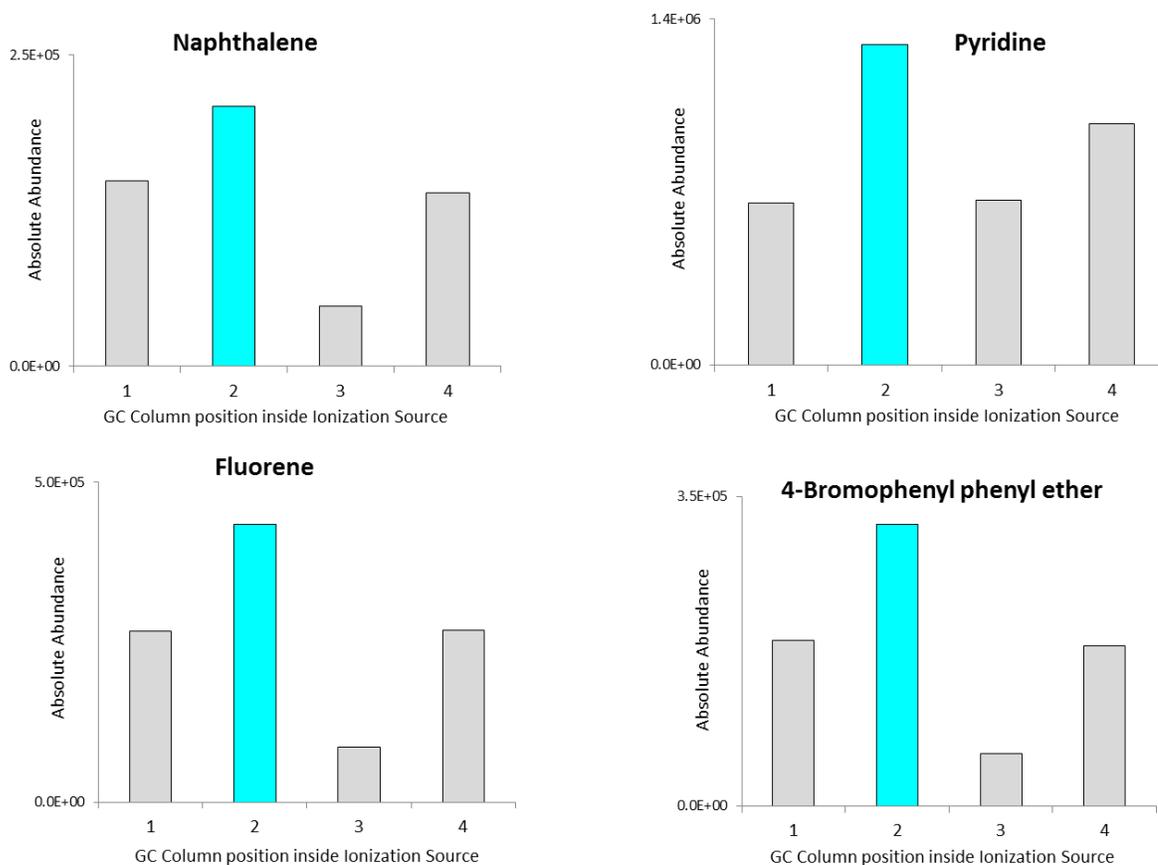
**Appendix B Optimization of GC column position in the ionization source in order to obtain the maximum ionization.**

**B.1 GC column positions tested**

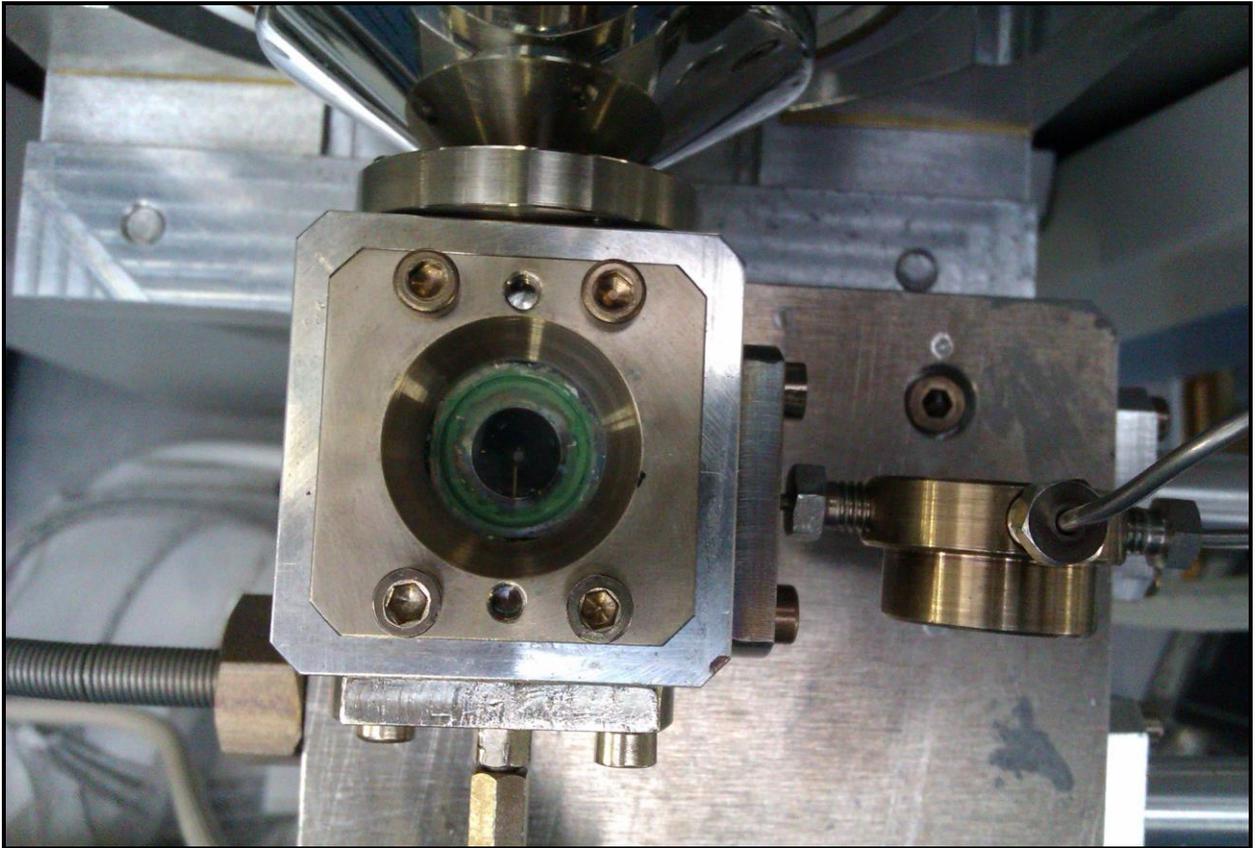


**Figure B.1** Four tested positions for GC column inside the ionization source. Positions 1, 2, 3, and 4 refer to the positions right at the entrance, 1 mm before the tip of the cone, right above the cone tip, and 1 mm after the tip of the cone, respectively

## B.2 The optimized position for the GC column inside the ionization source.



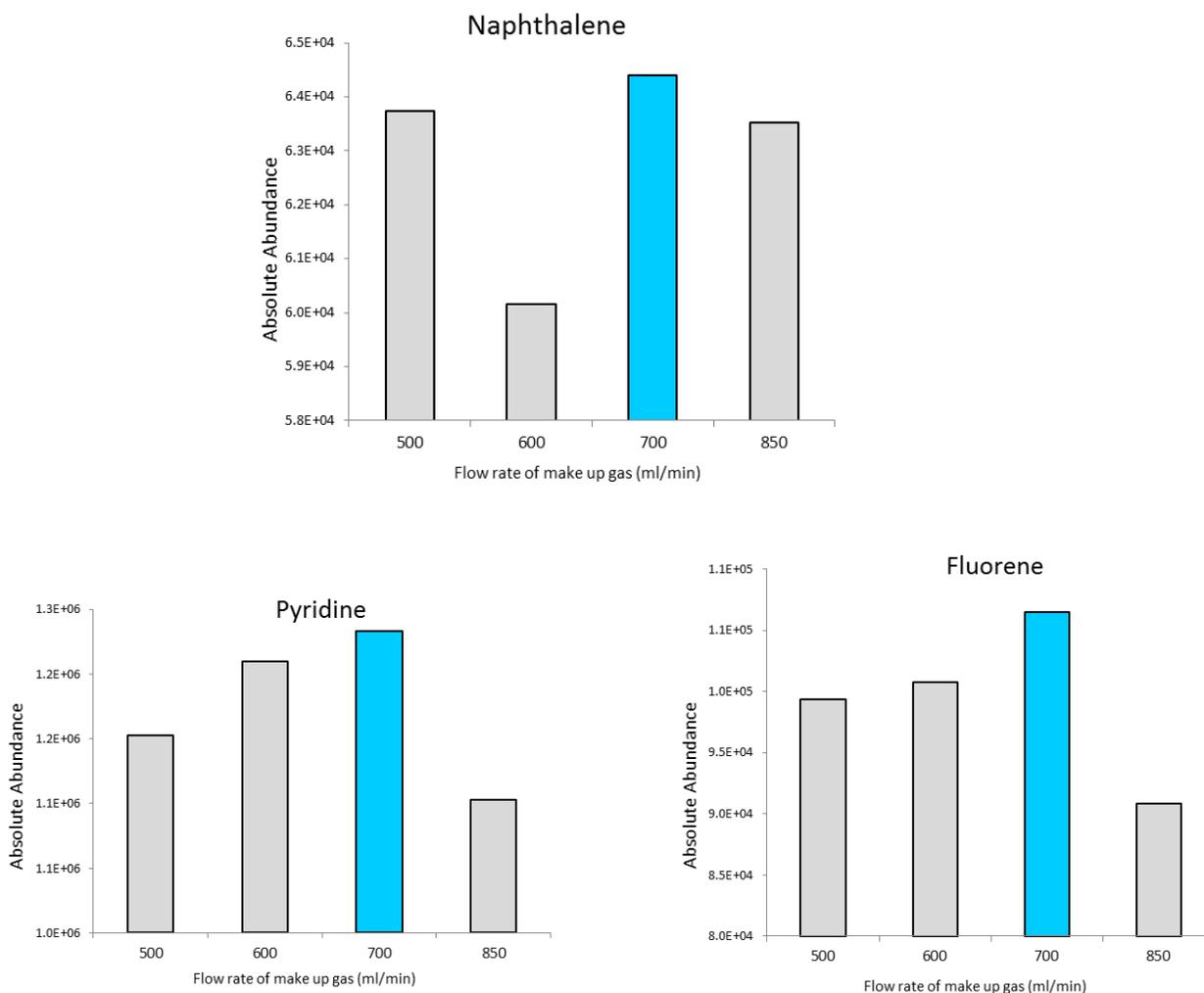
**Figure B.2** The results obtained for four compounds in the EPA mixture with each ionization position, when 1  $\mu\text{L}$  of EPA mixture at a concentration of 100  $\text{pg}/\mu\text{L}$  was injected and ionized with direct PI APPI. The results show that the optimized position is position number 2 (1 mm before the cone tip), which gives the best sensitivity towards vortex flow and radiation.



**Figure B.3** The optimized GC column position inside the ionization source, which was used in all experiments with GC-APPI/APLI MS.

## Appendix C Optimization of the make-up gas flow rate

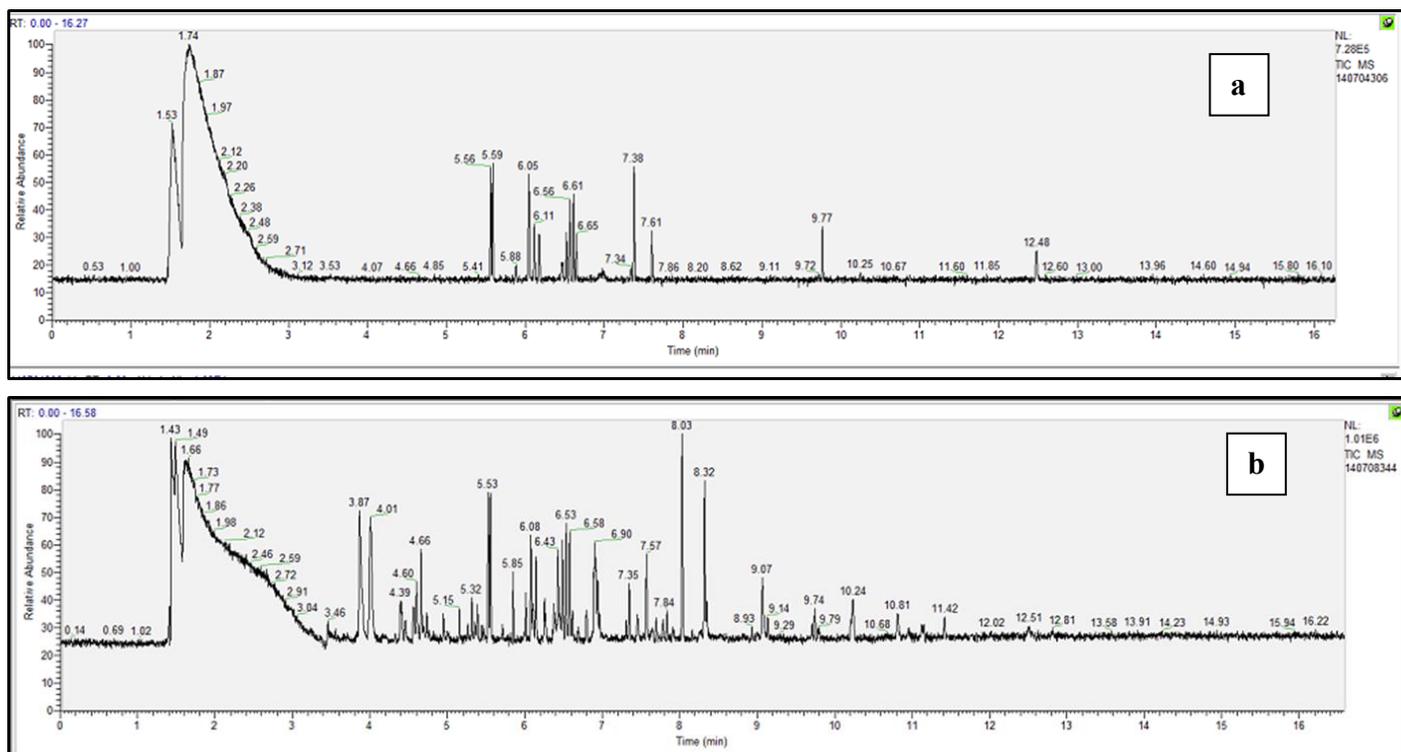
### C.1 Examination of four different flow rates (500, 600, 700, and 850 ml/min) for make-up gas (N<sub>2</sub>)



**Figure C.1** The results obtained for three compounds in the EPA mixture with each N<sub>2</sub> flow rate, when 1  $\mu$ L of EPA mixture at a concentration of 100 pg/ $\mu$ L was injected and ionized with direct PI APPI. The results show that the optimum flowrate is 700 ml/min, which gives the best vortex flow and in turn, best ionization efficiencies. Therefore, the flowrate of 700 ml/min was kept for all the experiments with GC-APPI/APPI MS.

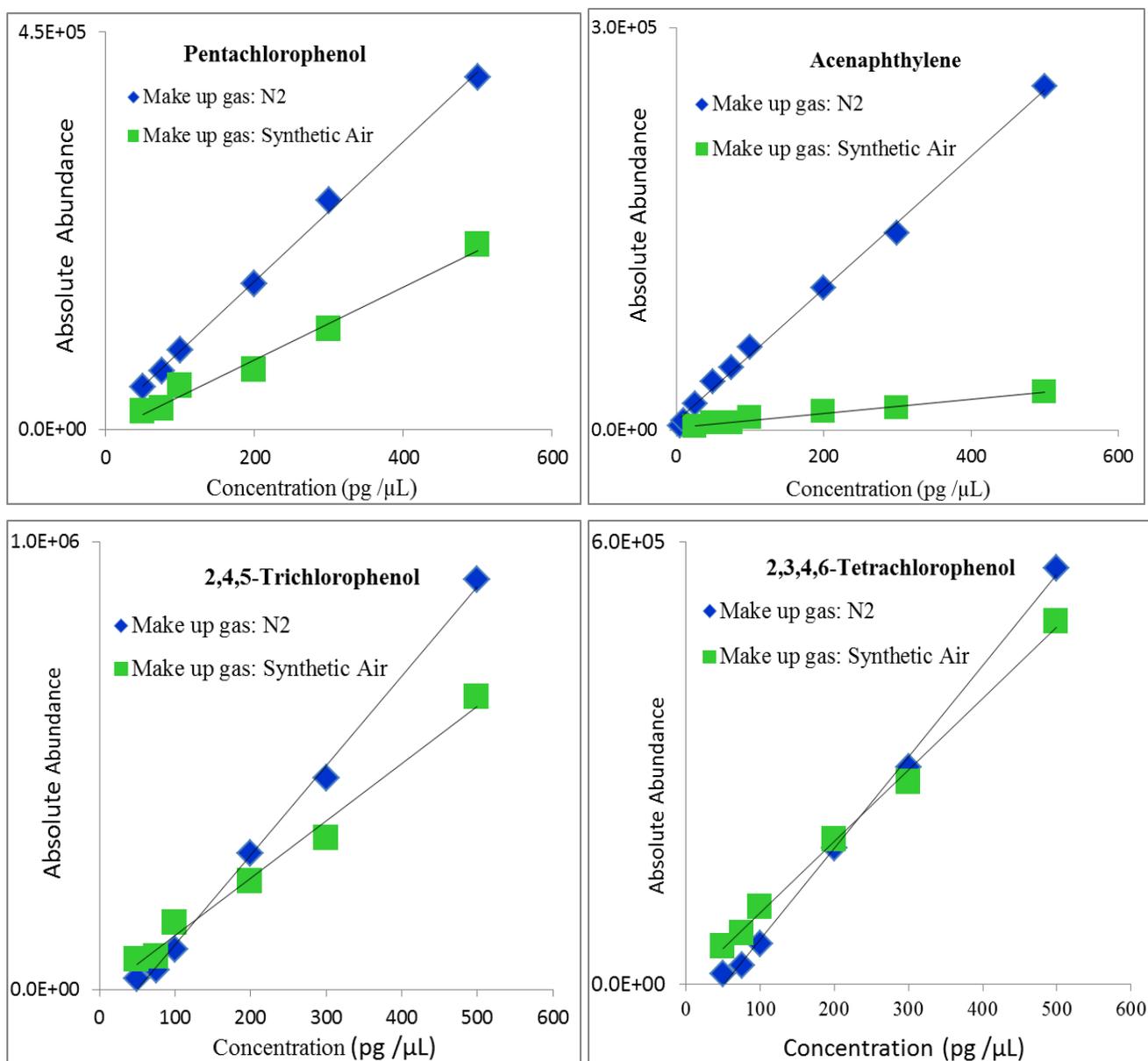
**Appendix D The preliminary results obtained using synthetic air instead of nitrogen as the make-up gas in negative ionization to examine the possible dopant function of oxygen in promoting negative ionization.**

### **D.1 TICs obtained with NI APPI using synthetic air and nitrogen as make-up gas**



**Figure D.1 TICs obtained with NI APPI using  $N_2$  (a) and synthetic air (b), when 1  $\mu$ L of EPA mixture at a concentration of 100 pg/ $\mu$ L was injected. As seen in the figure, utilization of synthetic air increased the number of detected compounds, which confirmed the potential dopant function of oxygen for NI.**

**D.2 A comparison of quantitative results obtained with N<sub>2</sub> and synthetic air as the make-up gas for NI APPI.**



**Figure D.2 A comparison of calibration curves obtained with NI-APPI using N<sub>2</sub> and synthetic air as the make-up gas. As seen, oxygen offers a dopant function in the lower concentrations.**

### D.3 Comparison of oxygen dopant function in NI-APPI in low and high concentrations of the EPA mixture.

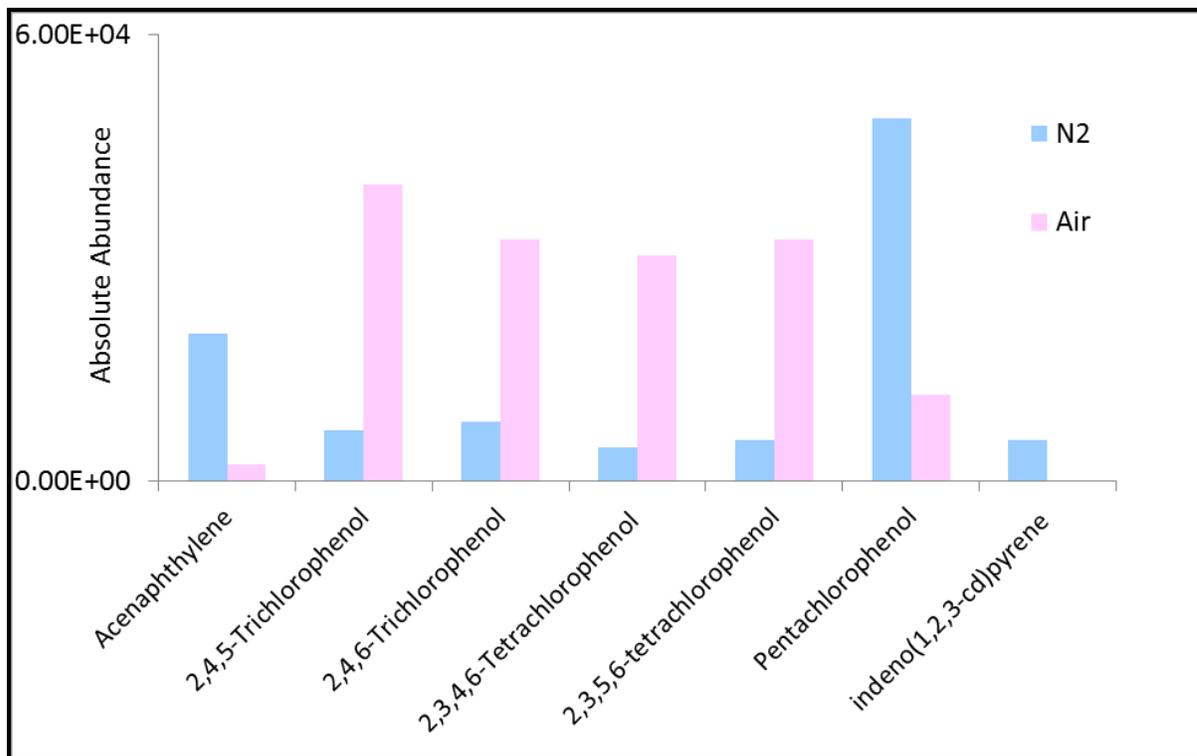
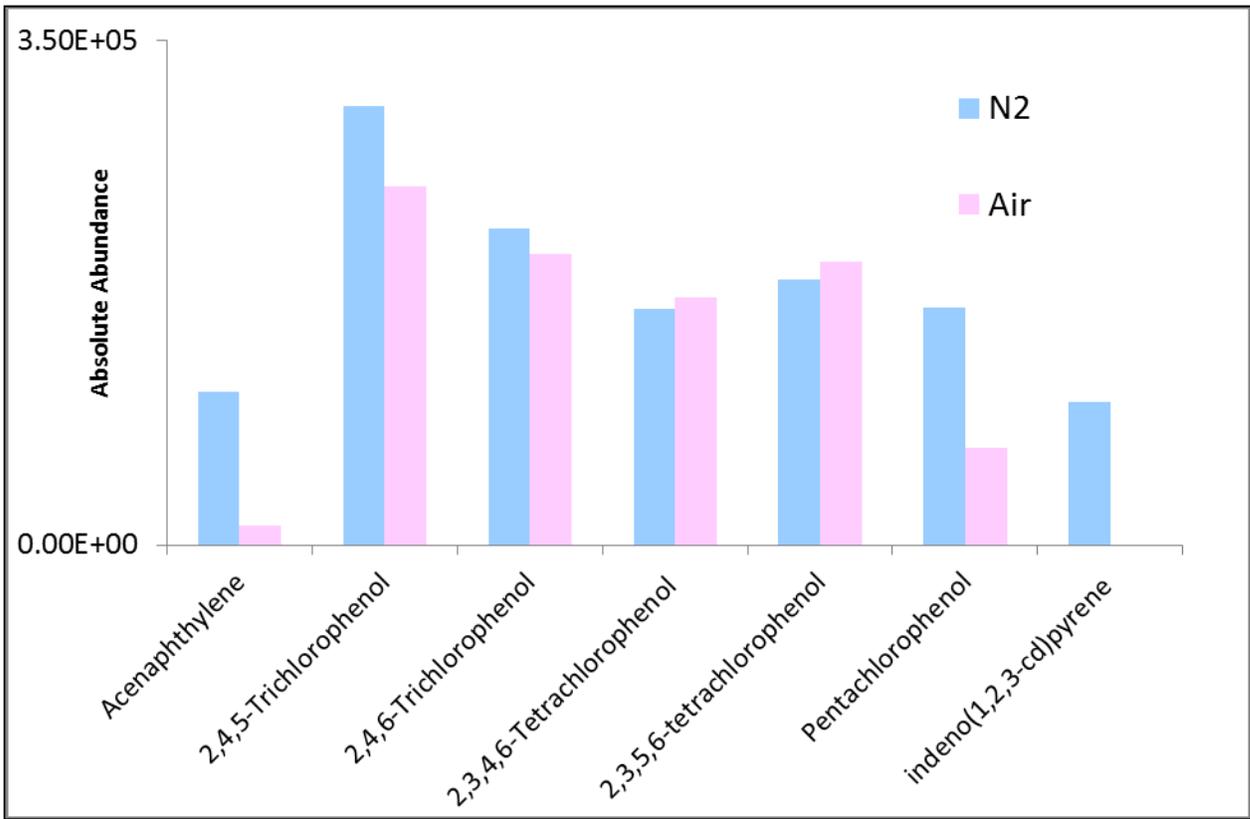
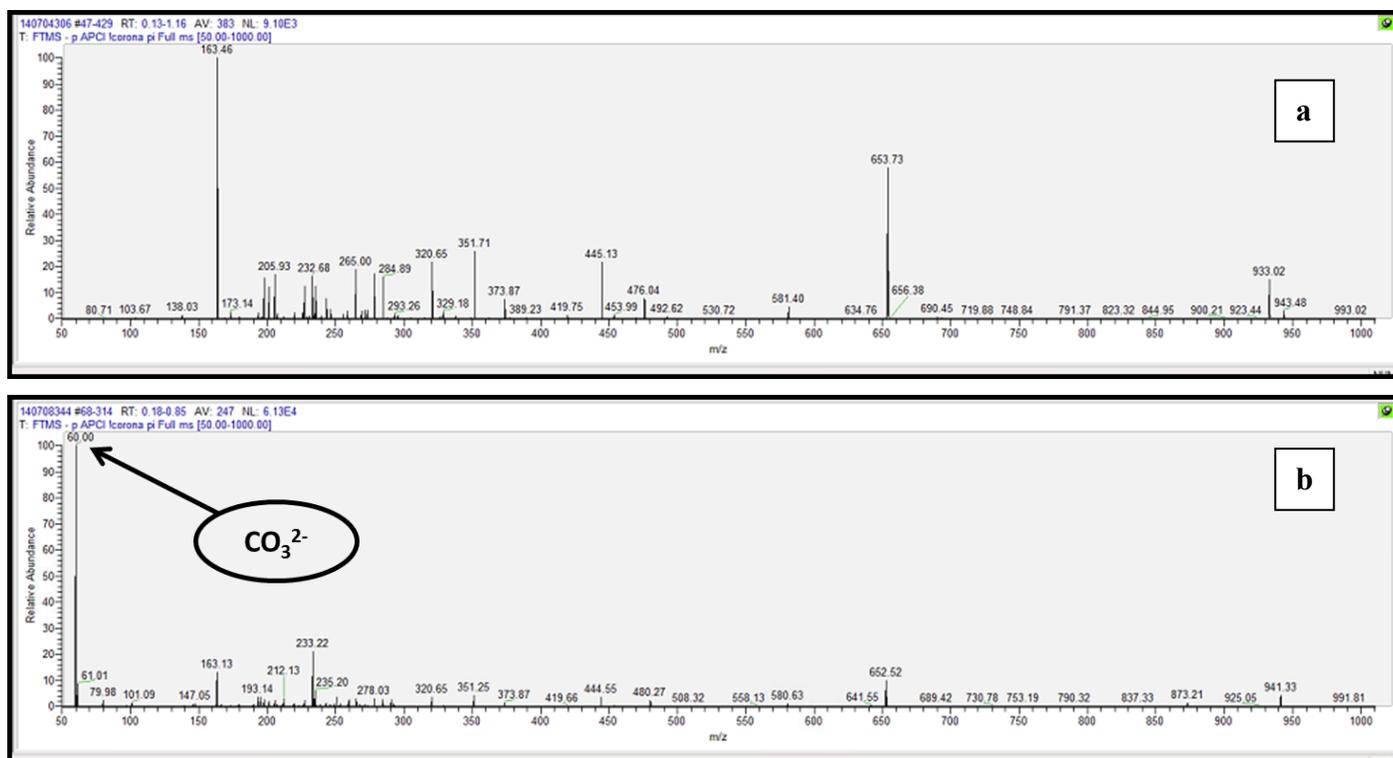


Figure D.3 A comparison of ionization responses obtained for seven compounds, when 1  $\mu\text{L}$  of the EPA mixture at a concentration of 25  $\text{pg}/\mu\text{L}$  was injected and ionized with NI APPI. As seen, oxygen offers a dopant function and enhanced the ionization responses of almost all studied compounds.



**Figure D.4** A comparison of ionization responses obtained for seven compounds, when 1  $\mu\text{L}$  of the EPA mixture at a concentration of 200  $\text{pg}/\mu\text{L}$  was injected and ionized with NI APPI. As seen, the dopant function of oxygen becomes less efficient in this higher concentration.

#### D.4 Background spectra obtained with NI-APPI using N<sub>2</sub> and synthetic air as make-up gas.



**Figure D.5** The background spectra obtained with N<sub>2</sub> (a) and synthetic air (b) as make-up gas with NI-APPI. In the background spectra, carbonate anion (CO<sub>3</sub><sup>2-</sup>, m/z 60) was observed with a considerable intensity, which originates from CO<sub>2</sub> impurity in the synthetic air bottle. CO<sub>2</sub> acts as a sink for thermal electrons and reduces the accessible electrons for analytes. Therefore, removing CO<sub>2</sub> from the synthetic air can further increase the efficiency of the negative ionization. Despite successful preliminary attempts to remove CO<sub>2</sub>, due to time restrictions, I could not finish these series of experiments. This path can be further explored and could lead to much more effective ionization in the negative mode.