# DEVELOPMENT OF MASS SPECTROMETRIC METHODS FOR THE CHEMICAL CHARACTERIZATION OF CONDENSATE OILS, THE DETECTION OF REACTION INTERMEDIATES IN SOLUTION AND FOR THE FAST IDENTIFICATION OF DRUG METABOLITES

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"All praise is due to GOD ALMIGHTY, for my success can only come from HIM. In GOD I trust, and unto HIM I look".

# "وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ عَلَيْهِ تَوَكَّنْتُ وَإِلَيْهِ أُنِيبُ" صدق الله العظيم

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# TABLE OF CONTENTS

LIST OF TABLES
LIST OF FIGURES
LIST OF SCHEMES 1
ABSTRACT 1
CHAPTER 1. INTRODUCTION 1
1.1 Introduction 1
1.2 Thesis Overview
1.3 References
CHAPTER 2. INSTRUMENTATION AND EXPERIMENTAL DETAILS FOR LINEA
QUADRUPOLE ION TRAP MASS SPECTROMETERS, HIGH RESOLUTION ORBITRA
MASS SPECTROMETER AND TWO-DIMENSIONAL GAS CHROMATOGRAPHY
(ELECTRON IONIZATION) HIGH RESOLUTION TIME-OF-FLIGHT MAS
SPECTROMETER
2.1 Introduction
2.2 Linear Quadrupole Ion Trap (LQIT) Mass Spectrometers
2.2.1 Instrumentation
2.2.2 The Functioning of the Linear Quadrupole Ion Trap
2.2.3 Ion Activation to Induce Ion-Source Collision-Activated Dissociation (ISCAD) 4
2.3 Orbitrap Mass Analyzer
2.3.1 Injection of Ions into the Orbitrap
2.3.2 Trajectories of Ions in the Orbitrap 4
2.3.3 Detection of Ions in the Orbitrap
2.4 Two-Dimensional Gas Chromatography/Electron Ionization High-Resolution Time-o
Flight Mass Spectrometry (GC×GC/(EI)TOF MS) 4
2.4.1 GC×GC Chromatograph
2.4.2 GC×GC Method Development
2.4.3 Folded Flightpath (FFP) Time-of-Flight Mass Spectrometer (TOF MS)5
2.5 References

CHAPTER 3. UTILIZATION OF THE DISTILLATION. PRECIPITATION, MASS FRACTIONATION SPECTROMETRY (DPF MS) METHOD FOR THE DETERMINATION OF THE CHEMICAL COMPOSITION OF UNCONVENTIONAL CONDENSATE-LIKE OIL SAMPLES WITH DIFFERENT API GRAVITIES ...... 59 3.1 3.2 3.2.1 3.2.2 3.3 Average wt% Determination for the Compounds in The Different Compound Classes 3.3.1 3.3.2 Determination of the Elemental Compositions of Compounds in The Five Condensate-3.3.3 Determination of the Elemental Compositions of Compounds in The Individual 3.3.4 Determination of the Average Molecular Weight (MW) of Compounds in The Condensate-Like Oil Samples from The Average Molecular Weight of Compounds in The 3.3.5 Determination of the Average Ring and Double Bond Equivalent (RDBE) Values of Compounds in The Individual Compound Classes and in The Whole Condensate-Like Oil 3.3.6 Determination of the Average Chain Length in the Side Chains of Alkyl Aromatic 3.5 CHAPTER 4. REAL-TIME MASS SPECTROMETRIC DETECTION OF REACTION **INTERMEDIATES** Formed DURING LASER-INDUCED  $UV/H_2O_2$ ADVANCED 4.1 4.2.1

4.2.2 Sample Preparation	05
4.2.3 Reaction Setup	06
4.2.4 Mass Spectrometry 1	07
4.2.5 Density-Functional Theory (DFT) Calculations	08
4.3 Results and Discussion	08
4.3.1 Results Obtained in the Negative-Ion Mass Spectrometry Experiments	12
4.3.2 Results Obtained in the Positive-Ion Mass Spectrometry Experiments 1	23
4.4 Proposed Ionization Mechanisms 1	33
4.5 Conclusions	36
4.6 References	37
CHAPTER 5. DEVELOPMENT OF GAS-PHASE ION-MOLECULE REACTION	NS
COUPLED WITH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR HIC	ΞH
THROUGHPUT IDENTIFICATION OF CARBOXYLIC ACID FUNCTIONALITIES	IN
PROTONATED ANALYTES	41
5.1 Introduction	41
5.2 Experimental 1	42
5.2.1 Chemicals	42
5.2.2 Mass Spectrometry 1	43
5.2.3 Ion-molecule Reactions	43
5.2.4 Reaction Kinetics	44
5.2.5 High Performance Liquid Chromatography (HPLC) 1	44
5.2.6 Computational Studies	45
5.3 Results and Discussion	45
5.4 Conclusions	58
5.5 References	59
PUBLICATIONS	62

# LIST OF TABLES

Table 3.1 List of Sigma-Aldrich obtained model compounds and their percent mass purities 62
Table 3.2 Positive-ion mass calibration: A) LQIT/orbitrap MS by using Pierce LTQ ESI positive- ion calibration solution. B) GC×GC/TOF MS by using perfluorotributylamine (PFTBA) as the calibration reagent
Table 3.3 Gravimetric weight percentages of the five different compound classes obtained usingthe DPF method for five condensate-like oil samples71
Table 3.4 The elemental composition types for the compounds in the different classes for five different condensate-like oils
Table 3.5 The average molecular weights of compounds in the different compound classes and their standard deviations (SD; based on two measurements) for five different condensate-like oils
Table 3.6 The RDBE values of compounds in the different compound classes of five different condensate-like oils
Table 3.7 The MW of the aromatic cores and the total number of carbons in the alkyl side chains of the alkyl aromatic hydrocarbons with averages and standard deviations from two experiments for the five condensate-like oils
Table 4.1 Exact measured $m/z$ -values, the derived elemental compositions and mass errors in ppm for the ions of the products and intermediates formed upon UV/H <sub>2</sub> O <sub>2</sub> advanced oxidation of 2-methylbenzoisothiazol-3-one and the fragment ions generated upon CAD on the product and intermediate ions detected in negative- and positive-ion detection modes
Table 5.1 Product ions, their branching ratios and the efficiencies for reactions of protonated compounds containing a carboxylic acid functionality with (isopropenoxy)trimethylsilane (ITS)
Table 5.2 Products, their branching ratios and the efficiencies for reactions of protonated sulfones, hydroxylamines, sulfonamides, ketones, amines, N-oxides, sulfoxides and carboxamides with ITS
Table 5.3 Structures and calculated proton affinities (B3LYP/6-311G++(d,p) level of theory) of a sulfone, sulfonamide and amide as well as the calculated proton affinities of the conjugate bases of the primary [adduct – acetone] <sup>+</sup> product ions
Table 5.4 Structures and calculated proton affinities (B3LYP/6-311G++(d,p) level of theory) of 2- methoxyphenol, phenol and methoxybenzene as well as the calculated proton affinities of the conjugate bases of the primary [adduct – acetone] <sup>+</sup> product ions

# LIST OF FIGURES

Figure 2.1 Steps required for a simple mass spectrometric experiment
Figure 2.2 Components of one of the LQIT mass spectrometers utilized in this research and the pressures of the main regions
Figure 2.3 Illustration of APCI ionization process in positive-ion detection mode
Figure 2.4 In quadrupole optics, the same RF voltage is applied to the opposite electrodes and 180° out-of-phase voltages are applied to the neighboring electrodes
Figure 2.5 The typical DC potential gradient applied to the different regions of the LQIT to facilitate the transmission of ions in the z-direction from the ion source into the ion trap. All indicated values for the applied voltages were relative to ground zero indicated by the central horizontal line
Figure 2.6 The three sections of the linear quadrupole ion trap and the side slits in the x-electrodes for ion ejection into the external electron multipliers for detection
Figure 2.7 A) An illustration of the ion detection system composed of a conversion dynode and an electron multiplier. The figure is not drawn in correct proportions. Ions ejected via the slits of the electrodes to the two conversion dynodes collided with the surface of the conversion dynode to generate opposite charge secondary particles. These secondary particles entered the electron multiplier to generate a cascade of electrons upon many collisions with the cathode surface, which eventually generated a measurable ion current in the anode cup. B) An actual picture of the ion trap and the conversion dynodes of the LQIT mass spectrometer used in this dissertation 31
Figure 2.8 Mathieu stability diagram describing the ion motion inside the ion trap. Ions of different $m/z$ values are illustrated as circles of different colors and sizes. The larger the circle, the greater the corresponding ion $m/z$ value. Ions will have stable trajectories inside the rhombus shaped region. Regions outside the rhombus shape, in which the ion trajectories are unstable, are also indicated
Figure 2.9 Illustration of the DC potential well created to regulate axial (z-axis) motion of ions by applying three DC trapping potentials, one for each section of the linear quadrupole ion trap electrodes. Larger DC potentials were applied to the front (DC1) and back sections (DC3) of the ion trap than to the center section (DC2)
Figure 2.10 A) The Mathieu stability diagram showing ions in the stable trajectory region. B) Only ions with $q_u$ values greater than 0.908 were ejected from the ion trap. C) A tailored RF waveform applied to the x-electrodes at the secular frequencies of all ions in the trap except the ions of interest, shown as a blue circle, caused the unwanted ions to be ejected out of the trap
Figure 2.11 (A) $\Delta$ greater main RF voltage amplitude increased the <i>a</i> values of the ions (R) When

Figure 2.11 A) A greater main RF voltage amplitude increased the  $q_u$  values of the ions. B) When the  $q_u$  value of the ion in purple reached 0.88, a supplementary RF voltage was applied to the xelectrodes. The frequency of motion of the ions whose  $q_u$  value is 0.88 was in resonance with the frequency of the supplementary RF voltage applied to the x-electrodes. C) These ions gained

Figure 2.12 Schematic illustration of the external reagent mixing manifold. The path the Helium/reagent mixture traveled to reach the ion trap is shown in green. The amount of Helium/reagent mixture entering the ion trap of the mass spectrometer was controlled by using a control valve (Granville-Phillips leak valve) and by diverting the excess mixture to exhaust. The standard He line and the line to the rough pump were closed when the manifold was in use...... 40

Figure 2.17 An illustration of the four steps of a modulation period. 1-4: A red circle represents an eluate that can be used as a reference point for the four individual steps. The green labels indicate which cold jet or hot jet was opened during each step. 1) In the first step, cold jet 1 was opened, allowing chilled nitrogen gas to cryo-focus eluates into the first stage of the modulator. Hot jet 2 was opened, allowing hot nitrogen gas to desorb any cryo-focused eluates from the second stage of the modulator. 2) In the second step, hot jet 1 was opened, allowing cold nitrogen gas to cryo-focus eluates in the first stage of the modulator. Cold jet 2 was opened, allowing cold nitrogen gas to cryo-focus eluates in the first stage of the modulator. 3) In the third step, both cold jets 1 and 2 were opened, allowing cold nitrogen gas to cryo-focus eluates in the first and second stages of the modulator. 4) In the final step, cold jet 1 was opened, allowing nitrogen gas to continue cryo-focusing of the eluates in the first stage of the modulator. Hot jet 2 was open, allowing hot nitrogen gas to desorb eluates in the first stage of the modulator. But jet 2 was open, allowing hot nitrogen gas to cryo-focus eluates in the first stage of the modulator. 50

Figure 2.18 An example of a modulation period of 3.5 seconds that was split between two stages, 1.75 seconds each, each with hot and cold pulses of 0.6 seconds and 1.15 seconds, respectively.

Figure 3.9 GC×GC/TOF chromatogram for the heavy saturated hydrocarbon fraction of oil 5...87

Figure 3.10 Color-mapped bubble plots of the RDBE values versus carbon number for ionized compounds derived from the four nonvolatile condensate-like oil fractions that were separated and characterized by using the DPF MS method. A) Heteroaromatic compound fraction. B) Saturated hydrocarbon fraction. C) Alkyl aromatic hydrocarbon fraction. D) Polar compound fraction. Bubble size indicates the relative abundance of the ions. The black bubbles represent ions containing only C and H. Red bubbles represent ions containing heteroatoms (N, O and/or S) along with C and H.

Figure 3.11 A) (+) APCI/N<sub>2</sub>/CS<sub>2</sub> low resolution mass spectrum of an equimolar (1 mM) mixture of two alkyl aromatic hydrocarbon model compounds. B) ISCAD mass spectrum of the mixture of the two ionized alkyl aromatic model compounds, illustrating the ability to use ISCAD to cleave off most of the alkyl substituents on the aromatic cores. C) (+) APCI/N<sub>2</sub>/CS<sub>2</sub> low resolution mass spectrum of 1 mM solution of indane. D) ISCAD mass spectrum of the ions shown in part C,

Figure 4.1 A schematic diagram of a top view of the experimental setup. f is the focal length. 107

Figure 4.5 Calculated enthalpies for the elimination of H<sub>3</sub>C-N=C=O (methyl isocyanate) and CO upon CAD from deprotonated 1-hydroxy-2-methyl-1,2-dihydro- $3H-1\lambda^4$ -benzo[*d*]isothiazol-3-one (*m*/*z* 182; deprotonated reaction intermediate B). All values are in kcal/mol and were calculated relative to the infinitely separated reactants at the M06-2X/6-311++G(d,p) level of theory ..... 117

Figure 4.7 Calculated enthalpies for the elimination of SO<sub>2</sub> upon CAD on deprotonated 2-(methylcarbamoyl)benzenesulfinic acid (reaction intermediate D2). All values are in kcal/mol and

were calculated relative to the infinitely separated reactants at the M06-2X/6-311++G(d,p) level of theory. The theoretical exact m/z-values are shown for D2 and the final fragment ion ...... 119

Figure 4.8 Positive-ion low-resolution mass spectrum of protonated reactants and reaction products formed in laser-induced UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation of 2-methylbenzoisothiazol-3-one. Ions corresponding to the protonated reactant A and protonated final products C, E1 and F2 were detected. Additionally, during positive ion experiments, ions of m/z 331 were observed ....... 124

Figure 5.1 a) The Lewis structure of (isopropenoxy)trimethylsilane (ITS). b) Mass spectrum measured after 30 ms reactions between protonated 3-aminobenzoic acid ( $[M+H]^+$ ) and ITS.. 146

# LIST OF SCHEMES

### ABSTRACT

Tandem mass spectrometry is a powerful technique for the chemical characterization of complex mixtures containing unknown compounds. Mass spectrometry cannot be used to directly determine chemical structures of charged compounds. However, the determination of the accurate mass-to-charge value of ions facilitates the identification of their elemental compositions. Further, mass spectrometry experiments wherein the ions are subjected to reactions can be used to elucidate the structures of ionized compounds. Finally, due to the high sensitivity and the large dynamic range of many mass spectrometers, they can be used to detect compounds present at trace levels in mixtures. These advantages, along with the availability of many different ionization methods and mass analyzers with dissimilar capabilities, have made tandem mass spectrometry a cutting-edge analytical technique for the analysis of complex mixtures in many fields.

This dissertation presents several examples of diverse applications of mass spectrometry. For example, high-resolution mass spectrometry was utilized for qualitative and quantitative chemical characterization of ionized compounds in petrochemical samples, specifically, in condensate-like oil samples. Further, the ability to use tandem mass spectrometry to rapidly detect trace amounts of short-lived reaction intermediates in solution reactions is demonstrated. Finally, selective tandem mass spectrometry methods based on diagnostic gas-phase ion-molecule reactions were developed for the identification of carboxylic acid functionalities in protonated analytes.

Chapter 2 describes the instruments and the atmospheric pressure chemical ionization method used to perform the research discussed in this dissertation. In Chapter 3, the success in the chemical characterization of condensate-like oil samples with different API gravities by using the Distillation, Precipitation, Fractionation Mass Spectrometry (DPF MS) method is discussed. Further, the determination of the average total number of carbon atoms in the alkyl side chains of the alkyl aromatic compounds in the condensate-like oil samples by using in-source collision-activated dissociation (ISCAD) in a linear quadrupole ion trap mass spectrometer is described. Chapter 4 describes the development of an online mass spectrometry-based method for the detection and identification of reaction intermediates generated during UV/H<sub>2</sub>O<sub>2</sub> induced oxidation of 2-methylbenzisothiazolin-3-one. Chapter 5 discusses the development of tandem

mass spectrometry experiments based on diagnostic gas-phase ion-molecule reactions for the identification of carboxylic acid functionalities in protonated analytes.

## CHAPTER 1. INTRODUCTION

### 1.1 Introduction

Tandem mass spectrometry is a powerful technique for the chemical characterization of complex mixtures containing unknown compounds. Mass spectrometry cannot be used to directly determine chemical structures of charged compounds. However, the determination of the accurate mass-to-charge value of ions facilitates the identification of their elemental compositions. Further, mass spectrometry experiments wherein the ions are subjected to reactions can be used to elucidate the structures of ionized compounds, this information can be obtained by using tandem mass spectrometry (MS<sup>n</sup>). A shared step between tandem mass spectrometry experiments involves isolation of ionized compounds of a specific mass-to-charge ratio by ejecting all other ions from the ion trap. Tandem mass spectrometry experiments can be based on collision-activated dissociation (CAD) or ion-molecule reactions. Additionally, high-resolution mass spectrometry can be used to determine the elemental compositions of the detected ions which has proven to be exceptionally powerful in identifying unknown compounds in a plethora of disciplines, including crude oil<sup>1</sup> and pharmaceutical research.<sup>2</sup>

For every mass spectral analysis, four main steps need to be accomplished: (1) sample evaporation, (2) ionization of the compounds in the sample, (3) mass-selective separation of the ions and (4) ion detection. Sample evaporation and ionization steps can occur either simultaneously, as in electrospray ionization,<sup>3</sup> or subsequently, as in atmospheric pressure chemical ionization.<sup>4</sup> The ionized compounds are transferred from the ion source into a mass analyzer where they are separated based on some physical variable related to their mass-to-charge (m/z) ratios. For instance, a scanning magnetic field<sup>5</sup> allows ion separation based on their momentum, while a time-of-flight mass filter allows ion separation based on their flight times.<sup>6</sup> A mass spectrum typically displays the m/z values of ions on the x-axis and their percent relative abundances on the y-axis.

Tandem mass spectrometry ( $MS^n$ ; n = the number of ion isolation/separation steps) experiments are used to extract information on the chemical structures of the detected ions. One of the most common commercially available tandem mass spectrometers contain a linear quadrupole ion trap (LQIT) mass analyzer. In this dissertation, structural information for ions was obtained by using collision-activated dissociation (CAD) in the linear quadrupole ion trap of an

LQIT instrument, and by using in source collision-activated dissociation (ISCAD) in the ion source area. Additionally, diagnostic gas-phase ion-molecule reactions were developed for the identification of specific functionalities in protonated analytes.

In CAD experiments, ions produced in the ion source are transferred into the ion trap, isolated by ejecting all other ions from the ion trap and subjected to energetic collisions with the helium buffer gas, which convert part of the ion kinetic energy into their internal energy, thus causing dissociation of the ions. In ISCAD experiments, all ions produced in the ion source are non-selectively, without ion isolation, subjected to a high voltage that accelerates the ions. Upon collisions with neutral atoms and molecules in the ion source area, part of the ion kinetic energy is converted into their internal energy, which results in ion dissociation. As in CAD, structural characterization of ions based on diagnostic ion-molecule reactions requires ion isolation, after which the isolated ions are allowed to react with neutral molecules.

### 1.2 Thesis Overview

The research described in this dissertation includes the development of high-resolution tandem mass spectrometry methods for the determination of the chemical compositions of complex mixtures, specifically, condensate-like oil samples, for the online tandem mass spectrometric detection of short-lived reaction intermediates in solution, and for the fast identification of functional groups in unknown drug metabolites by using tandem mass spectrometry based on diagnostic ion-molecule reactions coupled with high-performance liquid chromatography (HPLC).

Chapter 2 discusses fundamental aspects of the mass spectrometric instrumentation and ionization methods used in this research. The instruments used were linear quadrupole ion trap (LQIT), usually interfaced with HPLC, sometimes coupled to a high-resolution orbitrap mass spectrometer, and a two-dimensional gas chromatograph (GCxGC) coupled with electron ionization (EI) high-resolution time-of-flight (TOF) mass spectrometer (GCxGC/(EI)TOF MS).

Chapter 3 discusses the determination of the chemical compositions of five condensatelike oil samples with different API gravities by using the Distillation, Precipitation, Fractionation Mass Spectrometry (DPF MS) method. The samples were fractionated to five compound classes (volatile hydrocarbons, heavy saturated hydrocarbons, alkyl aromatic hydrocarbons, heteroaromatic compounds and polar compounds) and the average gravimetric weight percentage (wt%) of compounds in each compound class were determined. The chemical compositions of the compounds in each compound class and in the whole condensate-like oils were determined by using optimized ionization and high-resolution mass spectrometry methods. Data obtained using high-resolution mass spectrometry were used to determine the average molecular weights and the average ring- and double-bond equivalent values for compounds in each class, as well as the overall average molecular weight and ring- and double-bond equivalent values for the compounds in the entire condensate-like oils. Moreover, ISCAD was utilized to obtain information on the total number of carbons in the alkyl chains of alkyl aromatic hydrocarbons. Since the API gravity is generally used to make decisions about the quality and price of hydrocarbon products in petroleum industry, correlations between the API gravity and the chemical compositions of the condensate-like oils were examined. Correlations with the API gravity were found only for the wt% of the heteroaromatic compound and the heavy saturated hydrocarbon classes in the five oils. The wt% of the saturated hydrocarbons was found to directly correlate with the API gravity.

Examination of the different types of elemental compositions of compounds in the condensate-like oils and determination of their wt% by using high-resolution mass spectrometry revealed that compounds with the elemental composition type  $C_cH_h$  were generally the most abundant compounds in the condensate-like oils. Further, a direct correlation was found between the average MW of the alkyl aromatic hydrocarbons and the API gravity where the lighter condensate-like oils appeared to contain alkyl aromatic compounds with higher average MWs. Moreover, the average total number of carbon atoms in the alkyl chains of the alkyl aromatic hydrocarbons in the whole condensate-like oils was found to correlate with their API gravity. In general, these correlations can facilitate the development of a more accurate way to classify petroleum products than by using API gravity values. The work in this chapter has been recently published in Energy & Fuels journal.<sup>7</sup>

Chapter 4 describes a simple laser-based mass spectrometry method developed, in collaboration with the School of Aeronautics and Astronautics at Purdue University, for the online detection of short-lived reaction intermediates produced during laser-induced UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation of 2-methylbenzoisothiazol-3-one in solution. All previously proposed reaction intermediates and products were detected in either negative- or positive-ion mode mass spectrometry experiments. High-resolution mass spectrometry was used to determine the

elemental compositions of the detected ions, which were in agreement with the elemental composition of the previously proposed structures. Low- and high-resolution CAD experiments provided structural information for the observed ions, which were in agreement with the previously proposed structures. Density functional theory (DFT) calculations at the M06-2X/6-311++G(d,p) level of theory were carried out by a colleague, Erlu Feng, to examine the likely mechanisms of the fragmentation reactions of the ions.

Chapter 5 discusses the development of a method based on diagnostic gas-phase ionmolecule reactions for the fast identification of protonated molecules containing carboxylic acid functionalities in drug metabolite mixtures. The use of (isopropenoxy)trimethylsilane (ITS) as a reagent in the LQIT to identify carboxylic acid functionalities was an idea of an alumnus student in our laboratories, Joann P. Max. However, she had tested only a limited number of compounds containing carboxylic acids and all of them were aromatic carboxylic acids. Therefore, we decided to include a more diverse collection of analytes with carboxylic acid functionalities, including aliphatic, aromatic, and polyfunctional analytes, in order to expand the applicability of the method. Further, additional quantum chemical calculations were performed to provide the proton affinities of the previously tested analytes. Moreover, an investigation of the reaction kinetics and reaction efficiencies for all analytes was performed in the current study. The research described in this chapter has been recently published in the International Journal of Mass Spectrometry.<sup>2</sup>

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# CHAPTER 2. INSTRUMENTATION AND EXPERIMENTAL DETAILS FOR LINEAR QUADRUPOLE ION TRAP MASS SPECTROMETERS, HIGH RESOLUTION ORBITRAP MASS SPECTROMETER AND TWO-DIMENSIONAL GAS CHROMATOGRAPHY / (ELECTRON IONIZATION) HIGH RESOLUTION TIME-OF-FLIGHT MASS SPECTROMETER

#### 2.1 Introduction

Mass spectrometry is a powerful analytical tool that can be used to examine highly complex mixtures.<sup>1</sup> Recent improvements in mass spectrometric analyses and the rapid development of new instruments and ionization techniques have made it stand out among other analytical methods,<sup>2</sup> with applications in many different fields, including petroleomics,<sup>3</sup> proteomics,<sup>4</sup> pharmaceutical sciences,<sup>5</sup> environmental sciences<sup>6</sup> and many more.

Three main steps are required to achieve a simple mass spectrometric analysis. First, the compounds to be analyzed must be evaporated and ionized. Second, the generated gas-phase ions must be separated based on their mass-to-charge ratios (m/z). Third, the separated ions must be detected (**Figure 2.1**).



Figure 2.1 Steps required for a simple mass spectrometric experiment.

Various methods are available for the evaporation and ionization of compounds.<sup>7</sup> The most commonly used evaporation/ionization methods include atmospheric pressure ionization methods (API), such as electrospray ionization (ESI),<sup>8</sup> atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI).<sup>9,10</sup> It is worth noting that the ionization efficiencies of different compounds ionized by using the same ionization method can differ drastically depending on their chemical properties, polarities and sizes and the ionization method.<sup>11</sup>

Therefore, the choice of the proper evaporation/ionization method depends on the nature of the sample.<sup>12</sup> Often, more than one such method is needed to fully characterize the sample.

Unlike Fourier-transform infrared spectroscopy or nuclear magnetic resonance spectroscopy,<sup>13</sup> which provide structural information for compounds based on absorption of electromagnetic radiation by the analytes, mass spectrometry provides structural information based on the reactions, often fragmentation reactions, of the ionized analytes. However, for analysis of complex mixtures, it is beneficial to ionize each compound without fragmentation as to not further complicate the mixture. This is why tandem mass spectrometry ( $MS^n$ ; n = number of ion isolation/mass analysis steps) was developed.<sup>14</sup>  $MS^n$  experiments start with evaporation and ionization of all compounds in a mixture. The ions of interest are then isolated and are allowed to undergo reactions, usually collision-activated dissociation (CAD), by accelerating the ions and allowing them to collide with an inert target gas. The energetic collisions convert part of the ion kinetic energy into internal energy, which results in dissociation. All the ions are then detected.

Currently, two main types of mass spectrometric instruments are available, not ion trap mass spectrometers (scanning and pulsed instruments) where the events of ionization, ion separation and detection occur in different parts of the instrument, and ion trap mass spectrometers, where most events occur in the same space but at different times; also referred to as tandem-intime mass spectrometers.<sup>15</sup> The tandem mass spectrometric experiments discussed in this dissertation were tandem-in-time, performed in ion trap mass spectrometers; specifically, linear quadrupole ion traps, some of which were coupled with an orbitrap mass analyzer (LQIT/orbitrap MS). The MS<sup>n</sup> experiments discussed in this dissertation started with isolating the ions of interest by ejecting all other ions from the ion trap and allowing them to either fragment by colliding with a buffer gas in the ion trap often helium, nitrogen or argon,<sup>16</sup> or react with neutral reagents introduced into the ion trap using an external reagent mixing manifold, a technique referred to as gas-phase ion-molecule reactions.<sup>17</sup> Structural information, including the chemical functionalities in the analyzed ions, can sometimes be deduced from the fragmentation patterns obtained in CAD experiments and/or the product ions obtained in gas-phase ion-molecule reactions.<sup>17</sup> Other mass spectrometry experiments in this research dissertation were not MS<sup>n</sup> experiments and were performed using a two-dimensional gas chromatograph/electron ionization time-of-flight mass spectrometer (GC×GC/TOF MS). Below, a discussion on the instrumentation and the fundamental aspects of MS<sup>n</sup> experiments performed using the LQIT mass spectrometers sometimes coupled

with an orbitrap mass analyzer is followed by a discussion on the instrumentation and fundamentals of (GC×GC/TOF MS).

### 2.2 Linear Quadrupole Ion Trap (LQIT) Mass Spectrometers

Linear quadrupole ion trap mass spectrometers (LQIT) are among the most widely used ion trap tandem mass spectrometers in a plethora of academic<sup>18</sup> and industrial applications.<sup>19</sup> In the studies discussed in this dissertation, a Thermo Scientific LQIT mass spectrometer was used. There were four main regions in the LQIT mass spectrometers employed here, as illustrated in **Figure 2.2**.



Figure 2.2 Components of one of the LQIT mass spectrometers utilized in this research and the pressures of the main regions.

The ion source box was operated at atmospheric pressure (760 Torr) and it housed the API source, which was either an atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI) or electrospray ionization (ESI) source; however, the latter two were not used in the experiments described in this dissertation. The pressure in the API stack was maintained at approximately 1 Torr by using two Edwards E2M30 rotary-vane mechanical pumps with an evacuation rate of 650 L min<sup>-1</sup>. The ion optics region was kept at a pressure range of 0.5 -

 $1 \times 10^{-3}$  Torr. This was accomplished by a triple ported Leybold TW220/150/15S turbomolecular pump capable of evacuating air at rates of 25 L s<sup>-1</sup> and 300 L s<sup>-1</sup> in the first and second inlets of the mass spectrometer, respectively. The third inlet of the turbomolecular pump maintained the pressure reading in the ion trap chamber at approximately  $1 \times 10^{-5}$  Torr. A cathode ionization gauge was used to record the pressure in this region. However, the ion trap itself was filled with about  $1 \times 10^{-3}$  Torr of helium buffer gas that the ionization gauge was not able to detect due to the high ionization energy of helium. Therefore, the actual pressure in the ion trap region was about  $1 \times 10^{-3}$  Torr. Below, the operation of the different regions of the LQIT mass spectrometer are discussed.

#### 2.2.1 Instrumentation

### 2.2.1.1 Ion Source Box and API Stack

The ion source box was coupled with an ionization source (in this research, APCI) to evaporate and ionize analyte molecules before they entered the API stack. The principles and mechanisms of APCI are discussed in section 2.2.1.2. The generated ions were guided into the mass spectrometer under the influence of a potential difference of 0 - 20 V between the ion source box and the API stack. Additionally, nitrogen gas was used as the sheath and auxiliary gas (unless otherwise stated) to help guide the flow of ions into the ion trap via an ion transfer capillary. The ion transfer capillary was heated to facilitate the de-solvation of the ions and avoid condensation. The ions generated in the ionization source were drawn into the ion transfer capillary via a negative pressure gradient. To facilitate the ion transfer through the ion transfer capillary, a positive DC voltage was applied to the ion transfer capillary in positive-ion mode experiments and a negative DC voltage was applied to the ion transfer capillary in negative-ion mode experiments. The tube lens had a mass dependent potential applied to it to focus the ions toward the opening of the skimmer. When the parameters of the LQIT instrument were tuned, the tube lens offset voltage was adjusted to maximize sensitivity by balancing de-solvation with fragmentation. The motion of neutral molecules could not be influenced by the applied voltage and hence they were not transmitted into the ion optics region.

### 2.2.1.2 Atmospheric Pressure Chemical Ionization (APCI)

Atmospheric pressure chemical ionization (APCI) is an efficient method for evaporating and ionizing not only polar but also semipolar, nonpolar, nonvolatile and thermally stable analytes, like large organic hydrocarbons. The APCI source used in the studies in this dissertation was equipped with a fused silica transfer capillary maintained at a temperature of (300 - 500) °C via a ceramic heater. N<sub>2</sub> was used as the sheath and auxiliary gas (unless otherwise stated) and was blown through the probe, around the heated transfer capillary as the parallel dashed arrows pointing out of the APCI probe in **Figure 2.3** indicate. The analyte solutions were introduced via a Hamilton syringe into the fused silica transfer capillary. In the presence of N<sub>2</sub> as the nebulizing gas, fine droplets of the analyte and solvent molecules were formed near the corona discharge needle (**Figure 2.3**).



Figure 2.3 Illustration of APCI ionization process in positive-ion detection mode.

Although it was initially believed that APCI follows a gas-phase ionization mechanism, recent studies have suggested otherwise.<sup>11</sup> The corona discharge needle maintained at a potential of 2.0 - 4.0 kV relative to ground zero, resulted in the ionization of the most abundant compound present, the nebulizing gas (N<sub>2</sub>), producing nitrogen molecular cations as the primary ions. These primary ions underwent a series of ion-molecule reactions with the second-most abundant compounds present, the solvent molecules, to generate solvent ions. The solvent ions subsequently reacted with the analyte molecules (the least abundant compounds present) to form analyte ions in

a process similar to chemical ionization (CI). **Scheme 2.1A** shows an example where a protic solvent, water, was used to generate protonated analytes in the positive-ion detection mode.

In some of the APCI experiments discussed in this dissertation, an aprotic solvent, carbon disulfide (CS<sub>2</sub>), was used as the solvent for the analysis of alkyl aromatic hydrocarbons and heteroaromatic compounds as it allowed their ionization with minimal fragmentation.<sup>3</sup> Similar to what was discussed above, N<sub>2</sub> was utilized as the sheath and auxiliary gases and was blown through the probe around the heated transfer capillary to converge with the mist of CS<sub>2</sub> and the analyte molecules flowing from the heated capillary. The Corona discharge needle held at a potential of 2.0 - 4.0 kV, relative to ground zero, ionized the ambient N<sub>2</sub> gas to produce primary N<sub>2</sub><sup>++</sup> molecular ions. These ions reacted with the CS<sub>2</sub> solvent molecules to generate secondary ions (CS<sub>2</sub><sup>++</sup>) that reacted with the analyte molecules via electron abstraction to generate analyte molecular cations. Generally, molecular ions generated from aromatic analytes using the above approach do not fragment while those generated from aliphatic analytes experience extensive fragmentation.<sup>3</sup> Two schematics showing the cascade of ion-molecule reactions initiated by the corona discharge to generate analyte ions in the positive-ion detection mode are shown in **Scheme 2.1**.



Scheme 2.1 APCI ionization mechanism for: A) the generation of protonated analytes in positive-ion detection mode when using water as the solvent and B) the generation of molecular ions in positive-ion detection mode when using  $CS_2$  as the solvent.

### 2.2.1.3 Ion Optics Region

After the ions left the API stack region, they entered the ion optics region and passed through four multi-pole (MP) lenses. The DC and RF voltages applied to each lens enabled focusing of the ions through several ion guides, namely, two square quadrupole ion guides (MP00 and MP0) and a round-rod octupole ion guide (MP1).<sup>20</sup> The RF potential applied to the opposite electrodes of the ion guides restricted the motion of the ions in the x-y direction. An RF potential with the same amplitude and frequency was applied to the adjacent electrodes in the multipole but  $180^{\circ}$  out-of-phase (opposite sign voltage), while opposite electrodes were supplied with the same RF amplitude and frequency.<sup>21</sup> This generated an oscillating RF field analogous to a sine wave between the electrodes, causing ions with the same *m/z*-values to move as a tightly focused beam in a circular oscillatory, corkscrew-like motion (**Figure 2.4**).<sup>21,22</sup>



**Figure 2.4** In quadrupole optics, the same RF voltage is applied to the opposite electrodes and 180° out-of-phase voltages are applied to the neighboring electrodes.

Lenses in between the ion guides extracted and transferred the ions efficiently from one ion guide into another. Ions exited the multipole MP00 region with almost zero kinetic energy due to the high pressure in this region. The DC potential gradient illustrated in **Figure 2.5** drove the ions downhill to enter MP00 and continue towards the linear quadrupole ion trap.



**Figure 2.5** The typical DC potential gradient applied to the different regions of the LQIT to facilitate the transmission of ions in the z-direction from the ion source into the ion trap. All indicated values for the applied voltages were relative to ground zero indicated by the central horizontal line.

### 2.2.1.4 Ion Trap Region

The ion trap comprised of four parallel hyperbolic-shaped electrodes placed between front and back lenses. The ion beam exiting the ion guides entered the ion trap through the front lens. The hyperbolic electrodes were partitioned into three sections (front, center and back). The center sections in the x-electrodes had slits that allowed the ions to exit and reach the detectors<sup>23</sup> (**Figure 2.6**). The center sections of the y-electrodes had no slits, but they were dented to maintain a uniform field. RF and DC voltages were applied to the electrodes. Each opposing electrode pair was electrically connected. Thus, the RF voltages of the same amplitude and frequency were applied to the opposite electrodes while RF voltages 180° out-of-phase were applied to the adjacent electrodes. This allowed trapping the ions in the radial x-y direction.<sup>23</sup> RF potentials with variable frequencies and amplitudes were applied to the x-electrodes to enable ion excitation, isolation, and ejection for detection. In order to regulate the axial (z-direction) motion of ions, three axial DC potentials were used, one for each section of the electrodes. Initially, the ions are driven into the ion trap by applying a lower DC potential for the front section, compared to the front lens DC potential, and even lower potential for the center section. More details on controlling the ion motion in the z-direction is provided under section 2.2.2.



**Figure 2.6** The three sections of the linear quadrupole ion trap and the side slits in the x-electrodes for ion ejection into the external electron multipliers for detection.

### 2.2.1.5 Ion Detection Region

The ions were detected by two detectors located perpendicular to the z-axis outside the ion trap. Each detector consisted of a conversion dynode and an electron multiplier (**Figure 2.7**). A potential of -15 kV or +15 kV applied to the conversion dynodes attracted positive or negative ions ejected from the ion trap, respectively. When positively charged ions struck the negatively charged dynode, opposite charge secondary particles including electrons were ejected, but when negatively charged ions struck the positively charged dynode, positively charged secondary particles, positive ions, were ejected.<sup>24</sup> The concaved surface of the dynode focused the generated secondary particles toward the electron multiplier. An attractive electric field between the dynode and the electron multiplier pulled the secondary particles toward the electron multiplier. The secondary particles hit the surface of the funnel shaped cathode as they travelled further into the electron multiplier, causing the emission of more and more electrons. Thus, a cascade of electrons was created and finally a metal anode cup collected the stream of electrons at the exit of the detector where the current was measured. The measured current was proportional to the number of ions ejected from the ion trap.





### 2.2.2 The Functioning of the Linear Quadrupole Ion Trap

The functioning of the linear quadrupole ion trap can be summarized as ion trapping, ion isolation, ion activation, required for tandem mass spectrometry experiments, and ion ejection for detection.<sup>25</sup> These functions are discussed in detail below.

## 2.2.2.1 Ion Trapping

The DC and RF potentials applied on the ion trap electrodes allowed trapping the ions in the axial and radial directions. Low-energy collisions of the ions with the helium buffer gas in the ion trap allowed cooling of the ions and restricted their motion into the central section of the ion trap.

### 2.2.2.1.1 Ion Trapping in the Radial x-y Direction

For radial ion trapping, RF and DC potentials were applied to the front, center and back sections of the ion trap electrodes to generate a quadrupolar potential.<sup>26</sup> The quadrupolar potential ( $\Phi$ ) can be defined as given in equation 2.1 below:

$$\Phi = \Phi_0 \left(\lambda x^2 + \sigma y^2 + \gamma z^2\right) / r_0^2$$
(2.1)

where  $\Phi_0$  is the applied electric potential, and  $\lambda$ ,  $\sigma$  and  $\gamma$  are weighing constants for the *x*, *y* and *z* coordinates, respectively.  $r_0$  is a constant that defined the quadrupole as a mass filter or an ion trap. In any electric field, differential equations defined by the Laplace transform must satisfy the Laplace condition in which the second derivative of the electric potential must equal zero.<sup>26</sup> Hence the following expression for the weighing constants must be valid:

$$\lambda + \sigma + \gamma = 0 \tag{2.2}$$

For a linear quadrupole ion trap,  $\lambda$  and  $\sigma$  are equal to 1; therefore, in order for equation 2.2 to be valid,  $\gamma$  must equal -2.<sup>26</sup> Substituting the values of these constants in equation 2.1 produces equation 2.3, which describes the potential at any point inside the quadrupole field:

$$\Phi_{x,y,z} = \Phi_0 \left( x^2 + y^2 - 2z^2 \right) / r_0^2$$
(2.3)

Utilizing the standard transformations  $x = r\cos\theta$ ,  $y = r\sin\theta$  and z = z, equation 2.3 can be expressed as below for the polar coordinates:

$$\Phi_{r,z} = \Phi_0 \left( r^2 \cos^2 \theta + r^2 \sin^2 \theta - 2z^2 \right) / r_0^2$$
(2.4)

Applying the trigonometric identity  $cos^2\theta + sin^2\theta = 1$  to equation 2.4 gives equation 2.5:<sup>26</sup>

$$\Phi_{r,z} = \Phi_0 \left( r^2 - 2z^2 \right) / r_0^2 \tag{2.5}$$

The RF potential (defined as  $Vcos\Omega t$ , where V is the amplitude of the RF voltage with an angular frequency of  $\Omega$  for time t, expressed in rad s<sup>-1</sup>) and DC potential (U) combined define the quadrupole potential. Thus,  $\Phi_0$  can be expressed as:

$$\pm \Phi_0 = \pm (U - V \cos \Omega t) \tag{2.6}$$

Substituting equation 2.6 in equation 2.3 followed by differentiating with respect to x, the quadrupole potential becomes:

$$\frac{\delta \phi}{\partial x} = \frac{2x}{r_0^2} \left( U + V \cos \Omega t \right)$$
(2.7)

Therefore, the forces experienced by the ions in the x- and y-directions within a quadrupolar field can be expressed as shown below:

$$F_x = m\frac{d^2x}{dt^2} = -ze\frac{d\Phi}{dx}$$
(2.8)

$$F_{y} = m \frac{d^{2} y}{dt^{2}} = -ze \frac{d\Phi}{dy}$$
(2.9)

wherein *m* is the mass of the ion, *z* is the number of charges of the ion and *e* is the charge of an electron  $(1.602 \times 10^{-19} \text{ Coulombs})$ .

Substituting equation 2.7 into equation 2.8 and 2.9, the forces experienced by the ions in the xand y-direction can be written as:<sup>18</sup>

$$\frac{d^2x}{dt^2} + \frac{2ze}{mr_0^2} (U - V\cos\Omega t) x = 0$$
(2.10)

$$\frac{d^2 y}{dt^2} + \frac{2ze}{mr_0^2} (U - V \cos \Omega t) y = 0$$
(2.11)

Equations 2.10 and 2.11 define the stability of ion trajectories in a linear quadrupole ion trap and they resemble the Mathieu equation form given below:

$$\frac{d^2u}{d\xi^2} + (a_u + 2q_u \cos 2\xi)u = 0$$
(2.12)

wherein  $\xi$  can be defined as:

$$\xi = \frac{\Omega t}{2} \tag{2.13}$$

Substituting equation 2.13 in equations 2.10 and 2.11, the ion trajectories in the quadrupole ion trap can be expressed in Mathieu equations as:

$$q_{u} = q_{x} = (-)q_{y} = \frac{4zeV}{mr_{o}^{2}\Omega^{2}}$$
(2.14)

$$a_{u} = a_{x} = (-)a_{y} = \frac{8zeU}{mr_{o}^{2}\Omega^{2}}$$
 (2.15)

wherein  $q_u$  and  $a_u$  are the Mathieu stability parameters, describing the ion motion.<sup>27</sup> As shown in equations 2.14 and 2.15. The Mathieu stability parameters  $q_u$  and  $a_u$  are inversely proportional to the mass of the ion. The Mathieu stability diagram is shown in **Figure 2.8**.<sup>22</sup>



**Figure 2.8** Mathieu stability diagram describing the ion motion inside the ion trap. Ions of different m/z values are illustrated as circles of different colors and sizes. The larger the circle, the greater the corresponding ion m/z value. Ions will have stable trajectories inside the rhombus shaped region. Regions outside the rhombus shape, in which the ion trajectories are unstable, are also indicated.<sup>22</sup>

Any ions with  $a_u$  and  $q_u$  values that fall within the rhombus shaped region of the Mathieu stability diagram will have stable trajectories in the ion trap. In contrary, ions with  $a_u$  and  $q_u$  values outside the rhombus region will have unstable trajectories and will not be trapped under these conditions. If  $a_u$  parameter is set to 0, the range of  $q_u$  is maximized, which enables trapping ions within the largest possible mass range. When  $a_u$  is set to 0, motion of the ions in the ion trap can be manipulated by varying the main RF potential amplitude. Increasing the main RF amplitude increases the  $q_u$  value. When the value of  $q_u$  for ions in the trap reaches 0.908,<sup>27</sup> the trajectories of the given ions become unstable in x- and y-directions, leading to the ejection of these ions from the ion trap. It is worth noting that because the mass of the ions and the  $q_u$  value are inversely proportional, ions are ejected in ascending order of m/z values, where ions of the smallest m/z values are ejected first.<sup>28</sup>

### 2.2.2.1.2 Ion Trapping in the Axial (z) Direction

For axial ion trapping, DC voltages were applied to the front, center and back of the ion trap electrodes (DC1, DC2 and DC3; **Figure 2.9**). To allow the ions to enter the trap, low DC potentials were applied to DC1 and DC2. To confine the ions axially in the ion trap, DC1 and DC3 potentials at the front and back of the ion trap were raised. By applying a lower potential to the center sections of the ion trap DC2, a potential energy well was created in the center of the ion trap wherein the ions were trapped (**Figure 2.9**).



**Figure 2.9** Illustration of the DC potential well created to regulate axial (z-axis) motion of ions by applying three DC trapping potentials, one for each section of the linear quadrupole ion trap electrodes. Larger DC potentials were applied to the front (DC1) and back sections (DC3) of the ion trap than to the center section (DC2).

### 2.2.2.2 Role of Helium as a Dampening Buffer Gas

Helium was used as a buffer gas inside the linear quadrupole ion trap. Ions underwent lowenergy collisions with the helium gas, which kinetically cooled the ions without inducing fragmentation, and moved the ions toward the center of the ion trap.<sup>29,30</sup> This process enhanced the sensitivity of the analysis by improving the trapping and detection efficiencies.<sup>25</sup>

### 2.2.2.3 Ion Isolation

The first step in tandem mass spectrometry experiments (MS<sup>n</sup>) is ion isolation. Therefore, the ions of interest are trapped while all other ions are ejected from the ion trap. Ions of specific m/z values oscillate at unique frequencies, which can be exploited to eject unwanted ions from the ion trap. In **Figure 2.10 A** three ions of different m/z values are illustrated as colored circles. Only ions with  $q_u$  values greater than 0.908 are ejected out of the ion trap;<sup>22</sup> ions illustrated as a red circle in **Figure 2.10 B**. To isolate the ion illustrated as a blue circle in **Figure 2.10 C**, a tailored RF waveform was applied to the x-electrodes of the ion trap with frequencies of 5 – 500 kHz (excluding the unique frequency of the ions of interest) to eject all ions except those of a selected m/z value. This process led to isolation of the ions of interest.


**Figure 2.10** A) The Mathieu stability diagram showing ions in the stable trajectory region. B) Only ions with  $q_u$  values greater than 0.908 were ejected from the ion trap. C) A tailored RF waveform applied to the x-electrodes at the secular frequencies of all ions in the trap except the ions of interest, shown as a blue circle, caused the unwanted ions to be ejected out of the trap.<sup>23</sup>

#### 2.2.2.4 Ion Activation to Induce Ion Trap Collision-Activated Dissociation (CAD)

The process called collision-activated dissociation (CAD) was employed to gain structural information for the ions by examining their fragmentation patterns.<sup>31</sup> Ion fragmentation was achieved by accelerating the ions and allowing them to collide repeatedly with the helium buffer gas in the ion trap for a specific period of time (the default was 30 ms). Collisions converted part of the ion kinetic energy into their internal energy. When they had gained enough internal energy to overcome the dissociation energy threshold, the ions underwent fragmentation. To carry out CAD experiments, the ions of interest were isolated, and the RF amplitude was reduced so that the

ions of interest had a  $q_u$  value of 0.25. Then a supplementary RF potential was applied to the xelectrodes with a frequency equal to the unique secular frequency of the isolated ions. This resulted in dipolar excitation and acceleration of the isolated ions. Reducing the  $q_u$  value of the ions of interest to 0.25, kept the ions of interest away from the apex and the ejection point of the Mathieu stability diagram so that the ions will not be ejected from the ion trap when they are activated to induce CAD.

#### 2.2.2.5 Resonance Ejection for Ion Detection

Ions can be ejected from the trap by increasing the RF amplitude until the  $q_u$  value exceeds 0.908 when their trajectories become unstable in the x and y-directions and they get ejected from the ion trap. However, this method of ion ejection was not ideal as only a few ions can reach the electron multiplier detectors on the sides of the ion trap. To improve ion ejection for detection, the LQIT utilized a technique called "x-electrode bipolar resonance ejection".<sup>32,33</sup> In this technique, ions are ejected at a  $q_u$  value of 0.88; under the apex of the Mathieu stability diagram (**Figure 2.11**). To achieve the ejection of ions at this  $q_u$  value, a supplemental RF voltage was applied to the x-electrodes of the linear quadrupole ion trap. By increasing the applied RF amplitude, the secular frequencies of oscillation of all trapped ions also increased. When ions'  $q_u$  value reaches 0.88, their oscillatory frequency became in resonance with the supplementary RF voltage. This resonance caused the ions to gain additional kinetic energy,<sup>22</sup> which lead to a fast increase in the ions' oscillation amplitude and to the eventual ejection of the ions from the ion trap. Since the supplementary frequency was applied selectively to x-electrodes, the ions ejected as a tight packet in the x-direction out of the slits in the x-electrodes.<sup>33</sup> The ejected ions were then directed towards the detectors for detection.



Figure 2.11 A) A greater main RF voltage amplitude increased the  $q_u$  values of the ions. B) When the  $q_u$  value of the ion in purple reached 0.88, a supplementary RF voltage was applied to the x-electrodes. The frequency of motion of the ions whose  $q_u$  value is 0.88 was in resonance with the frequency of the supplementary RF voltage applied to the x-electrodes. C) These ions gained kinetic energy and moved away from the center of the ion trap to exit through the slits in the x-electrodes to the detectors.<sup>23</sup>

#### 2.2.2.6 Ion-Molecule Reactions

Another type of tandem mass spectrometric experiments that is used extensively in our laboratories for structure elucidation of the ions is gas-phase ion-molecule reactions.<sup>17,34–36</sup> Similar to CAD experiments, the ions of interest are first isolated and then allowed to react with neutral reagent molecules introduced into the ion trap through pulsed-valves<sup>37</sup> or a leak valve in a reagent mixing manifold, which was first developed by Gronert and co-workers.<sup>38,39</sup> **Figure 2.12** shows a schematic illustration of the external reagent mixing manifold currently deployed in our laboratories to study ion-molecule reactions. The product ions formed facilitated the elucidation of the analyte structures.



**Figure 2.12** Schematic illustration of the external reagent mixing manifold. The path the helium/reagent mixture traveled to reach the reaction chamber; herein the ion trap, is shown in green. The amount of helium/reagent mixture entering the ion trap of the mass spectrometer was controlled by using a control valve (Granville-Phillips leak valve) and by diverting the excess mixture to exhaust. The standard helium line and the line to the rough pump were closed when the manifold was in use.

The liquid reagent was loaded into a Hamilton syringe that was driven continuously at a specific flow rate by an automated syringe pump (typically 3-10  $\mu$ L hr<sup>-1</sup>). As the reagent was pumped out of the syringe needle, it leaked into the ion trap through the helium line. In order to make sure that gaseous molecules of the reagent reached the ion trap, the syringe port and the surrounding areas of the manifold were heated via a heating tape to an appropriate temperature based on the boiling point of each reagent. The boiling points of the reagents used in this setup did not usually exceed 180 °C. A Granville-Phillips leak valve was used to control the flow of the helium/reagent mixture into the ion trap. The control valve allowed only the specified amount of the helium/regent mixture to reach the ion trap and diverted the excess into an exhaust line. After

completing the experiments, the manifold was isolated from the instrument and baked out to remove any remaining reagent molecules and prevent future contaminations.

During gas-phase ion-molecule reactions, a long-lived collision complex (shown in brackets in the below equation) is formed between a neutral reagent molecule and an ion due to the long-range ion-dipole and/or ion-induced dipole attractive forces, as illustrated below:

$$A^{+} + BC \xleftarrow{k_{c}}{k_{b}} \left[A^{+} ... BC\right]^{*} \xleftarrow{kp}{kp'} \left[AB ... C^{+}\right]^{*} \xrightarrow{k_{b}'} AB + C^{+}$$

where  $k_c$  is the collision rate constant,  $k_b$  is the dissociation rate constant for the formation of separated reactants,  $k_p$  and  $k_p$ ' are the forward and reverse reaction rate constants, respectively, and  $k_b$ ' is the dissociation rate constant for the formation of separated products. In order to rationalize the possible outcomes of ion-molecule reactions, Brauman proposed a double-well potential energy surface model (**Figure 2.13**).<sup>40</sup> According to this model, the long-range dipole and ion-induced dipole forces provide "solvation energy" to the complex and thus lower its potential energy. The collision complex can "borrow" this solvation energy to overcome reaction barriers (transition states) and endothermic reaction steps within the collision complex, but only overall exothermic reactions can take place. The rates of gas-phase ion-molecule reactions are mainly dictated by the energy difference between the separated reactants and the transition states ( $\Delta E$  in **Figure 2.13**). Unlike reactions in solution, the total energy in vacuum is conserved. The height of the central barrier dictates whether the reaction proceeds forward to generate the products or backward to form the reactants.

The transition state that leads to the formation of products is usually "tighter" (entropically less desirable) than that leading to separated products. Therefore, dissociation of the reactant complex to generate separated reactants is often faster than the product formation even when the net reaction is exothermic, and the energy barrier is low. Thus, many gas-phase ion-molecule reactions do not occur at the collision rate ( $k_c$ ).<sup>41</sup>



Figure 2.13 Brauman's double-well potential energy surface for ion-molecule reactions in the gas phase.

#### 2.2.3 Ion Activation to Induce Ion-Source Collision-Activated Dissociation (ISCAD)

Ion-source collision-activated dissociation (ISCAD) was initially developed for declustering ionized proteins in triple-quadrupole mass spectrometers.<sup>42</sup> Later, it was utilized to gain structural information on ions.43,44 The ISCAD experiments conducted for research in this dissertation were different from the CAD experiments in three main aspects. First, no ion isolation was performed to conduct ISCAD; therefore, ISCAD was not considered a tandem mass spectrometry experiment. Second, all ions generated in the ion source were subjected to dissociation. Third, ISCAD did not take place in the ion trap region of the instrument but rather, as the name suggests, in the ion source region. A DC voltage up to 100 V was applied to the multipoles and lenses of the ion optics region so that all the ions generated in the APCI source were accelerated. The pressure between the skimmer region and the first multipole ion optics region of the ion source was about 50 mTorr. The collisions of the ions with the atoms and molecules in the ion optics region converted part of the ion kinetic energy into internal energy and caused them to fragment. ISCAD was previously shown to effectively cleave off all alkyl side chains (sometimes without one methylene group) from the stable cores of the molecular ions of alkyl aromatic hydrocarbon.<sup>45</sup> In the research reported in this dissertation, ISCAD was utilized on molecular ions of alkyl aromatic hydrocarbons to determine the average number of carbon atoms in the alkyl substituents of alkyl aromatic hydrocarbons.

#### 2.3 Orbitrap Mass Analyzer

The general concept of orbital trapping of ions around a central wire stretched along the axis of a cylindrical electrode was first introduced by Kingdon in 1923.<sup>46</sup> In the Kingdon trap, a DC voltage was applied between outer coaxial cylindrical and end-cap electrodes and an inner thin-wire central electrode. This trapping device was not very successful and allowed trapping of singly charged ions only for a few seconds.<sup>46</sup> Knight modified the shape of the Kingdon trap in 1981 in order to introduce an axial quadrupole potential.<sup>47</sup> Although the device had better performance than the Kingdon trap, it did not gain popularity because the ion resonance in both axial and radial directions was considerably weak, broadened and shifted in frequency. Makarov introduced orbitrap mass analyzers in 2000.<sup>48</sup> His design consisted of an outer barrel-like electrode and an inner spindle-like electrode.<sup>48</sup> The orbitrap utilized a dynamic ion trapping method based on a DC field to separate the ions. In orbitraps, dynamic ion trapping can be achieved by using one of three ways: linear trapping, ring trapping or orbital trapping.<sup>49</sup> The electrodes were individually supplied with external voltages.<sup>48</sup> The space between the electrodes comprised the ion trapping region and was connected to a vacuum pump. It is worth noting that orbitraps can be used alone as independent mass spectrometers or can be coupled to other mass spectrometers such as linear quadrupole ion trap mass spectrometers as in the research discussed in this dissertation. A schematic of the linear quadrupole ion trap coupled with an orbitrap detector that was utilized in the research discussed in this dissertation (Thermo Linear Quadrupole Ion Trap/Orbitrap) is shown in **Figure 2.14**. The instrument utilized in this research was equipped with an additional octupole collision cell (commercially known as HCD cell) in which additional fragmentation experiments called medium energy collision-activated dissociation (MCAD) can be performed although they were not performed in the experiments in this dissertation. Details on the aspects of ion transfer into the orbitrap, trajectories of the ions and ion detection in the orbitrap are provided below.



**Figure 2.14** Schematic illustration of an LQIT mass spectrometer coupled with an orbitrap mass analyzer (Thermo LTQ/orbitrap). The pressures in different areas of the instrument are indicated.

#### 2.3.1 Injection of Ions into the Orbitrap

Ions generated in the ion source were transferred into the linear quadrupole ion trap. From this ion trap, they were transferred into an RF-only quadrupole called the C-trap (**Figure 2.14**). The pressure in the C-trap was maintained at 1 mTorr and it contained  $N_2$  gas.<sup>50</sup> The nitrogen gas served as the dampening gas in the C-trap. The ions in the C-trap underwent collisional cooling with the  $N_2$  gas molecules and were as a result axially compressed into the center of the C-trap. The ions were then ejected from the C-trap through the ion optics as a tight ion packet and were accelerated to high kinetic energies before being injected at a position offset from the center of the orbitrap, the ion packet is usually deflected by a set of transfer lenses; since only ions can be deflected, neutral molecules did not enter from the C-trap into the orbitrap.<sup>50</sup>

#### 2.3.2 Trajectories of Ions in the Orbitrap

The outer and inner electrodes of the orbitrap are precisely machined to create a quadrologarithmic field with potential distribution U defined as:

$$U(\mathbf{r},z) = \frac{k}{2} \left( z^2 - \frac{r^2}{2} \right) + \frac{k}{2} (\mathbf{R}_{\rm m})^2 \ln \left[ \frac{r}{\mathbf{R}_{\rm m}} \right] + C$$
(2.16)

where r and z are the axial and horizontal cylindrical coordinates (z = 0 being the plane of symmetry), k is the field curvature,  $R_m$  is the radius of ion of mass m, and C is a constant.<sup>48,51,52</sup> Stable ion trajectories in this quadro-logarithmic field in the orbitrap have three characteristic frequencies, rotational around the central electrode ( $\omega_{\varphi}$ ), radial oscillations ( $\omega_r$ ), and axial oscillations along the z-axis( $\omega_z$ ). The equations of ion motion in polar coordinates ( $\varphi$ , r, z) can be defined as:

$$\frac{\partial^2 r}{\partial t^2} - r \left(\frac{\partial \varphi}{\partial t}\right)^2 = -\frac{q}{m} \frac{k}{2} \left[\frac{R_m^2}{r} - r\right]$$
(2.17)

$$\frac{d}{dt} \left[ r^2 \frac{\partial \varphi}{\partial t} \right] = 0 \tag{2.18}$$

$$\frac{\partial^2 z}{\partial t^2} = -\frac{q}{m}kz \tag{2.19}$$

where m/q is the mass-to-charge ratio of the ions.

Unlike the radial and rotational frequencies, the axial frequency is completely independent of the initial ion parameters, the position and energy of the ions. Therefore, the axial frequency is used to determine the m/q values of the ions. Ions of the same m/q continue to oscillate together along the z-axis while those of different m/q values oscillate off phase as separate rings from end to end along the z-axis of the spindle-like electrode. The ions of the same m/q value remain moving in phase for tens up to hundreds of thousands of oscillations. The axial frequency of oscillation ( $\omega_z$ ) of an ion can be defined as:

$$\omega_z = \sqrt{\left(\frac{q}{m}\right)k} \tag{2.20}$$

where k is proportional to the voltage applied between the inner and outer electrodes.

#### 2.3.3 Detection of Ions in the Orbitrap

Ions with the same m/q value moved as ion packets that exhibited coherent axial oscillations<sup>53,54</sup> (Figure 2.15). The ion rotational frequencies depend on the initial parameters employed, including the angle of entrance, initial positions and energies of the ions. The axial

frequencies of ion packets, each packet containing ions of a specific m/q value, generated measurable image currents and a Fourier transform operation was employed to convert the recorded time-domain data into m/q values and related abundances and therefore producing the mass spectrum.<sup>50</sup>



Figure 2.15 Illustration of the ion injection from the C-trap into the orbitrap in the Thermo Scientific LTQ/orbitrap.

### 2.4 Two-Dimensional Gas Chromatography/Electron Ionization High-Resolution Timeof-Flight Mass Spectrometry (GC×GC/(EI)TOF MS)

Gas chromatography (GC) is a powerful analytical technique for the separation of volatile compounds in complex mixtures. Two-dimensional gas chromatography (GC×GC) has a substantially higher resolving power than one-dimensional gas chromatography.<sup>55</sup> The higher resolution of GC×GC is ascribed to linking two GC columns consisting of different stationary phases. The fractions eluting from the first column are concentrated and introduced as a narrow band into the second GC column wherein the compounds in each band are further separated. The technique is regarded as comprehensive since all the fractions collected from the first column are transferred into the second column. The second column enhances the peak capacity of the

chromatogram by 20-fold as all compounds in a sample can be distributed within an area spanned by the first and second dimension rather than in just one dimension.<sup>56</sup>

In this research, a LECO Pegasus 4D GC×GC/(EI) TOF instrument equipped with an Agilent 7890B two-dimensional gas chromatograph (Agilent technologies, Santa Clara, CA) was used to determine the chemical compositions of the distillates of condensate-like oil samples containing volatile hydrocarbons. A schematic of the GC×GC/(EI)TOF is shown in Figure 2.16.<sup>57</sup> The samples were injected into the GC×GC with an auto injector (Agilent G4513A). The columns employed were a polar 60 m capillary column (ZB-35HT inferno, Zebron) located in the primary oven, followed by a nonpolar 2 m capillary column (ZB-1HT Inferno, Zebron) located in the secondary oven. These columns can withstand temperatures of up to 430 °C. A quad-jet dual stage thermal modulator united the two columns (see section 2.4.1 for details on the modulator). Eluates from the secondary column were ionized by using positive ion mode EI at 70 eV electron kinetic energy. All ions were analyzed by using a medium-resolution folded-flightpath TOF mass spectrometer with a resolution of 25,000 at m/z 219. Measured values included the analyte elution times in the first and second columns, peak areas, measured accurate m/z-values of the analyte molecular ions and their fragment ions, and a list of possible chemical formulas and structures proposed based on a comparison of the measured EI mass spectra to those in the Wiley (2011) and NIST (2011) mass spectral databases. Data collection, processing, and analysis were performed via LECO Visual Basic Scripting (VBS) software, ChromaTOF version 5.10.58.0.52262.



Figure 2.16 Components of the GC×GC/(EI) TOF MS used in this research.<sup>56</sup>

### 2.4.1 GC×GC Chromatograph

The GC×GC system consisted of an auto sampler, a main oven that enclosed the primary column, a secondary oven that enclosed the secondary column, and a thermal modulator that united the primary and secondary columns. The secondary oven was located in a region separated from the primary oven to allow the analyst to use different temperatures in the ovens.

Depending on the goal of the analysis and the components in the sample, the columns can be used in normal or reversed phase configurations. In the normal phase configuration, the first column is nonpolar, and the second column is polar. The reversed phase column configuration, which was used in the research discussed in this dissertation, had first a polar column and then a nonpolar column. This set-up was suitable for separating light saturated branched alkanes and cycloalkanes from each other in crude oil<sup>3</sup> and in petroleum-derived fuels and condensate oils.<sup>58,59</sup> The thermal modulator is a vital component of the  $GC \times GC$  system. The modulator performs three functions: it periodically samples the eluates from the primary column, it traps the eluates inside the modulator by cryo-focusing, and it finally injects the eluates into the secondary column.<sup>60</sup> A critical function of the thermal modulator is preserving the separation of the eluates coming from the primary column before being transferred into the secondary column.

The modulator in the GC×GC system used here was a quad-jet dual stage thermal modulator (**Figure 2.17**). The thermal modulator contained two stages, each with a cold and a hot jet. Liquid nitrogen was used for cooling the cold jets and heated nitrogen gas was used for the hot jets. Each modulation period was composed of four steps: 1) cold jet 1 was opened to allow chilled nitrogen gas to cryo-focus eluates into the first stage of the modulator; hot jet 2 was opened to allow heated nitrogen gas to desorb any cryo-focused eluates from the second stage of the modulator; 2) hot jet 1 was opened to allow heated nitrogen gas to cryo-focus eluates in the first stage of the modulator; cold jet 2 was opened to allow cold nitrogen gas to cryo-focus eluates in the second stage of the modulator; 3) both cold jets 1 and 2 were opened to allow cold nitrogen gas to cryo-focus eluates in the first and second stages of the modulator; 4) cold jet 1 was opened to allow nitrogen gas to continue cryo-focusing the eluates in the first stage of the modulator; hot jet 2 was opened to allow heated nitrogen gas to continue cryo-focusing the eluates from the second stage of the modulator. In the final stage, the eluates desorbed from stage 2 of the modulator and entered the secondary column.



**Figure 2.17** An illustration of the four steps of a modulation period. 1-4: A red circle represents an eluate that can be used as a reference point for the four individual steps. The green labels indicate which cold jet or hot jet was opened during each step. 1) In the first step, cold jet 1 was opened, allowing chilled nitrogen gas to cryo-focus eluates into the first stage of the modulator. Hot jet 2 was opened, allowing hot nitrogen gas to desorb any cryo-focused eluates from the second stage of the modulator. 2) In the second step, hot jet 1 was opened, allowing hot nitrogen gas to desorb eluates in the first stage of the modulator. Cold jet 2 was opened, allowing cold nitrogen gas to cryo-focus eluates in the second stage of the modulator. 3) In the third step, both cold jets 1 and 2 were opened, allowing cold nitrogen gas to cryo-focus eluates in the first and second stages of the modulator. 4) In the final step, cold jet 1 was opened, allowing nitrogen gas to continue cryo-focusing of the eluates in the first stage of the modulator. Hot jet 2 was open, allowing hot nitrogen gas to desorb eluates from the second stage of the modulator. In the final stage, the red circle (eluate reference point) desorbed from stage 2 of the modulator and entered the secondary column.

#### 2.4.2 GC×GC Method Development

To develop a proper GC×GC method for optimal separation of compounds in complex mixtures, several parameters should be adjusted, including the modulation time, the secondary oven offset temperature, temperature ramping rates, the modulator offset temperature and the division of the modulation time between the hot and cold jets. These parameters are discussed below.

#### 2.4.2.1 Modulation Time

The modulation time should be long enough to allow all eluates from the primary column to enter the modulator and then be injected into the secondary column before the second band of eluates emerge from the primary column. If the modulation period is too short, the retention time in the secondary column of an eluate would be greater than the modulation period and the compounds may co-elute with other compounds emerging from the next modulation period, a phenomenon referred to as the wrap-around. If the modulation period is too long, the separation of compounds in the primary column could be lost and the secondary column may be overloaded, which would decrease the peak capacity of the column. The peak capacity refers to the maximum number of analytes that can be separated with a column.<sup>61</sup> The modulation period was separated into two stages as shown in **Figure 2.18**; a stage with hot and cold jet pulses for each column.



Figure 2.18 An example of a modulation period of 3.5 seconds that was split between two stages, 1.75 seconds each, each with hot and cold pulses of 0.6 seconds and 1.15 seconds, respectively.

#### 2.4.2.2 Hot and Cold Jet Pulse Times During Modulation

The hot and cold jet pulse times refer to how long the hot and cold jets pulse hot and cold  $N_2$  gas into the first and second modulation stages. Volatile analytes get cryo-focused when the cold jet is pulsed and desorb from the column surface when the hot jet is pulsed. Longer hot jet pulse times give sharp peaks and results in better resolution in the secondary column. However, if it is too long, analytes may not get adequately cryo-focused, resulting in coelution of analytes. If the hot jet pulse time is too short, the analytes may not have enough time to desorb before the next modulation period, resulting in wrap-around of the analytes.

#### 2.4.2.3 Modulator Offset Temperature

The modulator offset temperature is the temperature difference between the secondary oven and the modulator. The modulator temperature influences the temperature of the  $N_2$  gas coming from the hot jets. A greater modulator offset temperature gives sharper peaks in the GC×GC chromatogram. Therefore, it is highly recommended to use a higher modulator offset temperature as long as it does not exceed the maximum operational temperature of the column, which was 430 °C for the columns used in this dissertation research.

#### 2.4.2.4 Secondary Oven Offset Temperature

The temperature difference between the primary and secondary ovens is referred to as the secondary oven offset temperature. A high offset temperature could decrease the eluate retention times and the peak resolution because the eluates would travel faster through the secondary column. However, if the analytes were strongly retained in the secondary column, it may be necessary to increase the secondary oven offset temperature to prevent wrap-around of the analytes.

#### 2.4.2.5 Temperature Ramp Rate

Temperature ramp rate is the rate at which the temperature is increasing with time in the primary and secondary ovens and in the modulator. At high ramp rates, the temperature increases faster, and the analytes move faster through the columns and modulator and hence their retention

times are shorter. This decreases the resolution of the chromatographic separation in both columns and might result in coelution of analytes.

#### 2.4.3 Folded Flightpath (FFP) Time-of-Flight Mass Spectrometer (TOF MS)

GC×GC system detectors have to have fast acquisition rates. This is why time-of-flight mass analyzers are often used as the detectors. The TOF MS used in this research has a folded-flightpath (FFP) design that uses nonlinear electrostatic fields in gridless mirrors to provide a long flight path for the ions to travel through before they strike the detector. The ions were refocused by central lenses before moving into the next electrostatic mirror. The FFP design minimized ion loss and improved the resolution of the instrument.<sup>62</sup> Flight paths of 20 m and 40 m (when the ions travel through the folded-flight-path twice) had resolutions of approximately 25,000 and 50,000, respectively.

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# CHAPTER 3. UTILIZATION OF THE DISTILLATION, PRECIPITATION, FRACTIONATION MASS SPECTROMETRY (DPF MS) METHOD FOR THE DETERMINATION OF THE CHEMICAL COMPOSITION OF UNCONVENTIONAL CONDENSATE-LIKE OIL SAMPLES WITH DIFFERENT API GRAVITIES

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#### 3.1 Introduction

The worldwide decline in API gravity of crude oil in the last few decades, has introduced challenges during the transportation, separation, and processing of crude oil.<sup>1,2</sup> The API gravity is used as a measure of the crude oil density, and according to the definition of the American Petroleum Institute, API gravity is a measure of the density of the crude oil relative to that of water at 60  $^{\circ}$ F;<sup>3</sup> the denser the crude oil, the lower is its API gravity.<sup>4,5</sup> One of the solutions to lower the viscosity of heavy crude oil, to improve its mobility, and thereby tackle the challenges faced during crude oil transportation and processing is crude oil dilution by blending with lighter crude oil and/or condensate oils.<sup>6</sup>

Condensate oils exist in natural gas reservoirs, but are recovered as liquids in gasprocessing plants.<sup>7</sup> Condensate oils are unconventional liquid hydrocarbons that are composed of highly volatile and light hydrocarbons.<sup>8</sup> Blending a heavy crude oil with condensate oils has been shown more efficient in lowering the viscosity of a heavy crude oil than blending with a light crude oil.<sup>9</sup> Possibly, due to the lower viscosity of condensate oils compared to light crude oil.<sup>9</sup> The API gravities of condensate oils depend on their chemical composition as well as the temperature and the pressure at which they were recovered and hence, can range between 45-75 °API,<sup>10</sup> while crude oils with API gravities greater than 31.1 °API are categorized as light crude oils.<sup>11</sup>

Although both condensate oils and light crude oils can be used as diluents for heavy crude oil dilution,<sup>8</sup> the stability of the crude oil blend, i.e. the rate of asphaltenes precipitation, depends on the chemical composition of the diluent.<sup>1</sup> Particularly, the presence of polar heteroaromatic compounds in the diluent can prevent the flocculation of the asphaltenes in the heavy crude oil.<sup>1</sup> Generally, the presence of a higher concentration of light hydrocarbons and heteroaromatic compounds in the diluent, prevents the aggregation of asphaltenes in heavy crude oils.<sup>7,9</sup> This is

partially due to the ability of the polar heteroaromatic compounds to form hydrogen bonds that disrupt the asphaltene/asphaltene interactions.<sup>9-12</sup>

Knowing the chemical composition of hydrocarbon products can help in predicting their physical properties,<sup>13</sup> and since the exact chemical compositions of condensate oils are still largely unknown,<sup>14</sup> such predictions can be faulty. Hence, the rational selection of a condensate oil for crude oil blending is complicated, as the physical properties of the condensate oil and/or the crude oil blend cannot be easily predicted.<sup>15</sup> In most previous studies, condensate oil samples have been analyzed, without fractionation, by using two-dimensional gas chromatography coupled with a flame ionization detector (FID).<sup>16,17</sup> Unfortunately, the API gravities of the tested samples were not reported.<sup>16</sup> In one study, the compounds in a condensate oil sample were fractionated into different combinations of compound classes by using what is known in petroleum industries as the SARA method; the saturated, aromatic, resins and asphaltenes method.<sup>6</sup> By mass, 81.43% of the compounds found in the condensate oil were saturated hydrocarbons, followed by 15.39% naphthenic compounds, while aromatic compounds corresponded to only 3.11%. Olefins and undefined compounds corresponded to 0.07%.<sup>6</sup> However, it was previously shown by our group that the fractions obtained by using the SARA method are not separate chemical compound classes but rather complex mixtures of compounds. For example, our group found that the fraction referred to as saturated hydrocarbons obtained by the SARA method contains a large amount of aromatic compounds.18

A new method called the distillation, precipitation, fractionation mass spectrometry method (DPF MS) was developed in our laboratories in 2018, for the fractionation and detailed characterization of crude oils.<sup>18,19</sup> This method involves the separation of crude oil constituents into six chemical compound classes namely, volatile hydrocarbons, asphaltenes, heavy saturated hydrocarbons, alkyl aromatic hydrocarbons, heteroaromatic compounds and polar compounds.<sup>18</sup> After obtaining each compound class, it is then analyzed by using an optimized mass spectrometric method. In this study, the DPF MS method was utilized to determine the types of compounds in five condensate-like oil samples. The analyses involved the gravimetric wt% of each compound class, the average molecular weight (MW) and ring and double bond equivalent (RDBE) values for compounds in each compound class and in the entire condensate-like oils.

Collision-activated dissociation (CAD) was deployed in a plethora of literature reported studies for the structural elucidation of ionized aromatic compounds in heavy petroleum products.<sup>20</sup> In many among these studies, dealkylation was reported as the prevailing fragmentation pathway for ions containing stable aromatic cores.<sup>20-23</sup> However, CAD experiments require prior knowledge of the m/z values of the ions of interest, as the ions are first isolated in the ion trap and then subjected to collision energies with the buffer gas.<sup>24</sup> A benefit of using in-source collision-activated dissociation (ISCAD) is that prior knowledge of the m/z values of the ions of interest is not required because ISCAD is applied to all the ions generated in the ion source and no ion isolation step is involved in the process. Therefore, ISCAD was utilized in this study to determine the average alkyl chain length of the compounds in the alkyl aromatic hydrocarbon class.

Many research groups have performed attempts to find possible correlations between the bulk physical properties of crude oils, including the density, and their chemical composition.<sup>19,25,26</sup> Correlations between the API gravity and the chemical compositions of the oil samples are of interest, as the API gravity is a major parameter for the classification, quality assessment and market price assignment in petrochemical inductries<sup>27,28</sup>.

The samples selected for this study were unconventional, they had properties of both condensate oils and light crude oils. Like condensate oils, they were recovered from natural gas fields and had a condensate oil-like appearance. However, their API gravities were within the light crude oil API range. For these reasons, in this chapter these samples will be referred to as condensate-like oil samples.

#### **3.2** Materials and Methods

#### 3.2.1 Materials

*n*-Hexane (HPLC grade), dichloromethane (HPLC grade), 2-propanol (HPLC grade), and carbon disulfide (>99.9%) were purchased from Sigma-Aldrich. High purity *n*-pentane (>99%) was obtained from Acros-Organics. **Table 3.1** provides names and mass purities of the 16 model compounds used in this study that were purchased from Sigma-Aldrich. All chemicals were used as received without further purification. Whatman PTFE membrane filters (TE 36) were purchased from GE Healthcare Life Sciences. Solid phase extraction kit (Si/CN-S-1.5 g) was purchased from Interchim. RediSep Rf Gold normal phase silica flash columns (40 g) were purchased from Teledyne Isco. The condensate-like oil samples (37.50, 41.61, 41.62, 42.40 and 43.00 °API), referred to as 1 - 5, respectively, throughout the chapter, were generously provided by Chevron

corporation. The sample 3 (41.62 °API) originated from the Appalachian Basin, a region that encompasses the north eastern seaboard of the United States and the sample 2 (41.61 °API) originated from the Permian Basin located in the southwestern part of the United States. The samples 1 (37.50 °API), 4 (42.40 °API) and 5 (43.00 °API) all originated from the Western Canadian Sedimentary Basin. The API gravity values were determined using equation 3.1:<sup>29</sup>

$$API = \frac{141.5}{d_r(\frac{60}{60})} - 131.5 \tag{3.1}$$

where  $d_r$  is the relative density of the crude oil with respect to water, measured at 60 °F. Measurements of  $d_r$  were performed using an SVM 3001 Stabinger Viscometer (Anton Paar).

Table 3.1 List of Sigma-Aldrich obtained model compounds and their percent mass purities.

I

Model compounds							
Chemical	Percent mass purity						
<i>n</i> -hexylamine	99%						
Diethyl disulfide	99%						
Ethyl benzoate	$\geq$ 99%						
Butyl sulfone	99%						
3-ethylcarbazole	97%						
1,3-diphenylisobenzofuran	97%						
2-methylthiophene	98%						
Cholestane	$\geq 97\%$						
Nonylcyclohexane	95%						
Decylcyclohexane	95%						
Heptadecylbenzene	95%						
p-xylene	$\geq 99\%$						
1,3,5-isopropylbenzene	95%						
Hexaethylbenzene	95%						
1,3,5-triethylbenzene	$\geq 97\%$						
Indane	95%						

#### 3.2.2 Methods

## 3.2.2.1 Separation of The Condensate-Like Oil Samples into Fractions of Different Chemical Compound Classes

The DPF method<sup>18</sup> was performed on the five condensate-like oils of different API gravities to fractionate them into six major compound classes: volatile hydrocarbons, asphaltenes, heavy saturated hydrocarbons, alkyl aromatic hydrocarbons, heteroaromatic compounds and polar compounds. The first step was room temperature vacuum distillation during which the sample was placed in a round bottom flask connected to a distillation setup that is held under vacuum by connecting it to a rough pump. In the distillation setup no heat was provided and the volatile hydrocarbons that typically boil at temperatures lower than 170 °C, including volatile cyclic and naphthenic compounds, were evaporated, condensed and collected in a receiving flask, immersed in acetone-dry ice bath, connected to the end of the distillation setup. After removal of the volatile compounds, the asphaltenes were precipitated by mixing the sample with ten times its volume nhexane.<sup>30</sup> The sample-*n*-hexane mixture was sonicated for 20 minutes and then left undisturbed overnight in a tightly closed flask to allow the asphaltenes, if present, to precipitate.<sup>28</sup> In the next morning, the sample-n-hexane mixture was filtered through a 0.45 µm Whatman PTFE cellulose membrane filter to collect the asphaltenes. Note: nylon or synthetic plastic membrane filters should not be used since they dissolve in *n*-hexane and might contaminate the sample and complicate the results. In this study, none of the condensate-like oil samples contained a detectable amount of asphaltenes. Before the next step, n-hexane was removed from the sample by means of rotary evaporation at 30 °C. 500 mg of the remaining filtrate, referred to as maltenes, was chromatographically fractionated into three compound classes based on their solubility in three different solvents, n-hexane, dichloromethane and 2-propanol by using a Combi-flash RF 200 auto column system (Teledyne Isco, Inc). The n-hexane-eluted fraction contained heavy saturated, cyclic and alkyl aromatic hydrocarbons, while the dichloromethane and the 2-propanol fractions contained the heteroaromatic and polar compounds, respectively. Finally, since the *n*-hexaneeluted fraction is a complex mixture of saturated and small alkyl aromatic hydrocarbons, it was simplified through further fractionation by using gravitational solid phase extraction in a Si/CN-S-1.5 g solid phase cartridge (Interchim) to obtain the saturated hydrocarbons and the alkyl aromatic hydrocarbons separately. The gravimetric wt% of each recovered compound class was determined relative to the mass of the sample subjected to fractionation.

Sample loss during transfer and processing was inevitable. Potential reasons for sample loss during processing include the incomplete elution of compounds from the auto column system or the solid phase extraction cartridge with the solvents used. In the future, it would be useful to conduct further studies on the improvement of the DPF method to reduce sample loss. The sample losses were determined after each step and they ranged from 10 - 13%, which was similar to the values reported in the literature by our group (8% - 17%) when the DPF method was used to fractionate crude oil samples of different API gravities.<sup>18,19</sup> Therefore, we do not think that the losses observed in this study were sample type related.

# 3.2.2.2 Optimized Linear Quadrupole Ion Trap/Orbitrap APCI Mass Spectrometry (LQIT/Orbitrap APCI MS)

All obtained compound classes except the volatile hydrocarbons class, were analyzed using a Thermo Scientific LQIT/orbitrap HRMS coupled to an atmospheric pressure chemical ionization (APCI) source. The APCI conditions used were as previously described.<sup>18</sup> For the chemical class containing heavy saturated hydrocarbons, *n*-hexane was used as the solvent and APCI reagent, while oxygen was employed in place of nitrogen as the sheath and auxiliary gas ((+) APCI/ $O_2/n$ hexane). It is worth noting here, that special care should be taken while performing these experiments since all requirements for the generation of flame are present, i.e. fuel, oxygen and the electric current from the APCI needle. The ((+) APCI/O<sub>2</sub>/n-hexane) conditions are known to predominantly produce [M-H]<sup>+</sup> ions due to protonation of the saturated hydrocarbons followed by elimination of H<sub>2</sub>.<sup>31,39</sup> For the chemical classes containing alkyl aromatic hydrocarbons and heteroaromatic compounds, CS<sub>2</sub> was used as the solvent and APCI reagent, while nitrogen was used as the sheath and auxiliary gas ((+) APCI/N<sub>2</sub>/CS<sub>2</sub>). These conditions mostly produce stable molecular ions [M<sup>+•</sup>]. Polar compounds were ionized in the APCI source by using 3:1 methanol:*n*hexane solution as the solvent/reagent and N2 as the sheath and auxiliary gas ((+) APCI/N2/3:1 methanol:*n*-hexane). This approach predominantly generates stable [M+H]<sup>+</sup> ions for the polar analytes. Methanol and *n*-hexane are immiscible solvents; however, once the polar compounds are added to the solvent mixture, they become completely miscible. These optimal APCI conditions for each compound class were used to achieve optimum ionization while avoiding substantial fragmentation.<sup>18</sup> 30 mg mL<sup>-1</sup> of each compound class fraction was dissolved in the appropriate solvent and the solution was introduced into the APCI source through direct injection by using a 500 µL Hamilton syringe at the specific flow rates previously reported; in details, 10, 3, 3 and 1 µL min<sup>-1</sup> for the saturated hydrocarbons, alkyl aromatic hydrocarbons, heteroaromatic compound and polar compound classes, respectively.<sup>18</sup> All ions were transferred into the orbitrap for high-resolution analysis (100,000 resolution at m/z 400). Average mass spectra from 20 microscans were collected in each experiment. Mass calibration and tuning were conducted on regular basis by using Pierce LTQ ESI positive ion calibration solution (covers m/z range 150-2000; obtained from Thermo Scientific) and Agilent LC/MS tuning mix for APCI (covers m/z range 50-2000). The mass accuracies of the calibration reagent ions are provided in **Table 3.2A** and **Figure 3.1A**.

 Table 3.2 Positive-ion mass calibration: A) LQIT/orbitrap MS by using Pierce LTQ ESI

 positive-ion calibration solution. B) GC×GC/TOF MS by using perfluorotributylamine (PFTBA) as the calibration reagent.

A) LQIT/orbitrap MS Mass Calibration Table								
Expected $m/z$	Observed $m/z$	Error in measured $m/z$ (ppm)						
195.08762	195.08763	0.05126						
524.26496	524.26494	-0.03815						
1121.99702	1121.99805	0.91801						
1221.99064	1221.99043	-0.17185						
1321.98425	1321.98430	0.03782						
1421.97786	1421.97708	-0.54853						
1521.97148	1521.97283	0.88701						
1621.96509	1621.96573	0.39458						
1721.95870	1721.95769	-0.58654						
1821.95231	1821.95404	0.94953						
B) GC×GC/TOF MS Mass Calibration Table								
Expected $m/z$	Observed $m/z$	Error in measured $m/z$ (ppm)						
68.99	68.99	- 0.05						
118.99	118.99	0.25						
130.99	130.99	- 0.17						
218.99	218.99	- 0.36						
263.99	263.99	0.24						
413.98	413.98	0.36						
501.97	501.97	0.01						



**Figure 3.1** A) LQIT/orbitrap FTMS spectrum of the positive ions detected in the Pierce LTQ ESI calibration solution. B) GC×GC/TOF MS spectrum of perfluorotributylamine (PFTBA) calibration reagent.

To determine the average chain length, the average total number of carbons, in the alkyl side chains of the alkyl aromatic hydrocarbons, in-source collision-activated dissociation (ISCAD) was deployed to cleave off the alkyl side chains from the aromatic cores of the molecular ions. For ISCAD, 100 V electric potential was applied to the multipoles and lenses of the ion optics so that all the ions generated in the APCI source were accelerated without the need for prior ion isolation.<sup>19</sup> The collision of the ions with the atoms and molecules in the ion optics region of the LQIT held at 50 mTorr, caused them to fragment. ISCAD can effectively cleave off all alkyl side chains (sometimes without one methylene group) from the stable cores of the molecular ions of alkyl aromatic hydrocarbons.<sup>32,33</sup>

Xcalibur software was used for data interpretation including the assignment of chemical formulas for all detected ions based on their accurately measured masses. The general elemental formula that was used in Xcalibure for the detected ions in the condensate-like oil samples was  $C_cH_hN_nO_oS_s$  where c and h are positive non-zero integers and n, o and s are positive integers from zero to three.<sup>11</sup> Compound formulas with mass accuracies between -10 ppm and 10 ppm were considered. Data were exported to Microsoft Excel 2010 for further data analysis. Equation 3.2 was inputted to Microsoft Excel in order to determine the ring and double bond equivalent (RDBE) values for all the detected ions:<sup>5</sup>

$$RDBE = c - \frac{h}{2} + \frac{n}{2} + 1 \tag{3.2}$$

where *c*, *h* and *n* are the number of carbon, hydrogen and nitrogen atoms in the ion, respectively.

### 3.2.2.3 Medium-Resolution Two-Dimensional Gas Chromatography/Time-of-Flight Electron Ionization Mass Spectrometry (GC×GC/(EI)TOF MS)

The distillates obtained from the first DPF step, containing the volatile hydrocarbons, were analyzed using a LECO Pegasus 4D GC×GC/(EI)TOF instrument equipped with an Agilent 7890B two-dimensional gas chromatograph (Agilent technologies, Santa Clara, CA). A method was developed for the analysis of the volatile hydrocarbons.<sup>19</sup> In detail, the volatile hydrocarbons were dissolved in high purity *n*-pentane (>99%) to generate solutions of 1.0 wt/wt% concentration. An auto sampler (Agilent G4513A) was used to inject 0.5 µL of the solutions into a split/splitless injector with a split ratio of 1:20 that was held at 260 °C under a constant flow of helium carrier gas (1.25 mL min<sup>-1</sup>). The compounds were separated using two capillary columns; polar 60 m capillary column (ZB-35HT inferno, Zebron) followed by a nonpolar 2 m capillary column (ZB-1HT Inferno, Zebron). These columns can withstand temperatures up to 430 °C. Note: high temperature ferrules should be installed to be able to operate at higher temperatures than 200 °C. The primary oven contained the polar column and was set at 35 °C and was held at that temperature for one minute. After that, the oven was heated to 55 °C at a rate of 1 °C min<sup>-1</sup>, followed by heating at a rate of 3 °C min<sup>-1</sup> to a maximum temperature of 175 °C. The oven was held at the maximum temperature of 175 °C for 3 min. A quad-jet dual stage thermal modulator placed between the two columns consisted of two hot jets and two cold jets, one hot and one cold jet pair for each stage

(details were provided in chapter 2). Liquid nitrogen was used for cooling the cold jets and heated nitrogen gas was used for heating the hot jets. The total modulation time was 3.5 s which was split equally between the two stages (1.75 s each), 0.6 s of which was used for the hot jet pulse and 1.15 s for the cold jet pulse; as shown in Figure 2.18 in chapter 2. The modulator temperature was 80 °C greater than the temperature of the secondary oven containing the nonpolar column. The secondary oven offset was set at a temperature 10 °C greater than that of the primary oven and was subjected to the same temperature program as the primary oven. The transfer line between GC×GC and the TOF MS was held at 300 °C throughout the analysis. The MS ion source was heated to 250 °C. The eluting compounds were ionized by EI using 70 eV electron kinetic energy. All ions were transferred into a TOF MS for medium-resolution analysis (25,000 resolution at m/z 219). Mass spectra were collected at an acquisition rate of 200 Hz. Mass calibration and tuning were conducted daily before performing any experiments by using perfluorotributylamine (PFTBA) as the calibration reagent. The mass accuracies of the calibration reagent ions are provided in **Table 3.2B** and Figure **3.1B**. Data collection, processing, and analysis were performed via LECO Visual Basic Scripting (VBS) software, ChromaTOF version 5.10.58.0.52262. Wiley (2011) and NIST (2011) mass spectral databases were used for compound identification. The peak picking criteria were: a signal to noise ratio threshold of  $\geq 25$  and a library match factor threshold of > 800 were used.18,19,34

# 3.2.2.4 The Average Molecular Weight (MW) Determination for Compounds in The Five Condensate-Like Oil Samples

In this study, the average MW of compounds in the entire condensate-like oils were obtained by substituting the average MW determined for the compounds in the individual compound classes and their gravimetric wt% in equations 3.3-3.7 which are based on the major ion types generated in the mass spectrometric analysis methods.<sup>18</sup> Equation 3.3 was used to determine the average MW for heteroaromatic compounds and alkyl aromatic hydrocarbons, equation 3.4 was used for the heavy saturated hydrocarbons and equation 3.5 was utilized to determine the experimental average molecular weight of polar compounds. The average MW of the volatile hydrocarbons analyzed by using GC×GC/(+)EI TOF MS was determined by using equation 3.6 below.<sup>18</sup> Due to EI induced fragmentation, most of the compound ions detected in the volatile hydrocarbons fraction did not include a molecular ion; therefore, the ChromaTOF software

was used to predict the molecular ion m/z values based on the compound identification obtained from the EI mass spectral library match. The overall average MW of compounds in the condensatelike oil was obtained from substituting the average MW values obtained from equations 3.3-3.6 in equation 3.7 below.

Experimental Avg MW = 
$$\frac{\sum((ion m/z) \times abundance)}{\sum all abundances}$$
 (3.3)

Experimental Avg MW = 
$$\frac{\sum(((ion m/z) + 1) \times abundance)}{\sum Abundances}$$
 (3.4)

Experimental Avg MW = 
$$\frac{\sum(((ion m/z) - 1) \times abundance)}{\sum Abundances}$$
 (3.5)

Experimental Avg MW = 
$$\frac{\sum (molecular \ ion \ m/z \ \times \ GC \ peak \ area)}{\sum GC \ peak \ area}$$
 (3.6)

$$Overall Avg MW of compounds in a condensate - like oil = \frac{\sum(Average MW of compounds in each individual fraction \times wt\%)}{\sum(wt\%)}$$
(3.7)

#### 3.3 Results and Discussion

The distillation, precipitation, fractionation mass spectrometry (DPF MS) method<sup>18</sup> was used for the detailed characterization of five condensate-like oil samples with different API gravities. This method involved separating the compounds in the condensate-like oil samples into six classes: volatile hydrocarbons, asphaltenes, heavy saturated hydrocarbons including cyclic hydrocarbons, alkyl aromatic hydrocarbons, heteroaromatic compounds, and polar compounds. No measurable amounts of asphaltenes were recovered in the filtration step for all the condensate-like oil samples. Therefore, only five compound classes were obtained from each condensate-like oil sample, which were analyzed by using the optimized high-resolution mass spectrometric analysis methods. Below, is a discussion of the characteristics of the condensate-like oil samples including the gravimetric wt% of the compound classes and their chemical compositions. Further, correlations between the chemical compositions of compounds in the condensate-like oil samples and their API gravities were examined. A linear correlation was assigned only if the goodness of the fit, R<sup>2</sup> value, was  $\geq 0.9$ . This is followed by a discussion on the average molecular weight and

ring and double bond equivalent values of the compounds in the different compound classes and the average chain lengths of the alkyl substituents in the alkyl aromatic hydrocarbon class.

# **3.3.1** Average wt% Determination for the Compounds in The Different Compound Classes in The Five Condensate-Like Oil Samples

After fully fractionating the condensate-like oil samples, the mass of the compounds in each class was determined and the mass percentage, average gravimetric wt%, of compounds was obtained by dividing the mass of the compounds in the individual compound class by the mass of all compounds in the sample subjected to fractionation; multiplied by 100%. The experiments were performed in duplicates and the results reported in this chapter were highly reproducible. Detailed data and statistical analyses of the averages (avg.), standard deviations (SD) and 95% confidence intervals (95% CI at  $\alpha = 0.05$ ) are provided in **Table 3.3**. For all statistical analyses reported herein, the data were assumed to be normally distributed.<sup>35,3</sup>

-	Gravimetric weight percent (wt %)										
-	5 (43 00 ° API)				$\frac{g_{\text{III}}}{4(42.40 \circ A \mathbf{D})}$						
Chemical Classes	$\frac{5 (45.00 \text{ Arr})}{T1 \text{ T2 SD Arg 05% CI}}$			$\frac{1}{1} \frac{4 (42.40 \text{ Art})}{1}$							
Volatile Hydrocarbons	0.76	0.76	0.00	0.76	)5% CI	1 31	1 31	0.00	1 31	)570 CI	
Heterogrometic Compounds	0.70	0.70	0.00	0.70	0 37 0 53	0.59	0.63	0.00	0.61	0.58 0.64	
Polar Compounds	0.41	0.49	0.00	0.45	0.57-0.55	1 30	0.05	0.02	1.06	0.58-0.04	
Saturated Hydrocarbons	81 08	81.00	0.02	81 04	80.96.81.12	78 44	77.69	0.32	78.06	77 53 78 50	
Alley Aromatic Hydrocarbons	7.02	6.68	0.00	6 96	6 51 7 21	6.05	71.09	0.56	6.61	5 92 7 20	
Aikyi Aloinatic Hydrocarbons	10.55	0.08	0.23	0.80	0.31-7.21	0.05	12.44	0.50	12 22	J.03-7.39 12 19 12 49	
LOSS	10.55	10.87	0.25	10.71	10.39-11.03	12.22	12.44	0.11	12.55	12.10-12.40	
10tai	100.00	100.00	0.00	100.00	-	100.00	100.00	0.00	100.00	-	
-	Gravimetric wei				Gravimetric weig	ight percent (wt %)					
	3 (41.62 °API)				2 (41.61 °API)						
Chemical Classes	T1	Τ2	SD	Avg.	95% CI	T1	Τ2	SD	Avg.	95% CI	
Volatile Hydrocarbons	1.64	1.64	0.00	1.64	-	1.45	1.45	0.00	1.45	-	
Heteroaromatic Compounds	1.02	1.00	0.01	1.01	1.00-1.02	0.91	1.09	0.10	1.00	0.86-1.14	
Polar Compounds	0.28	0.22	0.04	0.25	0.19-0.31	0.81	0.91	0.05	0.86	0.79-0.93	
Saturated Hydrocarbons	76.10	75.74	0.25	75.92	75.57-76.27	76.55	76.16	0.20	76.36	76.08-76.64	
Alkyl Aromatic Hydrocarbons	13.21	10.93	1.61	12.07	9.84-14.30	8.10	8.64	0.27	8.37	8.00-8.74	
Loss	7.76	10.47	1.92	9.11	6.45-11.77	12.19	11.75	0.22	11.97	11.67-12.27	
Total	100.00	100.00	0.00	100.00	-	100.00	100.00	0.00	100.00	-	
	Gravimetric weight percent (wt %)										
	1 (37.50 °API)										
Chemical Classes	T1	T2	SD	Avg.	95% CI						
Volatile Hydrocarbons	2.07	2.07	0.00	2.07	-						
Heteroaromatic Compounds	3.27	3.11	0.11	3.19	3.04-3.34						
Polar Compounds	1.68	1.49	0.13	1.59	1.41-1.77						
Saturated Hydrocarbons	67.81	71.89	2.88	69.85	65.86-73.84						
Alkyl Aromatic Hydrocarbons	10.81	9.83	0.69	10.32	9.36-11.28						
Loss	14.35	11.61	1.94	12.98	10.29-15.67						
Total	100.00	100.00	0.00	100.00	-						

**Table 3.3** Gravimetric weight percentages of the five different compound classes obtained using the DPF method for five condensate-like oil samples. Each experiment was performed twice. API gravities are shown in parenthesis.

For all five condensate-like oil samples, when the average gravimetric wt% of different types of compounds were considered, the heavy saturated hydrocarbons corresponded to the majority of the compounds (**Table 3.3**; 81.0% in the lightest condensate-like oil sample 5 (with the greatest API gravity) vs. 69.9% in the heaviest condensate-like oil sample 1 (with the lowest API gravity)), the second most abundant compound class was the alkyl aromatic hydrocarbons (**Table 3.3**, 6.9% in the lightest condensate-like oil sample 5 vs. 10.3% in the heaviest condensate-like oil sample 1. The gravimetric wt% of compounds in the volatile hydrocarbon, heteroaromatic compound and polar compound classes were each found to be less than 4.0%. Similarly, in a previous study on light crude oil samples conducted by our group, the largest constituent was the heavy saturated hydrocarbons with wt% of (44.3-48.2%) followed by alkyl aromatic hydrocarbons with a wt% of (17.5-21.2%), while the volatile saturated hydrocarbons, heteroaromatic compounds and polar compounds were all with wt% values lower than 10.0%.<sup>19</sup>

Plots of the average gravimetric wt% of compounds in each compound class in the five condensate-like oil samples as a function of their API gravities were generated in OriginPro software. Linear correlations were only found for the heteroaromatic compound and the heavy saturated hydrocarbon classes (**Figure 3.2**). The heavier condensate-like oil samples with smaller API gravities contained more heteroaromatic compounds ( $R^2 = 1.00$ ; **Figure 3.2A**) and less heavy saturated hydrocarbons ( $R^2 = 0.94$ ; **Figure 3.2B**) than the condensate-like oil samples with greater API gravities. A similar correlation between the API gravity and average gravimetric wt% of heteroaromatic compounds obtained by using the DPF method on heavy, medium and light crude oil samples was previously reported.<sup>19</sup> Additionally, condensate-like oil sample 1 with the lowest API gravity; 37.50 °API, contained more alkyl aromatic hydrocarbons than the other condensate-like oil samples. Generally, aromatic compounds are denser that saturated hydrocarbons of the same molecular weight. Thus, heavier crude oils tend to contain more aromatic compounds and less saturated hydrocarbons than lighter crude oils.<sup>37</sup>


Figure 3.2 A) Plot showing the inverse correlation between the average wt% of the class of heteroaromatic compounds as a function of the API gravity for five different condensate-like oil samples. B) Plot showing the direct correlation between the average wt% of compounds in the saturated hydrocarbon class as a function of API gravity for five different condensate-like oil samples.

# 3.3.2 Determination of the Elemental Compositions of Compounds in The Five Condensate-Like Oil Samples

For all the compound classes derived from the five condensate-like oil samples, highresolution mass spectrometry measurements were obtained in order to derive semi-quantitative information on the percentage of compounds with different elemental composition types in each class and in the whole condensate-like oil sample (data and statistics are reported in **Table 3.4**). Semi-quantitative measurements were based on the assumption that the ionization methods utilized in this study resulted in minimal fragmentation of the ions and that compounds belonging to a specific compound class have similar ionization efficiencies.<sup>18</sup> Although studies from our group have previously provided support to this assumption, (**Figure 3.3**). The consolidated data for each compound class to further support this assumption, (**Figure 3.3**). The consolidated data for the percent distribution of compounds with each elemental composition type in the whole condensate-like oil samples (**Table 3.4**) were determined by substituting the ion abundances of the compounds belonging to a certain elemental composition type in the individual compound classes and the gravimetric wt% of the compounds in each compound class in equation 3.8:<sup>19,38</sup>

The consolidated wt% of compounds with a certain elemental composition type

$$= \sum_{x=1}^{5} \left( \frac{\Sigma i_x \, abundance}{\Sigma x \, abundance} * wt\%(x) \right)$$
(3.8)

where x is one of the five compound classes in each condensate-like oil sample, *i* is a certain elemental composition type of an ionized compound,  $\Sigma x$  abundance is the total ion abundance for all compounds in each compound class and wt%(x) is the gravimetric wt% of the compounds in that class in the condensate-like oil sample.

Figure 3.3 Low-resolution, positive ion-mode mass spectra of equimolar (1 mM) mixtures of model compounds. Model compounds in red were analyzed in a previous study.<sup>18</sup> A)
APCI/N<sub>2</sub>/n-hexane, methanol (25:75 v/v) mass spectrum of model compounds representing the polar compound class. Peak labelled with a green asterisk (\*) at *m/z* 65 was due to protonated dimers of methanol and peak labelled with a black asterisk at *m/z* 85 was due to protonation of hexane followed by elimination of H<sub>2</sub>. B) APCI/N<sub>2</sub>/CS<sub>2</sub> mass spectrum of model compounds representing the heteroaromatic compound class. Peak labelled with a blue asterisk at *m/z* 76 was due to molecular ions of CS<sub>2</sub>. C) APCI/O<sub>2</sub>/n-hexane mass spectrum of model compounds representing the saturated and cyclic hydrocarbon class. Peak labelled with a red asterisk was due to an impurity and peak labelled with a black asterisk at *m/z* 85 was due to protonation of hexane followed by elimination of H<sub>2</sub>.<sup>39</sup> D) APCI/N<sub>2</sub>/CS<sub>2</sub> mass spectrum of model compounds representing the alkyl aromatic hydrocarbon class. Peak labelled with a blue asterisk at *m/z* 76 was due to an impurity and peak labelled with a black asterisk. Peak labelled with a black asterisk at *m/z* 85 was due to protonation of hexane followed by elimination of H<sub>2</sub>.<sup>39</sup> D) APCI/N<sub>2</sub>/CS<sub>2</sub> mass



Compounds with the elemental composition type  $C_cH_h$  were the most abundant (57%-77%) in all the condensate-like oil samples (**Table 3.4**). A similar finding was reported for the elemental composition type  $C_cH_h$  of two light crude oil samples (45.8%, 57.3%; the corresponding API gravities were 35.40 °API and 32.10 °API, respectively)<sup>19</sup> analyzed using the DPF MS method.<sup>18</sup> However, the  $C_cH_h$  content in most of the studied condensate-like oil samples was greater than that in the previously studied crude oil samples.<sup>18,19</sup>

The densest condensate-like oil sample 1 (37.50 °API) was found to contain more compounds with the elemental composition types  $C_cH_hS_s$  and  $C_cH_hN_nO_oS_s$  than the lighter condensate-like oil samples 2-5 (**Table 3.4**). The content of  $C_cH_hS_s$  compounds was found to correlate with the API gravity; in fact, more  $C_cH_hS_s$  compounds were found with the increase in the condensate-like oil sample densities ( $R^2 = 0.95$ , **Figure 3.4A**). However, no linear correlations were found between the API gravity and the average wt% of compounds with the elemental composition types  $C_cH_hN_n$ ,  $C_cH_hO_o$ ,  $C_cH_hO_oS_s$ ,  $C_cH_hN_nO_oS_s$  ( $R^2 = 0.13$ , 0.00, 0.01, 0.24 and 0.50, respectively).

**Table 3.4** The elemental composition types for the compounds in the different classes for five different condensate-like oils. All data reported are averages of two trials.

			Semi-qı	antitative average	wt %				
Condensate- like Oil Sample	Elemental Composition Type	Volatile Hydrocarbons	Heavy Saturated Hydrocarbons	Heteroaromatic Compounds	Alkyl Aromatic Hydrocarbons	Polar Compounds	Total in the Condensate- like Oil	SD of the Total Content of Compounds with a Specific Elemental Composition Type	95% CI
1		1.40	53.10	0.00	10.30	0.10	65.00	1.30	63.2-66.8
2		1.20	52.10	0.60	3.50	0.10	57.60	0.10	57.46-57.74
3	$C_cH_h$	1.20	58.80	0.70	4.80	0.10	65.70	0.00	-
4		1.00	69.20	0.50	2.60	0.10	73.40	0.80	66.21-80.59
5		0.60	73.70	0.40	1.30	0.10	76.10	0.40	75.55-76.65
1		0.02	0.03	0.00	0.80	0.00	0.91	0.30	0.49-1.33
2		0.06	0.00	0.01	0.20	0.00	0.30	0.10	0.16-0.44
3	$C_cH_hS_s$	0.03	0.03	0.02	0.30	0.00	0.40	0.20	0.12-0.68
4		0.09	0.00	0.00	0.20	0.00	0.30	0.10	0.16-0.44
5		0.01	0.00	0.01	0.15	0.00	0.20	0.10	0.06-0.34
1		0.03	0.49	0.02	0.00	0.10	0.60	0.10	0.46-0.74
2		0.00	1.87	0.00	0.20	0.20	2.30	0.00	-
3	$C_cH_hN_n$	0.02	0.25	0.01	0.20	0.30	0.80	0.10	0.66-0.94
4		0.02	0.40	0.00	0.20	0.10	0.70	0.20	0.42-0.98
5		0.02	1.09	0.00	0.30	0.10	1.50	0.40	0.95-2.05

1	~	0.20	1.10	0.70	0.30	0.10	2.30	0.10	2.16-2.44
2	$C_cH_hO_o$	0.00	19.20	0.00	0.60	0.10	19.90	0.10	19.76-20.04
3		0.10	2.10	0.10	0.70	0.10	3.00	0.40	2.45-3.55
4		0.10	0.30	0.10	0.30	0.00	0.80	0.10	0.66-0.94
5		0.00	0.50	0.00	0.60	0.00	1.20	0.20	0.92-1.48
1		0.03	0.04	0.12	0.08	0.03	0.29	0.10	0.15-0.43
2		0.00	0.02	0.02	0.08	0.00	0.11	0.00	-
3	$C_cH_hO_oS_s$	0.08	0.04	0.01	0.07	0.04	0.24	0.10	0.10-0.38
4		0.03	0.01	0.00	0.08	0.01	0.14	0.00	-
5		0.00	0.07	0.01	0.29	0.01	0.39	0.10	0.25-0.53
1		0.20	12.10	0.20	1.20	0.70	14.40	1.40	12.46-16.34
2		0.10	3.00	0.20	2.30	0.40	6.00	0.20	5.72-6.28
3	$C_{c}H_{h}N_{n}O_{o}$	0.10	13.20	0.20	2.60	0.30	16.30	0.90	15.05-17.8
4		0.10	7.00	0.10	2.40	0.60	10.20	1.20	8.54-11.86
5		0.10	5.50	0.10	2.70	0.20	8.60	0.20	8.32-8.88
1		0.10	0.80	0.70	0.90	0.10	2.60	0.10	2.46-2.74
2		0.00	0.00	0.10	1.10	0.10	1.40	0.00	-
3	$C_cH_hN_nO_oS_s$	0.10	0.70	0.10	1.20	0.10	2.10	0.20	1.82-2.38
4		0.10	0.60	0.00	0.80	0.10	1.50	0.10	1.36-1.64
5		0.00	0.30	0.10	1.60	0.00	2.00	0.40	1.45-2.55

 Table 3.4 continued



Figure 3.4 A) Plot showing the inverse correlation between the average wt% of compounds with the elemental composition type C<sub>c</sub>H<sub>h</sub>S<sub>s</sub> with the API gravity for the five different condensate-like oil samples. B) Plot showing the inverse correlation between the average wt% of compounds with the elemental composition type C<sub>c</sub>H<sub>h</sub> in the alkyl aromatic hydrocarbon with the API gravity for the five different condensate-like oil samples.

Although samples 2 (41.61 °API) and 3 (41.62 °API) have similar API gravities and show similarities in the average gravimetric percentages of compounds in the different compound classes (**Table 3.3**). However, they contain different amounts of compounds of specific elemental composition types, especially those with the elemental composition types  $C_cH_hO_o$  (20.0% vs. 3.0%) and  $C_cH_hN_nO_o$  (6.0 vs. 16.3%) as shown in **Table 3.4**. This finding suggests that it is hard to draw general conclusions on the chemical composition of condensatelike oils solely based on their API gravities. They might have similar API gravities and yet different amounts of compounds with similar elemental composition types. It is important to remember here that, sample 2 was obtained from the Permian Basin while sample 3 was obtained from the Appalachian Basin. Thus, the observed differences may be related to their different origins as the chemical composition of petroleum products is highly affected by the type of organic matter they originated from and the source rock from which they were extracted.<sup>2,19</sup>

# **3.3.3 Determination of the Elemental Compositions of Compounds in The Individual** Compound Classes Derived from The Condensate-Like Oil Samples

Additional correlations were found with the API gravity when the content of compounds with specific elemental composition types was considered for the individual compound classes of the condensate-like oil samples (**Table 3.4**). For the alkyl aromatic

compound class, the average wt% of compounds with the elemental composition type  $C_cH_hN_nO_o$  increased with the increase in the API gravity ( $R^2 = 0.94$ ; Figure 3.5).



**Figure 3.5** Plot showing the direct correlation between the average weight percent of compounds with the elemental composition type C<sub>c</sub>H<sub>h</sub>N<sub>n</sub>O<sub>o</sub> in each alkyl aromatic compound class as a function of API gravity of five different condensate-like oil samples.

On the other hand, for the alkyl aromatic hydrocarbon class, as the API gravity increased the average wt% of compounds with the elemental composition type  $C_cH_h$  or  $C_cH_hS_s$  decreased ( $R^2 = 0.97$  and 0.96, respectively; Figures 3.4B and 3.6).



Figure 3.6 Plot showing the inverse correlation between the the average weight percent of compounds with the elemental composition type  $C_cH_hS_s$  in each alkyl aromatic hydrocarbon class as a function of API gravity of five different condensate-like oil samples.

Similarly, inverse correlations with the API gravities were found for the wt% of compounds with the elemental composition types  $C_cH_hN_nO_oS_s$ ,  $C_cH_hO_oS_s$  and  $C_cH_hO_o$  in the heteroaromatic compound classes ( $R^2 = 0.95$ , 0.94 and 0.94, respectively). Thus, more information on the oil samples were obtained when the proportion of compounds with a specific elemental composition type in the individual compound classes was considered compared to a limited consideration of the proportion of all compounds with specific elemental composition types in the entire condensate-like oil samples.

# **3.3.4** Determination of the Average Molecular Weight (MW) of Compounds in The Condensate-Like Oil Samples from The Average Molecular Weight of Compounds in The Individual Compound Classes

Many of the physical properties of hydrocarbon oils including the API gravity and viscosity are thought to be determined by the average MW of the compounds.<sup>40</sup> Determination of the average MW is a common practice in petroleum industries. Typically, methods used for MW determination are freezing point depression, GC simulated distillation techniques or gel permeation chromatography.<sup>18,41-44</sup> However, a previous study on model compound mixtures showed that freezing point depression or GC simulated distillation techniques underestimate the actual average MW values<sup>18</sup> while the values obtained by using the DPF MS method were much more accurate.<sup>18</sup>

In our study, the average MWs of the compounds in the whole condensate-like oils ranged between 253 and 327 Da (data from two experiments and statistical data are shown in **Table 3.5**). While lower average MW, ranging from 228 to 243 Da, for compounds in condensate-like oils (API gravity not provided) determined by using gel permeation chromatography were reported in previous literature studies.<sup>44</sup> Possible reasons for the inaccuracy of the gel permeation method in providing average MW values for heavy petroleum compounds, include complications in the elution behavior caused by intramolecular hydrogen bonding and the lack of suitable reference compounds.<sup>45,46</sup> Indeed, the measured values are usually associated with large errors of > 10%,<sup>47</sup> compared to typical errors of < 3% for the values determined by the DPF MS method.<sup>18</sup> Therefore, we believe that the values obtained here using DPF MS method are likely to be more accurate.

**Table 3.5** The average molecular weights of compounds in the different compound classes and their standard deviations (SD; based on twomeasurements) for five different condensate-like oils. The average molecular weights were determined based on the molecular weightsdetermined for compounds in each individual compound class and the gravimetric weight percentage of each class.

	Average molecular weight (Da)											
	-	5	5 (43.00	) °API)		4 (42.40 °API)						
Chemical Classes	T1	T2	SD	Avg.	95% CI	T1	T2	SD	Avg.	95% CI		
Volatile Hydrocarbons	229.3	231.6	1.6	230.5	228.3-232.7	225.5	223.1	1.7	224.3	221.9-226.7		
Heteroaromatic Compounds	339.5	336.5	2.1	338.0	335.1-340.91	332.6	330.7	1.3	331.7	329.9-333.5		
Polar Compounds	216.2	216.8	0.4	216.5	215.9-217.1	286.3	285.4	0.6	285.8	285.0-286.6		
Heavy Saturated Hydrocarbons	269.8	278.5	6.2	274.2	265.6-282.8	320.2	325.8	4.0	323.0	317.5-328.5		
Alkyl Aromatic Hydrocarbons	410.8	422.0	7.9	416.4	405.5-427.4	388.4	386.5	1.3	387.5	385.7-389.3		
Overall Average Molecular Weight of Condensate-like Oil	280.6	289.4	6.2	285.0	276.4-293.6	323.6	328.4	3.4	326.0	321.3-330.7		

	Average molecular weight (Da)										
			3 (41.62	2 °API)		2 (41.61 °API)					
Chemical Classes	T1	T2	SD	Avg.	95% CI	T1	T2	SD	Avg.	95% CI	
Volatile Hydrocarbons	228.5	228.9	0.3	228.7	228.3-229.1	225.2	227.9	1.9	226.6	224.0-229.2	
Heteroaromatic Compounds	453.5	461.0	5.3	457.2	449.9-464.5	475.5	472.0	2.5	473.8	470.3-477.3	
Polar Compounds	240.1	236.8	2.3	238.4	235.2-241.6	397.8	391.0	4.8	394.4	387.7-401.1	
Heavy Saturated Hydrocarbons	312.6	313.1	0.3	312.9	312.5-313.3	234.7	236.3	1.1	235.5	234.0-237.0	
Alkyl Aromatic Hydrocarbons	379.1	363.0	11.4	371.0	355.2-386.8	387.4	383.0	3.1	385.2	380.9-389.5	
Overall Average Molecular Weight of the Condensate-like Oil	321.4	319.7	1.2	320.6	318.9-322.3	253.5	254.3	0.6	253.9	253.1-254.7	
		Average	molecu	ılar weigh	nt (Da)	_					
			1 (37.5	0 °API)							
Chemical Classes	T1	T2	SD	Avg.	95% CI	_					
Volatile Hydrocarbons	231.3	234.2	2.1	232.8	229.9-235.7						
Heteroaromatic Compounds	450.8	455.0	3.0	452.9	448.7-457.1						
Polar Compounds	398.6	399.5	0.7	399.1	398.1-400.1						
Heavy Saturated Hydrocarbons	307.3	308.0	0.5	307.6	306.9-308.3						
Alkyl Aromatic Hydrocarbons	304.8	307.9	2.2	306.3	303.3-309.3						
Overall Average Molecular Weight of the Condensate-like Oil	311.8	313.3	1.1	312.6	311.1-314.1						

The average MWs of the compounds in all the condensate-like oil samples examined in this study, were found to be lower than the MW, previously determined by using the DPF MS method, for a medium-light crude oil sample (31.14 °API).<sup>18</sup> The MWs of the compounds in samples 1-5 ranged from 253 to 327 Da compared to (473 Da) for the medium-light crude oil sample. Crude oils tend to contain large compounds like asphaltenes and large polar compounds.<sup>2</sup> The lower average MWs of compounds in the condensate-like oil samples may be resulting from the existence of a lower amount of such compounds.<sup>48</sup>

No correlations were found between the API gravities and the overall average MWs of the compounds in the condensate-like oil samples ( $R^2 = 0.04$ ; Figure 3.7). However, the average MW of the alkyl aromatic hydrocarbons in the condensate-like oils was found to directly correlate with the API gravity ( $R^2 = 0.96$ ; Figure 3.8), which we were not able to explain in this study since oils with a low API gravity are expected to contain more large compounds than lighter oils.<sup>40</sup>



Figure 3.7 The overall average molecular weight of the condensate-like oil samples as a function of API gravity.



**Figure 3.8** The average MW of the alkyl aromatic hydrocarbons as a function of the API gravity for the five different condensate-like oils. As the API gravity increases, the average MW of the alkyl aromatic hydrocarbons increases.

# **3.3.5** Determination of the Average Ring and Double Bond Equivalent (RDBE) Values of Compounds in The Individual Compound Classes and in The Whole Condensate-Like Oil Samples

High-resolution data obtained from the DPF MS method were utilized in the determination of the average ring and double bond equivalent (RDBE) values for compounds in individual compound classes of the five condensate-like oil samples (**Table 3.6**) by using Eq. 2. Then, the RDBE values from individual compound classes were utilized to determine the average RDBE value for the condensate oil sample as a whole. The RDBE value describes the level of unsaturation in the compounds, i.e. the number of rings and multiple bonds. When the values obtained were plotted versus the API gravities of the condensate-like oil samples, no correlations were found (**Tables 3.6**). However, the heteroaromatic compounds and the alkyl aromatic hydrocarbons in the densest condensate-like oil sample 1 had the lowest degree of unsaturation, average RDBE values, among all studied condensate-like oil samples.

	Average RDBE values												
			5 (43	.00 °API)		4 (42.40 °API)							
Chemical Classes	T1	T2	SD	Avg.	95% CI	T1	T2	SD	Avg.	95% CI			
Volatile Hydrocarbons	1.9	1.5	0.3	1.7	1.3-2.1	2.1	2.1	-	2.1	-			
Heteroaromatic Compounds	8.8	8.8	0.0	8.8	-	10.2	9.2	0.7	9.7	8.7-10.7			
Polar Compounds	2.8	2.4	0.3	2.6	2.2-3.0	6.0	5.9	0.1	5.9	5.8-6.0			
Heavy Saturated Hydrocarbons	1.9	1.9	0.0	1.9	-	2.1	2.0	0.1	2.0	1.9-2.1			
Alkyl Aromatic Hydrocarbons	7.2	7.4	0.1	7.3	7.2-7.4	6.4	5.4	0.7	5.9	6.9-4.9			
Overall Average RDBE Values of the Condensate-like Oil	2.4	2.4	0.0	2.4	-	2.5	2.4	0.1	2.4	2.3-2.5			

**Table 3.6** The RDBE values of compounds in the different compound classes of five different condensate-like oils. The average RDBE values of each individual compound class were determined based on the RDBE value of the ions and their abundances.

	Average RDBE values										
			3 (41.0	62 °API	)			2 (41.61	°API)		
Chemical Classes	T1	T2	SD	Avg.	95% CI	T1	T2	SD	Avg.	95% CI	
Volatile Hydrocarbons	2.5	2.5	0.1	2.5	2.4-2.6	2.6	2.1	0.3	2.4	2.0-2.8	
Heteroaromatic Compounds	9.0	9.2	0.1	9.1	9.0-9.2	10.0	9.7	0.2	9.9	9.6-10.2	
Polar Compounds	3.3	3.3	0.0	3.3	-	4.0	3.7	0.2	3.9	3.6-4.2	
Heavy Saturated Hydrocarbons	2.8	2.8	0.0	2.8	-	1.6	1.6	0.0	1.6	-	
Alkyl Aromatic Hydrocarbons	5.0	5.3	0.2	5.2	4.9-5.5	5.5	5.8	0.2	5.6	5.3-5.9	
Overall Average RDBE Values of the Condensate-like Oil	3.1	3.2	0.0	3.1	-	2.1	2.1	0.0	2.1	-	
		Ave	erage F	RDBE va	alues	_					
			1 (37.	50 °API	)	_					
Chemical Classes	T1	T2	SD	Avg.	95% CI	_					
Volatile Hydrocarbons	3.3	2.5	0.6	2.9	2.1-3.7						
Heteroaromatic Compounds	6.6	6.8	0.1	6.7	6.6-6.8						
Polar Compounds	2.3	2.2	0.1	2.2	2.1-2.3						
Heavy Saturated Hydrocarbons	2.8	2.8	0.0	2.8	-						
Alkyl Aromatic Hydrocarbons	4.6	4.6	0.1	4.6	4.5-4.7						
Overall Average RDBE Values of the Condensate-like Oil	3.3	3.3	0.0	3.3	-						

The RDBE values measured for the volatile and heavy saturated hydrocarbons were greater than 1; thus, it clearly demonstrated that these fractions contained cyclic compounds. In **Figure 3.9** the GC×GC/TOF chromatogram obtained from the heavy saturated hydrocarbon fraction of the condensate-like oil sample 5 demonstrates that not only linear and branched hydrocarbons were present in the heavy saturated hydrocarbon fractions, but also cyclic hydrocarbons were present. For a better illustration, the RDBE values for the ionized compounds derived from the four nonvolatile fractions in the condensate-like oil samples were plotted against the carbon number; the color-mapped bubble plots are shown in **Figure 3.10**.



**Figure 3.9** GC×GC/TOF chromatogram for the heavy saturated hydrocarbon fraction of oil 5.



Figure 3.10 Color-mapped bubble plots of the RDBE values versus carbon number for ionized compounds derived from the four nonvolatile condensate-like oil fractions that were separated and characterized by using the DPF MS method. A) Heteroaromatic compound fraction. B) Saturated hydrocarbon fraction. C) Alkyl aromatic hydrocarbon fraction. D)
Polar compound fraction. Bubble size indicates the relative abundance of the ions. The black bubbles represent ions containing only C and H. Red bubbles represent ions containing heteroatoms (N, O and/or S) along with C and H.



# Figure 3.10 continued

# 3.3.6 Determination of the Average Chain Length in the Side Chains of Alkyl Aromatic Hydrocarbons

Based on the gravimetric percentages shown in **Table 3.3**, the second most abundant class of compounds in all the condensate-like oil samples in this study, after the heavy saturated hydrocarbon class, was the alkyl aromatic hydrocarbon class. Thus, the molecular ions obtained for the alkyl aromatic hydrocarbon fractions were further investigated using in-source collision-activated dissociation (ISCAD). In many of our group's previous studies, ISCAD has been proven effective in cleaving off all alkyl side chains, possibly without one methylene group, from the stable aromatic cores.<sup>18</sup> Further proof was provided in this study by using ISCAD on three alkyl aromatic model compounds for which the mass spectra are given in **Figure 3.11**.



Figure 3.11 A) (+) APCI/N<sub>2</sub>/CS<sub>2</sub> low resolution mass spectrum of an equimolar (1 mM) mixture of two alkyl aromatic hydrocarbon model compounds. B) ISCAD mass spectrum of the mixture of the two ionized alkyl aromatic model compounds, illustrating the ability to use ISCAD to cleave off most of the alkyl substituents on the aromatic cores. C) (+) APCI/N<sub>2</sub>/CS<sub>2</sub> low resolution mass spectrum of 1 mM solution of indane. D) ISCAD mass spectrum of the ions shown in part C, including indane molecular ions. Peaks at *m/z* 76 designated with a blue asterisk (\*) are due to the molecular ion of CS<sub>2</sub>.

During ISCAD experiments, all ions generated in the ion source were accelerated from the skimmer region to the first multipole ion optics region of the ion source. No prior ion isolation was required. The accelerated ions fragmented as a result of colliding with the atoms and molecules in the ion optics region. The ISCAD mass spectra measured for all ionized compounds in the alkyl aromatic hydrocarbon classes of the condensate-like oil samples 1-5 (**Figure 3.12**) were used to determine the average total number of carbon atoms in the alkyl chain substituents (as shown in Eq. 3.9).<sup>32</sup>



**Figure 3.12** (+) APCI/CS<sub>2</sub> mass spectra after ISCAD for the ionized compounds in the alkyl aromatic hydrocarbon classes of the five condensate-like oils from top to bottom in this order: 4, 5, 3, 2, and 1.

To determine the average total number of carbon atoms in the alkyl chain substituents, the difference between the average MW of the alkyl aromatic hydrocarbons before and after ISCAD was used to estimate the number of carbons lost upon ISCAD. It is noteworthy that the average RDBE values did not significantly differ after ISCAD; thus, the difference was majorly

attributed to the loss of carbons from the alkyl chains rather than from the fragmentation of the aromatic cores. **Figure 3.13** the plot of the RDBE values versus the carbon number for condensate-like oil 1 before and after ISCAD was used as an example.



**Figure 3.13** Ring and double bond equivalent (RDBE) versus carbon number plot for APCI mass spectrum (black) and ISCAD spectrum (red) of the alkyl aromatic hydrocarbon fraction of condensate-like oil 1.

Average total number of carbon atoms in the alkyl side chains of the compounds in the alkyl aromatic hydrocarbon classes =

$$\frac{Average \, MW \, of \, compounds - average \, MW \, of \, the \, aromatic \, cores}{14} + 1 \tag{3.9}$$

Interestingly, a direct correlation was found between the average total number of carbon atoms in the alkyl chain substituents of compounds in the alkyl aromatic hydrocarbon classes and the API gravity of the condensate-like oil samples ( $R^2 = 0.97$ , **Figure 3.14**; **Table 3.7**). In other words, in the lighter condensate-like oil samples the compounds had a larger average number of carbons in the alkyl chain substituents than in the denser condensate-like oil samples. Surprisingly, previous reports on alkylbenzenes and alkylnaphthalenes showed similar trends in density when it comes to the number of carbons in the side chains.<sup>49,50</sup>



**Figure 3.14** The average total number of carbons in the side chains of the alkyl aromatic hydrocarbons as a function of API gravity for the five different condensate-like oils. As the API gravity increases, the average approximate total number of carbons in the alkyl side chains increases.

# **Table 3.7** The MW of the aromatic cores and the total number of carbons in the alkyl side chains of the alkyl aromatic hydrocarbons withaverages and standard deviations from two experiments for the five condensate-like oils. API gravities are shown in parenthesis.

	The average MW of the aromatic cores and the average total number of carbons in the alky											
			5 (43 (	)() ° A PI)	side	$\frac{4 (42 40 \circ \text{API})}{4 (42 40 \circ \text{API})}$						
	T1	T2	<u>5 (45.0</u> SD	Avg.	95% CI	T1	T2	SD	Avg.	95% CI		
MW of the Aromatic Cores (Da)	125.9	125.8	0.1	125.8	125.7-125.9	127.1	127.6	0.4	127.4	126.8-128.0		
Total Number of Carbons in the Alkyl Side Chains	22.2	21.4	0.6	21.8	21.0-22.6	19.7	19.5	0.1	19.6	19.5-19.7		
	The average MW of the aromatic cores and the average total number of carbons in the chains of the alkyl aromatic hydrocarbons											
			3 (41.6	52 °API)		2 (41.61 °API)						
	T1	T2	SD	Avg.	95% CI	T1	T 2	SD	Avg.	95% CI		
MW of the Aromatic Cores (Da)	126.1	126.2	0.0	126.2	-	112.6	113.2	0.4	112.9	112.3-113.5		
Total Number of Carbons in the Alkyl Side Chains	19.1	17.9	0.8	18.5	17.4-19.6	20.6	20.3	0.3	20.4	20.0-20.8		
	The a	verage M	W of th	e aromati	c cores and the							
	av	verage tota	al numł	per of carl	bons in the							
	al	kyl side c	hains o	of the alky	aromatic							
			hydro	carbons		_						
			1 (37.5	50 °API)								
	T1	T2	SD	Avg.	95% CI	_						
MW of the Aromatic Cores (Da)	123.8	124.4	0.4	124. 1	123.5-124.7							
Total Number of Carbons in the Alkyl Side Chains	13.9	14.1	0.1	14.0	13.9-14.1							

### 3.1 Conclusions

In this study, we have proven the applicability of the DPF MS method, not only on crude oil samples of different API gravities, but also on other unconventional hydrocarbon products; condensate-like oil samples. The chemical compositions of five condensate-like oil samples of different API gravities were successfully determined by using the DPF MS methodology. No measurable amounts of asphaltenes were found in any of the tested condensate-like oil samples. This explains the greater API gravity of these condensate-like oil samples compared to heavier crude oils.<sup>3</sup> The absence of asphaltenes renders these condensate-like oil samples less viscous than crude oils, since the viscosity of crude oils is largely determined by the asphaltenes content.<sup>51</sup> Thus, these unconventional oils could be more favorable for crude oil dilution. However, this would need further investigation because many other factors might affect the precipitation of asphaltenes in crude oil blends.

For each condensate-like oil sample, five compound classes were obtained using the DPF method; namely, volatile saturated hydrocarbons, heavy saturated hydrocarbons, alkyl aromatic hydrocarbons, heteroaromatic compounds and polar compounds. The gravimetric percentages for the five compound classes obtained using the DPF method were determined and examined for correlations with the API gravity. Correlations were only found between the wt% of the heteroaromatic compound and the heavy saturated hydrocarbon classes in the five oils and the API gravity. Where the wt % of heteroaromatic compounds was found to inversely correlate with the API gravity while the wt% of the saturated hydrocarbons was found to directly correlate with the API gravity.

The elemental composition types of the compounds in the condensate-like oil samples and their wt% were obtained by using high-resolution mass spectrometry. Compounds with the elemental composition type  $C_cH_h$  were generally the most abundant compounds in all the condensate-like oil samples, with an approximate wt% between 57-77%. More information was revealed upon the examination of the molecular composition types of compounds in the individual compound classes in each condensate-like oil sample. For instance, compounds with the elemental composition types  $C_cH_hS_s$  and  $C_cH_hN_nO_oS_s$  were more abundant in the densest condensate-like oil sample 1 (37.50 °API). However, no trends or correlations were found between the API gravity

and the average wt% of compounds with the elemental composition types  $C_cH_hN_n$ ,  $C_cH_hO_o$ ,  $C_cH_hO_oS_s$ ,  $C_cH_hN_nO_o$  and  $C_cH_hN_nO_oS_s$ .

Based on average MW results for the compounds in the whole condensate-like oil samples, as well in each compound class in the condensate-like oil samples, the condensate-like oil samples in the current study had lower average MW values than medium-light crude oils tested previously by using the DPF MS method.<sup>18</sup> The absence of asphaltenes and the low amount of large polar compounds in the condensate-like oils is a likely explanation. Moreover, a direct correlation was found between the average MW of the alkyl aromatic hydrocarbons and the API gravity where the lighter condensate-like oils appeared to contain alkyl aromatic compounds with higher average MWs. additionally, the average total number of carbon atoms in the alkyl chain substituents of the alkyl aromatic hydrocarbons in the whole condensate-like oils was found to correlate with their API gravity. In general, these correlations would facilitate the development of more accurate ways to classify petroleum products than by solely using API gravity values.

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# CHAPTER 4. REAL-TIME MASS SPECTROMETRIC DETECTION OF REACTION INTERMEDIATES FORMED DURING LASER-INDUCED UV/H<sub>2</sub>O<sub>2</sub> ADVANCED OXIDATION OF 2-METHYLBENZOISOTHIAZOL-3-ONE

#### 4.1 Introduction

Advanced oxidation processes induced by  $UV/H_2O_2$  have been adopted for the degradation of a wide range of emerging water pollutants, including various pharmaceutical and antimicrobial agents.<sup>1</sup> Advanced oxidation was recently applied on isothiazolinones, including benzoisothiazol-3-one, used both in domestic and industrial applications.<sup>2</sup> These compounds have been reported to be a class of emerging pollutants in wastewater and in surface water.<sup>2,3</sup> The experimental approach was based on a microcapillary reactor with a UV light source into which an aqueous solution of H<sub>2</sub>O<sub>2</sub> and benzoisothiazol-3-one was introduced.<sup>4-6</sup> The final products of the UV/H<sub>2</sub>O<sub>2</sub> induced advanced oxidation of benzoisothiazol-3-one were detected and identified based on negative-ion collision-activated dissociation (CAD) results obtained by using an HPLC/high-resolution quadrupole-time-of-flight tandem mass spectrometer coupled with a negative-ion electrospray ionization source ((-)ESI).<sup>4</sup> Based on the observed reaction products, careful literature studies on oxidation reactions of sulfur compounds, and kinetic models, mechanistic pathways were proposed for the degradation reactions.<sup>4,7,8</sup> The proposed reaction pathways are shown in Scheme 4.1, wherein the identified reaction products are colored in red.<sup>4</sup> The reactions were proposed to be initiated by the formation of hydroxyl radicals upon treatment of H<sub>2</sub>O<sub>2</sub> with UV light with a wavelength of 254  $\text{nm}^{4,9-13}$  as isothiazolinones have been reported to be unreactive toward H<sub>2</sub>O<sub>2</sub> in the absence of UV light.<sup>4</sup> The UV-generated hydroxyl radicals were proposed to subsequently react with the sulfur atom of benzoisothiazol-3-one to produce 1-hydroxy-1,2-dihydro-3H-1 $\lambda^4$ benzo[d]isothiazol-3-one as a reaction intermediate (compound B in Scheme 4.1), that then reacts with  $O_2$  to produce benzo[d]isothiazol-3(2H)-one 1-oxide (compound C in Scheme 4.1). This compound reacts with a hydroxyl radical (pathway 1) or two hydroxyl radicals (pathway 2) and a H atom donor to generate additional short-lived intermediates, proposed to be 1-hydroxy-1,2dihydro-3*H*-1 $\lambda^4$ -benzo[*d*]isothiazol-3-one 1-oxide, 2-carbamoylbenzene-sulfinic acid and 2-(dihydroxy(oxo)- $\lambda^6$ -sulfaneyl)benzamide (labelled in Scheme 4.1 as D1, D2 and E2, respectively). with These products were proposed to react  $O_2$ to form saccharine and/or

2-carbamoylbenzenesulfonic acid as the final products (designated in **Scheme 4.1** as E1 and F2, respectively).<sup>4</sup> However, no quantum chemical calculations were performed to test the feasibility of the proposed mechanisms, and none of the proposed short-lived reaction intermediates were detected.<sup>4</sup> The lack of more certain knowledge on the reaction mechanisms involved renders process optimization challenging.<sup>14,15</sup>



Scheme 4.1 The previously proposed reaction pathways for UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation of benzoisothiazol-3-one.<sup>4</sup> None of the short-lived reaction intermediates (in brackets) were detected. Compound C was called "a transformation product". High-resolution mass spectrometry was used to determine the elemental compositions of the final products shown in red and negative-ion CAD tandem mass spectrometry was deployed to determine their structures. Molecular weights (MW) provided throughout this chapter are monoisotopic MW values, as only these are relevant to mass spectrometric measurements.

Due to the high sensitivity of mass spectrometry, this method has been used previously to detect minor reaction intermediates in solution.<sup>15</sup> The goal of the study reported in this chapter was to develop a mass spectrometry based device for studying short-lived reaction intermediates in order to improve the understanding of important organic reactions, and the  $UV/H_2O_2$  advanced oxidation of 2-methylbenzoisothiazol-3-one was used as the test case. Herein, a method that employs a pulsed  $UV_{266}$  laser and a linear quadrupole ion trap (LQIT) mass spectrometer coupled with a high-resolution orbitrap detector is introduced for the online detection and characterization of the reaction intermediates and products formed in laser-induced  $UV/H_2O_2$  advanced oxidation of 2-methylbenzoisothiazol-3-one. Since 2-methylbenzoisothiazol-3-one is a simple derivative of benzoisothiazol-3-one, the advanced oxidation pathways for 2-methylbenzoisothiazol-3-one are likely to be similar to the pathways previously reported for benzoisothiazol-3-one (Scheme 4.1).<sup>4</sup>

# 4.2 Experimental

### 4.2.1 Materials

A benzoisothiazol-3-one derivative, the 2-methylbenzoisothiazol-3-one (98% mass purity), and aqueous hydrogen peroxide solution (30% v/v) were purchased from Sigma Aldrich. N-Methyl saccharin (98% mass purity) was purchased from Millipore Sigma. Acetonitrile, water and methanol (LC/MS grade) and acetone (HPLC grade) were purchased from Fisher Scientific. All chemicals were used as received without purification.

# 4.2.2 Sample Preparation

In previous publications on the advanced oxidation of isothiazolinones, water was used as the solvent.<sup>4,16</sup> In this study, acetonitrile was used as the solvent as the same products were detected as when using water but it provided a better mass spectrometric signal and did not generate background ions that would interfere with the expected reaction intermediates or products as when using water (or the other solvents tested). A solution of 2-methylbenzoisothiazol-3-one and  $H_2O_2$  in acetonitrile was prepared with final concentrations of 10 ppm and 50 ppm, respectively. All solutions were freshly prepared before analysis and stored in vials wrapped in aluminum foil to prevent any ambient light induced reactions.

# 4.2.3 Reaction Setup

The laser-induced UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation setup consisted of a syringe pump that held a 500  $\mu$ L Hamilton syringe. The syringe was loaded with the reagent solution, 2methylbenzoisothiazol-3-one and H<sub>2</sub>O<sub>2</sub> in different solvents, including water. The use of water generated similar results to those obtained when acetonitrile was used as a solvent. However, a lower signal was obtained when water was the solvent. The use of 50:50 v/v water-methanol and neat methanol as solvents improved the signal but generated an impurity ion at m/z 182 in the negative-ion experiments, which would interfere with the ionized reaction product of m/z 182. Therefore, methanol containing solvents were not used. The use of acetone was challenging due to the inability to control the formation of a stationary solution droplet at the needle tip (as described in the experimental setup below), possibly due to the low boiling point and low surface tension of this solvent. Finally, acetonitrile generated a good signal in both low- and highresolution experiments, did not produce impurity ions that could interfere with the results and was easy to control for the formation of the stationary solution droplet. Therefore, only results from the experiments with acetonitrile as the solvent are reported in this chapter.

The syringe pump was operated at a rate of 5  $\mu$ L min<sup>-1</sup> to generate and maintain a 2 mm droplet of the reagent solution for a few seconds at the tip of the syringe needle at a distance of approximately 1/8 inch (3.2 mm) from the tip of the ion transfer capillary tube of the spray cone of the mass spectrometer. No voltage was applied to the syringe needle. A Quantel Q-Smart 850 Nd:YAG laser was used to initiate the reactions as well as for the evaporation and ionization of the generated reaction intermediates and products. Without harmonic generators, the laser emits photons with a wavelength of 1064 nm and an energy of 850 mJ per pulse. For the experiments discussed here, the laser was equipped with two harmonic generators to emit photons with a wavelength of 266 nm and an energy of 100 mJ per pulse. The collimated laser beam was focused using a plano-convex lens with a focal length of 750 mm into a diameter of 0.03 mm at the focal point. The laser and the lens were aligned such that the focal point of the resulting laser beam was positioned in the space between the droplet at the tip of the syringe needle and the heated capillary tube of the experimental setup and depicts the position of the focal point of the laser beam relative to both the heated capillary tube and the stationary droplet.



Figure 4.1 A schematic diagram of a top view of the experimental setup. f is the focal length.

# 4.2.4 Mass Spectrometry

A Thermo Scientific LTQ linear quadrupole ion trap (LQIT) mass spectrometer coupled with a high-resolution orbitrap analyzer (model: LTQ/orbitrap, model number: 01894 B) was used for all experiments and was operated in both negative- and positive-ion detection modes. Before use, the mass spectrometer was calibrated using Pierce electrospray ionization (ESI) negative-ion and Pierce LTQ ESI positive-ion calibration solutions obtained from Thermo Scientific.

For the laser experiments, the ion transfer capillary tube temperature was set to 275 °C. Mass spectra were collected in a continuous mode by using a 100 ms maximum ion injection time (time that the ions accumulate in the ion trap during each scan), and a mass spectrum was acquired every 120 ms. The generated ions were detected at low resolution by using the linear quadrupole ion trap and at high resolution (100,000 resolution at m/z 400) by using the orbitrap.

MS<sup>2</sup> experiments based on collision-activated dissociation (CAD) were performed on ions detected in the linear quadrupole ion trap. The ions were isolated by ejecting all other ions from the ion trap. A normalized collision energy of 5-35 (arbitrary units), a q value of 0.25 and an activation time of 30 ms were used. The collision gas was the helium buffer gas. The fragment ions were detected at low and high resolution. Thermo XCalibur 2.0 software was used for all data collection and processing.

### 4.2.5 Density-Functional Theory (DFT) Calculations

For proton affinity calculations, Maestro 7.0.113 was used to determine the lowest energy conformers of the neutral as well as the protonated/deprotonated compounds. Structural optimization and single point energy calculations were conducted using Gaussian 6.0.16 at the B3LYP/6-311G++(d,p) level of theory.<sup>17</sup>

Density functional theory (DFT) calculations of transition states were performed at the M06-2X/6-311++G(d,p) level of theory by using the Gaussian 16 software. All transition state structures were determined to possess a vibration with one negative frequency, while energy minima did not possess vibrations with negative frequencies. Intrinsic reaction coordinate (IRC) calculations were performed to confirm that the transition state structures connected the correct reactant and product structures. The free energies used to construct the potential energy surfaces were calculated using ideal gas statistical mechanics. All calculations were performed at 298.15 K.

#### 4.3 **Results and Discussion**

In this study, a method based on tandem mass spectrometry was developed for the realtime detection and characterization of the solution reaction intermediates and products formed upon UV/H<sub>2</sub>O<sub>2</sub> induced advanced oxidation of 2-methylbenzoisothiazol-3-one. In the experiment discussed here a 266 nm six-nanosecond laser was used to initiate the reaction through generation of hydroxyl radicals from H<sub>2</sub>O<sub>2</sub> in microdroplets (discussed in more detail in section 4.4) before facilitating the desorption and ionization of the generated intermediates and products so that they can be detected and characterized by a tandem mass spectrometer. Benzoisothiazolinones absorb UV light in the range 200-250 nm while H<sub>2</sub>O<sub>2</sub> absorbs UV light in the range 200-300 nm;<sup>4</sup> therefore, UV wavelength of 266 nm was selected to generate hydroxyl radicals from H<sub>2</sub>O<sub>2</sub> while preventing direct photolysis of the benzoisothiazolinone.<sup>11</sup>

To perform the experiments, the reagent mixture in several different solvents, water, methanol, 50:50 v/v water-methanol, acetonitrile and acetone, was loaded into a syringe that was placed on the top of a syringe pump. The pump provided the force required to push the liquid out and form a 2 mm stationary droplet at approximately 3 mm from the heated capillary tube of the mass spectrometer to minimize the distance it takes for ions to enter the heated capillary tube and
travel into the low-pressure region of the mass spectrometer.<sup>18</sup> Minimizing the distance between the stationary droplet and the mass spectrometric ion transfer capillary tube shortens the sampling time and reduces the possibility of secondary reactions between ionized and neutral reaction intermediates and products and the neutral molecules residing in the space between the sample source, the stationary droplet, and the capillary tube.<sup>19</sup> To initiate the reaction, a focused highpower laser beam was pulsed through the microdroplets released from the stationary droplet into the 3 mm gap between the stationary droplet (held in place by adhesion to the tip of the stainlesssteel needle) and the heated capillary tube of the mass spectrometer. The laser beam also facilitated the evaporation and ionization of any short-lived reaction intermediates and final products in the reacting microdroplets (details discussed later in section 4.4). Once the ionized intermediates and products entered the mass spectrometer, they experienced a decreasing pressure gradient going from atmospheric pressure to 1 Torr in the tube lens region, 50 mTorr beyond the skimmer, and about 2 mTorr in the quadrupole mass analyzer.<sup>20</sup> Pressure reading in the orbitrap compartment was typically below  $5x 10^{-10}$  Torr.<sup>21</sup> Therefore, once the ionized intermediates and products enter the mass spectrometer, they stop undergoing reactions.

It is noteworthy that at first, an aqueous solution of 2-methylbenzoisothiazol-3-one and  $H_2O_2$  was used for the mass spectrometric experiments. However, this experiment yielded so low mass spectrometric signal that high-resolution mass measurements became challenging. Therefore, several more mass spectrometry-friendly solvents were tested for suitability for the experiments. A better signal was obtained when more volatile solvents with higher vapor pressure, lower boiling point and lower surface tension than water were used, such as 50:50 v/v water-methanol, methanol, acetone and acetonitrile. The use of methanol-water and methanol as solvents yielded similar mass spectrometric results as water but an impurity background ion of m/z 182 was detected in the negative-ion detection mode, which would interfere with the ionized reaction products of m/z 182 (intermediate B in Scheme 4.2). Therefore, these solvents were not used. When acetone was tested as a solvent, a stable droplet was difficult to maintain at the tip of the syringe and as a result, acetone was not used. Finally, acetonitrile proved to be the best solvent in these experiments as it was easy to control to form a stable droplet and yielded a high mass spectrometric signal that was enough to carry out high-resolution experiments. This is possibly related to the higher vapor pressure, lower boiling point and lower surface tension of acetonitrile than water.

Only the results obtained using acetonitrile solvent are discussed in detail below. The mass spectrometric measurements were performed in the negative- and positive-ion modes at low- and high-resolution. Each detected ion was isolated in the linear quadrupole ion trap by ejecting all other ions out and was subjected to collision-activated dissociation (CAD) in order to obtain structural information. Results obtained in the negative-ion mode experiments will be discussed first. A similar discussion on the ions observed in the positive-ion mode will follow. Finally, the proposed activation, evaporation and ionization mechanisms will be discussed. Molecular weights (MW) provided throughout this chapter are monoisotopic MW values, as only these are relevant to mass spectrometric measurements. In the figures below, the provided exact m/z values for the proposed structures of ions are theoretical monoisotopic values. Measured monoisotopic exact m/z values are provided in text and in **Table 4.1**.



Scheme 4.2 Reaction pathways proposed for UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation of 2methylbenzoisothiazol-3-one based on literature.<sup>4</sup> Molecular weights (MW) provided throughout this chapter are monoisotopic MW values, as only these are relevant to mass spectrometric measurements. All reaction intermediates are shown in brackets.

#### 4.3.1 Results Obtained in the Negative-Ion Mass Spectrometry Experiments

Four ions that were believed to be related to the laser-induced UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation of 2-methylbenzoisothiazol-3-one were detected in the negative-ion experiments, namely, ions with m/z values of 182, 198, 216 and 214 (for a low-resolution mass spectrum, see **Figure 4.2**). Based on the elemental compositions determined for these ions using high-resolution mass spectrometry, the detected ions may correspond to deprotonated compounds B, D1 and/or D2, E2 and F2 in **Scheme 4.2**, respectively. Negative ions corresponding to the reagent, 2-methylbenzoisothiazol-3-one (structure A, Error! Reference source not found.**4.2**), were not observed, which is not surprising since 2-methylbenzoisothiazol-3-one is not a Brønsted acid and hence cannot be readily deprotonated. The examination of the structures of the above ions is discussed next. High-resolution m/z values of the detected ions, their elemental compositions and mass errors in ppm are provided in **Table 4.1**.

**Table 4.1** Exact measured m/z-values, the derived elemental compositions and mass errors in ppm for the ions of the products and intermediates formed upon UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation of 2-methylbenzoisothiazol-3-one and the fragment ions generated upon CAD on the product and intermediate ions detected in negative- and positive-ion detection modes.

				Accurate mass	
	Product ion <b>symbol</b> ( <i>m</i> / <i>z</i> )	Accurate mass based elemental composition (mass error in ppm)	m/z of the ions formed upon $CAD (MS^2)$	hased elemental	Neutral losses
				composition of the	
				fragment ions (mass	
			CAD (MD)	error in ppm)	
	<b>B</b> (182 02693)	$C_{0}H_{0}NO_{0}S(-0.52)$	118 02986	$C_{\rm T}H_{\rm A}NO(0.21)$	$CH_{2}S(H)=0$
Negative-ion detection mode [M-H] <sup>-</sup> ions	D(102.02093)	$C_8H_8NO_2S(0.10)$	134.06136		<u>SO</u>
	<b>E2</b> (216.03235)	$C_8H_{10}NO_4S$ (-0.72)	154.00150	$C_8H_8NO_2(-0.65)$	H-SO2
			152 07164	$C_8H_8HO_2(-0.05)$	112502 SO:
			152.07104	$C_{811_{10}} O_2(-0.03)$	
			93.03458	C <sub>6</sub> H <sub>5</sub> O (-0.04)	H <sub>2</sub> SO <sub>2</sub> CH <sub>3</sub> N=C=O
	<b>F2</b> (214.01788)	C <sub>8</sub> H <sub>8</sub> NO <sub>4</sub> S (-0.32)	156.99649	$C_6H_5O_3S(0.01)$	CH <sub>3</sub> N=C=O
			150.05605	$C_8H_8NO_2(-0.04)$	SO <sub>2</sub>
			93.03468	C <sub>6</sub> H <sub>5</sub> O (1.02)	CH <sub>3</sub> N=C=O
					SO <sub>2</sub>
			79.95734	SO <sub>3</sub> (-0.33)	C <sub>8</sub> H <sub>8</sub> NO
Positive-ion detection mode [M+H] <sup>+</sup> ions	A (166.03229)	C <sub>8</sub> H <sub>8</sub> NOS (1.06)	151.00859	C <sub>7</sub> H <sub>5</sub> NOS (-0.30)	CH <sub>3</sub>
			137.00546	C <sub>7</sub> H <sub>5</sub> OS (-0.73)	CH <sub>2</sub> =NH
			123.02607	C <sub>7</sub> H <sub>7</sub> S (0.16)	HN=C=O
			109.01047	$C_6H_5S(-1.65)$	CH <sub>3</sub> N=C=O
	<b>B</b> (184.04344)	$C_8H_{10}NO_2S$ (4.16)		NA	
	C (182.02702)	C <sub>8</sub> H <sub>8</sub> NO <sub>2</sub> S (-0.01)	153.00044	C <sub>7</sub> H <sub>5</sub> O <sub>2</sub> S (-0.22)	CH <sub>2</sub> =NH
	<b>D1/D2</b> (200.03850)	$C_8H_{10}NO_3S$ (4.54)	NA		
	<b>E1</b> (198.02190)	C <sub>8</sub> H <sub>8</sub> NO <sub>3</sub> S (-0.22)	168.99516	C <sub>7</sub> H <sub>5</sub> O <sub>3</sub> S (-1.35)	CH <sub>2</sub> =NH
			151.99257	C <sub>7</sub> H <sub>4</sub> O <sub>2</sub> S (-0.54)	CH <sub>2</sub> =NH
					OH
			134.05978	C <sub>8</sub> H <sub>8</sub> NO (-1.91)	SO <sub>2</sub>
			121.02825	$C_7H_5O_2(-1.27)$	CH <sub>3</sub> N=S=O
			106.06492	C <sub>7</sub> H <sub>8</sub> N (-1.95)	$SO_2$
					CO
	<b>F2</b> (216.03239)	C <sub>8</sub> H <sub>10</sub> NO <sub>4</sub> S (-0.51)	199.00578	C <sub>8</sub> H <sub>7</sub> O <sub>4</sub> S (-0.87)	NH <sub>3</sub>
			166.03185	C <sub>8</sub> H <sub>8</sub> NOS (-1.60)	$H_2O_3$
			151.00594	C <sub>4</sub> H <sub>7</sub> O <sub>4</sub> S (-0.13)	C <sub>4</sub> H <sub>3</sub> N
			184.99042	$C_7H_5O_4S(0.60)$	CH <sub>3</sub> NH <sub>2</sub>
	(331.05682)	$C_{16}H_{15}N_2O_2S_2(-0.37)$	166.03194	C <sub>8</sub> H <sub>8</sub> NOS (-1.05)	C <sub>8</sub> H <sub>7</sub> NOS

# 4.3.1.1 Reaction Intermediate B

A hydroxyl radical attack on the sulfur atom of compound A as shown in **Scheme 4.2** may generate reaction intermediate B with the molecular weight 183 Da. Therefore, the negatively charged ions of m/z 182 detected by mass spectrometry (**Figure 4.2**) may correspond to the deprotonated hydroxylated 2-methylbenzoisothiazol-3-one (intermediate B). In order to determine

the elemental compositions of these ions, they were transferred into the orbitrap for high-resolution measurements. Ions of m/z 182 were measured to have the exact m/z value of 182.02693, which is within -0.52 ppm from the theoretical mass of a negative ion with the elemental composition of C<sub>8</sub>H<sub>8</sub>NO<sub>2</sub>S (**Table 4.1**), the elemental composition of the deprotonated reaction intermediate B in **Scheme 4.2**.



**Figure 4.2** Negative-ion low-resolution mass spectrum measured for the products and intermediates formed upon UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation of 2-methylbenzoisothiazol-3-one. The detected ions with their exact measured m/z-values and the derived elemental compositions correspond to all the four deprotonated reaction intermediates proposed in Scheme 4.2 and the final product of pathway 2. Background ions designated with a red asterisk (\*) correspond to deprotonated nitric acid (NO<sub>3</sub><sup>-</sup>) at *m*/*z* 62 and deprotonated percarbonic acid (CHO<sub>4</sub><sup>-</sup>) at *m*/*z* 77. NO<sub>3</sub><sup>-</sup> and CHO<sub>4</sub><sup>-</sup> ions have been previously reported to be formed upon corona discharge of air and plasma discharge of air (discussed further in section 4.4).<sup>22</sup>

To examine the structure of the ions of m/z 182 (possibly ionized reaction intermediate B), they were isolated in the linear quadrupole ion trap by ejecting out all other ions and subjected to CAD with the helium buffer gas. Among the observed fragment ions were ions of m/z 154 generated via the loss of CO. Ions from losses of H<sub>3</sub>C-N=C=O (methyl isocyanate) and CO produced the most abundant fragment ions of m/z 97 (**Figure 4.3A**). These fragmentations are in agreement with the hypothesis that the ions of m/z 182 have the structure of the ionized intermediate B. Quantum chemical calculations supporting the mechanisms of formation of ions of m/z 154 and m/z 97 upon fragmentation of deprotonated intermediate B are shown in **Figures 4.4** and **4.5**, respectively. Other fragment ions observed upon CAD of the ions of m/z 182 included

ions of m/z 118 with the elemental composition of C<sub>7</sub>H<sub>4</sub>NO (determined using high resolution) corresponding to the loss of a molecule with MW 64 Da with the elemental composition CH<sub>4</sub>OS (all high-resolution CAD results are provided in **Table 4.1**). The observation of this fragmentation is in agreement with the structure proposed above for the ions of m/z 182. A mechanism proposed for the loss of CH<sub>3</sub>S(H)=O from the ions of m/z 182 is shown in **Figure 4.6**.



Figure 4.3 Low-resolution negative-ion mode CAD mass spectra (normalized collision energy 30) measured for the isolated negative ions of A) m/z 182, B) m/z 198, and C) m/z 216, corresponding to the ionized reaction intermediates B, D1/D2 and E2 (Scheme 4.2). D) Low-resolution negative-ion mode CAD mass spectrum measured for ions of m/z 214 corresponding to the ionized final reaction product F2 (Scheme 4.2). Ions for which the elemental compositions were verified by using high-resolution mass spectrometry are marked with a blue asterisk (\*).



**Figure 4.4** Calculated enthalpies for the elimination of CO upon CAD from deprotonated 1hydroxy-2-methyl-1,2-dihydro-3H- $1\lambda^4$ -benzo[*d*]isothiazol-3-one (*m/z* 182; deprotonated reaction intermediate B). All values are in kcal/mol and were calculated relative to the infinitely separated reactants at the M06-2X/6-311++G(d,p) level of theory.



**Figure 4.5** Calculated enthalpies for the elimination of H<sub>3</sub>C-N=C=O (methyl isocyanate) and CO upon CAD from deprotonated 1-hydroxy-2-methyl-1,2-dihydro- $3H-1\lambda^4$ -benzo[*d*]isothiazol-3-one (*m*/*z* 182; deprotonated reaction intermediate B). All values are in kcal/mol and were calculated relative to the infinitely separated reactants at the M06-2X/6-311++G(d,p) level of theory.



**Figure 4.6** Calculated enthalpies for the elimination of a molecule with the elemental composition CH<sub>4</sub>OS upon CAD from deprotonated 1-hydroxy-2-methyl-1,2-dihydro- $3H-1\lambda^4$ -benzo[*d*]isothiazol-3-one (*m/z* 182; deprotonated reaction intermediate B). The theoretical exact *m/z*-values are shown for B and the final fragment ion. Measured exact *m/z*-values are provided in **Table 4.1**.

# **4.3.1.2** Compound C

A compound analogous to the reaction intermediate B (generated from benzoisothiazol-3one) discussed above has been proposed to react with oxygen to convert the hydroxylated sulfur atom to a sulfoxide group to generate compound C (**Scheme 4.1**) that was detected and structurally characterized by negative-ion CAD-based tandem mass spectrometry.<sup>4</sup> An analogous compound may be generated from 2-methylbenzoisothiazol-3-one studied here. However, like the reactant A (**Scheme 4.2**), product C is not a Brønsted acid. Therefore, it is not surprising that deprotonated C was not observed in the negative-ion mode mass spectrometry experiments. However, as discussed below, protonated C was detected in the positive-ion mode mass spectrometry experiments.

#### 4.3.1.3 Reaction Intermediates D1 and D2

In the reaction pathway shown in **Scheme 4.2**, a hydroxyl radical is proposed to attack the sulfur atom in the sulfoxide group in compound C, which may produce either 1-hydroxy-2-methyl-1,2-dihydro- $3H-1\lambda^4$ -benzo[*d*]isothiazol-3-one 1-oxide with an intact hetero-cyclic ring or 2-carbamoylbenzenesulfinic acid with an opened ring structure (intermediates D1 and D2, respectively; **Scheme 4.2**). Ions of *m*/*z* 198 observed in the negative-ion mode experiments (**Figure 4.2**) may correspond to deprotonated D1 and/or D2. Based on high-resolution measurements, the exact *m*/*z* value of these ions is *m*/*z* 198.02306 (**Table 4.1**), which indicates that the elemental composition of these ions is C<sub>8</sub>H<sub>8</sub>NO<sub>3</sub>S (as the measured value is within 0.10 ppm of the theoretical value). This elemental composition matches the elemental composition of the deprotonated D1 and D2 (**Scheme 4.2**).

The structure of the ions of m/z 198 was probed by isolating these ions in the ion trap and subjecting them to CAD. The ions generated major fragment ions of m/z 180 and 134 (**Figure 4.3B**). The former fragment ions were formed by loss of water. This loss is in agreement with the structure proposed for D1 as deprotonated D1 is likely to eliminate water upon CAD (which is not the case for D2). On the other hand, the fragment ions of m/z 134 are formed by the loss of a molecule with MW 64 Da; these ions were determined to have an elemental composition C<sub>8</sub>H<sub>8</sub>NO. Thus, these ions were generated through elimination of a sulfur dioxide molecule. This fragmentation reaction is in agreement with structure D2 as deprotonated D2 is likely to eliminate

SO<sub>2</sub>, possibly as shown in **Figure 4.7**. The elimination of SO<sub>2</sub> through heterolytic cleavage of the carbon-sulfur bond in benzenesulfinic acid anions was previously reported in the literature.<sup>23</sup>



**Figure 4.7** Calculated enthalpies for the elimination of SO<sub>2</sub> upon CAD on deprotonated 2-(methylcarbamoyl)benzenesulfinic acid (reaction intermediate D2). All values are in kcal/mol and were calculated relative to the infinitely separated reactants at the M06-2X/6-311++G(d,p) level of theory. The theoretical exact m/z-values are shown for D2 and the final fragment ion. Measured exact m/z-values are provided in **Table 4.1**.

#### 4.3.1.4 Compound E1

In the reaction pathway shown in **Scheme 4.2**, further oxidation of reaction intermediate D1 is proposed to produce 2-methylbenzo[d]isothiazol-3(2H)-one 1,1-dioxide (product E1, **Scheme 4.2**). Ions corresponding to the deprotonated product E1 **Scheme 4.2** were not observed in the negative ion-mode experiments for the same reason as ionized compounds A and C were not observed. However, protonated E1 was detected in the positive-ion mode experiments discussed in section 4.3.2.

#### 4.3.1.5 Reaction Intermediate E2

As shown in **Scheme 4.2**, intermediate D2 has been proposed to react with a hydroxyl radical to generate the reaction intermediate E2 (2-(dihydroxy(oxo)- $\lambda^6$ -sulfaneyl)benzamide; MW 217 Da). As expected, negative ions of m/z 216 were observed although they had a much lower abundance than the other intermediate or product ions observed in the negative-ion mode (**Figure 4.2**). These ions were determined to have an elemental composition C<sub>8</sub>H<sub>10</sub>NO<sub>4</sub>S through high-

resolution experiments, which agrees with the elemental composition of the deprotonated compounds with the proposed structure E2 (**Scheme 4.2**). When the ions of m/z 216 were subjected to CAD, the most abundant fragment ions had an m/z value of 150 (**Figure 4.3C**) and they were formed via the loss of a molecule with MW 66 Da. The fragment ions of m/z 150 were identified by high resolution mass spectrometry to have the elemental composition C<sub>8</sub>H<sub>8</sub>NO<sub>2</sub> (**Table 4.1**). Therefore, these fragment ions were generated via the loss of H<sub>2</sub>SO<sub>2</sub> from the precursor ions of m/z 216. This fragmentation is in agreement with the structure of the proposed reaction intermediate E2. A mechanism proposed for the loss of H<sub>2</sub>SO<sub>2</sub> from the ions of m/z 216 upon CAD to generate the fragment ions of m/z 150 is shown in **Scheme 4.3**.



Scheme 4.3 The proposed mechanisms for the elimination of H<sub>2</sub>SO<sub>2</sub> (MW 66 Da) and SO<sub>2</sub> (MW 64 Da) upon CAD on deprotonated 2-(dihydroxy(oxo)- $\lambda^6$ -sulfaneyl)benzamide (*m/z* 216). The theoretical exact *m/z*-values are shown for E2 and the fragment ions. Measured exact *m/z*-values are provided in **Table 4.1**.

The second most abundant fragment ions of the ions of m/z 216 were the ions of m/z 159 (**Figure 4.3C**) formed via the elimination of a molecule with MW 57 Da (the elemental composition of these ions was not determined). This loss is likely to correspond to the elimination of methyl isocyanate (H<sub>3</sub>C-N=C=O), observed for several ions discussed above. This elimination is in agreement with the structure proposed for intermediate E2 (**Scheme 4.2**).

Other observed fragment ions had m/z values 152, 134 and 93 (**Figure 4.3C**). The fragment ions of m/z 152 were determined to have the elemental composition C<sub>8</sub>H<sub>10</sub>NO<sub>2</sub>, which means that

these ions were formed via the elimination of SO<sub>2</sub> from the ions of m/z 216. Similarly, the expulsion of SO<sub>2</sub> was reported as a common feature during CAD experiments on deprotonated aromatic sulfonic acids and their derivatives<sup>24–26</sup> through a rearrangement process discussed further in section 4.3.1.6 and shown in **Scheme 4.4** below. In this rearrangement step a carbon-sulfur bond is cleaved and a new carbon-oxygen bond is formed, possibly as shown in **Scheme 4.3**.<sup>24–26</sup> Fragment ions of m/z 134 (whose elemental composition could not be determined) may correspond to elimination of H<sub>2</sub>SO<sub>3</sub> from the ions of m/z 216, which would be in agreement with the structure E2. Moreover, phenoxide anions of m/z 93.03458 (-0.04 ppm from the theoretical value) were detected. These ions were formed via the elimination of methyl isocyanate (CH<sub>3</sub>-N=C=O) from the fragment ions of m/z 150 shown in **Scheme 4.3** as was proven by MS<sup>3</sup> experiments on ions of m/z 150. All these observations are in agreement with the detected ions of m/z 216 having the structure of the deprotonated intermediate E2 (**Scheme 4.2**).

### 4.3.1.6 Compound F2

The final product F2 of pathway 2 (Scheme 4.2), 2-(methylcarbamoyl)benzenesulfonic acid (MW 215 Da), is proposed to be formed by oxidation of the intermediate E2. Indeed, ions of m/z 214 were found to be the most abundant among the five negative ions detected by mass spectrometry in the UV/H<sub>2</sub>O<sub>2</sub> induced advanced oxidation reaction (Figure 4.2) and were thought to be formed by deprotonation of the product F2. The accurate m/z-value and the elemental composition of the ions of m/z 214 were determined to be m/z 214.01788 and C<sub>8</sub>H<sub>8</sub>NO<sub>4</sub>S, in agreement with the elemental composition of the deprotonated product F2 of pathway 2. Upon CAD, the ions of m/z 214 yielded three major fragment ions (Figure 4.3D). The most abundant fragment ions of m/z 150 were determined to have the elemental composition C<sub>8</sub>H<sub>8</sub>NO<sub>2</sub>. Therefore, these fragment ions were generated via elimination of SO<sub>2</sub> from the ions of m/z 214. This finding supports the proposed structure F2 for the ions of m/z 214 as loss of SO<sub>2</sub> has been reported in the literature for deprotonated sulfonic acid derivatives.<sup>27</sup> This reaction may occur in a similar manner as that shown in Scheme 4.4. Additionally, ions of m/z 214 fragmented to generate fragment ions of m/z 157, which were identified by high-resolution mass spectrometry to have the elemental composition C<sub>6</sub>H<sub>5</sub>O<sub>3</sub>S. Therefore, they were formed by the loss of a molecule with the elemental composition C<sub>2</sub>H<sub>3</sub>NO, corresponding to methyl isocyanate (H<sub>3</sub>C-N=C=O) elimination also

observed for several ions discussed above. The loss of methyl isocyanate was followed by the loss of sulfur dioxide to generate phenoxide anions ( $C_6H_5O^-$ ) of m/z 93. Performing CAD in an MS<sup>3</sup> experiment on the fragment ions of m/z 157 generated fragment ions of m/z 93 with an elemental composition C<sub>6</sub>H<sub>5</sub>O<sup>-</sup>. Therefore, these ions were formed via the loss of sulfur dioxide from ions of m/z 157. Moreover, the fragment ions of m/z 79.95734 corresponding to SO<sub>3</sub> radical anions (with measured m/z-value within -0.33 ppm from the theoretical value) were observed. A similar fragmentation pattern was reported for deprotonated compound F2 shown in Scheme 4.1,<sup>16</sup> which differs from that of deprotonated compound F2 in Scheme 4.2 only by the absence of the methyl group on the nitrogen. Therefore, elimination of CHNO, instead of C<sub>2</sub>H<sub>3</sub>NO, was observed followed by loss of  $SO_2$ . Additionally, formation of phenoxide anions and  $SO_3$  radical anions have been reported in the literature upon CAD on deprotonated 2-carbamoylbenzenesulfonic acid (structure F2 in Scheme 4.1). The formation of phenoxide ions and SO<sub>3</sub> radical anions has been reported in the literature when subjecting deprotonated aromatic sulfonic acids including deprotonated benzene sulfonic acid to CAD.<sup>26</sup> SO<sub>3</sub> radical anions were proposed to be formed by carbon-sulfur bond homolysis as shown in Scheme 4.4. The mechanism of formation of phenoxide ions involved rearrangement and was proposed to be initiated by intramolecular nucleophilic addition of a sulfonate oxygen to the aromatic ring followed by a heterolytic cleavage of the carbon-sulfur bond to rearomatize the ring. The resulting anions, phenylsulfite anions, undergo elimination of SO<sub>2</sub> to generate phenoxide ions as shown in Scheme 4.4.<sup>26</sup> Literature reported DFT calculations at the B3LYP/6-31+G(2d,p) level of theory for the elimination of SO<sub>2</sub> during CAD on deprotonated benzenesulfonic acid to form the phenoxide anions showed that the initial step in this process is the isomerization of the benzenesulfonate anion via a three-membered transition structure (58.6 kcal/mol) to the thermochemically more stable phenylsulfite anion (-4.8 kcal/mol). The phenylsulfite anion undergoes cleavage of an O-S bond to afford the phenoxide anion and neutral SO<sub>2</sub> (31.7 kcal/mol).<sup>25</sup>



**Scheme 4.4** Literature proposed steps for the formation of phenoxide ions and SO<sub>3</sub> radical anions in CAD experiments on deprotonated benzenesulfonic acid.<sup>26</sup>

#### 4.3.2 Results Obtained in the Positive-Ion Mass Spectrometry Experiments

Four major ions that were believed to be related to the ionized reactants or reaction products generated upon laser-induced UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation of 2-methylbenzoisothiazol-3-one were detected in the positive-ion experiments: ions of m/z 166, 182, 198, and 216 (**Figure 4.8**). These ions are likely to correspond to protonated compounds A, C, E1 and F2 (**Scheme 4.2**), respectively. Other ions of m/z 184 and 200 were observed when zooming closely into the mass spectrum. These ions likely correspond to protonated intermediates B and D1/D2 (**Scheme 4.2**). Another ion of m/z 331 was also detected and determined by high-resolution mass spectrometry (with a measured accurate mass of 331.05682 and a mass accuracy of -0.37 ppm) to have the elemental composition C<sub>16</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub>S<sub>2</sub>. These ions correspond to the proton-bound dimer of 2-methylbenzoisothiazol-3-one (compound A in **Scheme 4.2**). CAD on the ions of m/z 331 produced solely fragment ions of m/z 166 (**Table 4.1**), which corresponded to protonated 2-methylbenzoisothiazol-3-one.



**Figure 4.8** Positive-ion low-resolution mass spectrum of protonated reactants and reaction products formed in laser-induced UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation of 2-methylbenzoisothiazol-3-one. Ions corresponding to the protonated reactant A and protonated final products C, E1 and F2 were detected. Additionally, during positive ion experiments, ions of m/z 331 were observed.

#### **4.3.2.1** Compound A

The most abundant among the detected ions were those of m/z 166. These ions may correspond to the protonated reactant 2-methylbenzoisothiazol-3-one (compound A in **Scheme 4.2**). In order to verify this, the ions were transferred into the orbitrap for high-resolution measurements (**Table 4.1**). The exact m/z value of these ions (m/z 166.03229, which was within 1.06 ppm from the theoretical value) revealed an elemental composition C<sub>8</sub>H<sub>8</sub>NOS, in agreement with protonated compound A.

To probe the structure of the ions of m/z 166, they were isolated in the linear quadrupole ion trap and subjected to CAD (the low-resolution CAD mass spectra are shown in **Figure 4.9** and the high-resolution CAD results are provided in Table **4.1**). The most abundant fragment ions had an m/z value of 151 and an elemental composition of  $C_7H_5NOS$  (based on high-resolution mass spectrometry). Therefore, the fragment ions of m/z 151 were likely formed via the elimination of a methyl group from the precursor ions of m/z 166, in agreement with their proposed structure. Fragment ions of m/z 137 were generated via elimination of a neutral molecule with MW 29 Da. The elemental composition of the ions of m/z 137 was determined to be  $C_7H_5OS$  (with a mass accuracy of -0.73 ppm). Thus, the fragment ions of m/z 137 were formed via elimination of CH<sub>2</sub>=NH (methanimine). Further, the precursor ions of m/z 166 also fragmented to generate ions of m/z 123 via the loss of a neutral molecule of MW 43 Da. The elemental composition of the ions of m/z 123 (accurate m/z-value was measured to be 123.02607 which is within 0.16 ppm from the theoretical value) was determined to be C<sub>7</sub>H<sub>7</sub>S. Therefore, the fragment ions of m/z 123 were generated via elimination of HN=C=O (isocyanic acid) from the precursor ions of m/z 166. This fragmentation would require extensive rearrangement if the ions of m/z 166 had the structure of protonated A. Finally, fragment ions of m/z 109 (with measured accurate m/z of 109.01047, with a mass accuracy of -1.65 ppm) were determined to have the elemental composition C<sub>6</sub>H<sub>5</sub>S. Therefore, these fragment ions were generated by elimination of a molecule with an elemental composition C<sub>2</sub>H<sub>3</sub>NO, possibly CH<sub>3</sub>N=C=O. This fragmentation is in agreement with the structure proposed for the ions of m/z 166. It also should be noted that elimination of CH<sub>3</sub>N=C=O was observed for several related negatively charged ions as discussed above (**Figure 4.3**).

In order to confirm that the ions of m/z 166 correspond to protonated 2methylbenzoisothiazol-3-one, a 10 ppm solution of the commercially obtained neat 2methylbenzoisothiazol-3-one in acetonitrile was prepared and protonated via atmospheric pressure chemical ionization ((+)APCI). The protonated molecules were isolated and subjected to CAD (Error! Reference source not found.**Figure 4.10A**). The same four fragment ions as observed for the unknown ions of m/z 166 were observed for the protonated 2-methylbenzoisothiazol-3-one, and they had similar relative abundances, in high resolution measurements at normalized collision energy 20, and the same elemental compositions as those obtained for the fragment ions of the precursor ions of m/z 166 derived from the reaction mixture. These results confirmed the identification of ions of m/z 166 as protonated A. They also provided guidance on how related ions may fragment. For example, elimination of CH<sub>2</sub>=NH may be diagnostic for protonated compounds with the 2-methylisothiazol ring. Further, some protonated compounds containing this ring can eliminate CH<sub>3</sub>N=C=O. Finally, ions of this type can undergo fairly extensive rearrangement reactions upon fragmentation, such as elimination of HN=C=O from protonated A. These findings were used to facilitate the interpretation of the remaining detected positive ions.



Figure 4.9 Low-resolution positive-ion mode CAD mass spectra (collision energy 30 arbitrary units) measured for isolated ions of A) *m/z* 166, verified to correspond to protonated 2-methylbenzoisothiazol-3-one (A; Scheme 4.2), B) *m/z* 182, likely corresponding to protonated 2-methylbenzo[*d*]isothiazol-3(2*H*)-one 1-oxide (C; Scheme 4.2). C) *m/z* 198 likely corresponding to protonated N-methylsaccharine (E1; Scheme 4.2), and D) *m/z* 216 likely corresponding to protonated 2-(methylcarbamoyl)benzenesulfonic acid (F2; Scheme 4.2). Two structures are shown for protonated F2 as this compound has two protonation sites with nearly equal proton affinities (C=O: 209.5 kcal/mol; S=O: 209.3 kcal/mol) determined at the B3LYP/6-311G++(d,p) level of theory. Fragment ions for which the elemental composition was determined by using high-resolution mass spectrometry are indicated with a blue asterisk (\*).



**Figure 4.10** High-resolution mass spectra obtained at CAD 20 arbitrary units on APCI protonated model reference compounds. A) 2-methylbenzoisothiazol-3-one. B) N-methyl saccharin; only one elemental composition prediction was provided for ions of m/z 198, 151, 134, 121 or 106 up to 140 ppm mass tolerance.

# 4.3.2.2 Compound B

In the positive-ion mode experiments, ions of m/z 184 were detected, although not clearly observed among the five ion peaks in **Figure 4.8**. However, after zooming closely into the mass spectrum the ion peak was observable. To investigate the possibility of these ions corresponding to the protonated reaction product B (**Scheme 4.2**), the ions were transferred into the orbitrap and determined to have an exact m/z value of 184.04344 (within 4.16 ppm from the theoretical value) and therefore to have the elemental composition C<sub>8</sub>H<sub>10</sub>NO<sub>2</sub>S. This elemental composition matches the elemental composition of the protonated compound B. Unfortunately, MS<sup>2</sup> experiments could not be conducted on ions on m/z 184 due to signal loss caused by their low ion abundance.

# 4.3.2.3 Compound C

In the positive-ion mode experiments, ions of m/z 182 were among the five easily observed ions (**Figure 4.8**). To investigate the possibility of these ions corresponding to the protonated reaction product C (**Scheme 4.2**), the ions were transferred into the orbitrap and determined to have an exact m/z value of 182.02702 (within -0.01 ppm from the theoretical value) and therefore to have the elemental composition C<sub>8</sub>H<sub>8</sub>NO<sub>2</sub>S. This elemental composition matches the elemental composition of the protonated compound C.

To scrutinize the structure of the ions of m/z 182, they were isolated in the linear quadrupole ion trap and subjected to CAD. The only fragment ions observed corresponded to elimination of a neutral molecule with MW 29 Da. The elemental composition of these fragment ions (accurate measured m/z value 153.00044 was within -0.22 ppm from the theoretical value) was determined to be C<sub>7</sub>H<sub>5</sub>O<sub>2</sub>S. Therefore, the eliminated molecule with MW 29 Da corresponds to methanimine (CH<sub>2</sub>=NH). The same fragmentation was observed for the protonated compound A (see discussion above). These observations support the proposal that the ions of m/z 182 have the structure of protonated C.

According to quantum chemical calculations, the predominant protonation site of compound C (**Scheme 4.2**) is the oxygen atom of the sulfoxide group with a proton affinity of 210.5 kcal/mol as the calculated proton affinity of the carbonyl oxygen is only 206.4 kcal/mol while the nitrogen atom has a proton affinity of only 199.2 kcal/mol determined at the B3LYP/6-311G++(d,p) level of theory. Based on this result, a possible mechanism for the elimination of CH<sub>2</sub>=NH from the ions of m/z 182 is shown in **Figure 4.11** together with a potential energy surface obtained using quantum chemical calculations based on density functional theory (DFT) at the M06-2X/6-311++G(d,p) level of theory.



**Figure 4.11** Calculated enthalpies for the elimination of methanimine upon CAD from protonated 2-methylbenzoisothiazol-3-one 1-oxide (compound C). All values are in kcal/mol and were calculated relative to the infinitely separated reactants at the M06-2X/6-311++G(d,p) level of theory. The theoretical exact m/z-values are shown for C and the final fragment ion. Measured exact m/z-values are provided in **Table 4.1**.

# 4.3.2.4 Compounds D1/D2

In the positive-ion mode experiments, ions of m/z 200 were detected, although not clearly observed among the five ion peaks in **Figure 4.8**. However, after zooming closely into the mass spectrum the ion peak was observable. To investigate the possibility of these ions corresponding to the protonated reaction product D1/D2 (**Scheme 4.2**), the ions were transferred into the orbitrap and determined to have an exact m/z value of 200.03850 (within 4.54 ppm from the theoretical value) and therefore to have the elemental composition C<sub>8</sub>H<sub>10</sub>NO<sub>3</sub>S. This elemental composition matches the elemental composition of the protonated compounds D1/D2. Unfortunately, MS<sup>2</sup> experiments could not be conducted on ions on m/z 200 due to signal loss caused by their low ion abundance.

# 4.3.2.5 Compound E1

Ions of m/z 198 were also observed in the positive-ion experiments (**Figure 4.8**). These ions may be generated upon protonation of N-methylsaccharine (E1), the final product of reaction pathway 1 (**Scheme 4.2**). The ions (with an accurate measured m/z 198.02190 and a mass accuracy of -0.22 ppm) were determined to have the chemical formula C<sub>8</sub>H<sub>8</sub>NO<sub>3</sub>S. This elemental composition is in agreement with the elemental composition of protonated N-methylsaccharine (E1).

CAD experiments in the linear quadrupole ion trap were utilized to study the structure of the ions of m/z 198 (**Figure 4.9C**). The fragment ions of m/z 134 were the most dominant fragment ions. Their elemental composition was C<sub>8</sub>H<sub>8</sub>NO (with a mass accuracy of -1.91 ppm). The generation of these fragment ions therefore occurred via elimination of SO<sub>2</sub>, in agreement with structure E1. Performing CAD in an MS<sup>3</sup> experiment on the fragment ions of m/z 134 generated fragment ions of m/z 106 with an elemental composition C<sub>7</sub>H<sub>8</sub>N. Therefore, these ions were formed via the loss of CO. These fragmentations are in agreement with the ions of m/z 198 having the structure of protonated E1.

Ions of m/z 121 were the second most-abundant fragment ions of precursor ions of m/z 198 (**Figure 4.9C**). These fragment ions (with a measured accurate mass of 121.02825 and a mass accuracy of -1.27 ppm) were determined to have the elemental composition C<sub>7</sub>H<sub>5</sub>O<sub>2</sub>. Therefore, a molecule with the elemental composition CH<sub>3</sub>NOS was lost from the precursor ions of m/z 198 to form the fragment ions of m/z 121. The structure of the above neutral molecule is likely to be CH<sub>3</sub>N=S=O, which supports the proposal that the ions of m/z 198 correspond to protonated E1.

Fragment ions of m/z 169 (with an accurate m/z-value of 168.99516 and a mass accuracy of -1.35 ppm) of precursor ions of m/z 198 were determined to have the elemental composition C<sub>7</sub>H<sub>5</sub>O<sub>3</sub>S and are therefore generated upon the loss of methanimine (CH<sub>2</sub>=NH). The same fragmentation was observed for protonated A and C (as discussed above). Performing CAD in MS<sup>3</sup> experiments on fragment ions of m/z 169 generated fragment ions of m/z 152 (with an accurate m/z 151.99257 and a mass accuracy of -0.54 ppm) with the elemental composition C<sub>7</sub>H<sub>4</sub>O<sub>2</sub>S. This fragmentation involved the elimination of an OH radical. These fragmentations are in support of the structure proposed for the ions of m/z 198.

N-Methylsaccharin (compound E1, **Scheme 4.2**) is commercially available; therefore, it was obtained to verify the above structural characterization. A 10 ppm solution of N-

methylsaccharin in acetonitrile was injected into the mass spectrometer and subjected to (+)APCI. Protonated N-methylsaccharine of m/z 198 was transferred into the linear ion trap, isolated and subjected to CAD (**Figure 4.10B**). The detected fragment ions were the same and had similar relative abundances as those obtained upon CAD, in high-resolution measurements at collision energy 20, of the unknown ions of m/z 198 derived from the oxidation reaction. This finding confirms the structure of the ions of m/z 198 as protonated E1. The observed fragmentation patterns suggest that protonated compounds of this type containing a sulfone moiety may rapidly eliminate SO<sub>2</sub> upon CAD. It also supports the above hypothesis that the loss of methanimine (CH<sub>2</sub>=NH) may be diagnostic for protonated compounds with the 2-methylisothiazol ring. Further support was obtained for the above hypothesis that major rearrangements may occur upon the fragmentation of this type of protonated molecules. Finally, as the protonated compound A predominantly eliminated a methyl radical, the protonated compound C exclusively eliminated CH<sub>2</sub>=NH, and the protonated compound E1 predominantly eliminated SO<sub>2</sub>, the fragmentation pathways of this type of compounds are clearly very sensitive to the exact structures of the ions.

#### 4.3.2.6 Compound E2

Unlike ions for compounds B and D1/D2, ion peak corresponding to protonated compound E2 was not observed even when zooming closely into the mass spectrum. This can be explained by the lower abundance of compound E2 in the reaction mixture compared to the other compounds, which was clearly seen in negative-ion mode results (ions of m/z 216 corresponding to deprotonated E2 had the lowest abundance of all detected ions). Additionally, sulfonic and sulfinic acids and their derivatives have better ionization efficiencies in negative-ion mode than positive-ion mode,<sup>23,24</sup> and are hence rarely studied in their protonated forms; therefore, the lower abundance of compound E2 than the other compounds in the reaction mixture in addition to low protonation efficiency of compound E2 were perhaps the reasons for the difficulty in observing protonated E2 in positive-ion mode experiments.

#### 4.3.2.7 Compound F2

During the positive-ion experiments, ions of m/z 216 were also observed (Figure 4.8). These ions may be generated upon protonation of the final reaction product F2 of pathway 2 (Scheme 4.2). The ions (with an accurate m/z 216.03239 and an error of -0.51 ppm) were determined to have the elemental composition C<sub>8</sub>H<sub>10</sub>NO<sub>4</sub>S, which agrees with the elemental composition of protonated F2 (Scheme 4.2). Based on DFT calculations at the B3LYP/6-311G++(d,p) level of theory, F2 molecules can be protonated on either the oxygen atom of the carbonyl group (PA = 209.5 kcal/mol) or the oxygen atom of the sulfonic acid group (PA = 209.3), with a proton affinity difference of only about 0.2 kcal/mol. Therefore, both structures are likely to be formed as shown in **Figure 4.9D**.

CAD experiments in the linear quadrupole ion trap were utilized to study the structure of the ions of m/z 216. Fragment ions of m/z 199 were the most dominant. Their elemental composition was determined to be C<sub>8</sub>H<sub>7</sub>O<sub>4</sub>S (with a mass accuracy of -0.87 ppm). Therefore, elimination of ammonia likely occurred in this fragmentation, in agreement with the structure of protonated E1, possibly as shown in **Scheme 4.5**. In **Scheme 4.5**, the structure of F2 protonated at the sulfoxide oxygen is shown, as this structure is more likely to donate a proton to the amido group.



Scheme 4.5 A possible mechanism for the elimination of ammonia upon CAD on protonated 2-carbamoylbenzenesulfonic acid (F2). The theoretical exact m/z-values are shown for F2 and the final fragment ion. The measured accurate m/z-values of protonated F2 and the final fragment ion are shown in **Table 4.1**.

Fragment ions of m/z 185 were the second most-abundant fragment ions of the precursor ions of m/z 216. These fragment ions (with a measured accurate mass of 184.99042 and a mass accuracy of 0.60 ppm) were determined to have the elemental composition C<sub>7</sub>H<sub>5</sub>O<sub>4</sub>S. Therefore, a

molecule with the elemental composition  $CH_3NH_2$  was lost from the precursor ions of m/z 216 to form the fragment ions of m/z 185 (**Figure 4.9D**). A mechanism proposed for the formation of ions of m/z 185 is shown in **Scheme 4.5**.

# 4.4 Proposed Ionization Mechanisms

Since the ions detected in the positive- and negative-ion mass spectrometry experiments in this study were as expected based on the previously proposed<sup>4</sup> reaction intermediates and products of UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation of benzoisothiazol-3-one in solution, the reactions likely occurred in solution rather than in the gas phase. Moving the stationary droplet 0.5 inch from the laser beam so that mostly gaseous molecules would be reaching the laser resulted in observation of negative air ions including those of m/z 62, 77 and 125, but none of the ionized reaction intermediates or products were observed in negative-ion mode. A similar experiment in the positive-ion mode resulted in observing protonated 2-methylbenzoisothiazolin-3-one and the proton-bound dimer of 2-methylbenzoisothiazolin-3-one, yet none of the protonated reaction products were observed.

During the experiments, the laser beam did not directly hit the stationary droplet. Therefore, the laser beam must have hit microdroplets generated from the stationary droplet while they traveled toward the inlet of the mass spectrometer, which initiated the reactions via hydroxyl radical formation in the microdroplets. The generation of microdroplets from the suspended droplet may be a result of the shear from the drag induced by the mass spectrometer (pressure gradient).<sup>27,28</sup> The release of microdroplets is enhanced for solvents with a lower surface tension.<sup>29</sup> This likely explains the observed enhancement in the signal when solvents with lower surface tension than water were first tested in this study. The surface tension of water is 72.0 mN m<sup>-1</sup>, which is at least 2.5 times the surface tension of the other solvents that were tested in this study (acetonitrile 28.3, methanol 22.3, and acetone 22.7 mN m<sup>-1</sup>).<sup>30,31</sup>

Further break-down of the microdroplets due to the laser beam increased their surface area, thus enhanced heat transfer and increased evaporation rate<sup>27</sup> to eventually release reagent, reaction product and reaction intermediate molecules into the gas phase. Thus, the boiling point of the solvent in use affects the efficiency of solvent evaporation. This is likely to partially explain the enhanced signal when solvents with lower boiling points than water were used; the boiling point of water is 100.0 °C, while it is 81.7, 64.6, and 56.1 for acetonitrile, methanol and acetone, respectively.<sup>32</sup>

The generation of ions is a crucial step in mass spectrometric analyses. However, the mechanisms through which the ions are formed can be complex. In order to better understand the ionization mechanism(s) of reactants, reaction intermediates and products formed in the laser-induced UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation experiments in this study, mass spectra obtained during laser operation in air in the absence of the stationary droplet as well as in the presence of a stationary droplet containing the reaction mixture with the exception of the 2-methylbenzoisothiazol-3-one were examined. **Figure 4.12** shows a mass spectrum of the negative ions generated from ambient air by the laser beam (their elemental compositions were identified. The identity of the ion of m/z 127 is unknown at this time but an elemental composition CH<sub>3</sub>O<sub>7</sub> (error -1.2 ppm) was determined for it (possibly corresponding to a cluster HCO<sub>4</sub><sup>-</sup>, H<sub>2</sub>O, O<sub>2</sub>). Notably, these same ions have been reported in the literature as the negative ions formed upon corona discharge ionization of air.<sup>22</sup>



Figure 4.12 Low-resolution negative-ion mass spectrum showing the negatively charged ions generated by the laser beam from ambient air and their elemental compositions determined by using high-resolution experiments. No ions were observed above m/z 200.

When the same experiment was carried out in the presence of a stationary droplet containing 50 ppm H<sub>2</sub>O<sub>2</sub> in acetonitrile, the negative ions shown in **Figure 4.13** were detected. All the same ions were detected as observed in air, with the exception of the ions of m/z 127. Ions of m/z 125 corresponding to the deprotonated nitric acid dimer NO<sub>3</sub><sup>-</sup>(HNO<sub>3</sub>) dominated the mass

spectrum. Interestingly, the same was previously observed when introducing hydroxyl radicals into a corona discharge in air.<sup>22</sup> The domination of deprotonated nitric acid dimer is rationalized by the enhanced production of HNO<sub>3</sub> in the presence of hydroxyl radicals generated from H<sub>2</sub>O upon corona discharge. The formation of HNO<sub>3</sub> accelerates the conversion of primary negative ions formed from air to  $NO_3^-$  ions.<sup>22</sup> In the present study, hydroxyl radicals were produced by the exposure of the microdroplets containing H<sub>2</sub>O<sub>2</sub> to UV light. Based on the above results, the ionization mechanisms in the laser-based method used in this study may be similar to corona discharge ionization mechanisms.



Figure 4.13 Negative-ion mass spectrum of the ions generated by the laser beam from ambient air in the presence of a stationary droplet containing 50 ppm  $H_2O_2$  in acetonitrile. No ions were observed above m/z 200.

As mentioned above, the laser beam produces primary ions from air, that participate in the generation of negative air ions such as  $NO_3^-$  detected in the negative-ion mode. These ions are expected to undergo ion-molecule reactions in a chemical ionization-like process to deprotonate the reagent, intermediate and product molecules released into air from the microdroplets. Similarly, in the positive-ion experiments, the laser beam produces primary ions from air molecules. These ions likely undergo ion-molecule reactions with the solvent molecules to produce protonated solvent molecules (ions of m/z 83 correspond to the protonated dimer of acetonitrile were detected within the background ions in positive-ion mode experiments). The protonated solvent and solvent related molecules transfer a proton, in a chemical ionization-like process, to the reagent,

intermediate and product molecules released from the microdroplets. An illustration of the proposed ionization mechanism is provided in **Figure 4.14**, wherein negative-ion mode ionization is used as an example.



**Figure 4.14** A general illustration of the proposed negative-ion ionization process in a side view of the experimental setup (the figure is not drawn in correct proportions). Negatively charged ions from air are proposed to be formed first and to participate in the ionization of the reaction intermediates and products.

## 4.5 Conclusions

The final products of the UV/H<sub>2</sub>O<sub>2</sub> induced advanced oxidation of benzoisothiazol-3-one have been determined in the literature and reaction pathways with intermediates have been proposed but none of the intermediates were detected.<sup>4</sup> In this study, a new experimental strategy was developed for the detection and structural characterization of the short-lived reaction intermediates formed during UV/H<sub>2</sub>O<sub>2</sub> induced advanced oxidation of 2-methylbenzoisothiazol-3one. In this method, a stationary droplet of the reagent solution was formed at 3 mm distance from the ion transfer capillary tube of a mass spectrometer. To initiate the reaction, a focused highpower laser beam was pulsed through the microdroplets released from the stationary droplet into the 3 mm gap between the stationary droplet and the heated capillary tube of the mass spectrometer. The laser beam also facilitated the evaporation and ionization of any reagents, short-lived reaction intermediates and final products in the reacting microdroplets for online detection through the mass spectrometer.

All previously proposed<sup>4</sup> intermediates and products were detected in either negative- or positive ion-mode mass spectrometry experiments. High-resolution mass spectrometry was used to determine the elemental compositions of all the detected ions and they were found to be in agreement with the structures proposed in the literature.<sup>4</sup> Low- and high-resolution CAD experiments provided structural information for the observed ions. The data were in agreement with the structures proposed in the literature.<sup>4</sup>

The presented experimental method for detection of short-lived reaction intermediates in solution is simple and is not limited to  $UV/H_2O_2$  advanced oxidation reactions.

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# CHAPTER 5. DEVELOPMENT OF GAS-PHASE ION-MOLECULE REACTIONS COUPLED WITH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR HIGH THROUGHPUT IDENTIFICATION OF CARBOXYLIC ACID FUNCTIONALITIES IN PROTONATED ANALYTES

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# 5.1 Introduction

The identification of chemical functionalities in unknown drug metabolites, degradation products and impurities is a common requirement during drug manufacturing and development. Therefore, high throughput analytical methods that can facilitate the identification of chemical functionalities, including carboxylic acid functionalities, are of high importance.<sup>1-3</sup> Complex mixture separation by using high-performance liquid chromatography (HPLC) followed by analysis using tandem mass spectrometry based on collision-activated dissociation (CAD) has become the method of choice in the analysis of complex mixtures in pharmaceutical industries.<sup>4</sup> However, when using tandem mass spectrometry based on CAD, reference standards are needed to identify unknown compounds in complex mixtures.<sup>3,5,6</sup> Moreover, CAD often does not allow the identification of chemical functionalities. Furthermore, ionized isomeric compounds often generate identical fragmentation patterns.<sup>3,7–9</sup> In such cases, tandem mass spectrometry methods based on diagnostic gas-phase ion-molecule reactions may be a suitable alternative method for the characterization and structural elucidation of unknown ionized compounds.<sup>10</sup> During the past three decades, our research group has developed diagnostic gas-phase ion-molecule reactions for the identification of many chemical functionalities in ionized analytes.<sup>11–18</sup> For the unambiguous identification of chemical functionalities in complex analytes, it is useful to find more than one reagent that forms a diagnostic product ion when allowed to react with an ionized analyte containing a specific chemical functionality. For carboxylic acid functionalities, diethylmethoxyborane and trimethyl borate have been previously reported as suitable reagents for the identification of the carboxylic acid functionality in deprotonated compounds and their ammonium cation adducts, respectively.<sup>19,20</sup> To expand the data base of functional group-selective ion-molecule reactions, a method based on a diagnostic reaction of protonated analytes with a

carboxylic acid functionality with (isopropenoxy)trimethylsilane (ITS) was developed. In this chapter, gas-phase ion-molecule reactions of protonated monofunctional and polyfunctional model compounds with ITS are used to illustrate the ability to identify carboxylic acid functionalities in protonated analytes. This tandem mass spectrometric method was coupled with HPLC separation to demonstrate the applicability of the method for high throughput complex mixture analysis. It is worth mentioning that the idea of utilizing the neutral reagent ITS for the identification of carboxylic acids in gas-phase ion-molecule reactions was first tested by Joann P. Max, an alumnus from our group who graduated in 2018.<sup>21</sup> However, the tests were limited to a few monofunctional aromatic carboxylic acids, and there was a need to test the applicability of the method on a variety of aliphatic, aromatic and polyfunctional compounds. Furthermore, quantum chemical calculations were utilized to determine the proton affinities of the compounds previously tested by Joann P. Max as well as of the compounds containing aliphatic carboxylic acid functionality or several functionalities. Additionally, the reaction kinetics were investigated to determine the reaction efficiencies of all tested protonated compounds with ITS in the gas phase.

#### 5.2 Experimental

# 5.2.1 Chemicals

Benzoic acid, 3-aminobenzoic acid, 4-aminobenzoic acid, 4-acetamidobenzoic acid, 4methoxybenzoic acid, trans-cinnamic acid, trans-3-hydroxycinnamic acid, 4-hydroxybenzoic acid, hexanoic acid, 2-methylhexanoic acid, octanoic acid, oxcarbazepine, carbamazepine, 2-methyl-4nitropyridine N-oxide, 4-nitropyridine N-oxide, 4-methylpyridine N-oxide, diphenylamine, benzylamine, aniline, benzophenone, cyclohexanone, *N*-*N*,*N*-diethylhydroxylamine, cyclohexylhydroxylamine, butyl sulfone, 4-aminophenyl sulfone, benzenesulfonamide, 4-(2aminoethyl)benzenesulfonamide, methanesulfonamide, 4-hydroxybenzenesulfonamide, diphenyl sulfoxide, methyl phenyl sulfone, naproxen, clozapine N-oxide, celecoxib, L-methionine sulfone, 3,4-dihydroxyphenylacetic acid, 1,2-dihydroxybenzene, 1,4-dihydroxybenzene, guaiacol, anisole, acetamide, methyl benzoate, methyl propionate, benzaldehyde and (isopropenyoxy)trimethylsilane were purchased from Sigma Aldrich (Saint Louis, MO, USA) and used as received. High-performance liquid chromatography-mass spectrometry (HPLC/MS) grade water, acetonitrile and methanol as well as formic acid and benzaldehyde were purchased from

Fisher Scientific (Pittsburgh, PA, USA). Phenol (1-<sup>13</sup>C, 99%) was purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA).

### 5.2.2 Mass Spectrometry

All mass spectrometry experiments were performed using a Thermo Scientific LTQ linear quadrupole ion trap mass spectrometer (LQIT) modified with an external reagent mixing manifold.<sup>20</sup> The mass spectrometer was coupled with an atmospheric pressure chemical ionization (APCI) source and was operated in the positive-ion detection mode. All sample solutions were prepared at a 1 mM concentration in 50/50 (v/v) water/methanol solution. Model compound solutions were introduced into the ion source through direct injection via a 500  $\mu$ L Hamilton syringe at a flow rate of 20  $\mu$ L min<sup>-1</sup>. Typical APCI conditions were 300 °C vaporizer temperature, 30 (arbitrary unit) sheath gas (N<sub>2</sub>) flow, 15 (arbitrary unit) auxiliary gas (N<sub>2</sub>) flow, and 5  $\mu$ A discharge current.

#### 5.2.3 Ion-molecule Reactions

Protonated analytes were transferred from the APCI source into the linear quadrupole ion trap where they encountered the reagent ITS that was introduced into the linear quadrupole ion trap via a previously described external reagent mixing manifold.<sup>22,23</sup> A 500  $\mu$ L Hamilton syringe operated by a syringe pump was used to introduce ITS into the reagent mixing manifold at a flow rate of 10  $\mu$ L h<sup>-1</sup>, which directed the reagent into the helium flow line operated at a gas flow rate of 500 mL min<sup>-1</sup>. The ITS-helium mixture was directed into a controllable variable leak valve that allowed a fraction of the mixture to enter the ion trap at a flow rate of 2 mL min<sup>-1</sup> while the excess mixture was directed into an exhaust line. Analyte ions were isolated in the ion trap using an isolation width of 2 *m*/*z* units and a q value of 0.25. The isolated ions were allowed to react with ITS for different times that were increased in increments of 10 ms starting from the default time of 30 ms, until about 5% of the abundance of the isolated analyte ions remained in the ion trap at a q value of 0.25. At this time, all ions were determined. If a primary product underwent a secondary reaction, then the branching ratio of the primary product included the sum of the abundances of the primary product included the sum of the abundances of the primary product included the sum of the abundances of the primary product included the sum of the abundances of the primary product included the sum of the abundances of the primary product included the sum of the abundances of the primary product included the sum of the abundances.

### 5.2.4 Reaction Kinetics

In gas-phase ion-molecule reactions, the reaction efficiency is defined as the fraction of collisions that produces products. The method adopted here for the determination of reaction efficiencies in gas-phase ion-molecule reactions has been described previously.<sup>11</sup> The mass spectrometer was used to monitor the change in the abundances of the ionized analytes and the product ions as a function of time during the gas-phase reactions. Plots of ion abundance vs. time were generated and linearized by taking the natural logarithm of the ion abundances. The slope of a semi-logarithmic plot of the decay of the abundance of the reactant ions as a function of time corresponded to the second-order reaction rate constant, *k*, times the concentration of the reagent, ITS. The reaction efficiencies were derived by using Equation 5.1,

$$Efficiency = \frac{slope(IM)}{slope(PT)} * \left(\frac{M_i M_{(PT)} + M_n}{M_{(PT)}(M_i + M_n)}\right)^{\frac{1}{2}} * \left(\frac{P_{n(PT)}}{P_{n(IM)}}\right)$$
(5.1)

where *slope (IM)* was the slope of the decay of the abundance of the protonated analyte reacting with ITS, *slope (PT)* was the slope of the decay of the abundance of protonated acetone reacting with ITS (assumed to occur at collision rate),  $M_i$  was the mass of the analyte ion,  $M_{(PT)}$  was the mass of protonated acetone,  $M_n$  was the mass of the reagent (ITS), and  $P_{n(IM)}$  and  $P_{n(PT)}$  were the pressure readings of the ion gauge for the reagent during the reactions of the analyte ion and protonated acetone, respectively, with ITS. A variable leak valve was set to maintain the ion gauge pressure reading at  $0.75 \times 10^{-5}$  Torr. Therefore, the term including the ratio of  $P_{n(PT)}$  to  $P_{n(IM)}$  was equal to 1.

#### 5.2.5 High Performance Liquid Chromatography (HPLC)

All HPLC experiments conducted in this study were performed on a Surveyor Plus HPLC system that was interfaced with the LQIT mass spectrometer. Compounds were introduced into the HPLC/MS via an autosampler through a full-loop injection line of 25  $\mu$ L volume. A Zorbax SB-C18 column (4.6 mm x 250 mm, 5  $\mu$ m particle size) purchased from Agilent Technologies (Santa Clara, CA, USA) was employed. Mobile phase solutions A and B contained 0.1% (v/v) formic acid in water and 0.1% (v/v) formic acid in acetonitrile, respectively. The gradient mobile phase constituents were as follows: 0.00 min, 80% A and 20% B; 23.00 min, 25% A, 75% B; 24.00
min, 5% A, 95% B, 24.99 min, 5% A, 95% B, 25.00 min, 80% A, 20% B; and 35.00 min, 80% A, 20% B. The sample flow rate was 0.8 mL min<sup>-1</sup>. The column was placed in a temperature-controlled compartment maintained at 30 °C. Selected ions from protonated model compounds were monitored during HPLC runs, on which tandem mass spectrometric analyses were performed.

## 5.2.6 Computational Studies

All calculations were based on density functional theory (DFT) and were performed using the Gaussian 09 software package. Calculations involving transition states were performed by lab alumni Mckay Easton and Jacob R. Milton at the M06-2X/6-311++G(d,p) level of theory. All transition state structures were determined to contain exactly one negative frequency. Proton affinities of the tested analytes were calculated at the B3LYP/6-31G++(d,p) level of theory. The lowest-energy conformers of all neutral and protonated molecules were identified using the Maestro 7.0 Macromodel conformational search by using the MMFF94s force field.

#### 5.3 **Results and Discussion**

The previous use of the reagent (isopropenoxy)trimethylsilane (ITS; **Figure 5.1a**) in the derivatization of carboxylic acids for gas chromatographic analysis<sup>24</sup> in addition to its high volatility (boiling point 93 °C) made it an attractive reagent for the mass spectrometric identification of carboxylic acid functionalities.



**Figure 5.1** a) The Lewis structure of (isopropenoxy)trimethylsilane (ITS). b) Mass spectrum measured after 30 ms reactions between protonated 3-aminobenzoic acid ( $[M+H]^+$ ) and ITS.

**Figure 5.1b** shows a mass spectrum obtained after 30 ms reactions between ITS and protonated 3-aminobenzoic acid. Three product ions were observed: protonated ITS (m/z 131), an adduct that had lost an acetone molecule, [adduct – acetone]<sup>+</sup> (m/z 210), and a secondary product ion formed upon reaction of the primary product ion with a second ITS molecule followed by loss of a second acetone molecule,  $2^{\circ}$  [adduct – acetone]<sup>+</sup> (m/z 282). Similar products were observed for all protonated carboxylic acids tested in this study, regardless of the presence of other functionalities (**Table 5.1**). A plot generated by monitoring the abundances of protonated 4-aminobenzoic acid, the primary reaction product [Adduct-acetone]<sup>+</sup> and the secondary reaction product  $2^{\circ}$ [Adduct-acetone]<sup>+</sup> as a function of reaction time is provided in **Figure 5.2** below.



**Figure 5.2** A plot of the abundances of protonated 4-aminobenzoic acid, the primary reaction product  $[adduct - acetone]^+$  and the secondary reaction product  $2^\circ [adduct - acetone]^+$  versus reaction time.

In order to test the uniqueness of the reaction between ITS and protonated compounds containing carboxylic acid functionalities, various analytes containing other functional groups were ionized and allowed to react with ITS (**Table 5.2**). Protonated compounds containing *N*-oxo, sulfoxo, carboxamido, keto, amino, hydroxylamino, sulfone, phenol, nitro, amido or sulfonamido functionalities but no carboxylic acid functionality react with ITS to form only the proton transfer product ion and the primary [adduct – acetone]<sup>+</sup> product ion (**Table 5.2**). In contrary to analytes containing a carboxylic acid functionality, none of these analytes produced a secondary [adduct – acetone]<sup>+</sup> product ion even after long reaction times until roughly 5% of the reactant ions remained in the ion trap. Thus, the observation of the  $2^{\circ}$  [adduct – acetone]<sup>+</sup> product ions was considered diagnostic for protonated compounds with a carboxylic acid functionality.

A reaction mechanism proposed for the reaction between protonated compounds containing a carboxylic acid functionality, exemplified by benzoic acid, with ITS was examined experimentally and computationally to better understand why 2° [adduct – acetone]<sup>+</sup> formation

appeared to be selective for compounds containing a carboxylic acid functionality even in the presence of another, more basic/nucleophilic functionality.

Most functional group-selective ion-molecule reactions start with proton transfer from the protonated analyte to the neutral reagent.<sup>19</sup> **Figure 5.3** shows the optimized geometries of the neutral and protonated reagent ITS (calculated at the B3LYP/6-31G++(d,p) level of theory). Proton transfer was proposed to occur primarily at the terminal carbon of the C=C in ITS since this is calculated to be the most basic site (PA = 224 kcal/mol). ITS has the ability to deprotonate all protonated compounds containing a carboxylic acid functionality shown in **Table 5.1** since ITS has a greater proton affinity than these analytes. Many of these reactions are highly exothermic. Despite of the high exothermicity of the proton transfer reactions, a substantial amount of the proton transfer products undergoes further reactions with each other within the ion/molecule collision complex instead of yielding separated protonated ITS and a neutral compound containing the carboxylic acid functionality.

**Table 5.1** Product ions, their branching ratios and the efficiencies for reactions of protonated compounds containing a carboxylic acid functionality with (isopropenoxy)trimethylsilane (ITS) (proton affinity = 224 kcal/mol; calculated at the B3LYP/6-31G++(d,p) level of theory). NA: not applicable.

Reactant ( $m/z$ of M+H)	Proton affinity (PA, kcal/mol)	Product ions $(m/z)$ and branching ratios		Reaction efficiency	
	196 <sup>a</sup>	Adduct – acetone (195)	27%	94%	
ОН		Protonated ITS (131)	73%		
(123) H <sub>2</sub> N	207 <sup>a</sup>	Adduct – acetone (210) 2° Adduct – acetone (282)	56%	97%	
С С ОН		Protonated ITS (131)	44%		
(138)					
ОН	207 <sup>a</sup>	Adduct – acetone (210) 2º Adduct – acetone (282)	83%	45%	
H <sub>2</sub> N		Protonated ITS (131)	17%		
(138)					
О	208 <sup>b</sup>	Adduct – acetone (252) 2º Adduct – acetone (324)	95%	33%	
N N N		Protonated ITS (131)	5%		
H (180)					
$H_2N$ (138) $O$ $H_2N$ $O$	208 <sup>b</sup>	Adduct – acetone (252) <b>2º Adduct – acetone (324)</b> Protonated ITS (131)	95% 5%	33%	

#### Table 5.1 continued



<sup>a</sup>Experimentally determined PA value from reference 25. <sup>b</sup>Calculated PA value (B3LYP/6-31G++(d,p) level of theory) from reference 11. <sup>c</sup>Calculated PA value (B3LYP/6-31G++(d,p) level of theory) from supporting information of reference 26. <sup>d</sup>Calculated at the B3LYP/6-31G++(d,p) level of theory. <sup>e</sup>Both the reactant ion and protonated ITS have m/z of 131. Therefore, it is not possible to calculate the reaction efficiency or product branching ratio.

**Table 5.2** Products, their branching ratios and the efficiencies for reactions of protonated sulfones, hydroxylamines, sulfonamides, ketones, amines, N-oxides, sulfoxides and carboxamides with ITS (proton affinity = 224 kcal/mol; calculated at the B3LYP/6-31G++(d,p) level of theory).

Reactant ( $m/z$ of M+H)	Proton affinity (PA, kcal/mol)	Product ions $(m/z)$ and branching ratio	tios	Reaction efficiency	
,0 ,5' (179)	204ª	Adduct – acetone (251) Protonated ITS (131) Acetone adduct (237)	94% 6%	33%	
	218 <sup>b</sup>	Protonated ITS (131) Adduct – acetone (162)	88% 22%	45%	
(50) N ОН	216 <sup>a</sup>	Protonated ITS (131) Adduct – acetone (188)	90% 10%	47%	
(116) 0 S NH <sub>2</sub> 0	203°	Adduct – acetone (230) Protonated ITS (131)	76% 24%	41%	
(158) O S O NH2	215 <sup>f</sup>	Adduct – acetone (273)	100%	41%	
(201) (201)	206 <sup>d</sup>	Adduct – acetone (246) Protonated ITS (131)	87% 13%	99%	
(174) 0 0	211 <sup>b</sup>	Adduct – acetone (255) Protonated ITS (131)	96% 4%	75%	
(183) (99)	201 <sup>b</sup>	Adduct – acetone (171) Protonated ITS (131)	55% 45%	89%	
H (170)	214 <sup>c</sup>	Protonated ITS (131) Adduct – acetone (242)	86% 14%	82%	

# Table 5.2 continued

NH <sub>2</sub> (108)	218 <sup>b</sup>	Adduct – acetone (180) Protonated ITS (131)	74% 95% 26%
(94) NH <sub>2</sub>	211 <sup>b</sup>	Protonated ITS (131) Adduct – acetone (166)	86% 79% 14%
NO <sub>2</sub> N+ O <sup>-</sup> (141)	207 <sup>b</sup>	Adduct – acetone (213) Protonated ITS (131)	62% 85% 38%
(141) N+ O <sup>-</sup> (110)	226 <sup>e</sup>	Adduct – acetone (182)	100% 90%
0 " (203)	222ª	Adduct – acetone (275)	100% 92%
0 NH <sub>2</sub> (253)	215 <sup>f</sup>	Adduct – acetone (325)	100% 72%
(237)	221 <sup>f</sup>	Adduct – acetone (309)	100% 82%
0 NH <sub>2</sub> (60)	206 <sup>b</sup>	Adduct – acetone (132)	100% 24%

# Table 5.2 continued

OH (125)	203ª	Adduct – acetone (197) 2º Adduct – acetone (269) Protonated ITS (131)	75% 25%	45%
(96)	197 <sup>a</sup>	Protonated ITS (131) Adduct – acetone (168)	90% 10%	4%
(109)	204 <sup>a</sup>	Protonated ITS (131)	100%	24%
0 (137)	201 <sup>f</sup>	Adduct – acetone (209) Protonated ITS (131)	92% 8%	49%
0 (89)	194 <sup>f</sup>	Adduct – acetone (161) Protonated ITS (131)	59% 41%	48%
(107) 0	202 <sup>f</sup>	Adduct – acetone (179) Protonated ITS (131)	92% 8%	42%
ОН	195 <sup>g</sup>	Adduct – acetone (183) 2º Adduct – acetone (255) Protonated ITS (131)	87% 7%	2%
(111) OH (111) (111)	199 <sup>g</sup>	Adduct – acetone (183) 2° Adduct – acetone (255) Protonated ITS (131)	54% 37%	5%

<sup>a</sup>Calculated PA value (B3LYP/6-31G(d) level of theory) from reference18. <sup>b</sup>Experimentally determined PA value from reference 25. <sup>c</sup>Calculated PA value (B3LYP/6-31G++(d,p) level of theory) from reference 11. <sup>d</sup>Calculated PA value (B3LYP/6-31G(d) level of theory) from reference 27. <sup>f</sup>Calculated at the B3LYP/6-31G++(d,p) level of theory. <sup>g</sup>Calculated PA value (B3LYP/6-31G(d) level of theory) from reference 28.



**Figure 5.3** Optimized geometries of neutral ITS (left) and ITS protonated on carbon 7 (right). Proton affinities (calculated at the B3LYP/6-31G++(d,p) level of theory) are indicated for the most basic functionalities.<sup>21</sup>

Quantum chemical calculations performed on protonated benzoic acid supported the proposal that the mechanism for the formation of the primary [adduct – acetone]<sup>+</sup> product ions begins with proton transfer from the protonated analyte to the most basic sight, the terminal carbon in the C=C bond, in ITS (**Figure 5.4a**). Calculations further suggested that nucleophilic substitution of acetone at the silicon atom of protonated ITS by the carbonyl oxygen atom of the carboxylic acid is the likely route for production of the primary product [adduct – acetone]<sup>+</sup>. The barrier for the formation of [adduct – acetone]<sup>+</sup> for protonated benzoic acid is -24.7 kcal/mol relative to the separated reactants (**Figure 5.4a**). This primary product ion is proposed to react with another ITS molecule by proton transfer followed by nucleophilic substitution of a second acetone molecule, with a barrier of -15.7 kcal/mol relative to separated reactants ([adduct – acetone]<sup>+</sup> and ITS), to generate the diagnostic secondary [adduct – acetone]<sup>+</sup> product ions. All structures are shown in **Figure 5.4**.



**Figure 5.4** Calculated potential energy surfaces (enthalpies in kcal/mol) for the formation of a) the primary [adduct – acetone] product ion and b) the diagnostic secondary [adduct – acetone]<sup>+</sup> product ion for benzoic acid. All enthalpies were calculated at the M06-2X/6-311++G(d,p) level of theory.<sup>21</sup>

Based on the proposed mechanism, the presence of two nucleophilic atoms (oxygen atoms of the carboxylic acid moiety) in the analytes is required for the formation of the diagnostic product ions. However, other protonated analytes containing functionalities that have more than one nucleophilic atom, such as sulfones, amides and sulfonamides, failed to produce the diagnostic product ions upon reactions with ITS and only produced the primary [adduct – acetone]<sup>+</sup> product ions. For sulfones, the primary [adduct – acetone]<sup>+</sup> product ions does not react with a second ITS molecule due to the lack of acidic hydrogens (**Table 5.3**). For amides and sulfonamides, acidic hydrogens exist and therefore, secondary reactions could take place, yet they did not. Since the

formation of the  $2^{\circ}$  [adduct – acetone]<sup>+</sup> product ion involves proton transfer reaction from the primary [adduct – acetone]<sup>+</sup> product ion to a second ITS molecule, it is important to consider the proton affinities of the conjugate bases of the primary [adduct – acetone]<sup>+</sup> product ions. If ITS has a proton affinity that is lower than that of the conjugate base, then proton transfer will not occur, and the secondary reaction will not take place. Endothermic proton transfer can also occur, but only if the reaction products are lower in energy than the total energy of the system. Therefore, a comparison of the proton affinity of ITS (224 kcal/mol) and the proton affinities of the conjugate base of the primary [adduct – acetone]<sup>+</sup> product ions may help to explain the above observations. Indeed, as shown in **Table 5.3**, the conjugate bases of the primary [adduct – acetone]<sup>+</sup> product ions formed upon reactions between protonated sulfones, sulfonamides and amides with ITS have much greater proton affinities than ITS (224 kcal/mol). This explains why these analytes do not form the secondary [adduct – acetone]<sup>+</sup> product ions.

To challenge our method, two protonated dihydroxybenzenes, hydroquinone and pyrocatechol, as well as protonated 2-methoxyphenol (**Table 5.2**), were tested for their reactivity toward ITS. Although these protonated compounds do not contain a carboxylic acid functionality they reacted with ITS to form the  $2^{\circ}$  [adduct – acetone]<sup>+</sup> product ions. This observation was rationalized based on the acidity of the conjugate bases of the primary [adduct – acetone]<sup>+</sup> product ions of these analytes (**Table 5.4**). They have lower PAs than ITS (224 kcal/mol), which enables proton transfer from the primary [adduct – acetone]<sup>+</sup> products to ITS followed by the secondary addition/acetone elimination reaction (**Scheme 5.1**).

**Table 5.3** Structures and calculated proton affinities (B3LYP/6-311G++(d,p) level of theory) of a sulfone, sulfonamide and amide as well as the calculated proton affinities of the conjugate bases of the primary [adduct – acetone]<sup>+</sup> product ions (PA of ITS is 224 kcal/mol).<sup>21</sup>

Functionality	Structure ( <b>Protonation</b> Site)	PA (kcal/mol)	Primary [adduct – acetone] <sup>+</sup> Product Ion (Acidic Hydrogen)	PA of Conjugate Base (kcal/mol)
Sulfone		199	Si- O +S O H	255
Sulfonamide	O V S O H	196	Si-H ONH SNH	231
Amide	O N H	204	O <sup>SI</sup> H	232



**Scheme 5.1** Proposed mechanisms for the formation of the secondary product ions by protonated a) 1,2-dihydroxybenzene (that gets protonated on a hydroxyl group due to its ability to form a stabilizing intramolecular hydrogen bond) and b) 1,4-dihydroxybenzene (that gets protonated on a carbon based on the calculations shown in Table 5.4) upon reactions with ITS.<sup>21</sup>

Table 5.4 Structures and calculated proton affinities (B3LYP/6-311G++(d,p) level of theory) of

2-methoxyphenol, phenol and methoxybenzene as well as the calculated proton affinities of the conjugate bases of the primary [adduct – acetone]<sup>+</sup> product ions (PA of ITS is 224 kcal/mol).

Functionality	Structure ( <b>Protonation</b> Site)	PA (kcal/mol)	Primary [adduct – acetone] <sup>+</sup> Product Ion (Acidic Hydrogen)	PA of Conjugate Base (kcal/mol)
Ether, phenol	H C O O O O O O O O O O O O O O O O O O	203		210
Phenol	н-С	197	Si O + H	202

Therefore, a false positive result may be obtained for compounds with a phenol group and another nucleophilic functionality.

To illustrate the applicability of the method in high throughput identification of the carboxylic acid functionality by using HPLC separation followed by tandem mass spectrometric analysis based on ion-molecule reactions, an HPLC method was developed and an equimolar mixture containing 1 mM of each of 4-aminobenzoic acid, clozapine *N*-oxide, oxcarbazepine, diphenylsulfoxide, naproxen and celecoxib was used to test the HPLC separation followed by tandem mass spectrometric method (**Figure 5.5a**). The mobile phase gradient consisted of water and acetonitrile, and each solution contained 0.1% (v/v) formic acid. The eluted analytes were protonated by using APCI. The ions were transferred into the ion trap, isolated and allowed to react with ITS for 200 ms. Only the two protonated compounds containing a carboxylic acid functionality—4-aminobenzoic acid and naproxen—formed the diagnostic secondary product ion (**Figures 5.5b** and **5.5c**). Protonated diphenylsulfoxide, oxcarbazepine and celecoxib reacted to form only the primary products discussed above. No reactions were observed for protonated clozapine *N*-oxide. This was due to the much greater PA (237.3 kcal/mol calculated<sup>29</sup> at the B3LYP/6-31++G(d,p) level of theory) for clozapine *N*-oxide than ITS. This prevented the first step of the diagnostic reaction, proton transfer to ITS.



**Figure 5.5** a) Normalized total ion current HPLC/MS chromatogram for an equimolar mixture containing various model compounds. b)  $MS^2$  spectrum measured after reactions of the isolated protonated 4-aminobenzoic acid (*m*/*z* 138) with ITS for 200 ms. c)  $MS^2$  spectrum measured after reactions of the isolated protonated naproxen (*m*/*z* 231) with ITS for 200 ms.<sup>21</sup>

#### 5.4 Conclusions

Protonated analytes containing a carboxylic acid functionality were found to undergo a diagnostic reaction with ITS in the gas phase. This was true even in the presence of other, more basic functionalities in the analytes. With the exception of clozapine *N*-oxide, all examined protonated compounds in this study formed the primary product ion [adduct – acetone]<sup>+</sup> when allowed to react with ITS. However, with a few exceptions, only protonated compounds containing a carboxylic acid functionality reacted with ITS to form a diagnostic secondary product ion,  $2^{\circ}$  [adduct – acetone]<sup>+</sup>. Quantum chemical calculations and examination of the reactivity of protonated clozapine N-oxide indicated that the formation of the primary and secondary product ions was initiated by proton transfer. With the exception of clozapine *N*-oxide, all the protonated compounds studied were able to transfer a proton to ITS to form the primary product. However, a

secondary, diagnostic [adduct – acetone]<sup>+</sup> product was only observed for compounds with a carboxylic acid moiety, with a few exceptions. The mechanisms of the ion-molecule reactions were explored by calculations. Compounds containing a phenol group and an additional basic functionality were found to produce false positive results. This new tandem mass spectrometric method was coupled with HPLC to demonstrate that it can be used as a screening tool for compounds containing a carboxylic acid functionality in complex mixtures at the HPLC timescale.

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