

Characterization of Unsaturated Lipids Using Ambient Ionization Techniques (Paper Spray Ion Mobility-Mass Spectrometry (PS-IM-MS))

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Abstract

The study of lipid isomers is becoming of great importance as various diseases such as diabetes and cancer can be identified by discriminating the ratio of double bond lipid isomers in biological samples. This study focuses on the development of a direct, fast, low-cost, and reliable method applicable to biological matrices in differentiating double bond lipid isomers utilizing paper spray ion mobility-mass spectrometry (PS-IM-MS). The IM based separation alone cannot discriminate all three of the PC lipids analysed in a mixture, even using the multiplexing mode. Therefore, an epoxidation reaction with m-CPBA was performed on the various lipids to form epoxides at the double bond position. This product was further dissociated with PS-MS/MS that resulted in unique diagnostic ion pairs. The fragmentation of the di-epoxide product for PC 18:1(6Z)/18:1(6Z) resulted in the two diagnostic ion pairs with m/z of 634.4 and 660.4, respectively. The fragmentation of the PC 18:1(9Z)/18:1(9Z) di-epoxide product also resulted in the diagnostic ion pairs with m/z of 676.5 and 692.4. Although PS-MS/MS was successful in discriminating various double bond lipid isomers with diagnostic ion pairs, the PS-MS of the lipids already exhibited diagnostic ion pairs with no epoxidation or fragmentation. Further analysis of the lipid isomers with ion mobility and computational methods will determine if the nature of ambient ionization correlates to the diagnostic ion pairs in PS-MS.

Introduction

Lipids are vital parts of biological systems and can function as energy storehouses, hormone regulators, com-

ponents of cell membranes, chemical messengers, and much more (Ahmed, Saba, et al., 2018). Lipids can be classified as saturated or unsaturated as are structurally diverse with a variety of isomerism in the headgroup, chain length, sn-position, double bond positions, and geometry of double bonds (i.e., cis vs. trans) (Olajide, O. E., Donkor, B., & Hamid, A. M., 2022). Recent studies have shown that the characterization and identification of lipids are of great importance as cancerous and diseased tissue can be discriminated based on lipid structural differences, such as a higher presence of certain double bond configuration. Determining the double bond position in lipid isomers is beneficial considering that previous studies determined that a higher ratio of the double bond at position 11 to that of position 9 in PC (36:1) and PC (34:1) lipids can distinguish normal and cancerous tissue (Cao, Wenbo, et al., 2020).

Since lipids are structurally diverse with a variety of isomerism structural analysis is challenging only utilizing mass spectrometry (MS), especially double bond position isomers with the same mass over charge (m/z) ratio. Recent MS methods have been able to determine the double bond position in unsaturated lipids, such as ultraviolet-photodissociation (UVPD), ozone-induced dissociation (OzID), and many others but require modification to the mass spectrometers' setup (Williams, Peggy E., et al., 2017). To combat this, chemical methods including epoxidation with meta-Chloroperbenzoic acid (m-CPBA) will develop epoxides at the various double bond positions, and the product can be further dissociated with tandem mass spectrometry resulting in unique diagnostic ion pairs correlated to the double

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bond position. An illustration of this method can be seen in Figure 1. Utilizing ion mobility mass spectrometry (IM-MS) with epoxidation methods is of great advantage considering that discrimination of double bond isomerism is difficult only employing high resolution mass spectrometry.

Ion mobility mass spectrometry (IM-MS) is a very popular analytical technique used to separate ions in the gas phase depending on size, shape, and charge based on the balance of forces that effect the motion of an ion: the electric field and drag force created by collisions with the buffer gas, such as N_2 in this case (Burnum-Johnson, Kristin E., et al., 2019). Figure 2 shows the schematic illustration of the Agilent 6560 Ion Mobility- Quadrupole Time of Flight- Mass Spectrometer. This instrument is coupled with paper spray ambient ionization, where the sample is loaded onto a small triangular piece of paper and ions are generated directly for analysis by applying spray solvent and a high voltage to the wetted paper is of high interest (Liu, Jiang-jiang, et al., 2010). Figure 3 shows the photographic illustration of the paper spray ambient ionization inlet. By combining ion mobility mass spectrometry with ambient ionization, we hope to develop a direct, fast, low-cost, reliable method for discriminating double bond isomers applicable to biological matrices.

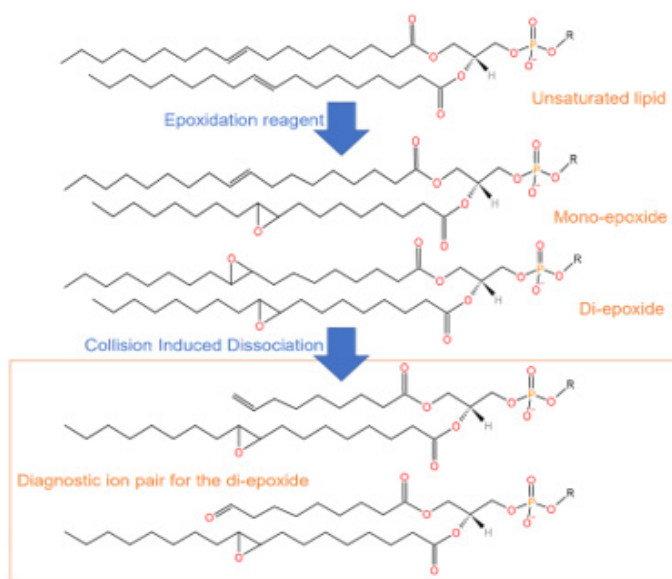


Figure 1. Schematic illustration of the epoxidation products and diagnostic ion pairs for an unsaturated lipid.

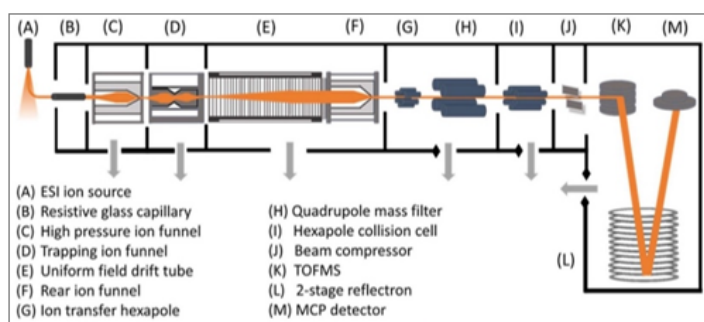


Figure 2. Schematic illustration of the Agilent 6560 Ion Mobility- Quadrupole Time of Flight- Mass Spectrometry instrument.

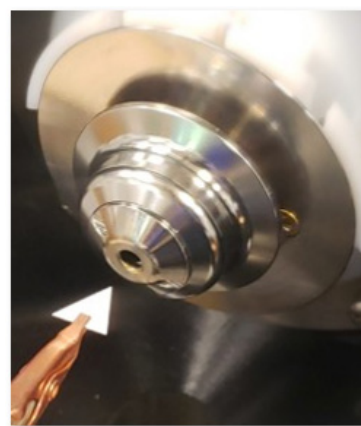


Figure 3. Photographic illustration of the paper spray ambient ionization inlet

Methods

The unsaturated lipids analyzed in this study include PC 18:1(9Z)/18:1(9Z), PC 18:1(6Z)/18:1(6Z), PC 18:1(9E)/18:1(9E), PG 18:1(9Z)/18:1(9Z), and PG 18:1(9E)/18:1(9E). Each unsaturated lipid sample standard was prepared in IPA/ACN/H₂O (2:1:1, v/v/v). For MS, IM-MS, and MS/MS, 2 μ L of 5 ppm unsaturated lipid standard was used with each trial performed, while IM-MS (multiplex) used 2 μ L of 1 ppm unsaturated lipid standard for each trial. Each trial used 15 μ L of IPA solvent for the ambient ionization. Tables 1 and 2 display the parameters and values for the optimized paper spray MS and IM-MS conditions.

Table 1 Optimized paper spray MS and IM-MS conditions.

Parameter	Value
Drying Gas Flow Rate	8 L/min
Drying Gas Temperature	250 °C
Fragmentor	400 V
Vcap (positive mode)	4,500 V
Vcap (negative mode)	3,500 V
Spray Shield	Multi bore vortex

Table 2 Continued optimized paper spray MS and IM-MS conditions.

Parameter	Value
Trap Release Time IM-MS (Single-Pulsed)	150 μ s
Trap Release Time IM-MS (Multiplex)	200 μ s
Trap Fill Time IM-MS (Single-Pulsed)	20,000 μ s
Trap Fill Time IM-MS (Multiplex)	3,900 μ s
Multiplex Sequence Length	4 bit
Paper Distance to MS Inlet	6 mm
Paper Size	8 mm x 10 mm (width x length)

For the epoxidation reactions the parameters and conditions mentioned above were used, but the unsaturated lipid was left to react with the m-CPBA on the triangular piece of paper. This epoxidation reaction was optimized based on the solvent system of the reagents, reaction time, and the volume and concentration of the epoxidation reagents. Table 3 and Figure 4 shows the parameters of the PS-IM-MS epoxidation optimization as well as the different reaction times and solvent systems.

Table 3 Parameters of PS-IM-MS epoxidation optimization.

Parameters	Value
Reaction Time	5 minutes
Solvent System of m-CPBA	IPA/ACN/H ₂ O (6:13:1, v/v/v/)
Volume of m-CPBA	2 μ L
Concentration of m-CPBA	10,000 ppm

Results and Discussion

The IM-MS (single-pulsed) of the three PC lipids along with the mixture found in Figure 6 shows the minimal difference in drift times for the double bond isomers. The PC 18:1(9Z)/18:1(9Z) and PC 18:1(9E)/18:1(9E) exhibits closer drift times to that of PC 18:1(6Z)/18:1(6Z). The IM-MS (single-pulsed) of the PC mixture in Figure 7 only has one peak for the drift time, which means that these double bond isomers cannot be discriminated only using IM-MS (single-pulsed). The IM-MS (multiplex) in Figure 8 demonstrates two peaks in the drift time. This is because the resolving power of the Agilent 6560 in single-pulse is \sim 60. With multiplex mode the IM data, after deconvolution through PNNL software, can be further processed via a high-resolution demultiplexing tool (HRdm) resulting in an increased resolution to enhance IM separation of the isomers with a resolving power around 200. Although this process resulted in a higher resolution, only one out of the three PC lipids could be separated reflecting structural similarities among the lipid isomers. For the epoxide reaction on the PC lipid, Figure 5 shows the highest intensity of the di-epoxide product, 818.6 m/z, for the PC 18:1(9Z)/18:1(9Z) at 5 minutes. This optimization of reaction time was used in the PS-MS/MS for PC 18:1(6Z)/18:1(6Z) and PC 18:1(9Z)/18:1(9Z) as well as the mixture for IM-MS. For the IM-MS the drift times are shown for the lipid, mono-epoxide, and di-epoxide in Figures 9 and 10. The drift time for the lipid as well as the epoxide product varies minimally, and the separation of these products would be difficult. The fragmentation of the di-epoxide product for PC 18:1(6Z)/18:1(6Z) resulted in the two diagnostic ion pairs with m/z of 634.4 and 660.4, respectively. The fragmentation of the PC 18:1(9Z)/18:1(9Z) di-epoxide product also resulted in the diagnostic ion pairs with m/z of 676.5 and 692.4. The conformation of unique diagnostic ion pairs for each PC lipid proves that the double bond lipid isomers can be differentiated using PS-MS/MS on the di-epoxide products.

Although PS-MS/MS of the di-epoxide products was successful, the PS-MS of the PC lipids exhibits some of the diagnostic ion pairs without any epoxidation reaction or fragmentation, shown in Figure 11. LC-MS was then used to further analyze the PC lipids and found that no diagnostic ion pairs were present. This indicates that the diagnostic ion pairs found in PS-MS might have been the result of the nature of ambient ionization.

Since ambient ionization occurs in atmospheric conditions and applies high voltages to ionize the analyte, it is possible that dissociation was induced because of the high voltages. Other investigations with PS-MS on the epoxidation and fragmentation of the lipids needs to be conducted. Further analysis of this reaction will be studied by comparing the drift times of the diagnostic ion pairs found in PS-MS and PS-MS/MS along with computational methods to predict the fragmentation patterns of the various lipids.

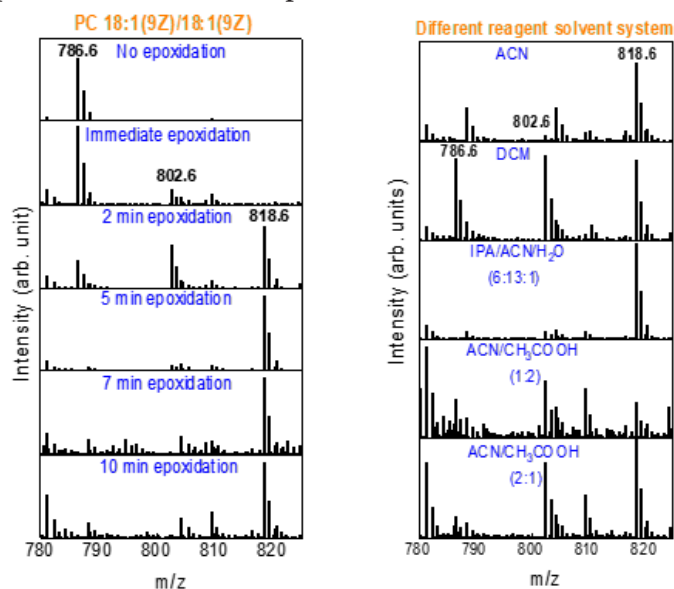


Figure 4. The optimization of the reaction times with m-CPBA and different solvent systems with PC 18:1(9Z)/18:1(9Z).

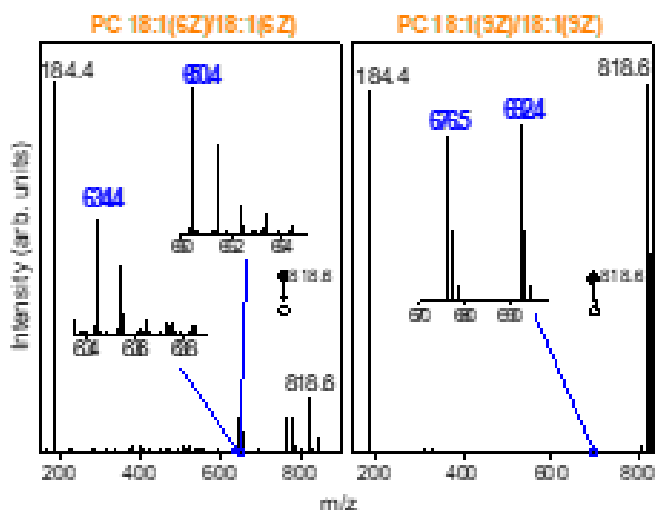


Figure 5. PS-MS/MS of the PC 18:1(6Z)/18:1(6Z) and PC 18:1(9Z)/18:1(9Z) of the diepoxide products.

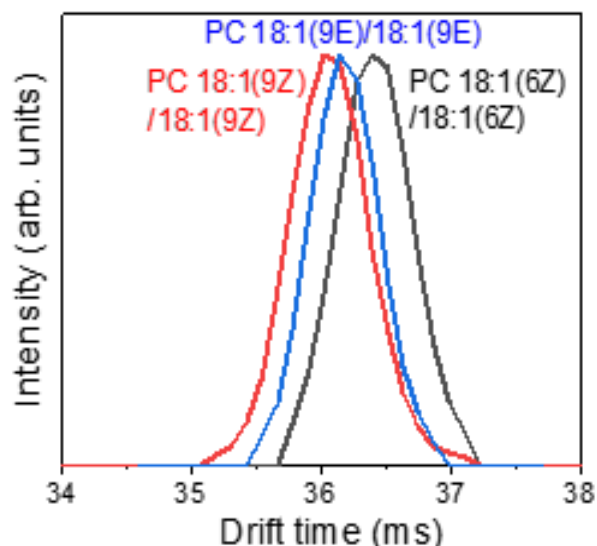


Figure 6 IM-MS (single-pulsed) PC 18:1(9Z)/18:1(9Z), PC 18:1(9E)/18:1(9E), and PC 18:1(6Z)/18:1(6Z).

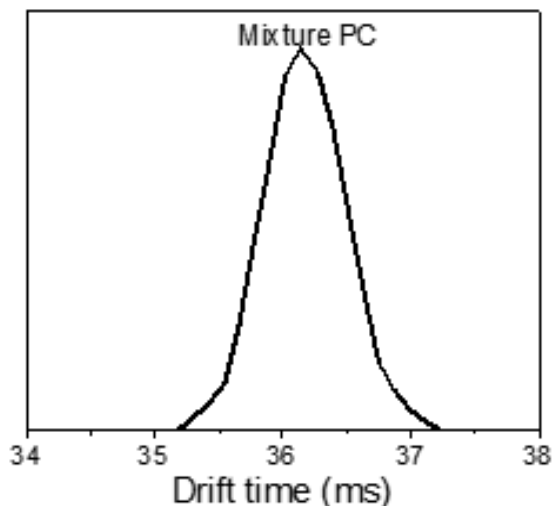


Figure 7 IM-MS (single-pulsed) of mixture of PC lipids.

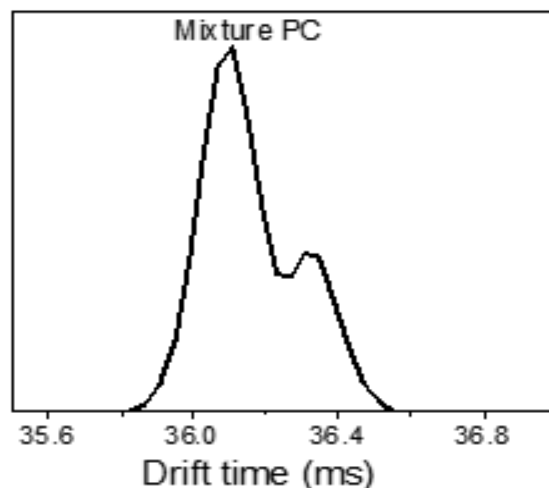


Figure 8 IM-MS (Multiplex) mixture of PC lipids.

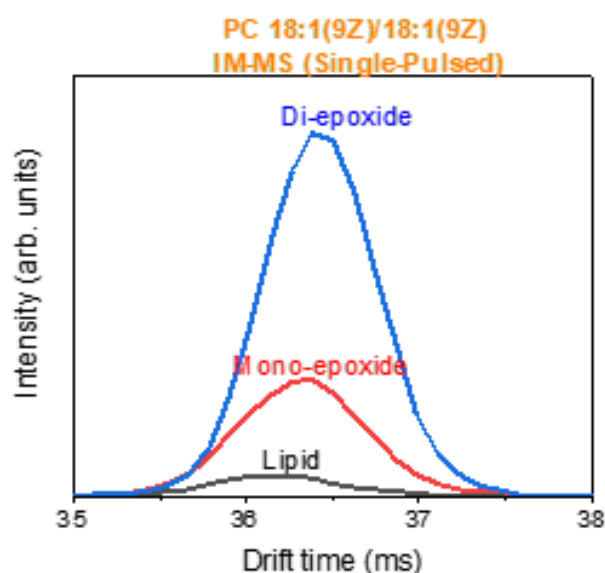


Figure 9 IM-MS (single-pulsed) of PC 18:1(9Z)/18:1(9Z) unsaturated lipid, mono-epoxide, and di-epoxide products.

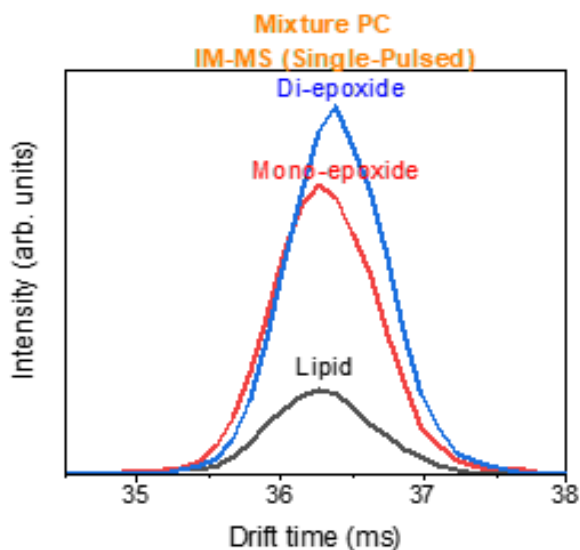


Figure 10 IM-MS (single-pulsed) of PC lipid, mono-epoxide, and di-epoxide mixture.

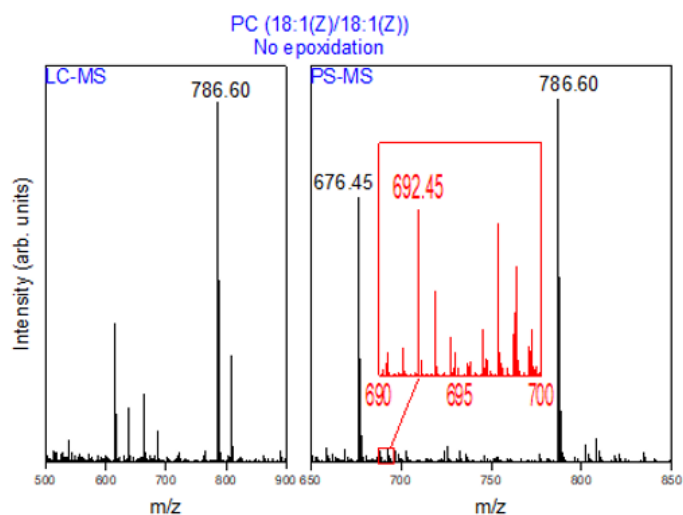


Figure 11. LC-MS and PS-MS of PC 18:1(9Z)/18:1(9Z) without performing epoxidation reactions. PS-MS shows diagnostic ion pairs of 676.45 m/z and 692.45 m/z along with the lipid, 786.60 m/z. LC-MS only exhibits the lipid, 786.60 m/z.

Statement of Research Advisor

Alexis's research studies were focused on the development and assessment of novel ambient ionization ion mobility mass spectrometry methods and their application in the study of epoxidation reaction of unsaturated lipids. She started with an extensive literature survey that led to the choice of several sets of lipid isomers that are challenging to analyze using the current mass spectrometry methods and implemented paper spray method developed in our laboratory in the differentiating them. Differentiating these lipid isomers can be used in the diagnosis of various diseases.

- Ahmed M. Hamid, Chemistry and Biochemistry, College of Sciences and Mathematics

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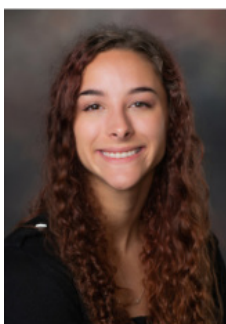
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Authors Biography



Alexis Toney is a senior-year undergraduate student pursuing a B.S in Chemistry with a minor in philosophy of religion at Auburn University. She has played a key role in the discrimination of various double bond position isomers using ambient ionization and computational methods in hopes to predict the fragmentation patterns of the lipids. She will be attending a chemistry graduate program after graduation.



Kimberly Kartowikromo is a second-year graduate student in the Department of Chemistry and Biochemistry at Auburn University. Her research focuses on environmental and clinical applications of ambient ionization ion mobility mass spectrometry in discriminating the double bond isomers in lipids.



Dr. Ahmed M. Hamid is an assistant professor in the Department of Chemistry and Biochemistry at Auburn University. His analytical research primarily focuses on environmental and clinical applications of ion mobility mass spectrometry, as well as developing portable novel mass spectrometry instruments.