# Aza-Prilezhaev Aziridination-Enabled Multidimensional Analysis of Isomeric Lipids via High-Resolution U-Shaped Mobility Analyzer-Mass Spectrometry

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ABSTRACT

Unsaturated lipids constitute a significant portion of lipidome, serving as players of multifaceted functions involving cellular signaling, membrane structure and bioenergetics. While derivatization-assisted liquid chromatography tandem mass spectrometry (LC-MS/MS) remains the gold standard technique in lipidome, it mainly faces challenges in efficiently labeling carbon-carbon double bond (C=C) and differentiating isomeric lipids in full dimension. This presents the need for new orthogonal methodologies. Herein, a metal- and additive-free aza-Prilezhaev aziridination (APA)-enabled ion mobility mass spectrometric method is developed for probing multiple levels of unsaturated lipid isomerization with high-sensitivity. Both unsaturated polar and nonpolar lipids can be efficiently labeled in the form of *N*-H aziridine without significant side reactions. The signal intensity can be increased by up to three orders of magnitude, achieving nM detection limit. Abundant site-specific fragmentation ions indicate C=C location and *sn*-position in MS/MS spectra. Better yet, stable mono-aziridination product is dominant, simplifying the spectrum for lipids with multiple double bonds. Coupled with a U-shaped mobility analyzer, identification of geometric isomers and separation of different lipid classes can be achieved. Additionally, a unique pseudo MS^3 mode with UMA-QTOF MS boosts the sensitivity for generating diagnostic fragments. Overall, the current method provides a comprehensive solution for deep-profiling of lipidome, which is valuable for lipid marker discovery in disease monitoring and diagnosis.
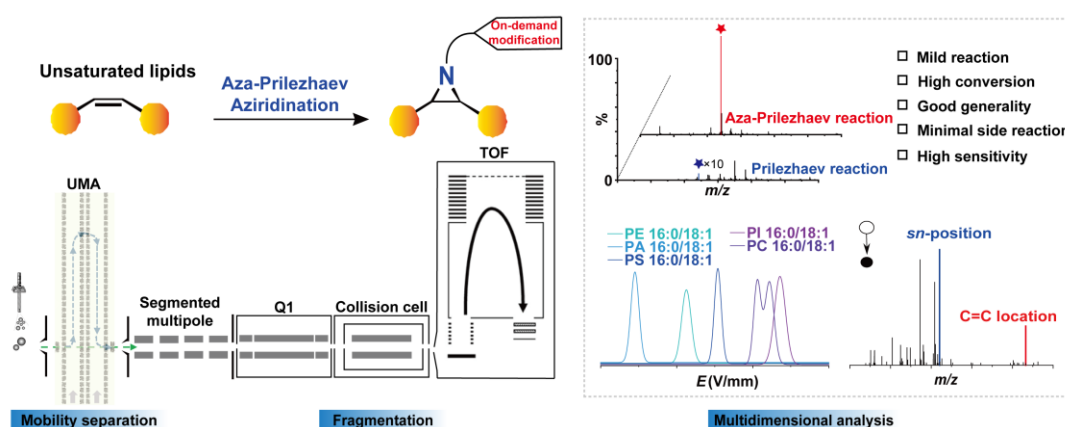
Keywords: lipid identification, derivatization, C=C location, *sn*-position, *cis* or *trans*, ion mobility mass spectrometry

INTRODUCTION

Lipids play a vital role in building block of biological membrane, cell signaling and energy reservoirs[1]. Contributing to the structural diversity, more than 48,000 unique lipids are involved in the LIPID MAPS Structure Database, considerable of which are isomers and isobars[2]. Unsaturated lipids often exist in isomeric structures that differ only in the position of carbon-carbon double bonds (C=C), the stereochemistry of C=C (*cis/trans*) or stereospecific numbering (*sn*) positions, fulfilling different biological roles[3,4]. They are increasingly recognized as physiologically active molecules and promising lipid markers[5,6]. Recent works revealed that disruption of lipid homeostasis is associated with many disorders such as cancer[7-14], diabetes[15-17], and Alzheimer's disease[18-20].

Over the past decades, the field of lipidomics exponentially grew through the use of a variety of approaches[21-26]. Conventional tandem mass spectrometry (MS/MS) methods allow to analyze the levels of lipid sum composition and the fatty acyl composition, but are blind to isomeric variants arising from different carbon-carbon bonding motifs[27,28]. Gas-phase ion activation methods including radical-directed dissociation (RDD)[29,30], ultraviolet photodissociation (UVPD)[31,32], ozone-induced dissociation (OzID)[33-35], electro impact excitation of ions from organic (EIEIO)[36,37] and oxygen attachment dissociation (OAD)[38,39] have been successfully employed in resolving isomeric lipids. However, taking membrane abundant glycerophospholipids (GP) as example, annotations of five levels in terms of structure information (lipid class, the length and desaturation degree of fatty acyl, fatty acyl *sn*-positions, and the location and stereochemistry of C=C in fatty acyl) remain a challenge in complex biological samples, due to low physiological content, wide polarity range of different subclasses, and interference from excess saturated lipids.

Derivatization-MS/MS methods entail a valuable step forward in identification of less abundant lipids. C=C derivatization, such as Paternò-Büchi (PB) reaction[40-43], epoxidation[44-47] and singlet oxygen-ene reaction[48], helps to transfer strong C=C to labile carbon-carbon single bond (C-C) in a high strained ring, following C-C cleavage across the oxirane or oxetane during CID. For high-sensitivity analysis, Yan[49], Guo[50], and Chen[51] used aziridination reaction instead of noncharged derivatization to introduce protophilic group and then improve ionization efficiency. The detection sensitivity has been successfully improved, especially for low polar lipids. However, these derivatization-MS/MS methods are difficult to simultaneously identify the five levels of structure information with the lipidomic settings of shotgun or liquid chromatography-mass spectrometry (LC-MS).

Ion mobility-mass spectrometry (IM-MS) can provide an additional dimension of information that supplements LC-MS/MS workflow. It has shown promising results in separation of lipid isomers and improvement on type II chemical interference[52-54]. In 2019, Fernandez-Lima and coworker realized the separation of C=C positional, geometric and *sn*-positional isomers of glycerophosphocholine (PC) and diacylglycerol at the IM dimension with specified IM parameters[55]. In 2023, Xia and coworker integrated PB reaction and IM-MS for deep structural annotation of phospholipidome. It successfully reduced type II chemical interference by using IM and pinpointed C=C location and *sn*-position during CID[56].

Current derivatization reactions still show several limitations in trace analysis of lipids, including low reaction rate and yield, poor substrate generality, low abundance of diagnostic ions, complicated work-up, harsh reaction conditions, serious side reactions and operational complexity, *etc*. Taking all these into consideration, we developed a universal and user-friendly platform combining aza-Prilezhaev aziridination (APA) with U-shaped mobility analyzer (UMA)-QTOF MS, which can rapidly form high strained rings for facilitating double bond cleavage, meanwhile introduce protophilic groups for unsaturated lipids under mild reaction conditions with high efficiency. Owing

to APA, the limits of detection was decreased by 1 ~ 3 orders of magnitude. It is compatible with most classes of lipids. During UMA-MS/MS analysis, different classes of lipids can be separated based on mobility in UMA first, thereby reducing the isotopic type II effect in the following MS measurement. Abundant diagnostic ions indicative of the C=C location and *sn*-position in aziridine-lipids can be obtained. Further improvement on the abundance of *sn*-specific fragments for GP has been realized by using UMA-based pseudo MS[3] mode. Overall, the integrated solution shows great potential for high-throughput isomeric lipid profiling.

EXPERIMENTAL METHODS

Lipid Nomenclature. The shorthand notations of lipids adhered to the guidelines of LIPID MAPS[57]. In brief, the number following colon represents the unsaturation degree of fatty acyl chain. The location of C=C was assigned by the number in bracket. *Z* and *E* represent *cis*- and *trans*-configurations of C=C, respectively. The underscore ("_") signified an unspecified *sn*-position, while the forward slash ("/") means that the *sn*-position is specified, in which the *sn*-1 fatty acyl is before "/" and the *sn*-2 fatty acyl is after "/". For example, PC 16:0/18:1 (9*Z*) indicates glycerophosphocholine (PC) containing a *sn*-1 saturated C16 fatty acyl and a *sn*-2 unsaturated C18 fatty acyl with one *cis*-C=C at C9-C10.

Chemicals. GP and SP standards were purchased from Avanti Polar Lipids. FA, GL and ST standards were obtained from Sigma-Aldrich (St. Louis, MO), Aladdin (Shanghai, China), TargetMol (Boston, USA), TCI (Tokyo, Japan), and Anpel (Shanghai, China). *N*-Boc-*O*-tosylhydroxylamine (TsONHBoc) was purchased from Aladdin. 3-Chloroperoxybenzoic acid (mCPBA) was purchased from Macklin (Shanghai, China). Hexafluoroisopropanol (HFIP) was purchased from Anpel (Shanghai, China). All reagents were used without further purification.

Sample Preparation. Lipid standards were dissolved in HFIP to 100 μg/mL as the stocking solution. A series of working solutions were prepared at different concentrations by diluting the stocking solution with HFIP. TsONHBoc was prepared at a final concentration of 10 mg/mL in HFIP. They were stored at 4 °C before further experiments.

Aza-Prilezhaev Aziridination of Unsaturated Lipids. Lipid working solutions were simply mixed with superstoichiometric TsONHBoc. For example, 20 μg/mL lipid solutions were mixed with equal volume TsONHBoc solution to prepare 10 μg/mL aziridine-lipids. After vortexing 60 s, the reaction mixture containing lipids and derivatization reagent was stirred at room temperature for at least 1 h to complete reaction. Alternatively, the reaction solution could be stirred at 50 °C for 10 min. Without complicated work-up, the final reaction solution was quenched and diluted by acetonitrile prior to shotgun analysis.

IM-MS/MS analysis. Experiments were performed on a prototype U-shaped mobility analyzer (UMA) coupled with a LCMS9030 QTOF mass spectrometer (Shimadzu, Kyoto, Japan). Details for UMA were described in a previous report[58]. Only a brief introduction is given here. UMA is a newly developed ion mobility analyzer (Figure S1), in which ions are separated according to their mobilities along a U-shaped trajectory. UMA can either select ions within a narrow mobility range to pass through in a so-called filter-SIM mode, or sequentially pass through all the ions from high to low mobility in a so-called filter-scan mode. Following UMA is a segmented multipole ion guide (SMP) which is operated at ~5 Pa. A DC gradient is formed in the SMP to avoid loss of mobility resolution. In this work, a DC difference between the exit of UMA and the entrance of SMP can be

applied to achieve collisional induced dissociation (CID) of ions eluted from UMA. All experiments were conducted under the following MS setting. Mass range, *m/z* 100-1600; ESI voltage, +4 kV; Nebulizer gas flow, 3 L/min N_2; Drying gas flow, 10 L/min N_2; Heating gas flow, 10 L/min N_2; Desolvation line temperature, 250 °C; Heat block temperature, 400 °C; and MS^2 CID energy, +25 ~ 40 eV. For conventional MS/MS analysis, the optimal parameters of UMA were as follows: UMA is in filter-scan mode for mobility separation; the DC voltage of UMA exit, 10 V; the DC voltage of SMP entrance, 0 V. For pseudo MS^3 analysis, the operating parameters of UMA were optimized as follows: UMA is in filter-scan mode for mobility separation; the DC voltage of UMA exit, 50 V; the DC voltage of SMP entrance, 0 V. Infusion speed was 20 μL/min utilizing a syringe pump (Harvard Apparatus, Holliston, MA, USA).

Data Processing. Data were analyzed with Origin Pro, presented as mean $\pm$ standard deviation for at least three replicates.

RESULTS AND DISCUSSION

Aza-Prilezhaev aziridination of C=C for deep-lipidotyping. From the perspective of proton affinity, aziridination reaction plays an important role on improving sensitivity. However, some limitations of previous aziridination methods exist and are described as follows. Metal catalyst dirhodium(ii) bis(α,α,α′,α′-tetramethyl-1,3-benzenedipropionate) [$Rh_2(esp)_2$] can catalyze stepwise nitrogen transfer to olefins *via* a rhodium-nitrene intermediates[59]. It is an effective method for *N*-H aziridination with excellent conversion, but serious overderivatization exists for polyunsaturated compounds, which complicated the spectrum of aziridination product and decreased its signal intensity[49]. Ketone can catalyze concerted nitrogen transfer to olefins *via* *N*-H oxaziridine intermediates[60], showing moderate conversion. It is a metal-free reaction without overderivatization, but needs abundant organic or inorganic base which makes severe ion suppression and matrix interference. Chloramine-T can catalyze stepwise nitrogen transfer to olefins *via* a nitrene intermediates.[50] It produces almost monoderivatization *N*-Ts aziridine product with showing moderate conversion. However, mass of oxidant reagent and inorganic salt in the reaction system causes serious side reaction, which makes severe matric interference and complicated the mass spectrum. In addition, *N*-Ts aziridine is difficult for further on-demand modification.
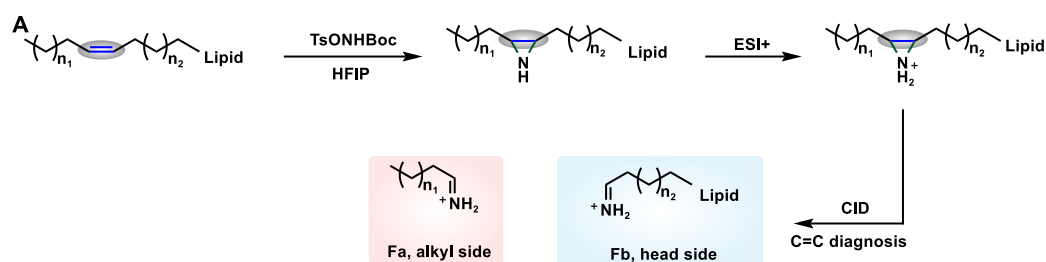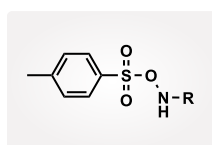
R group	Derivatization reagents	Derived lipids	Mass shift (Da)
t-Butyloxy carbonyl	TsONHBoc	*N*-H aziridine lipids	15
Methyl	TsONHMe	*N*-Me aziridine lipids	29
Benzoyl	TsONHBz	*N*-Bz aziridine lipids	119
Benzoyl-d_5	TsONHBz-d_5	d_5-*N*-Bz aziridine lipids	124
Benzyloxycarbonyl	TsONHCbz	*N*-Cbz aziridine lipids	149
...	...	...	...

Scheme 1. Scheme of (A) APA and site-specific fragmentation; (B) *N*-R *O*-tosylhydroxylamine analogues.

Inspired by the work reported by Jat et al[61], a metal- and additive-free APA reaction of C=C for lipids is applied for pinpointing C=C location and *sn*-position (Scheme 1). For unsaturated lipids, the user-friendly APA is expected to follow the mechanism wherein simultaneous formation of both new C-N bonds occurs *via* butterfly like transition state. The rationale for the design of the deep-lipidotyping method is as follows. Firstly, a protophilic group *N*-H aziridine is introduced into the chemical scaffold of lipids, thereby improving ionization efficiency. Secondly, it enables transferring high bond energy C=C into labile C-C among a high strained ring, in order to achieve C=C position information with low-energy CID. In addition, it provides amplification of structural difference in isomeric lipids to improve LC or IM resolution. To be noted, the structural difference between lipid isomers could be further increased through one-step derivatization with modified reaction reagent or two-step derivatization, achieving *N*-R aziridine-lipids (Figure S2). Desired fragmentation pathway was observed during tandem MS analysis of *N*-R aziridine-lipids (Figure S3). Thirdly, we speculated that *N*-R *O*-tosylhydroxylamine analogues (TsONHR) might serve as a series of mass tags, facilitating accurate qualitative and quantitative analysis with high-throughput.

Optimization of the APA conditions. A series of experiments were conducted to realize high-sensitivity analysis. Oleic acid was chosen as the model substrate to optimize the APA conditions. Among the different reagent used, TsONHBoc as aminating agent and hexafluoroisopropanol (HFIP) as solvent was found to be the most suitable combination producing aziridine-C18:1 in an excellent yield (Figure 1A-B). Superstoichiometric derivatization reagent were selected for high conversion. After 10 min reaction, the conversion reached to 50% at room temperature (Figure 1C), while nearly full conversion was obtained at 50 °C (Figure1D), which suggests that it can be potentially applied to online analysis for real-time monitoring.

Under the optimal conditions, C=C in a variety of unsaturated lipids can be efficiently labeled without serious side reaction and overderivatization, furnishing *N*-H aziridines in excellent yields. Mono-aziridination product is dominant (Figure S4). The derivatives are stable enough for different requirement experiments (Figure 1E). This clean reaction system (metal- and additive-free, no need for anhydrous solvent and inert atmosphere) is less susceptible to external disturbances. Reaction in solvent and in matrix containing solution are of comparable rate. Owing to a bare lone pair electron of the nitrogen atom, aziridination makes most classes of lipids ionize well in positive ESI mode. And the detection sensitivity of various lipids was increased by up to 3 orders of magnitude, especially for neutral lipids (Figure 1F). Moreover, no cleanup is required before shotgun analysis. The matrix effect was about 84.40%. Compared with Prilezhaev reaction-MS/MS method[47], aza-Prilezhaev reaction has higher sensitivity, higher reaction selectivity, less spectrum complexity and better substrate generality (Figure 2).
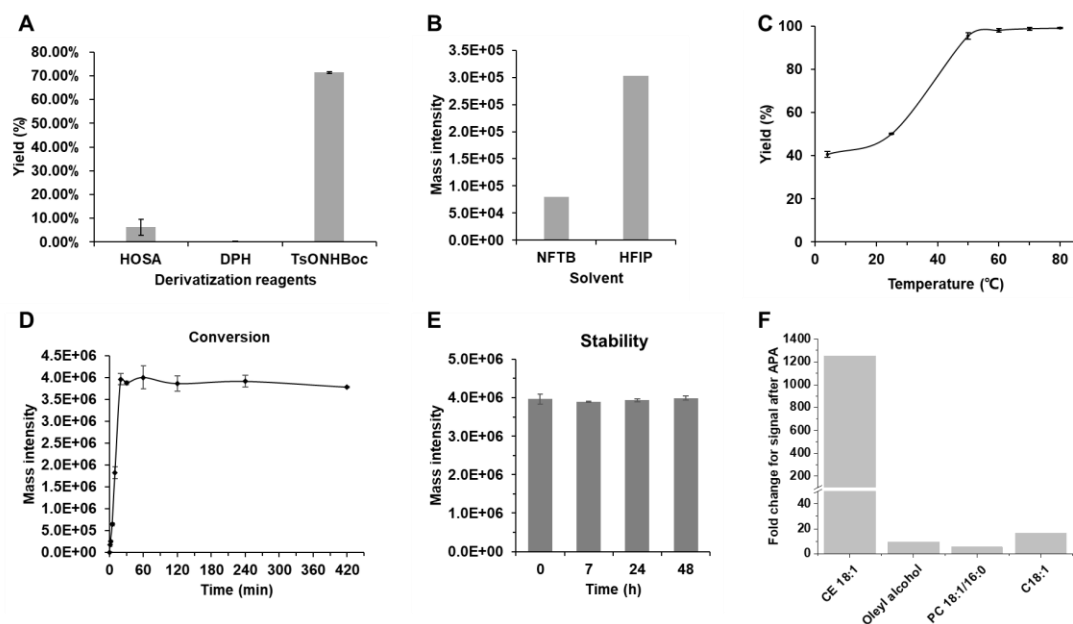
Figure 1. Optimization of the APA conditions, (A) derivatization reagents, (B) solvent, (C) reaction temperature; (D) Conversion at 50 °C; (E) Product stability; (F) Comparation of detection sensitivity before and after derivatization.
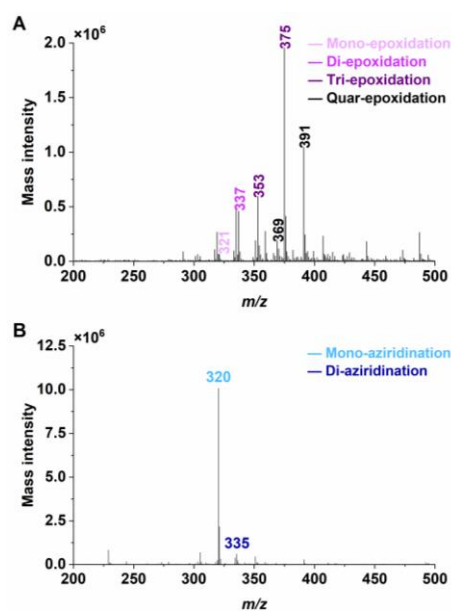

Figure 2. Mass spectra of Prilezhaev reaction and aza-Prilezhaev reaction product of polyunsaturated lipid. C20:4 (5Z, 8Z, 11Z, 14Z) was used as the model substrate.

Step 1 Derivatization	
Improve ionization efficiency	Amplify structural differences in isomeric lipids

Step 2 Mobility separation		
Profile lipids at sum composition	Reduce isotopic type II effect	Separate geometric isomers

Step 3 (Optional) Fragmentation in SMP
Generate abundant headgroup loss ions

Step 4 Fragmentation in CID
Convert the precursor ions into product ions

Step 5 MS analysis
Obtain diagnostic ions for C=C location and *sn*-position

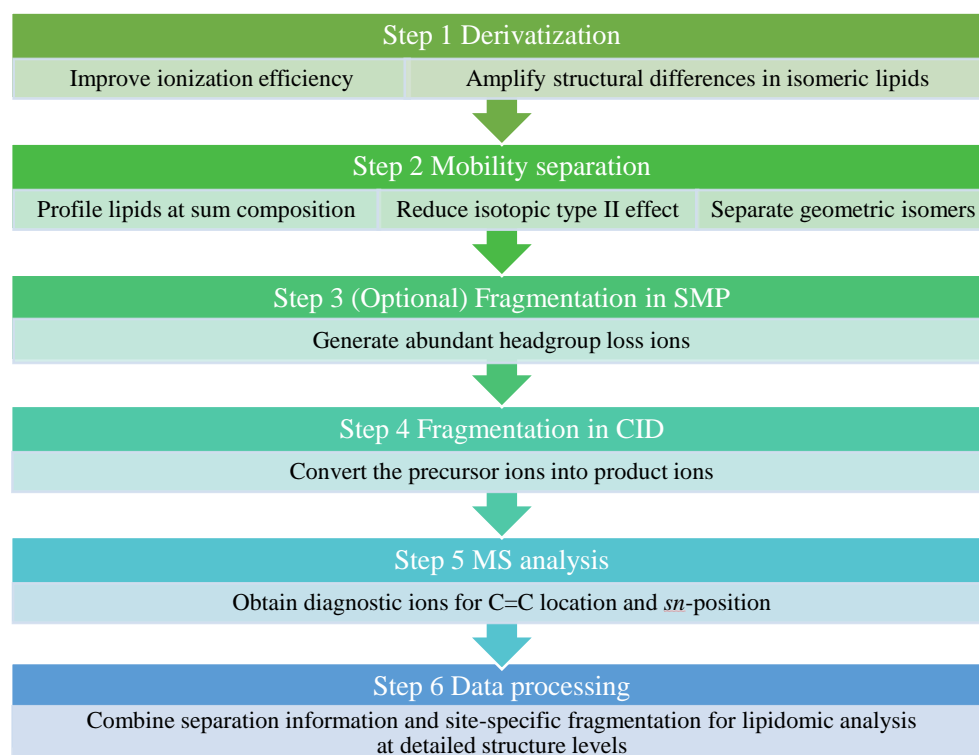| Step 6 Data processing |
|---|
| Combine separation information and site-specific fragmentation for lipidomic analysis at detailed structure levels |

Figure 3. Workflow of the APA-UMA-MS/MS method.

Multidimensional analysis of isomeric lipids. A systematic workflow has been developed as shown in Figure 3, in order to comprehensively characterize the structure of lipids, including lipid class, the combination of fatty acyl chains, C=C location and geometry, the relative substitution of acyl chains on the glycerol backbone (*sn*-position). Prior to analysis, the APA is used for forming the derivatives with improved ionization efficiency and amplified structural differences. Then, UMA is used to roughly distinguish different classes of lipids, separate geometric isomers, and reduce isotopic type II effect. Thereafter, QTOF-MS/MS is applied to achieve accurate *m/z* of diagnostic ions arising from cleavage across the aziridine moiety or the glycerol backbone. The last step is data analysis.

Differentiation of lipid type. APA provides the possibility for simultaneously analyzing abundant and less abundant polar/nonpolar lipids in positive ESI mode. Following ESI, we achieved rough separation and classification of different lipids including fatty acid (FA), cholesteryl ester (CE), sphingomyelin (SM), triacylglycerol (TG), and PC by UMA (Figure 4A). Moreover, we have evaluated the impact of charge polarity on separations *via* UMA using a mixture of GP standards. Under the same scan rate (0.4 V/mm/s) of UMA, better separations were achieved for the sodiated aziridine-GP, with an average resolving power of 150, likely due to forming more compact gas-phase conformation (Figure 4B). Without prior chromatographic separation in shotgun lipidomics, UMA separation demonstrates surprising effectiveness to reduce isobaric interference, thereby avoiding false annotation and uncovering neglected low abundance lipids. As shown in Figure 4C, without UMA separation, the mass peak of aziridine-PC 34:1 was embedded in the isotopic peak of aziridine-PC 34:2. By using UMA, the mobility peak of aziridine-PC 34:2 can be partially separated from that of aziridine-PC 34:1 (Figure 4D), where the cleaned mass spectrum of aziridine-PC 34:1 after such mobility separation is shown in Figure 4E.

Identification of C=C location. MS/MS experiments afford not only the identification of the head group and the fatty acyl substituents, but also differentiation of isomeric species. We chose C18:1 (Δ6), C18:1 (Δ9) and C18:1 (Δ11) as model substrates to validate the potency of APA-MS/MS method for pinpointing C=C position. During MS^2 CID, abundant diagnostic ions at *m/z* 184/112, 142/154, 114/182 arising from the cleavage across aziridine, indicated the C=C was located at Δ6, Δ9, Δ11, respectively (Figure 4F-H). Other lipids such as nine fatty acyls, two glycerolipids (GL), two sterol lipids (ST), ten GP and one sphingolipid (SP) were also compatible, achieving abundant site-specific fragments for C=C position assignments (Figure S5-6, Table S1).

Identification of sn-position. According to numerous reports[7,8,24], *sn*-positional isomers play a crucial role on human health, and some of them are even considered to be valuable disease-related biomarkers. Similarly, we chose PC 18:1 (Δ9)/16:0 and PC 16:0/18:1 (Δ9) to evaluate the ability of the developed method for generating *sn*-specific fragment ions. As expected, MS^2 CID of sodiated aziridine-PC 18:1 (Δ9)/16:0 standard produced *sn*-1 diagnostic ion at *m/z* 360.2873 (Figure 4I), while *sn*-2 diagnostic ion at *m/z* 290.2454 was generated from aziridine-PC 16:0/18:1 (Δ9) (Figure 4J). Actually, less abundant *sn*-2 diagnostic ion was also observed in the MS/MS spectra of aziridine-PC 18:1 (Δ9)/16:0 standard, because the corresponding regioisomeric impurities may exist in the commercial standards. Product ion at *m/z* 466.3891 was identified as C=C positional diagnostic ions, arising from the cleavage at Δ9 C=C in C18:1. Additional example, such as PC 18:1 (Δ9)_18:0, show similar fragmentation behavior, which further confirm the *sn*-specificity of the method (Figure S6).

Identification of C=C geometry. Although aziridination-MS/MS alone cannot identify geometric isomers, the employment of UMA allows differentiating the stereochemistry of the C=C (*cis* or *trans*) of aziridine-lipids. The peak-to-peak resolving power for the sodiated aziridine-C18:1 (9*Z*) and aziridine-C18:1 (9*E*) was R$_{pp}$ ~ 1.35 with a scan rate of 0.4 V/mm/s and gas flow rate of 1.0 L/min. The average resolving power was 100. The R$_{pp}$ was increased to 2.29 with a slower scan period (0.2 V/mm/s) and higher gas flow rate (2.0 L/min) as shown in Figure 4K. The average resolving power was 200 under these conditions.

These results suggest that the APA-UMA-MS/MS method could be a powerful tool for deep-profiling of lipidome.

Pseudo MS^3-enhanced indication of regioisomeric glycerophospholipids. Although aziridination-MS^2 method successfully maps C=C positional and *sn*-positional GP isomers, the efficiency of producing the *sn*-specific fragments can be further improved. Specifically, the tandem mass spectra of GP are dominated by headgroup ions or headgroup loss ions, thereby ions regarding to the structural information are of low abundance. To address this problem, Brodbelt and coworker developed a hybrid MS^3 method for deep structural annotation of GP by second generation of headgroup loss species[62]. According to a later work by Xia and coworker[8], high abundance of diagnostic ions can be achieved by charge-tagging derivatization coupled with MS^3.
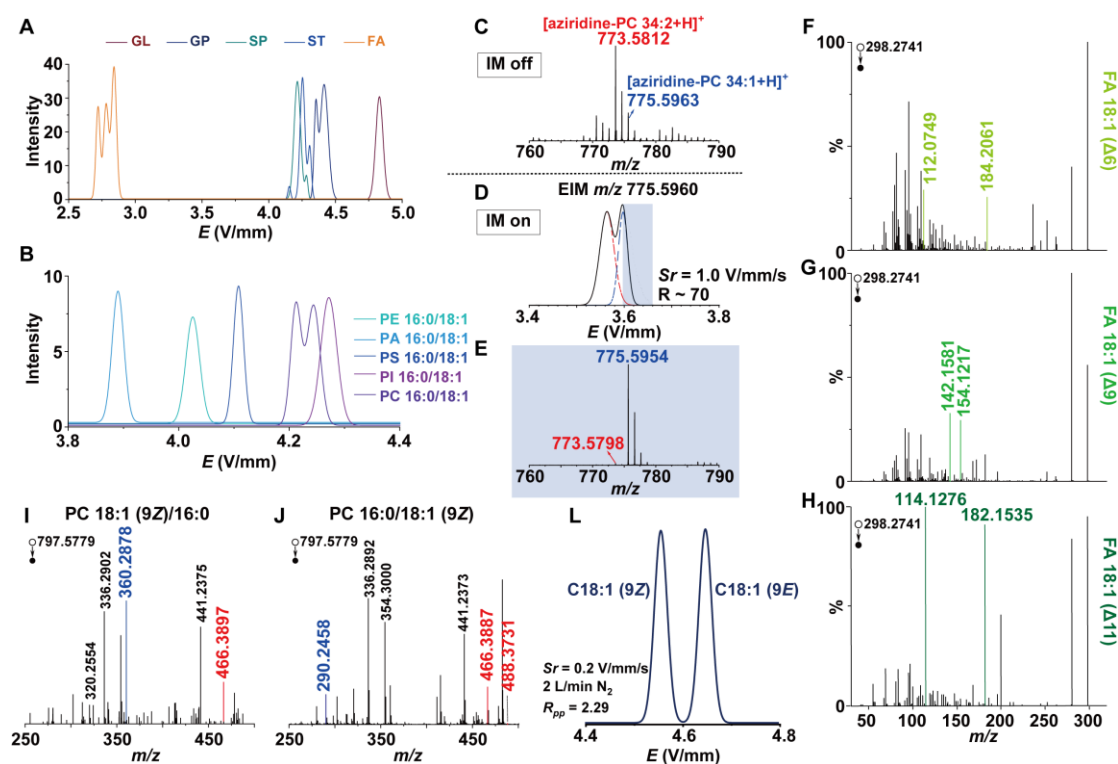
Figure 4. Detailed structural analysis of isomeric lipids by APA-UMA-MS/MS. EIM of (A) different classes of aziridine-lipids and (B) subclasses of aziridine-GP, detected as [M+Na]⁺. (C) MS spectrum of a 10:1 mixture of PC 34:2 and PC 34:1. (D) EIMs of *m/z* 775.5960. (E) MS spectrum isolated from *E* of 3.60–3.6640 V/mm. MS/MS spectra of (F) aziridine-C18:1 (Δ6), (G) aziridine-C18:1 (Δ9), (H) aziridine-C18:1 (Δ11), (I) aziridine-PC 18:1 (9*Z*)/16:0 and (J) aziridine-PC 16:0/18:1 (9*Z*), red trace represents C=C positional diagnostic ions, while blue trace represents *sn*-position diagnostic ions. (K) EIM of sodiated aziridine-C18:1 (9*Z/E*) with the scan rate of 0.2 V/mm/s and the gas flow rate of 2.0 L/min.

Accordingly, we established an UMA-based pseudo MS³ strategy to enhance site-specific fragment of regioisomeric GP. In brief, headgroup loss of precursor ions occurs in the segmented multipole ion guide (SMP, Figure S1) immediately following UMA to generate a dioxolane-type intermediate ion, as pseudo MS². The products ions with loss of headgroups were then selected by the quadrupole before being fragmented in the collision cell to generate abundant informative ions, arising from cleavage across the dioxolane moiety (as pseudo MS³). Taking PC as an example, compared with regular MS/MS (Figure 5A-B), the abundance of glycerol backbone structural ions originating from cross-ring cleavage across the dioxolane moiety are improved by around 1 order of magnitude using pseudo MS³ (Figure 5C-D). Ions regarding to the structural information are dominant in the spectra of pseudo MS³. Proposed fragmentation pathway of aziridine-PC 16:0_18:1 was shown in Figure 5E. Other subclasses of GP, including PG, PS, PE, PA 16:0/18:1 and PC 18:1_18:0 were also investigated. Their pseudo MS³ spectra reveal identical product ions to those observed for PC 16:0/18:1 (Figure S7, Table S2). The result is consistent with our assumption that pseudo MS³ enhances the backbone structure specific fragment by removing a highly polar headgroup during first dissociation step and fragmenting the glycerol backbone of GP during second dissociation step sequentially. This enhancement may be attributed to stepwise dissociation scheme

with differential collision energy, wherein loss of labile moiety (headgroup ions) in the first dissociation step will facilitate further internal energy release concentrating over the high energy bonds (derivatized double bonds) upon experiencing the second dissociation step. Using UMA for the first stage selection can render additional merit of lipid classification based on structural differences. Better quantitation can be achieved when UMA serves as a mobility filter for targeted analysis. In addition, unlike in methods with multiple stage low resolution MS such as ion trap, high resolution TOF provides higher confidence for both precursor and fragment identification of lipids.
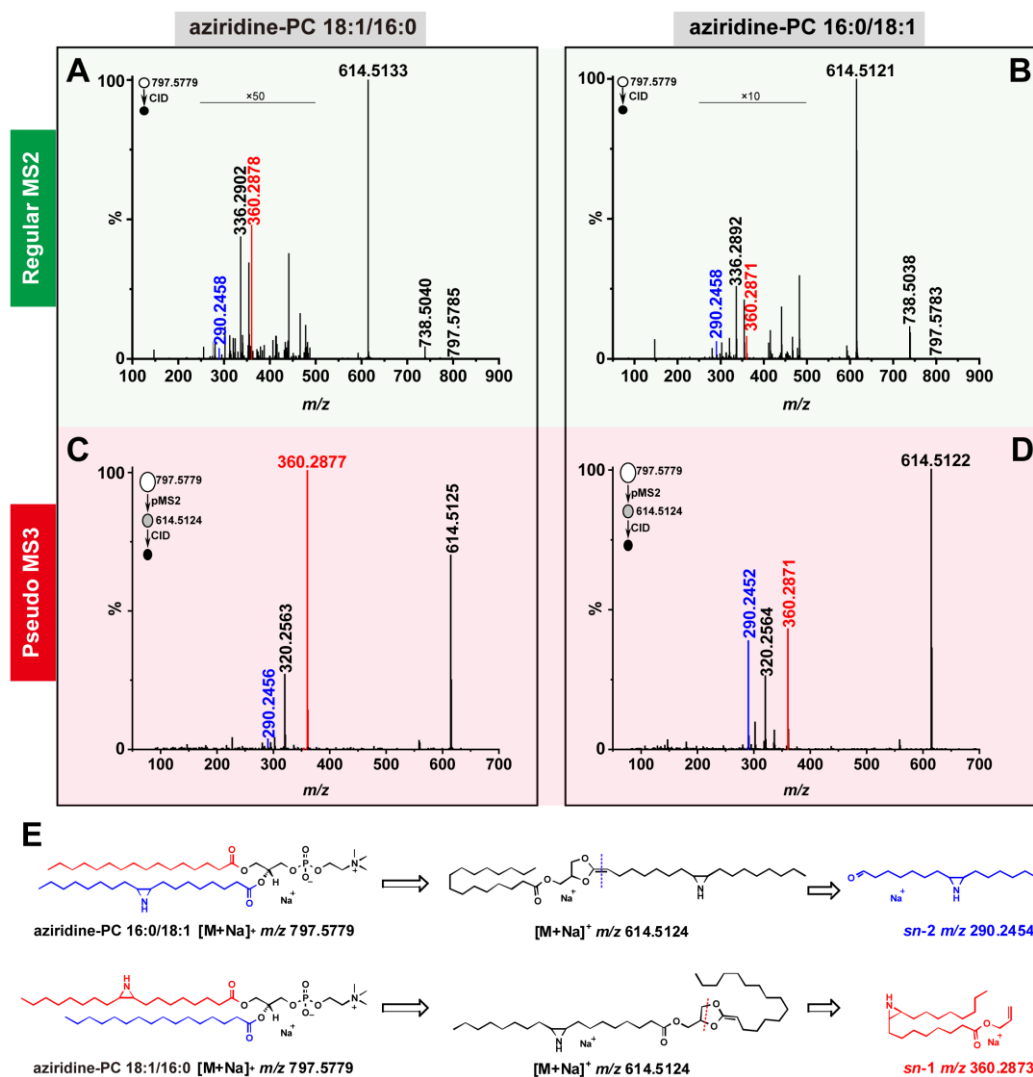

Figure 5. MS/MS spectra of (A) aziridine-PC 18:1/16:0 and (B) aziridine-PC 16:0/18:1; Pseudo MS^3 spectra of (C) aziridine-PC 18:1/16:0 and (D) aziridine-PC 16:0/18:1; (E) Proposed fragmentation scheme of aziridine-PC 16:0_18:1.

CONCLUSIONS

In summary, we have established an entire workflow by coupling APA with UMA-MS/MS for comprehensive structural annotation of polar and nonpolar lipids, achieving high level in multiple aspects simultaneously (sensitivity, specificity and efficiency). Aziridination considerably promotes lipid ionization with wide polarity and abundance range in positive ESI mode, particularly for neutral lipids. Following high-throughput separation of geometric isomers, highly specific cleavage can be performed to generate abundant C=C location and *sn*-position diagnostic ions. Additionally,

UMA-based pseudo MS^3 mode allows enhancement of the backbone structure-specific fragments, thus further improving detection sensitivity and increasing lipid coverage. In general, it is a versatile and convenient solution for simultaneous identification of C=C location/geometry and *sn*-position as well as lipid classes. Excellent performance in terms of conversion and sensitivity provides the possibility for online analysis. An integrated platform LC-APA-UMA-MS/MS and corresponding applications are still undergoing and will be reported later.

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This manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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