SHIMADZU

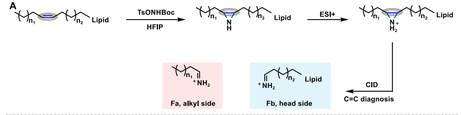
MP 477

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

Yuling Li[1], Yiming Wang[1], Kang Guo[1], Kuofeng Tseng[1], Xiaoqiang Zhang[1], Wenjian Sun[1]
(1) Shimadzu Research Laboratory (Shanghai) Co., Ltd.

1. Introduction

- While derivatization-assisted liquid chromatography tandem mass spectrometry 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.
- We have established an entire workflow by coupling a metal- and additive-free aza-Prilezhaev aziridination (APA)[1] with U-shaped ion mobility analyzer (UMA)[2]-MS/MS for comprehensive structural annotation of polar/nonpolar isomeric lipids, achieving high level in multiple aspects simultaneously (sensitivity, specificity and efficiency) (**Fig. 1**). The user-friendly APA with high conversion, minimal side reaction and good substrate generality is developed to improve the signal intensity by up to 3 orders of magnitude. This method was successfully used to identify C=C location, fatty acyl length and *sn*-position, headgroup composition and geometric configuration in a single run[3].



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
...	...	...	...

Fig. 1 Scheme of (A) APA and site-specific fragmentation; (B) *N*-R *O*-tosylhydroxylamine analogues used for on-demand modification.

2. Methods

- Lipid solutions were simply mixed with superstoichiometric APA reagent. Without complicated work-up, the final reaction solution was quenched and diluted by acetonitrile prior to UMA-QTOF MS/MS analysis (**Fig. 2**).
- Two operating modes: Offline & Online
- Multiple analyzing modes:

  MS^2: UMA → Quad mass selection → CID → TOF
  Pseudo MS^3: UMA → SMP for 1st fragmentation → Quadrupole mass selection → c.c. for 2nd fragmentation → TOF

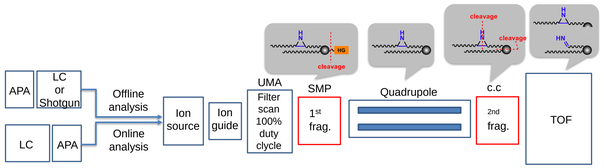


Fig. 2 Workflow of this research. SMP, segmented multipole; c.c., collision cell; HG, headgroup.

3. Results

- Compared with Prilezhaev epoxidation[4], APA has higher sensitivity, higher reaction selectivity, less spectrum complexity and better substrate generality (**Fig. 3**).
- Nearly full conversion was obtained at 50 ℃ for about 10 min. Stable mono-aziridination product is dominant, simplifying the spectrum for polyunsaturated lipids.
- Protophilic group was efficiently introduced for unsaturated lipids *via* APA. The LODs of lipids were decreased by 1 ~ 3 orders of magnitude, achieving nM detection limit.

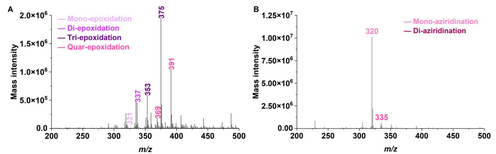


Fig. 3 Mass spectra of (A) Prilezhaev epoxidation and (B) APA products of polyunsaturated lipid.

- For shotgun analysis, UMA was successfully applied to roughly distinguish different classes and subclasses of lipids (**Fig. 4A-B**), reduce isotopic type II effect (**Fig. 4C-E**) and separate geometric isomers (**Fig. 4K**).
- Abundant site-specific fragmentation ions indicate the C=C location of C18:1 in MS/MS spectra (**Fig. 4F-H**). Most classes of lipids were also compatible.
- *sn*-Positional isomers were MS^2 CID of sodiated aziridine-PC 18:1 (Δ9)/16:0 standard produced *sn*-1 diagnostic ion at *m/z* 360.2873 (**Fig. 4I**), while *sn*-2 diagnostic ion at *m/z* 290.2454 was generated from aziridine-PC 16:0/18:1 (Δ9) (**Fig. 4J**).

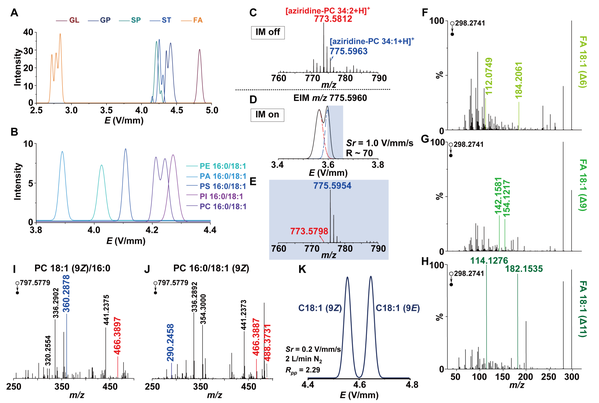


Fig. 4 Detailed structural analysis of isomeric lipids by APA-UMA-MS/MS. EIM of (A) different classes and (B) subclasses of aziridine-lipids. (C) MS spectrum of a 10:1 mixture of PC 34:2 and PC 34:1. (D) EIMs of *m/z* 775.5960. (E) MS spectrum isolated from *E* of 3.60–3.6640 V/mm. MS/MS spectra of (F) aziridine-C18:1 (Δ6), (G) aziridine-C18:1 (Δ9), (H) aziridine-C18:1 (Δ11), (I) aziridine-PC 18:1 (9Z)/16:0 and (J) aziridine-PC 16:0/18:1 (9Z), red trace represents C=C positional diagnostic ions, while blue trace represents *sn*-position diagnostic ions. (K) EIM of sodiated aziridine-C18:1 (9Z/E).

- We established an UMA-based pseudo MS^3 strategy to further enhance site-specific fragment of regioisomeric glycerophospholipids. Compared with MS/MS (**Fig. 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 MS^3 (**Fig. 5C-D**). 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.
- For real sample analysis, APA combined with mobility separation is feasible to reduce matrix effect. A total of 70 low polar lipids were detected in olive oil extracts. Without hydrolysis and complicated work-up, considerable number of triacylglycerols, diacylglycerols and sterol esters were identified at deep structure level.

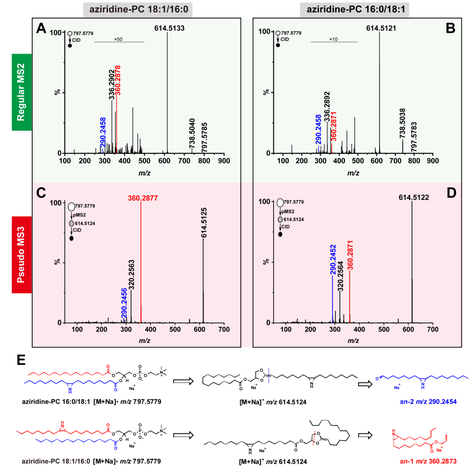


Fig. 5 MS/MS spectra of (A) aziridine-PC 18:1/16:0 and (B) aziridine-PC 16:0/18:1; Pseudo MS^3 spectra of (C) aziridine-PC 18:1/16:0 and (D) aziridine-PC 16:0/18:1; (E) Proposed fragmentation scheme of aziridine-PC 16:0_18:1.

4. Conclusion

- APA considerably promotes ionization of lipids with wide polarity and abundance range in positive ESI mode.
- Following high-throughput separation of geometric isomers, highly specific cleavage can be performed to generate abundant C=C location and *sn*-position diagnostic ions.
- UMA-based pseudo MS^3 mode allows enhancement of the backbone structure-specific fragmentation, thus further improving detection sensitivity and increasing lipid coverage.
- Development of an integrated platform LC-APA-UMA-MS/MS and corresponding applications are undergoing.

Reference
1) Synthesis (2022) 54, 4513-4520. Jat *et al.*
2) Anal. Chem. (2020) 92, 8356-8363. Wang *et al.*
3) Anal. Chem. (2024) 96, 7111-7119. Li *et al.*
4) Anal. Chem. (2019) 91, 11905-11915. Kuo *et al.*