Toxicological Centre
University of Antwerp

18th Annual Conference of the Metabolomics Society
METABOLOMICS 2022

Investigating the Potential of Ion Mobility To Oxidized Lipids using High Resolution Demultiplexing

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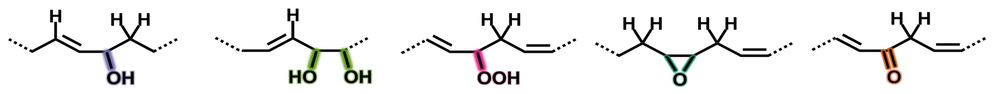
INTRODUCTION

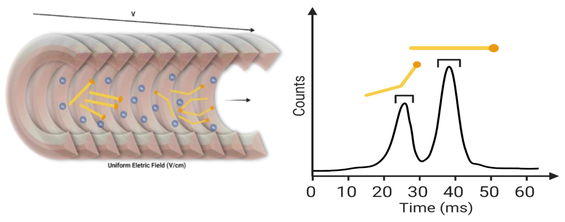
Oxidized lipids are class of modified lipids involved in numerous biochemical processes from cell differentiation to death. Diversity of modification types and their positions translates in different functionalities of oxidized lipids in biological systems.



Hydroxy Dihydroxy Hydroperoxy Epoxy Oxo

However, this structural diversity impose challenges in LC-MS/MS analysis of oxidized lipids due to large number of isomeric species and wide range of concertation. Thus, additional techniques that can improve peak capacity and increase the confidence in annotation can be of substantial benefit.

Ion mobility spectrometry (**IMS**) allows the separation of ions by their charge, shape, and size, thus providing an orthogonal separation to liquid chromatography- mass spectrometry (LC-MS) and reports **collision cross section (CCS)** values as additional annotation metric.

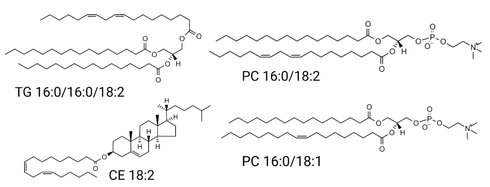


Objective

Investigate the separation of isomeric oxidized lipids using drift tube ion mobility spectrometry (DTIMS)

WORKFLOW

In vitro oxidation of lipids



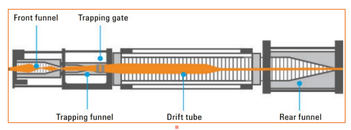
1. Prepare liposomes and micelles
- 0.5 mg of PC 16:0/18:2 (**Liposome**)
- 0.5 mg of TG 16:0/16:0/18:2 + 0.15 mg of PC 16:0/18:1 (**Micelle TG:PC, 3:1**)
- 0.5 mg of CE 18:2 + 0.19 mg of PC 16:0/18:1 (**Micelle CE:PC, 3:1**)

2. Add 500 µL of 3 mM NH_4HCO_3

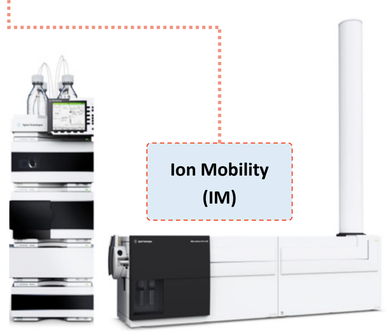
3. Sonicate for oxidize with $CuSO_4 \cdot 5H_2O$ and Na-L-ascorbate at 37°C for 24 h

4. Extract lipids with $CHCl_3$:MeOH, 2:1

Instrumentation



Quadrupole Time-of-Flight (QTOF) MS



Liquid Chromatography (LC)

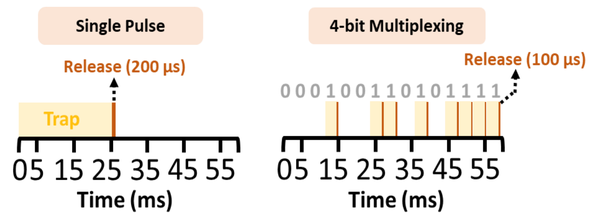
Ion Mobility (IM)

ESI (+) and ESI(-)	
LC column	Accucore C18 column (150 x 2.1 mm: 2.6 µm, 150 Å)
LC mobile phase	**A**: MeCN/H_2O (1/1, v/v) with 5 mM $HCOONH_4$ and 0.1% (v/v) HCOOH
	B: i-PrOH/MeCN/H_2O (85/10/5, v/v/v) with 5 mM $HCOONH_4$ and 0.1% (v/v) HCOOH

Data acquisition and processing

I. Full scan DTIM-MS methods

Trap fill time
Single pulse (25 ms)
4-bit multiplexing (3.9 ms)
Electric field of 12 and 17 V/cm



II. LC-IM-MS (all ions fragmentation (AIF))
Alternating frames between low (0 eV) and ramped collision energy (10-50 eV)

III. Data processing

IM Reprocessor
Mass calibration

PNNL PreProcessor
Interpolate drift bins*
Demultiplexing
Noise reduction and Spike removal

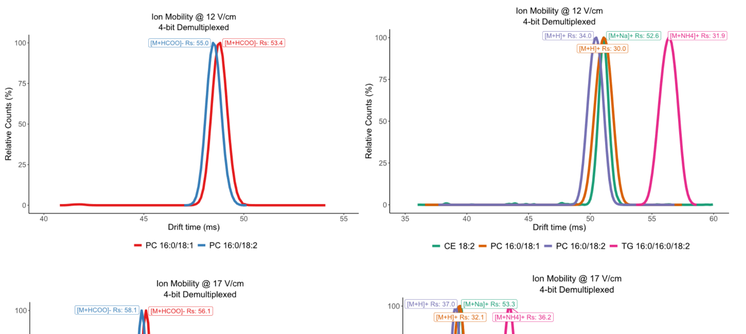
IM-MS Browser 10.0
Single field CCS calibration
Feature lists

HRdm 2.0
High resolution demultiplexing

* Necessary for HRdm

RESULTS

Effect of the electric field (E) on Resolution (R)



Increase in electric filed (E) leads to the increase in resolving power (Rs) and minimize peak broadening for DTIMS separation of unmodified lipid standards

Figure 1. The arrival time distribution of unmodified lipid standards (PC 16:0/18:1, PC 16:0/18:2, CE 18:2 and TG 16:0/16:0/18:2) ions using electric filed of 12 vs 17 V/cm.

High resolution demultiplexing (HRdm) allows to obtain resolving power up to 220 and potential base line separation of isomeric oxidized lipids.

Here, a longer temporal separation obtained by lowering the electric field allowed for improved separation using HRdm.

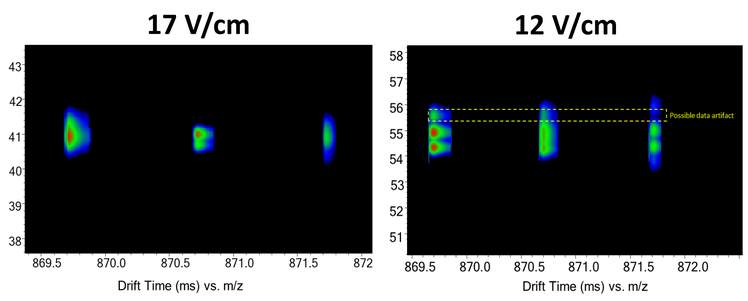


Figure 2. HRdm separation of a sodium adduct ion of a triacylglycerol with addition of one oxygen TG(50:2<O>) using electric filed of 17 vs 12 V/cm.

Ion species effect on gas phase conformation

Alkali metal adducts of (isomeric) oxidized lipids favor different gas phase conformations and thus can help in resolving isomeric species even without HRdm

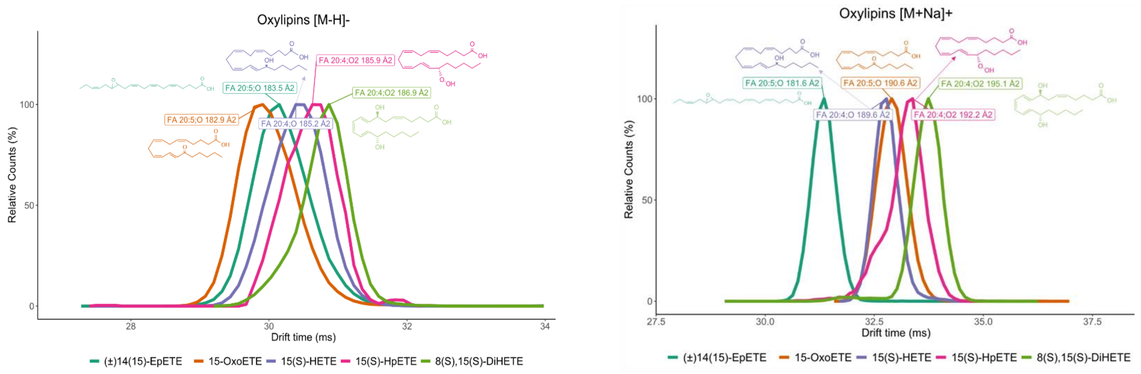


Figure 3. The arrival time distribution of five different oxylipins with different modifications types as negatively (deprotonated) and positively (sodiated) charged ions.

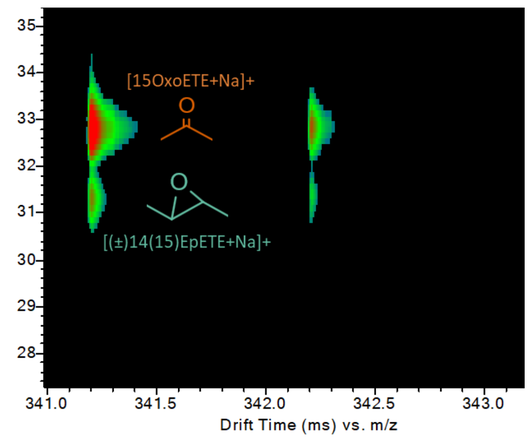


Figure 4. The sodium adducts of the isomeric oxylipins 15-oxoeicosatetraenoic acid (15OxoETE) and 14(15)-epoxyeicosatetraenoic acid ((±)14(15)EpETE) resulted in a ΔCCS of 5% and base peak separation in single pulse experiment at 12 V/cm electric filed.

CONCLUSIONS

- DTIMS allow separation of isomeric oxidized lipids which are poorly resolved by conventional LC-MS/MS
- Modulation of electric filed allows to improve resolving power of DTIMS
- High resolution multiplexing provides significant improvement in resolution
- Differential adducts of oxidized lipids might facilitate separation of multiple isomeric species

ACKNOWLEDGMENTS

epilipidnet
Pan-European Network in Lipidomics and EpiLipidomics

Universiteit Antwerpen
BOF DOCPRO 4

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY