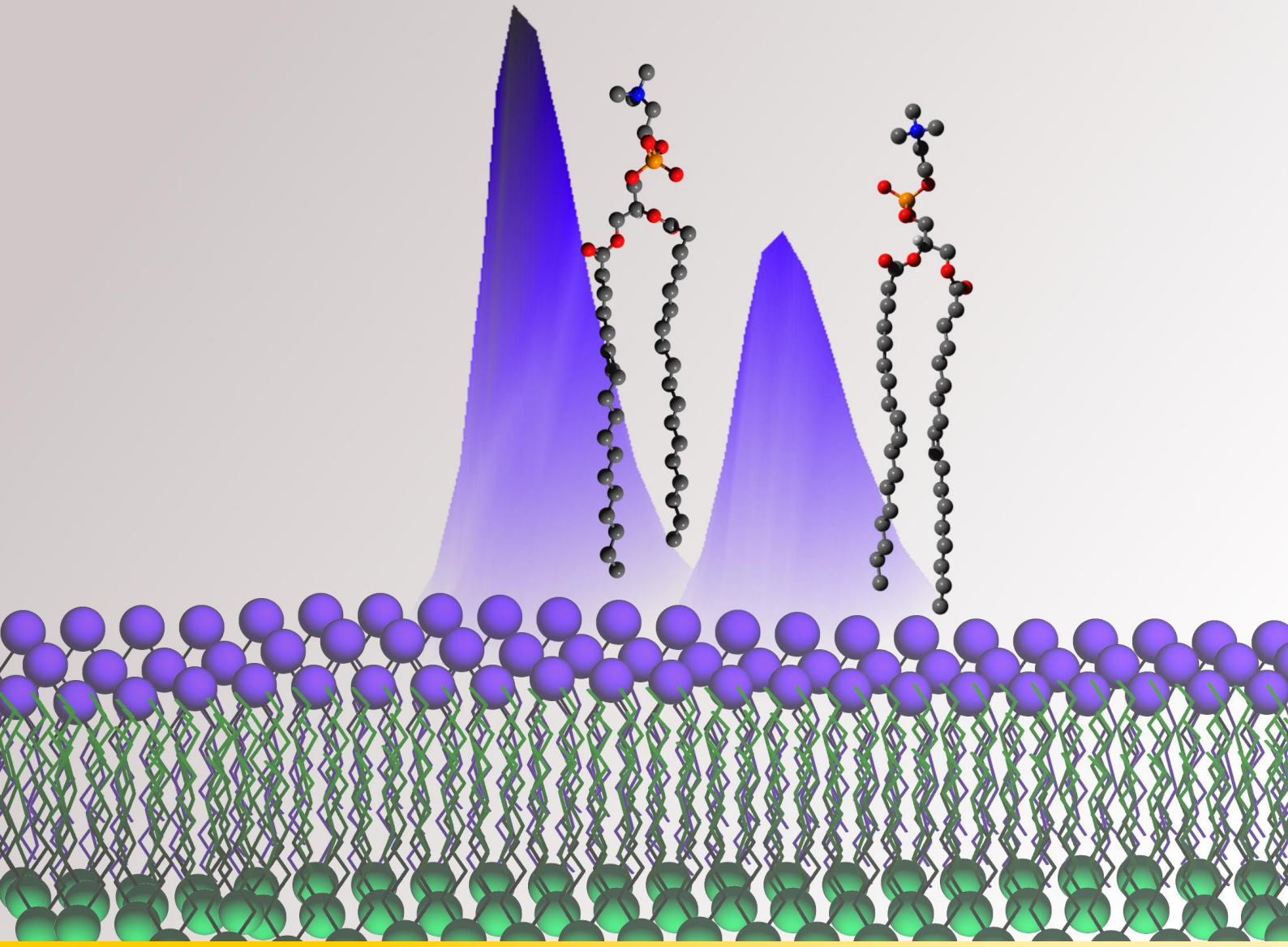

TOFWERK

Application Note

IMS-TOF 2015-1

Separation of isomeric lipids by ion mobility-time of flight mass spectrometry

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Keywords:

IMS-TOF

Lipidomics

Isomers

Ion Mobility

Overview

- LC-MS and direct infusion high-resolution MS usually fail to resolve isomers in complex biological samples such as lipid extracts
- High resolution ion mobility spectrometry (IMS) can be used for separation of isomers without adding experimental complexity
- IMS resolution can be further improved by operation above atmospheric pressure and novel post-processing algorithms

Introduction

Separation of isomeric lipids has always been a laborious task. Commonly employed techniques include chromatographic methods such as normal and reversed phase liquid chromatography, supercritical fluid chromatography, hydrophilic interaction chromatography and gas chromatography mostly using mass spectrometers as detectors. Yet, these techniques suffer from drawbacks such as poor separation efficiency, long separation times or tedious sample preparation procedures. Here we show how **high resolution ion mobility** spectrometry resolves these separation issues without adding experimental complexity or increasing analysis times.

From a biological point of view, detection and characterisation of lipid isomers is an extremely "hot" topic as differences in the position and geometry of double bonds as well as the acyl chain length and relative acyl chain position are thought to play a pivotal role for their biological functions [1].

This application note focuses on phosphatidylcholines (PCs) to demonstrate the capabilities of a high resolution ion mobility-time of flight mass spectrometer (IMS-TOF) for the separation and detection of isomeric lipids. This lipid class is a major constituent of eukaryotic cell membranes and highly abundant in plasma; PCs are involved (directly or indirectly) in many enzymatic reactions in blood circulation and in tissues [2].

Additionally, the TOFWERK IMS-TOF uses multiplexing technology which provides a dramatic increase in sensitivity and allows the utilization of novel post-processing algorithms. Superior IMS resolving power, far in advance of other commercially available systems, is achieved by the operation of the IMS drift cell at up to 1400 mbar.

Experimental

All measurements were carried out on a TOFWERK IMS-TOF. The system, Figures 1 and 2, comprises an ESI source, a 10 cm desolvation tube, a 20 cm drift tube (both made from resistive glass) and a TOFWERK HTOF (TOFMS). Desolvation and drift tube were thermostated at 150°C with nitrogen as the buffer gas. Ion mobility separation was carried out at a field strength of ca. 400 V/cm. Drift-tube pressure was set between atmospheric and 1402 mbar (nitrogen).

Lipid extracts and standards were obtained from Avanti Polar Lipids and diluted in methanol: isopropanol 1:1 (*v/v*) with 0.1% formic acid prior to measurement in positive ion mode. All other chemicals (analytical grade) were obtained from Sigma-Aldrich and used without further purification. Raw IMS-TOF data was post-processed using IMS Viewer and Tofware (TOFWERK, Switzerland), LipidXplorer (Shevchenko Lab, MPI-CBG) was used for lipid identification.

IMS resolving power *R* is defined as

$$R = \frac{t_d}{\Delta t_d}$$

where t_d is the IMS drift time of a peak, and Δt_d is it's width (FWHM).

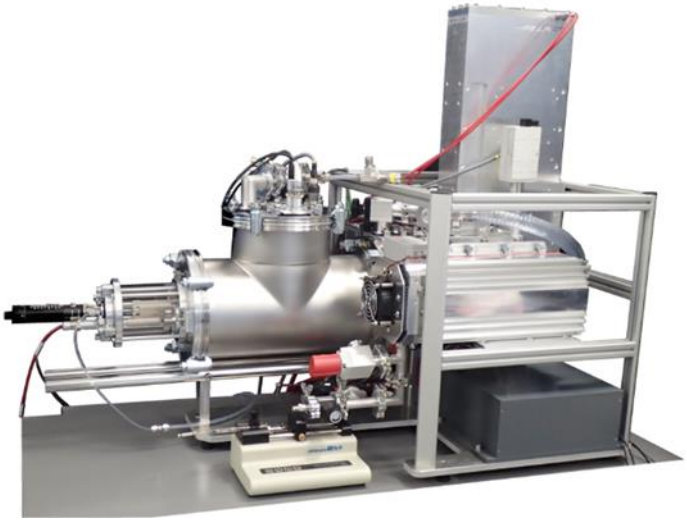


Figure 1. IMS-TOF without panels

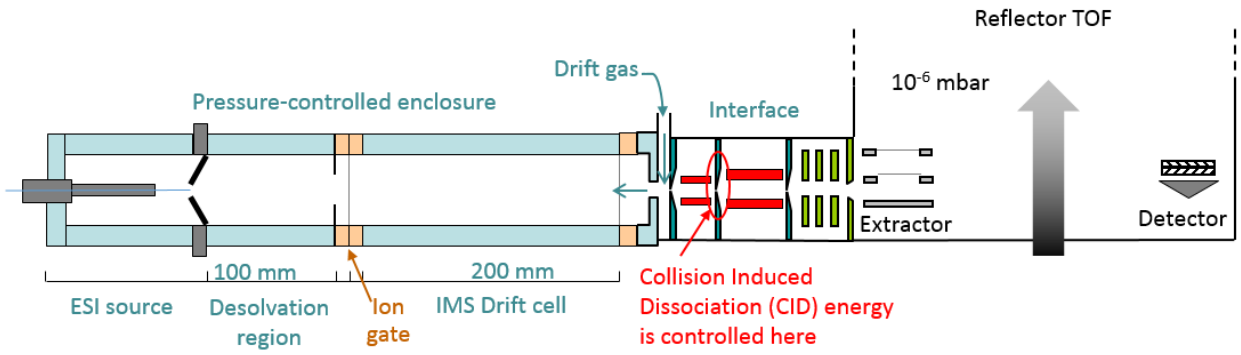


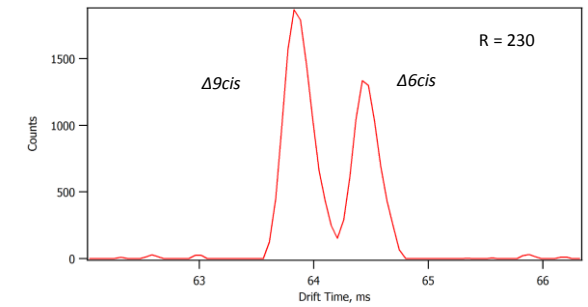
Figure 2. Schematic of the IMS-TOF

Results

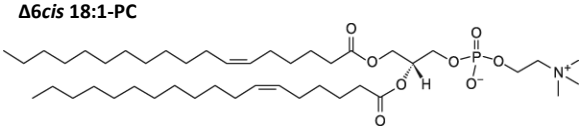
Position of the double bond

Within biological systems, PCs are processed by specific enzymes to produce second lipid messengers such as lysophosphatidylcholines (LPCs). As the signalling properties of LPCs are influenced by the position of the double bond, separation of PCs that differ in the position of the double bond in the fatty acyl chain is of high interest [2].

In Figure 3, we demonstrate the separation of 18:1/18:1 PCs with the double bond either in Δ6 or Δ9 position (*cis* geometry in both cases). This is enabled by the high IMS resolving power R = 230 that results from the use of a high pressure system and novel post-processing algorithms. The peaks are almost baseline separated.



Δ6*cis* 18:1-PC



Δ9*cis* 18:1-PC

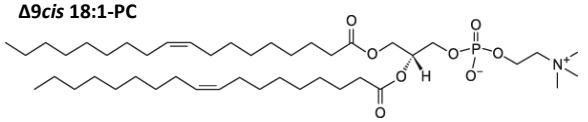
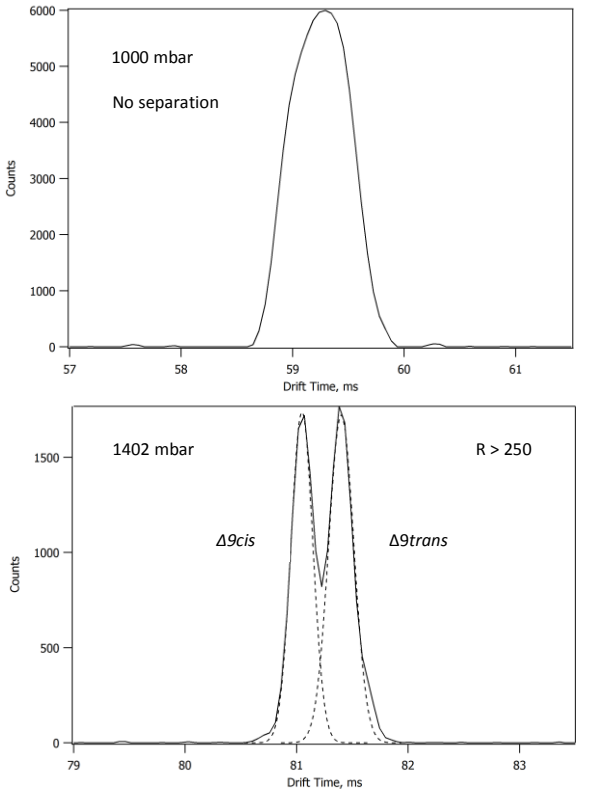


Figure 3. Separation of Δ6*cis* and Δ9*cis* 18:1 PC.

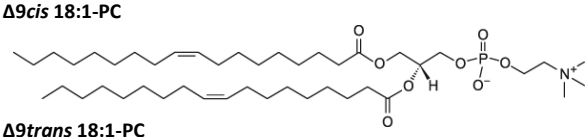
Geometry of the double bond (cis/trans isomers)

In naturally occurring eukaryotic lipids, the geometry of double bonds in fatty acids is strictly *cis*. This geometry is tightly controlled by specific enzymes and consequently, alterations in the geometry severely impacts their membrane functions and signalling properties and are thought to play a major role in aging and health impairments. [3]; the change from *cis* to *trans* geometry is evoked by peroxidation and involvement in electrophilic free radical reactions. Therefore, detection of *cis/trans* isomerism is an important aspect in current biological and medical lipid research.

The separation of *cis/trans* isomers represents a significant analytical challenge and usually requires the use of multiple techniques or derivatization. Due to the small differences in collision cross sections (in the range of 1%), separation by ion mobility is normally not feasible. Yet, using high resolution ion mobility at elevated pressure (1402 mbar) and post-processing, a resolving power R > 250 can be reached. As shown in Figure 4, this allows good (though not baseline) separation of *Δ9cis* and *Δ9trans* isomers of PC 18:1, which cannot be accomplished at atmospheric pressure. The dependence of IMS resolving power on pressure has already been described by Hill and co-workers [4].



Δ9*cis* 18:1-PC



Δ9*trans* 18:1-PC

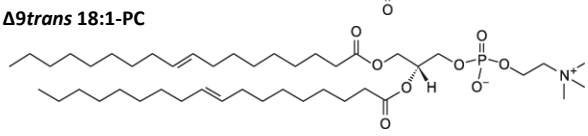


Figure 4. Δ9*cis* and Δ9*trans* 18:1 PC separation; atmospheric pressure (above), elevated pressure (below).

Position of the acyl chain (regioisomers)

The position of the acyl chain in phospholipids is thought to by an essential structural factor for their specific activity. Currently, enzymatic methods that selectively hydrolyze the ester moiety in *sn*-1 or *sn*-2 position are used for differentiation of these regioisomers. This approach does not allow structural assignments in complex lipids mixtures and therefore requires cumbersome purification and therefore large amounts of sample.
In Figure 5 we show the baseline separation of regioisomeric 16:0-18:1 PC and 18:1-16:0 PC as silver adducts. In contrast to enzymatic method, IMS-MS allows a quick and straightforward determination of the relative amounts of each regioisomer.

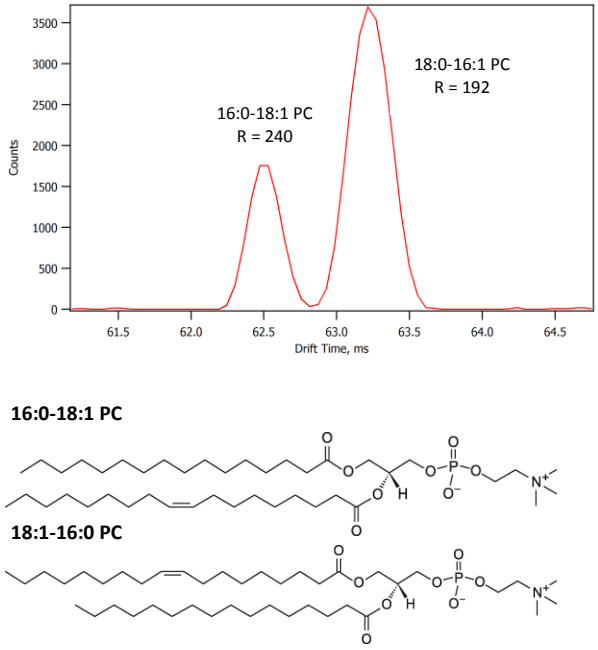


Figure 5. Separation of regioisomeric 16:0-18:1 PC and 18:1-16:0 PC

Conclusions

Using the high resolution IMS-TOF instrument, separation of isomeric lipids is now feasible. This includes positional isomers (position of the double bond), geometrical isomers (*cis/trans*) and regioisomers (position of the acyl chain). Superior IMS resolving powers are achieved by operating the instrument at super-atmospheric pressure and using novel post-processing algorithms.

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