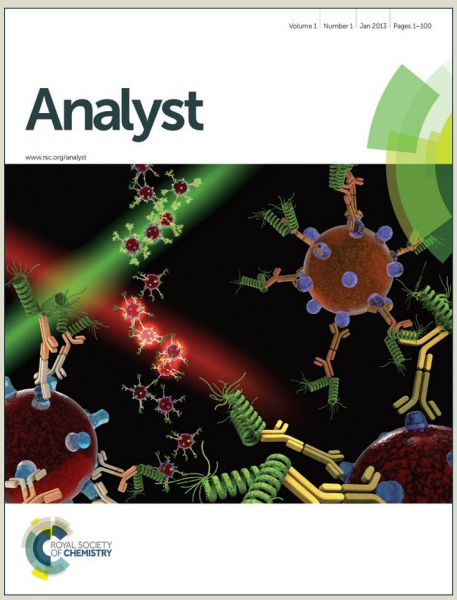
Analyst

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Journal Name

ARTICLE

High resolution ion mobility-mass spectrometry for separation and identification of isomeric lipids

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

M. Groessl*[a], S. Graf[a], R. Knochenmuss*[a]

Lipidomics is a particularly difficult analytical challenge due to the number and importance of isomeric species that are known or postulated in biological samples. Current separation and identification techniques are too often insufficiently powerful, slow or ambiguous. High resolution, low field ion mobility coupled to mass spectrometry is shown here to have sufficient performance to represent a new alternative for lipidomics. For the first time, drift-tube ion mobility separation of lipid isomers that differ only in position of the acyl chain, position of the double bond or double bond geometry is demonstrated. Differences in collision cross sections of less than 1% are sufficient for baseline separation. The same level of performance is maintained in complex biological mixtures. More than 130 high–precision reduced mobility and collision cross section values were also determined for a range of lipids. Such data can be the basis of a new lipidomics workflow, as the appropriate libraries are developed.

Introduction

Over the last decade, mass spectrometry based lipidomics has enabled the identification and quantification of lipids on a system wide level.[1–3] Like other *-omics* disciplines, the field has profited tremendously from advances in instrumentation, data processing and bioinformatics. Due to the key role of lipids in many cellular processes, lipidomics has been widely applied in various fields ranging from molecular biology and lipid biochemistry to drug and biomarker discovery, as well has health and nutritional sciences.[3–5]

It is estimated that the eukaryotic lipidome includes on the order of 100000 individual molecular species from a few hundred classes.[1] In order to cope with this enormous diversity, different analytical approaches have been developed. Shotgun lipidomics relies on the direct infusion of the samples combined with high resolution FT-MS and MS/MS for rapid identification, yet usually fails to characterise isomeric species.[6–8] Normal phase LC, reversed phase LC and silver-ion LC allow separation of lipid classes and, to some extent, also isomers, yet cannot be used for all lipid classes. Usually these methods also require long run times, or are not compatible with ESI-MS.[9–11]

More recently, ion mobility coupled to mass spectrometry (IM-MS) has been investigated as an alternative method for lipid analysis.[12,13] The major advantage of IM-MS is its ability to separate isobaric and isomeric analytes by hydrodynamic "shape," or more precisely ion-neutral collision cross sections Separation of lipid classes by IM-MS has been demonstrated on several occasions, including the determination of reduced mobilities and collision cross sections for database-aided identification of lipids.[14–16] Yet, to the best of our knowledge, there are only two reports on separation of regioisomers (position of the fatty acid chain)[17,18] whereas separation of positional isomers (differing in the position of the double bond) or geometrical isomers (*cis/trans* double bond geometry) by IM-MS have not been reported. This is due to the fact that these type of isomers usually differ by less than 2% in their collision cross sections and therefore require ion mobility resolving power beyond 100 for separation, a range which is not accessible by travelling wave ion mobility and commercial low pressure drift tube instruments. From a biological point of view, molecular characterisation of lipid species is highly desirable since, as with most biomolecules, their function is strongly coupled to their structure.[3]

For example, the effect of different double-bond positions in the fatty acid chains of phosphatidylcholines (PCs) on cellular membrane fluidity and function has been studied for several decades.[19,20] Additionally, it has been suggested more recently that double-bond positional isomers in naturally occurring lipids can have significant impact on the development of diseases.[21] Even though only *cis* (Z) isomers are formed during the biosynthesis of fatty acids in eukaryotic cells, *trans* (E) fatty acids can be introduced in food, synthesised by microorganisms, or converted from *cis* species through radical-based isomerization.[22] *Trans* geometry in lipids can lead to changes in membrane function and even inhibition of lipid enzymatic pathways and determination of *trans* lipids is therefore of great interest. Similarly, the position of the acyl chain (either *sn-1* or *sn-2*) on phospholipids can have a strong

[a] Tofwerk AG, Uttigenstr. 22, 3600 Thun, Switzerland. E-Mail addresses: groessl@tofwerk.com, kochenmuss@tofwerk.com
Electronic Supplementary Information (ESI) available. See DOI: 10.1039/x0xx00000x

biological impact as it affects the properties of secondary messengers upon specific enzymatic cleavage of one of the fatty acids.[23]

To date, the only mass spectrometric technique that allows determination of double-bond positions for lipidomics applications is called ozone-induced dissociation (OzID).[21,24] OzID involves selective ozonolysis of double bonds within the mass spectrometer followed by MS/MS. The drawback of this approach is that it requires handling of highly toxic ozone as well as modification of instrumentation, as no commercial product supports OzID. Also, the technique does not provide any information on the geometry of the double bond.

Herein, we demonstrate the advantages of using a high resolution IM-MS instrument for lipid analysis. Resolving power exceeding 250 enabled separation of isomeric lipids, including double-bond positional and geometrical isomers, even in complex biological samples. Also, true low-field conditions enable high precision determination of reduced mobilities (K_0) and collision cross sections (CCS), molecular descriptors which can complement accurate mass, fragmentation spectra and LC retention times for improved identification of analytes.[15,25] K_0 and CCS are physical molecular properties and should therefore be more robust and reproducible than LC retention times. Theory is also available to derive them from first principles, though it is not yet sufficiently refined for routine use. However, to be useful for unambiguous compound identification, it is crucial that K_0 and CCS are determined with high accuracy and precision.

Experimental

Materials

Polar lipid extracts of yeast (*S. cerevisiae*), bovine heart, porcine brain, egg yolk and *E. coli* as well as synthetic lipid standards were obtained from Avanti Polar Lipids (Alabaster, USA). Extracts were diluted to a total lipid concentration of 25 µg/ml in isopropanol:methanol:chloroform 4:2:1 (*v/v*) with 1 mM sodium acetate prior to lipidomics profiling. For determination of regioisomers, silver nitrate (0.1 mM) was added instead of sodium acetate. Measurements of synthetic lipid standards were performed using 1 µM solutions. All chemicals (analytical grade or higher) were obtained from Sigma-Aldrich (Switzerland) and used without further purification.

Instrumentation

All measurements were carried out on a Tofwerk IMS-TOF (Thun, Switzerland). The system comprises an ESI source, a 10 cm desolvation tube, a 20 cm drift tube (both made from resistive glass) and a Tofwerk HTOF (TOF-MS) with two-stage interface to the IMS. Desolvation and drift tubes were thermostated at 105°C with nitrogen as the buffer gas. Ion mobility separation was carried out at a field strength of ca. 400 V/cm (reduced electric field strength ca. 2 Td). Drift-tube pressure was set between atmospheric and 1400 mbar (nitrogen). Samples were introduced directly into the ESI source at 1 µl/min. The instrument was operated from m/z 100 to 1800 in both positive and negative ion mode with a total measurement time of 10 min for profiling of lipid extracts. The mass spectrometer was calibrated externally using a mixture of tetraalkylammonium salts and Ultramark 1621. Raw IMS-TOF data was post-processed using IMS Viewer and Tofware (Tofwerk, Switzerland).

The instrument uses a Hadamard-type multiplexing method.[26] In addition to increasing sensitivity, properties of the time domain data can be exploited to increase both signal to noise ratio and effective resolution. The latter can significantly exceed the diffusion limit which applies to conventional pulsed-mode drift tube IMS.[27]

Reduced mobility and collision cross section measurements

Measured ion mobility drift times have to be corrected for time spent outside the drift tube (*i.e.*, the interface region of the mass spectrometer) for precise determination of reduced mobilities (K_0) and collision cross sections (CCS). Corrected drift times are determined from a plot of the measured drift time versus the inverse drift voltage. Drift time measurements were carried out at five different drift potentials (from 8 to 12 kV) and the y-intercept of the plot vs. 1/field strength (the "nonmobility" component of the drift time) subtracted from the measured drift time to obtain the corrected drift time (see supplementary information). Reduced mobilities and collision cross sections can then be directly calculated from the corrected drift times, and no external calibration is needed. A modified zero-field equation, called the momentum transfer scan law, was used to correct for field-dependent momentum transfer and collision frequency (supplementary information).[28,29] Corrected drift times, K_0 and CCS are determined automatically by the post processing software.

Lipid identification

LipidXplorer (Shevchenko Lab, MPI-CBG) was used for lipid identification.[30] Both MS and mobility selected fragmentation spectra (IMS-CID-MS) were imported as mzml files and searched against selected glycerophospholipid classes, allowing a maximum error in mass accuracy of 20 ppm. Positive ion mode data was searched for phosphatidylcholines (PC), 1-O-alkyl–2-acyl-glycerophosphatidylcholines (PC-O), lysophosphatidylcholines (LPC), phosphatidylethanolamines (PE) and 1-O-alkyl-2-acylglycerophosphatidylethanolamines (PE-O). Negative ion mode data was searched for phosphatidylethanolamines (PE), lysophosphatidylethanolamines (LPE), phosphatidic acids (PA), lysophosphatidic acids (LPA), phosphatidylserines (PS), phosphatidylglycerols (PG), lsyophosphatidylglycerols (LPG) phosphatidylinositols (PI), lysophosphatidylinositols (LPI) cardiolipins (CL) and monolysocardiolipins (mLCL). Molecular lipid were annotated with the number of carbon atoms and double bonds of the fatty acid in *sn*-1 position followed by the number of carbon atoms and double bonds of the fatty acid in *sn*-2 position. When the position and geometry of the double bond is known, it is given in parentheses after the number of double bonds according to recommendation of the Lipid MAPS

consortium and Liebisch et al.[31,32] In cases where the exact composition of fatty acid moiety was unknown, lipid species were annotated with the total number of fatty acid carbon atoms and double bonds.

Results and Discussion

Separation of double bond positional and geometrical isomers

PCs were used to test whether high resolution IM-MS can differentiate double bond positional and geometrical isomers. Since cationization with alkali metals such as sodium and potassium can have a positive effect on the separation of isomeric species[33], K_0 and CCS values of PC 18:1(6Z)/18:1(6Z), PC 18:1(9Z)/18:1(9Z) and PC 18:1(9E)/18:1(9E) were determined for various adducts.

As can be seen in Table 1, the differences in K_0 and CCS (which reflect the degree of separation) are dependent on the type of adduct. RSD values were ≤ 0.2% in all cases, meaning that differences by more than 0.001 cm^2/Vs in K_0 or 0.6 $Å^2$ in CCS can be considered significant for these compounds. Assuming a resolving power of 250 and identical peak widths, compounds that differ by less than 1% in CCS (i.e. 3.0 $Å^2$ for PC 18:1/18:1 species) can be baseline separated, as shown in Figure 1. Whereas the difference between the 6Z and 9Z configuration is 1.0% for the protonated form, it rises to 1.2% for both sodiated and potassiated forms. On the contrary, the protonated forms of the 9Z and 9E configuration differ by 0.4% whereas the difference is less than 0.1% and therefore not significant for the potassiated forms. This shows clearly that adduct formation with alkali metals has an impact on the conformation of double bond isomers and indicates interaction with the fatty acid moieties of these lipids. It can be assumed that protonation takes place on PC head group, therefore not altering the conformation of the fatty acid tails and conserving the conformational differences between E and Z configuration.

In contrast, cationization slightly improves separation of 6Z and 9Z as well as of 6Z and 9E, indicating the position of the double influences the coordination of the alkali metal ion and consequently leads to a more pronounced change in lipid ion structure. The fact that the 6Z configuration exhibits the largest CCS of the group reflects the higher flexibility of the fatty acid chain due to higher number of carbon atoms between the double bond and the methyl end. Overall, these results indicate that sodiated species are preferential for efficient ion mobility separation of PC isomers.

Next, we wanted to confirm that the IM resolving power of the instrument is actually sufficient to distinguish mixtures of these isomers in complex samples. To this end, commercially available polar lipid extracts of bovine heart, porcine brain and yeast were analysed and K_0 and CCS values of naturally present PC 18:1/18:1 determined (Table 1). The results clearly point to the presence of PC 18:1(9Z)/18:1(9Z). To confirm this finding, the extracts were spiked with synthetic standards of the three isomers. Extracted ion mobilograms for PC 18:1/18:1 of the spiked bovine heart extract are shown in Figure 1.

For the sample spiked with PC 18:1(6Z)/18:1(6Z), analyte peaks are baseline separated while for 18:1(9E)/18:1(9E) no separation was obtained under standard conditions (ambient pressure), independent of the monitored adduct. The IMS-TOF instrument has a pressure-tight drift cell which offers the possibility to increase the pressure above atmospheric, thus further increasing the resolving power (above 250 in this case) due to increased number of ion-molecule collisions. As depicted in Figure 1, lower trace, raising the pressure to 1400 mbar led to partial separation of 9Z and 9E isomers. The increase in ion mobility resolution is accompanied by a loss in sensitivity of about 25% (1.5 ions per ToF extraction at 1400 mbar compared to 2 ions per ToF extraction at 1000 mbar). Separation was also confirmed by mixing the synthetic standards of the different isomers in pure solvents (not shown).

Table 1. Reduced low field mobilities and field-corrected collision cross sections of adducts of double bond positional and geometrical isomers of PC 18:1/18:1. The RSD was less than0.2% in all cases (n = 4). Bovine, porcine and yeast PC 18:1/18:1 refer to the naturally occurring species found in polar lipid extracts.

	H^+		Na^+		K^+	
	K_0 (cm^2/Vs)	CCS ($Å^2$)	K_0 (cm^2/Vs)	CCS ($Å^2$)	K_0 (cm^2/Vs)	CCS ($Å^2$)
PC 18:1(6Z)/18:1(6Z)	0.601	304.4	0.596	306.5	0.591	309.1
PC 18:1(9Z)/18:1(9Z)	0.607	301.3	0.603	302.9	0.598	305.4
PC 18:1(9E)/18:1(9E)	0.605	302.5	0.602	303.7	0.598	305.7
Bovine PC 18:1/18:1			0.603	302.8		
Porcine PC 18:1/18:1			0.603	302.9		
Yeast PC 18:1/18:1			0.604	302.7		

Journal Name

ARTICLE

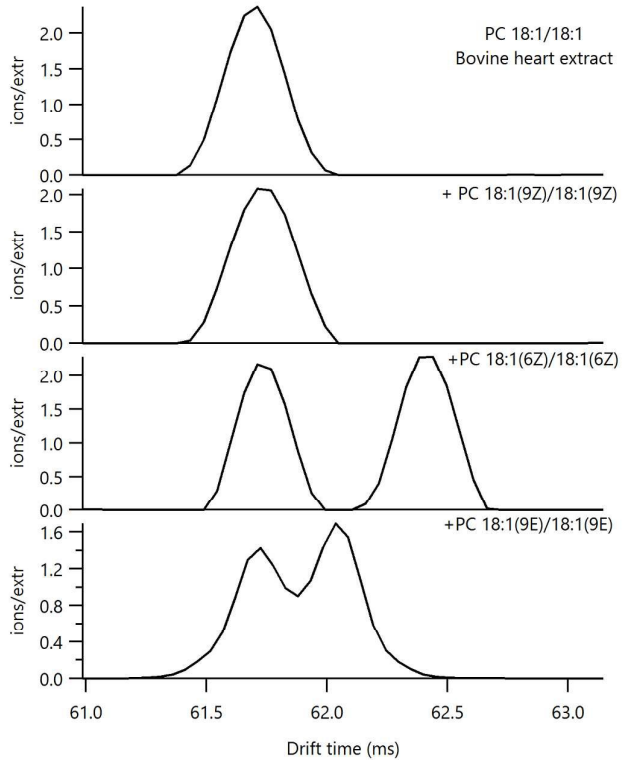


Figure 1. Extracted ion mobilograms for PC 18:1/18:1 for bovine heart polar lipid extract spiked with different isomers. The drift time axis for 9E spike was rescaled to compensate for the pressure, which was 1400 mbar rather than ambient as for the other traces.

Separation of acyl-chain regioisomers

As already discussed for isomers based on differences in double-bond geometry and position, the position of the acyl chain can also have a strong impact on the biological function of phospholipids. For example, PCs can be processed by specific enzymes such as phospholipase A2 to produce the lipid second messengers LPCs upon cleavage of the fatty acid in *sn*-2 position.[23] Consequently, regioisomers with different fatty acids in *sn*-1 and *sn*-2 position will have different biological effects.

Initially, protonated, sodiated and potassiated species were investigated for optimal separation of PC 16:0/18:1 and PC 18:1/16:0 regioisomers. As can be seen in Table 2, the difference in K_0 and CCS values is larger for sodiated (0.3%) than potassiated species. Even though these values differ significantly (RSD ≤ 0.2%), meaning that each species can be confidently identified based on K_0 and CCS as single species, the difference is not large enough for separation of mixtures that contain both isomers.

Separation of the regioisomeric lipids has been reported in the past using silver-ion chromatography, which exploits selective binding of transition metals, in this case that of Ag^+ to olefinic double bonds. Analogously, Campbell et al. recently reported the use of silver adducts to separate PC 16:0/18:1 from PC 18:1/16:0 by differential mobility spectrometry (DMS) coupled to an ion trap MS.[18] Silver adduction is also a useful strategy in drift-tube IMS, as can be seen in Table 2. The K_0 and CCS of Ag^+ adducts differ much more (0.7%) than do those of the other investigated cations. This is sufficient to enable separation of mixtures.

Additionally, for identification and quantitation of regioisomeric phospholipids, MS/MS and MS^3 methods have been proposed.[18,34] Such fragmentation-based methods rely on the assumption that isomer-specific fragments are formed which can be used for quantification. Precursor ions are isolated using a quadrupole or ion trap, allowing fragmentation of ions that fall within a defined m/z window. In contrast, in the IMS-TOF all ions are fragmented simultaneously. Since dissociation occurs after the mobility separation (IMS-CID-MS), fragment ions can be assigned to their precursor based on drift times (see supplementary information). Note that different instrumental geometries can potentially lead to different fragmentation pathways.

As can be seen in Figure 2, no isomer-specific fragments are found in the IMS-CID-MS spectra of an equimolar mixture of PC 16:0/18:1 and PC 18:1/16:0. IMS peaks corresponding to both isomers can be seen in the IMS traces for m/z 551.173 and m/z 525.152 (corresponding to neutral loss of the 16:0 and 18:1 fatty acids from $[M+Ag\text{-}N(CH_3)_3]^+$), demonstrating that the neutral loss of the fatty acid moiety is favoured for the *sn*-1 position, but is not exclusive to this isomer. Interestingly, even the ion mobilogram for the main fragment at m/z 683.417 (neutral loss of the PC head group from $[M+Ag]^+$) shows unequal distribution between the two isomers. The data indicate that multiple dissociation pathways take place which are not exclusive to a single isomer. In this example, the 1:1 ratio of the isomers is only correctly reflected in the ion mobilogram of the intact lipids. Note that the intensity of the ion at m/z 525.152 is only ca. 0.5% of the base peak at m/z 683.417, yet extraction of high-quality IMS spectra is still feasible.

Table 2. Low field reduced mobilities and field-corrected collision cross sections for PC 16:0/18:1 and PC 18:1/16:0. RSD ≤ 0.2% in all cases (n = 4).

	H^+		Na^+		K^+		Ag^+	
	K_0 (cm^2/Vs)	CCS ($Å^2$)	K_0 (cm^2/Vs)	CCS ($Å^2$)	K_0 (cm^2/Vs)	CCS ($Å^2$)	K_0 (cm^2/Vs)	CCS ($Å^2$)
PC 16:0/18:1	0.617	296.6	0.611	299.2	0.609	299.9	0.624	292.5
PC 18:1/16:0	0.617	296.2	0.613	298.2	0.610	299.4	0.619	294.7

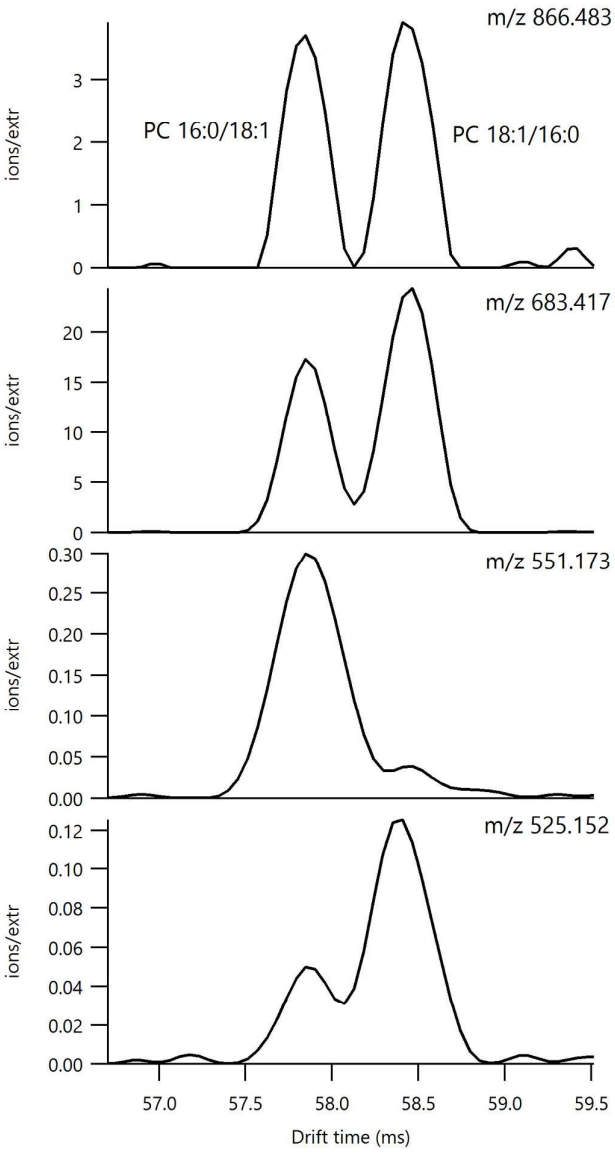


Figure 2. Extracted ion mobilograms for precursor and fragment ions obtained from IMS-CID-MS of an equimolar mixture of PC 16:0/18:1 and PC 18:1/16:0 (silver-ion adductsNone of the fragments is exclusive to a single isomer, making fragment-based identification unreliable. The ion at m/z 683.417 corresponds to neutral loss of the PC head group from $[M+Ag]^+$, while m/z 551.173 and m/z 525.152 correspond to neutral loss of the 16:0 and 18:1 fatty acids from $[M+Ag-N(CH_3)_3]^+$, respectively.

To verify that this approach also enables identification of these regioisomers in biological samples, polar lipid extracts of bovine heart, porcine brain and yeast were investigated. As shown in Figure 3, both regioisomers were unambiguously detected in the porcine brain extract, with PC 18:1/16:0 accounting for 12% of the peak area of the corresponding IMS trace. For yeast, 4% correspond to PC 18:1/16:0, whereas only traces of PC 18:1/16:0 are present in the bovine heart extract (Table 3 and supplementary information). Furthermore, to determine the concentration of each isomer in porcine brain and yeast extracts, standard addition experiments were carried out (Table 3). Correlation coefficients R^2 of ≥0.99 demonstrate the linear response of the instrument (supplementary information). At the concentrations found in the sample, a ratio of 1:50 between the two regioisomers can still be quantified accurately. The linear range for phospholipids is > 3 orders of magnitude, with a limit of detection of approximately 4 fmol/min (supplementary information).

Isotope correction for overlaps of IMS traces with isobaric lipids, *i.e.* $[PC\ 34:2+^{109}Ag]^+$, was performed analogously to isotope correction implemented in shotgun lipidomics software for mass spectra and as suggested in the literature (see supplementary information).[18,30]

The results show that separations of the regioisomers on a high-resolution drift-tube based IMS-MS are comparable to DMS-MS spectra reported in the literature. The drift-tube instrument offers the advantage of direct calculation of K_0 and CCS values from the drift times as well as the higher mass resolving power and accuracy of the mass analyser, which is crucial for lipid identification in lipidomic screens. Also, the drift-tube instrument does not require any special tuning to optimise the separation of a specific isomeric pair but is operated with the same settings for all analytes.

A 20-fold drop in ion current has been reported for the DMS-MS instrument when activating the DMS cell while the difference in ion current between MS and IMS-MS mode on the IMS-TOF is only a factor of 2-3 thanks to multiplexing.[26] In contrast, the reported DMS-MS instrument allows MS^3 fragmentation experiments, whereas the IMS-TOF is limited to drift-time selective IMS-CID-MS.

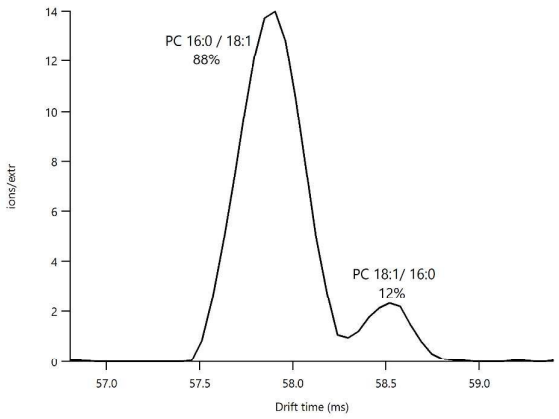


Figure 3. Separation of naturally occurring PC 16:0/18:1 and PC 18:1/16:0 regioisomers in a porcine brain polar lipid extract.

Table 3. Concentrations of PC 16:0/18:1 and PC 18:1/16:0 in commercially available porcine brain and yeast polar lipid extracts as determined by standard addition. Concentrations (% wt/wt) are based on a total lipid content of 25 mg/ml as indicated by the supplier.

	Porcine Brain				Yeast			
	Peak Area (%)	*c (uM)*	*c (ug /ml)*	*% wt/wt*	*Peak Area (%)*	*c (uM)*	*c (ug /ml)*	*% wt/wt*
PC 16:0/18:1	88	420	319	1.28	96	117	89	0.35
PC 18:1/16:0	12	58	44	0.18	4	5	4	0.02

Accurate determination of K_0 and CCS values in biological samples

Phospholipids from five biological sources (bovine heart, porcine brain, yeast, egg yolk and *E. coli*; supplementary information) were investigated as a further test of practical utility. Including only those previously identified in the literature, over 130 species from 14 classes were found. When multiple IMS peaks were present for a single MS peak, substances were included only if the identity of the lipids could be unambiguously assigned using fragmentation data or IMS trend lines for lipids of the same class.

An example of how IMS provides powerful information for lipid class identification is shown in Figure 4. PC-O and PE lipids are not isomers, but the small differences in mass (0.03 Da) of the pairs shown in Fig. 4 would require an MS resolving power >70000 for baseline separation, which is not attainable using ToF-MS. However, these isobars are separated by high resolution IMS as shown in the left panel of Fig. 4. The right panel shows the lipid-class specific trend lines used for assignment of the peaks and their congeners in the left panel. The assignments were further confirmed by post-IMS CID (not shown). Whereas the addition of a double bond to an unsaturated PC-O leads to a change in CCS of 1%, this relative difference between unsaturated PEs is only 0.5% per additional double bond. In these samples, the use of IMS-MS led to an increase of more than 60% in the number of detected peaks, compared to MS only (supplementary information).

Accuracies of <0.5% should be possible, but currently CCS values reported in literature vary by much more, and are therefore of questionable use for substance identification. Almost all available CCS values for lipids have been determined either by low pressure drift tube or travelling wave ion mobility instrument spectrometry (TWIMS) instruments.[15,16] Low-pressure drift instruments have been preferred for this purpose as they allow, in principle, the direct determination of CCS from drift times. However, most of these instruments do not operate under low (approx. 2 Td) but rather mid to high field conditions (8-20 Td). Unless the determinations are conducted under low field conditions, the commonly used zero field ("Mason-Schamp") equation always overestimates CCS (typically about 2% at 16 Td) and thereby introduces a systematic error.[28,29] Also, it has been recently reported that the use of fields ≥8 Td leads to a loss of ion mobility resolution for biomolecules probably caused by conformational unfolding[35], thereby decreasing the accuracy and utility of the measurement.

TWIMS instruments are even more susceptible to subtle and systematic error, which may or may not be correctable using elaborate calibration based on drift tube data. Also, it has been reported to be crucial that TWIMS is calibrated with compounds of the same chemical class as the analytes, further complicating correct CCS determination.[36] In general, the errors in precision and accuracy of TWIMS K_0 and CCS values reported to date appear to be typically around 2%,[16,37] plus whatever error is transferred from the reference drift tube data. Figure 5 shows the correlation between the CCS of 30 phospholipids as determined on a TWIMS instrument and the IMS-TOF. A substantial and systematic deviation from the line with unit slope and zero offset is observable, with the TWIMS values being 1.8 % larger on average.

The instrument in this study operates under true low field conditions, <2 Td, so systematic errors are expected to be minimal. Furthermore, the best available theoretical correction for the drift field has been applied, in the form of

the momentum transfer theory of Siems and Viehland.[28,29] The uncertainties associated with experimental parameters such as pressure, temperature, drift voltage and drift time were combined with the experimental reproducibility (< 0.3% RSD) to estimate the combined accuracy to be 1.3% (supplementary information), compared to 2% reported by McLean et al.[16] Yet, as discussed by Hill et al.[37], other parameters should also be taken in account, such as accuracy of the length of the drift tube, inhomogeneity of the electric field, water content of the drift gas and error of extrapolation for determination of corrected drift times. Systematic and thorough evaluation of the influence of these parameters on the accuracy of K_0 and CCS values is beyond the scope of this study, and will be the subject of future research.

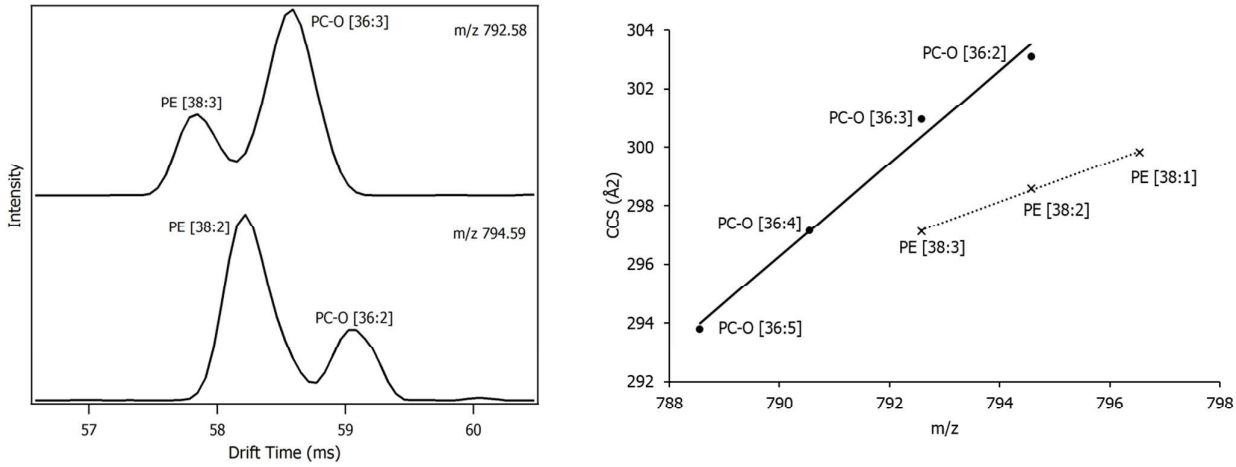


Figure 4. *Left*: Ion mobilograms for MS peaks at m/z 792.58 and m/z 794.59. These cannot be unambiguously assigned based on MS information only. IMS reveals the presence of at least two compounds in each case. IMS trend lines can be used to assign these to specific lipid classes, as shown in the right panel.

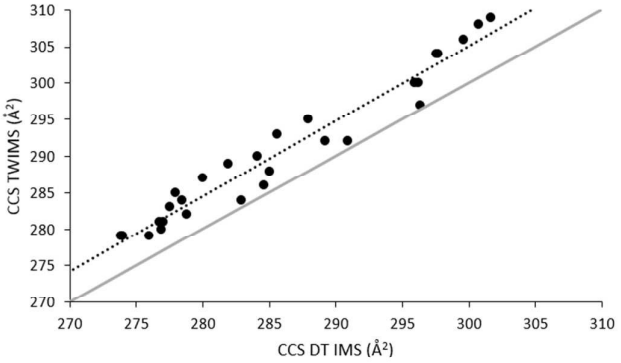


Figure 5. Comparison of CCS obtained in this study using a low-field drift-tube IMS (DT IMS) and values reported in literature using a travelling wave instrument (TWIMS).[15] Even though there is a rather good correlation (R^2 = 0.95; dotted line), there is a clear, systematic offset from the line representing perfect correlation (solid line with slope 1 and intercept zero).

Conclusions

High resolution (>250), low-field (2 Td) multiplexed drift-tube IM-MS has been used to rapidly and efficiently separate lipid isomers that differ only in position of the acyl chain, position of the double bond or double bond geometry. This was demonstrated both in test samples and in complex biological mixtures. Since differences in CCS of 1% or less were found between isomeric phospholipids, such performance is necessary for K_0 and CCS determinations to be become standard analytical techniques in lipidomics.

In addition to raw resolution, high precision and accuracy are needed. Evidence presented suggest that the technology used here has advantages compared to current alternatives. This is critical for generation of reliable reference data and libraries. As these become available lipidomics will finally have the tools for rapid system-wide identification and quantification of lipids.

References

1 M. R. Wenk, *Cell*, 2010, **143**, 888–95.

2 P. T. Ivanova, S. B. Milne, D. S. Myers and H. A. Brown, *Curr. Opin. Chem. Biol.*, 2009, **13**, 526–531.

3 R. W. Gross and X. Han, *Chem. Biol.*, 2011, **18**, 284–291.

4 T. Vihervaara, M. Suoniemi and R. Laaksonen, *Drug Discov. Today*, 2014, **19**, 164–170.

5 T. Hyötyläinen, I. Bondia-Pons and M. Orešič, *Mol. Nutr. Food Res.*, 2013, **57**, 1306–18.

6 K. Schuhmann, R. Herzog, D. Schwudke, W. Metelmann-Strupat, S. R. Bornstein and A. Shevchenko, *Anal. Chem.*, 2011, **83**, 5480–7.

7 K. Schuhmann, R. Almeida, M. Baumert, R. Herzog, S. R. Bornstein and A. Shevchenko, *J. Mass Spectrom.*, 2012, **47**, 96–104.

8 R. Almeida, J. K. Pauling, E. Sokol, H. K. Hannibal-Bach and C. S. Ejsing, *J. Am. Soc. Mass Spectrom.*, 2014, **26**, 133–148.

9 M. Lísa and M. Holcapek, *J. Chromatogr. A*, 2008, **1198-1199**, 115–130.

10 M. Lísa, E. Cífková and M. Holčapek, *J. Chromatogr. A*, 2011, **1218**, 5146–5156.

11 E. Cifkova, M. Holcapek, M. Lisa, M. Ovcacikova, a Lycka, F. Lynen and P. Sandra, *Anal Chem*, 2012, **84**, 10064–10070.

12 M. Kliman, J. C. May and J. a McLean, *Biochim. Biophys. Acta*, 2011, **1811**, 935–45.

13 G. Paglia, M. Kliman, E. Claude, S. Geromanos and G. Astarita, *Anal. Bioanal. Chem.*, 2015, available online.

14 T. P. I. Lintonen, P. R. S. Baker, M. Suoniemi, B. K. Ubhi, K. M. Koistinen, E. Duchoslav, J. L. Campbell and K. Ekroos, *Anal. Chem.*, 2014, 9662–9669.

15 G. Paglia, P. Angel, J. P. Williams, K. Richardson, H. J. Olivos, J. W. Thompson, L. Menikarachchi, S. Lai, C. Walsh, A. Moseley, R. S. Plumb, D. F. Grant, B. O. Palsson, J. Langridge, S. Geromanos and G. Astarita, *Anal. Chem.*, 2015, **87**, 1137–1144.

16 J. C. May, C. R. Goodwin, N. M. Lareau, K. L. Leaptrot, C. B. Morris, R. T. Kurulugama, A. Mordehai, C. Klein, W. Barry, E. Darland, G. Overney, K. Imatani, G. C. Stafford, J. C. Fjeldsted and J. a McLean, *Anal. Chem.*, 2014, **86**, 2107–16.

17 A. A. Shvartsburg, G. Isaac, N. Leveque, R. D. Smith and T. O. Metz, *J. Am. Soc. Mass Spectrom.*, 2011, **22**, 1146–55.

18 A. T. Maccarone, J. Duldig, T. W. Mitchell, S. J. Blanksby, E. Duchoslav and J. L. Campbell, *J. Lipid Res.*, 2014, **55**, 1668–1677.

19 C. Stubbs and A. D. Smith, *Biochim. Biophys. Acta*, 1984, **779**, 89–137.

20 H. Martinez-Seara, T. Róg, M. Pasenkiewicz-Gierula, I. Vattulainen, M. Karttunen and R. Reigada, *Biophys. J.*, 2008, **95**, 3295–3305.

21 T. W. Mitchell, H. Pham, M. C. Thomas and S. J. Blanksby, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.*, 2009, **877**, 2722–2735.

22 C. Ferreri and C. Chatgilialoglu, *ChemBioChem*, 2005, **6**, 1722–1734.

23 G. van Meer, D. R. Voelker and G. W. Feigenson, *Nat. Rev. Mol. Cell Biol.*, 2008, **9**, 112–124.

24 M. C. Thomas, T. W. Mitchell, D. G. Harman, J. M. Deeley, J. R. Nealon and S. J. Blanksby, *Anal. Chem.*, 2008, **80**, 303–311.

25 G. Paglia, J. P. Williams, L. Menikarachchi, J. W. Thompson, R. Tyldesley-Worster, S. Halldórsson, O. Rolfsson, A. Moseley, D. Grant, J. Langridge, B. O. Palsson and G. Astarita, *Anal. Chem.*, 2014, **86**, 3985–3993.

26 Z. Zhang, R. Knochenmuss, W. F. Siems, W. Liu, S. Graf and H. H. Hill, *Anal. Chem.*, 2014, **86**, 1661–1670.

27 R. Knochenmuss, S. Graf, K. Fuhrer and M. Gonin, in *Proceedings of the 61st ASMS Conference on Mass Spectrometry and Allied Topics*, 2013, p. WP745.

28 W. F. Siems, L. Viehland and H. H. Hill, in *Proceedings of the 62nd ASMS Conference on Mass Spectrometry & Allied Topics*, 2014, p. ThP 480.

29 W. F. Siems, L. A. Viehland and H. H. Hill, *Anal. Chem.*, 2012, **84**, 9782–9791.

30 R. Herzog, K. Schuhmann, D. Schwudke, J. L. Sampaio, S. R. Bornstein, M. Schroeder and A. Shevchenko, *PLoS One*, 2012, **7**, 15–20.

31 E. Fahy, S. Subramaniam, R. C. Murphy, M. Nishijima, C. R. H. Raetz, T. Shimizu, F. Spener, G. van Meer, M. J. O. Wakelam and E. a Dennis, *J. Lipid Res.*, 2009, **50**, S9–S14.

32 G. Liebisch, J. A. Vizcaíno, H. Köfeler, M. Trötzmüller, W. J. Griffiths, G. Schmitz, F. Spener and M. J. O. Wakelam, *J. Lipid Res.*, 2013, **54**, 1523–30.

33 Y. Huang and E. Dodds, *Anal. Chem.*, 2013, **85**, 9728–9735.

34 K. Ekroos, C. S. Ejsing, U. Bahr, M. Karas, K. Simons and A. Shevchenko, *J. Lipid Res.*, 2003, **44**, 2181–2192.

35 J. C. May and J. A. Mclean, *Proteomics*, 2015, available online.

36 W. B. Ridenour, M. Kliman, J. A. McLean and R. M. Caprioli, *Anal. Chem.*, 2010, **82**, 1881–1889.

37 C. L. Crawford, B. C. Hauck, J. a. Tufariello, C. S. Harden, V. McHugh, W. F. Siems and H. H. Hill, *Talanta*, 2012, **101**, 161–170.