ANALYSIS OF LIPID SIGNALING CLASS ANALYTES USING A TRAVELLING WAVE CYCLIC ION MOBILITY SEPARATOR

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SELECT SERIES
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INTRODUCTION

Lipid signaling analytes represent a diverse group of biomolecules that have essential roles in structural, storage and biochemical processes in living systems. Class separation is readily achieved using liquid chromatography and MS based identification techniques; however, structural elucidation remains challenging due to the chemical structure diversity and isobaric nature of these types of compounds. The addition of ion mobility enhances system peak capacity and improves the resolution of isobaric compounds. IM separation was achieved using a multi-pass travelling-wave cyclic IM (cIM) device, where increasing the number of passes around the device increases both the mobility resolution and ion residence time. MS and low-energy CID fragmentation data were obtained on IM separated precursor ions followed by TOF mass measurement.

METHODS

IM-MS

Data were collected on a SELECT SERIES Cyclic IMS (Q-cIM-oaTOF) instrument. Ion mobility separation was achieved using a multi-pass travelling-wave cIM separator, where increasing number of passes results in an increase in both mobility resolution and ion residence time. MS and CID fragmentation data were obtained post IMS on the precursors of separated lipids, followed by TOF mass measurement. The cIM device[1], is shown in Figure 1, consisting of a 98 cm path length RF ion guide comprising over 600 electrodes around which T-Waves circulate to provide mobility separation.

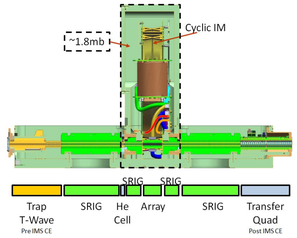


Figure 1. Geometry and design of the cyclic ion mobility region.

The circular path minimizes instrument footprint whilst providing a longer, variable separation that results in higher ion mobility resolutions. The multi-pass capability provides significantly higher resolution over a selected mobility range. The device can either be enabled for mobility separation or by-passed if not required. The multifunctional ion entry/exit array can eject species within a range of mobility values, providing additional functionality such as IMS[2] . The separation performance of the cIM device is illustrated in Figure 2.

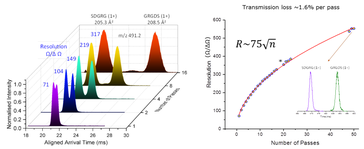


Figure 2. Resolution and performance characteristics of the cyclic ion mobility platform.

Sample preparation

Fatty acid (FA) and glycerophosphocholines (PC) standards were obtained from Nu-Chek Prep, Elysian, MN and Avanti Polar Lipids, Alabaster, AL and dissolved in chloroform/methanol (1:1, v/v). Stock solutions were diluted in MeOH/water (1:1, v/v) + 0.1% formic acid or MeOH/ water (1:1, v/v) and measured in both positive or negative ion ESI. The *cis/trans* oriented isomeric species were pairwise combined and sample concentrations adjusted to provide an equivalent response.

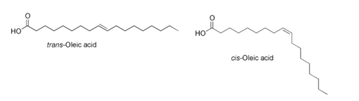
Steroid standards were donated by Clinical and Forensics, Scientific Operations, Waters Corporation and the prostaglandin standards purchased from Cayman Chemical, Ann Arbor, MI. Both were dissolved in methanol, diluted in MeOH/water (1:1, v/v) + 0.1% formic acid, or MeOH/ water (1:1, v/v), and measured in positive or negative ion ESI.

RESULTS

Chain length *vs.* resolution

Unsaturated FA standards, differing in chain length and number of *cis/trans* conformations, summarized in Figure 3, were chosen to determine the degree of IM separation required to separate lipid isomers. FA's represent the simplest class of lipid components, and are a core structural component of lipids.

FA's with *cis*-double bond orientations were found to be more compact than those with *trans*-orientations. Moreover, the *cis*- and *trans*-orientations for the monounsaturated FA's were distinguishable. A different number of cycles through the cyclic IM separator were required to achieve a similar degree of IM separation for mono unsaturated FAs of differing chain length.



# carbons/	# double bond(s)	double bond position(s)
C14	1	Δ 9 cis/Δ 9 trans
C15	1	Δ 10 cis/Δ 10 trans
C16	1	Δ 9 cis/Δ 9 trans
C17	1	Δ 10 cis/Δ 10 trans
C18	1	Δ 6 cis/Δ 6 trans
C22	1	Δ 13 cis/Δ 13 trans
C18	2	Δ 9 cis 12 cis/Δ 9 trans 12 trans
C18	3	Δ 9 cis 12 cis 15 cis/Δ 6 cis 9 cis 12 cis

Figure 3. Chemical structure example and free fatty acids double bond positions.

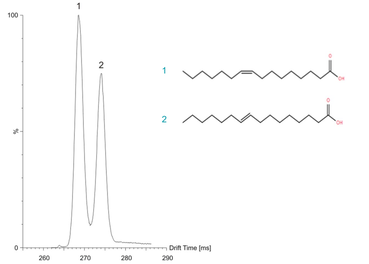


Figure 4. Cyclic ion mobility separation of protonated free fatty acids with distinct double bond orientations..

Shown above in Figure 4 is the cyclic IM separation of *cis* and *trans* oriented 9-palmitoleic acid (9Z/E-hexadecenoic acid), which required 20 cycles, equating to an estimated IM resolution of 335 (Ω/ΔΩ) to separate the conformational pair to 10% valley.

All other single double bond FFA's listed in Figure 3 were analyzed in a similar fashion, *i.e.* the number of cycles was varied to achieve a comparable degree of separation. The results summary in Figure 5 suggest a correlation between length/double bond position and orientation, affecting the analyte gas-phase structure, and the IM resolution required to separate *cis/trans* configurations.

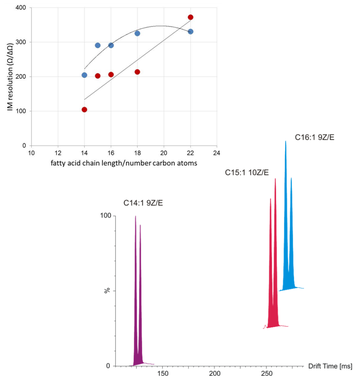


Figure 5. Cyclic IM separation of protonated fatty acids with distinct double bond orientations as a function of chain length and double bond position. Blue circles = observed IM resolution; red circles = observed IM resolution (Ω/ΔΩ)/ separation resolution (R_s) (measure for the required IM resolution to separate a cis/trans FA pair).

Multi-double bond FA and PC cyclic IM analysis

An example cyclic IM separation of FFA's comprising two double bonds is shown in Figure 6, with similar results obtained for the other multiple bond FA's and PC's tested (data not shown). Broader, non-resolved IM arrival time distributions, as previously reported[2,3], were typically observed. Moreover, species in the *trans* configuration ionized less efficiently.

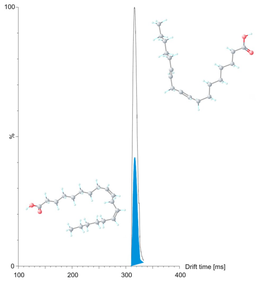


Figure 6. Cyclic ion mobility separation of protonated fatty acids with multiple double bonds. Blue = Δ 9 trans 12 trans C18:2, white = Δ 9 cis 12 cis C18:2.

Cyclic ion mobility - CID

The results shown in Figures 7 to 10 demonstrate the ability of the multifunction cyclic IM device to collect product ion spectra post IM separation where analyte activation was achieved in the (transfer) region between the cyclic IM cell and the oa-ToF entrance region for various isomeric mixtures of representative lipid signaling classes as well as steroids.

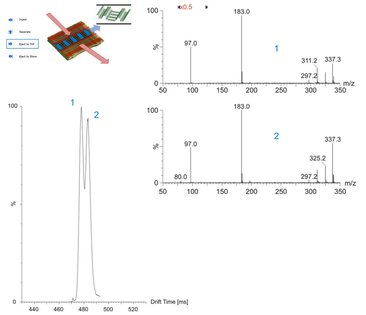


Figure 7. Partial cyclic ion mobility separation (6 cycles; Ω/ΔΩ ~ 125) of 13Z/E-docosenoic (C22:1) and the subsequent resolved post cIM CID spectra of the two cis/trans orientations of C22:1.

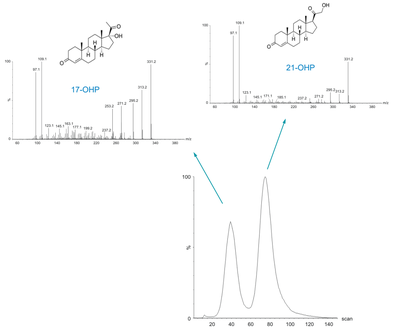


Figure 8. Cyclic ion mobility separation and CID spectra of protonated 17-hydroxyprogesterone (17-OHP) and 21-hydroxyprogesterone (21-OHP) at Ω/ΔΩ > 200.

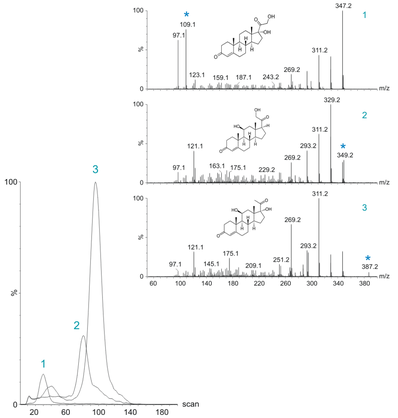


*Figure 9. Cyclic ion mobility separation and CID spectra of protonated 21-deoxycortisol (1), corticosterone (2), and 11-deoxycortisol (3). Arrival time distributions were extracted for the * marked product/adduct ions.*

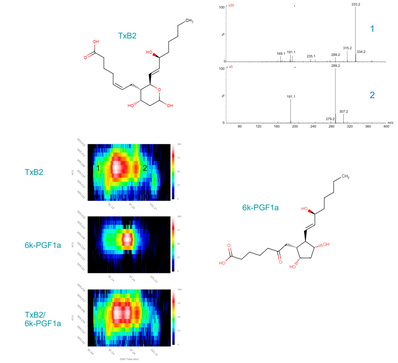


Figure 10. 2D cyclic IM-MS separations of Thromboxane B2 (TxB2), 6-Keto-Prostaglandin F1alpha (6k-PGF1a) and a TxB2/ 6k-PGF1a mixture (bottom) at Ω/ΔΩ > 125. Shown top right and bottom are the production spectra of deprotonated TxB2 and a TxB2 contamination (spectra 1 and 2) and a 1D representation of the cyclic IM-MS analysis of the TxB2/6k-PGF1 PG mixture, respectively.

CONCLUSION

- **The Select Series Cyclic IMS has been characterized and successfully applied for the IM separation of isomeric, *cis/trans* oriented, mono unsaturated FA's, steroids and prostaglandin sample mixtures.**
- **The required IM resolution, as afforded by variable resolution cIM, was found to be a function of FA chain length. Shorter, more compact and more rigid FA's required reduced resolution, as well as longer chain mono unsaturated FA's, as a result of partial chain back-folding. Multiple double bond FA's and PC's were not resolved.**
- **The majority of the investigated isomeric steroid and prostaglandin mixtures were fully or partially separated, requiring about half of the IM resolving power compared to free FA's.**
- **CID fragmentation was successfully obtained following IM separation of all analyte types, affording the structural elucidation of isomeric species.**

References

1. Giles K, Wildgoose JL, Pringle S, Garside J, Carney P, Nixon P, Langridge DJ. In 62nd Annual ASMS Conference on Mass Spectrometry and Allied Topics, Baltimore, MD, June 15–19, 2014.
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3. Kyle JE, Zhang X, Weitz KK, Monroe ME, Ibrahim YM, Moore RJ, Cha J, Sun X, Lovelace ES, Wagoner J, Polyak SJ4, Metz TO, Dey SK, Smith RD, Burnum-Johnson KE, Baker ES. Uncovering biologically significant lipid isomers with liquid chromatography, ion mobility spectrometry and mass spectrometry. Analyst. 2016 Mar 7;141(5):1649-59

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