LIPID ISOMER SEPARATION USING HIGH RESOLUTION CYCLIC ION MOBILITY MASS SPECTROMETRY

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INTRODUCTION

- **Galactosylceramide (GalCer) and glucosylceramide (GlcCer) are isomers and only differ in the position of the hydroxy group at the C-4 (Figure 1A).**
- **Ganglioside GD1a and GD1b differ in the sequence of sialic acid also called N-acetylneuraminic acid (NANA); (Figure 1B).**
- **A slight difference in chemical composition and molecular conformation contribute to profound differences in their physicochemical properties and biological functions.**
- **Therefore, it is very important to separate these isomers to understand their biological role and function.**
- **Here we demonstrate, the complete separation of these lipid isomers only possible with the multi-pass capability of the SELECT SERIES™ Cyclic™ ion mobility spectrometer (cIMS), Figure 2 [1, 2].**

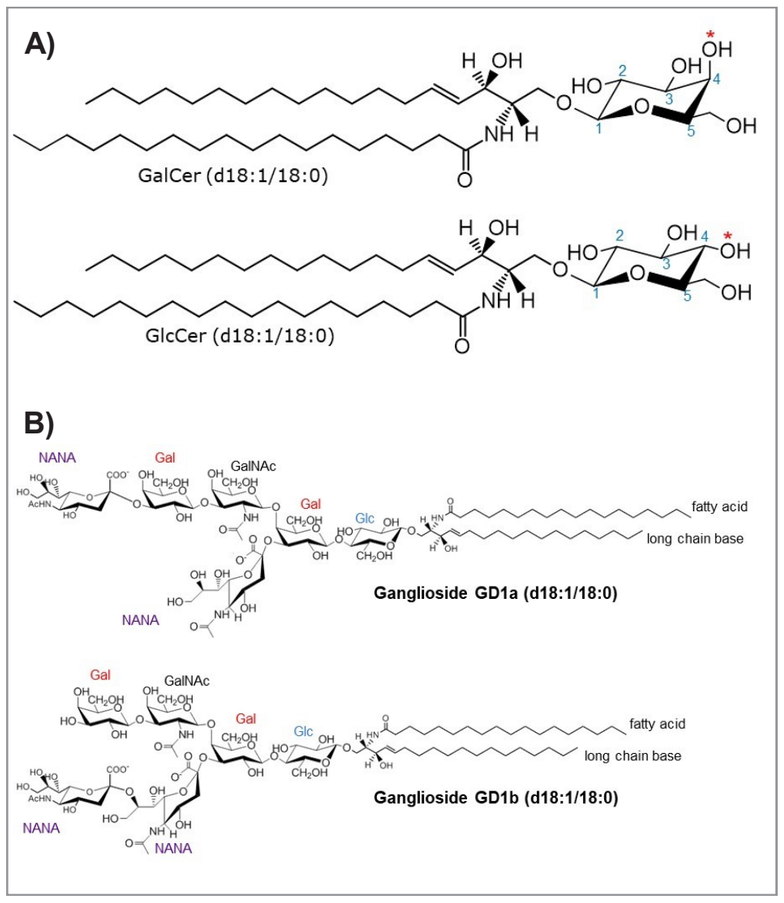

Figure 1. *Chemical structure of the analyzed lipid isomers.*

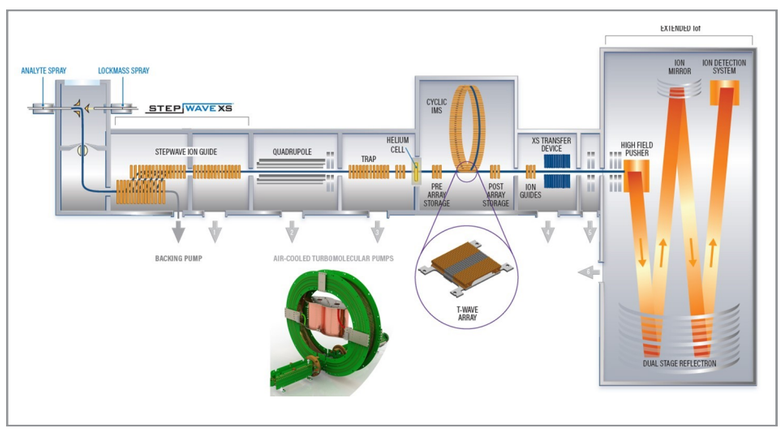

Figure 2. *Schematic of the cyclic IMS QTOF instrumentation. It contains three main regions: The trap region, the cyclic ion mobility device and the transfer region.*

METHODS

- GalCer d18:1/18:0, GlcCer d18:1/18:0, ganglioside GD1a (d18:1/18:0) and GD1b (d18:1/18:0) were purchased from Avanti Polar Lipids and a final concentration of 1ng/µL was prepared. Samples were infused at 5µL/min into the ESI source of the cIMS.
- Different adduct ions were selected in the quadrupole and transferred to the cyclic mobility cell for multiple passes.
- The deprotonated ion at m/z 726.54 $[M-H]^-$ (for Galcer and GlcCer) and The doubly-charged deprotonated ion at m/z 917.4875 $[M-2H]^{-2}$ (for ganglioside GD1a and GD1b) were selected in the quadrupole with low trap and transfer collision energy.
- The isolated ion was transferred to the cyclic mobility cell for multiple passes. The separate setting on the instrument was adjusted according to the number of passes required.

RESULTS

GalCer and GlcCer Isomer Separation

An equimolar mixture of the two ceramides was infused into the cIMS mass spectrometer at a flow rate of 5µL/min.

An initial single pass of the ion mobility cell, with a resolution of approximately 65 Ω/DΩ, resulted in an Arrival Time Distribution (ATD) of 22 msec with no separation of the ceramide isomers, *m/z* 726.54 $[M-H]^-$; **Figure 3A**.

Increasing the number of passes of the IMS cell to 5 (IMS resolution around 145 Ω/DΩ), produced marginal separation of the two deprotonated species, with ATD of 61.91 and 62.96 msec, **Figure 3B**.

Increasing the number of passes to 10 (IMS resolution around 205 Ω/DΩ) resulted in separation of the two lipids with a 15% valley and ATD of 111.47 and 113.58 msec, **Figure 3C**.

Complete resolution of the deprotonated GalCer and GluCer was achieved by increasing the number of passes of the IM cell to 20 passes (IMS resolution around 290 Ω/DΩ), **Figure 3D** with ATD of 210.53 and 214.49 msec.

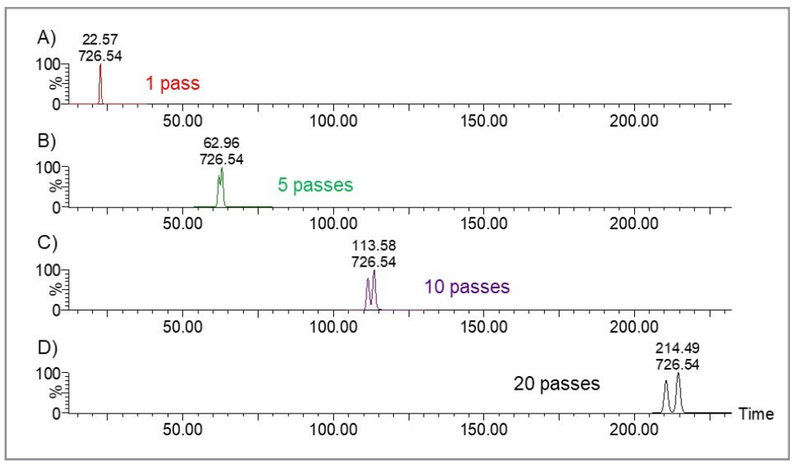

Figure 3. *Arrival Time Distribution for the separation of GalCer (d18:1/18:0) and GlcCer (d18:1/18:0) [M-H]- m/z 726.5440 mixtures using 1(A), 5(B), 10(C), and 20(D) passes of the ion mobility device.*

The data displayed in **Figure 4** shows separation obtained following the infusion of either GalCer (3A), GluCer (3B) or the equimolar mixture of the two ceramides (3C) using 20 passes of the IM cell.

The data obtained shows that GalCer had the shortest arrival time of 210.53 msec with GlcCer (B) having a longer arrival time of 214.63 msec.

As can be seen from both the individual infusion of GalCer and GlcCer sphingolipids there is evidence of the other isomer in the obtained spectra. This is most likely due to a small amount of impurity present in the stock solution. This shows the potential of the Cyclic IMS for the determination of stereoisomer impurities.

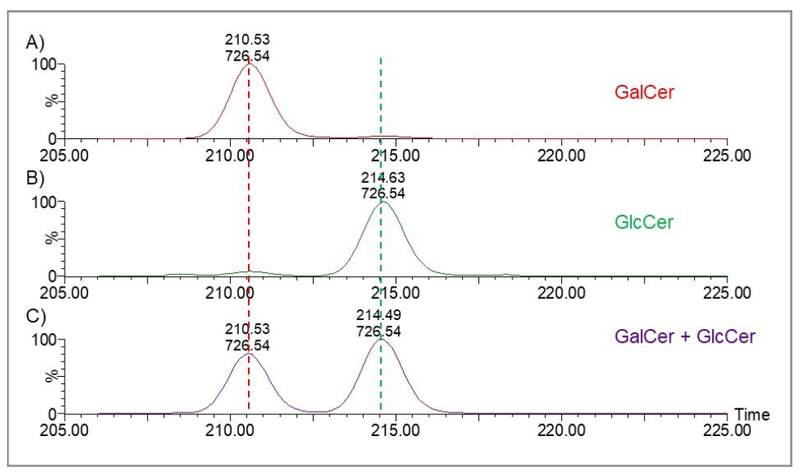

Figure 4. *Arrival Time Distribution for the separation of individual GalCer (A), GlcCer (B) and the equimolar mixture of the two ceramides (C) using 20 passes of the ion mobility device.*

References

1. Isaac G, Olivos H and Plumb RS. Separation of Galactosyl and Glucosylceramide Isomers Using the SELECT SERIES™ Cyclic™ IMS. Waters Application Note 720007539, February 2022.
2. Isaac G, Olivos H and Plumb RS. Separation of Ganglioside Isomers Using the SELECT SERIES™ Cyclic™ IMS . Waters Application Note 720007592, April 2022 .

RESULTS

Ganglioside GD1a and GD1b Isomer Separation

Similarly the GD1a and GD1b ganglioside mixture was subjected to either one, two, three, four or five passes of the cyclic ion mobility cell, **Figure 5**.

As the number of passes of the IMS cell is increased to three (IMS resolution ~110 Ω/DΩ) it is evident that there are two species, being resolved with an 80% valley.

With five passes (IMS resolution ~145 Ω/DΩ) the GD1b (d18:1/18:0) and GD1a (d18:1/18:0) are baseline resolved with ATDs centered on 41.22 and 42.58 msec, respectively.

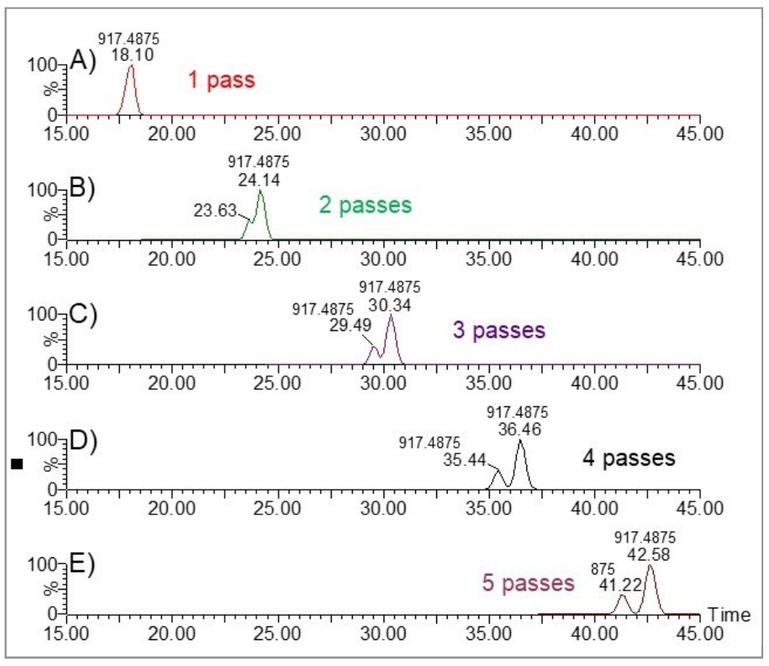

Figure 5. *Arrival time distribution for the separation of GD1a (d18:1/18:0) and GD1b (d18:1/18:0) at m/z 917.488 [M-2H]-2 mixtures using (A) 1 pass, (B) 2 passes, (C) 3 passes, (D) 4 passes, and (E) 5 passes of the ion mobility device.*

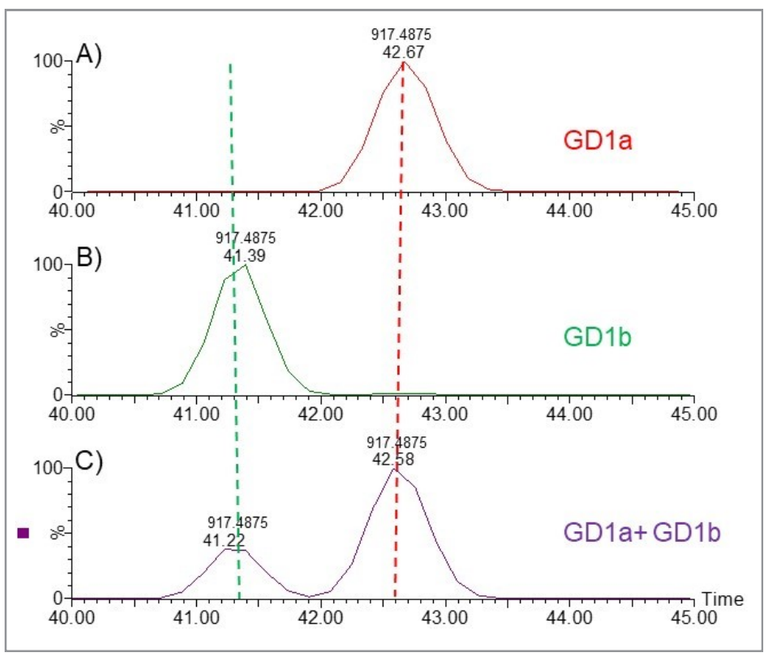

Figure 6. *Arrival time distribution for the separation of individual (A) GD1a (d18:1/18:0), (B) GD1b (d18:1/18:0), or (C) the equimolar mixture of the two ganglioside isomers at m/z 917.488 [M-2H]-2 using five passes of the ion mobility device.*

CONCLUSION

- **The cIMS has a unique multi-pass cyclic ion mobility capability, to scale ion mobility resolution to meet a given challenge to separate lipid isomers.**
- **The GalCer (d18:1/18:0) and GlcCer (d18:1/18:0) isomers were base line resolved using twenty passes of the IM cell, with IMS resolution of 290 Ω/ΔΩ.**
- **The ganglioside isomers GD1a (d18:1/18:0) and GD1b (d18:1/18:0) were successfully resolved using 5 passes of the IM cell with IMS resolution of 145 Ω/DΩ.**
- **The data presented illustrates the power of the SELECT SERIES Cyclic IMS in the separation of lipid isomers for lipidomics studies.**

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