

Nota applicativa

## Separation of Galactosyl and Glucosylceramide Isomers Using the SELECT SERIES™ Cyclic™ IMS

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### Abstract

Galactosylceramide (GalCer) and glucosylceramide (GlcCer) are involved in many physiological and pathological phenomena. Correctly classifying and characterizing these lipids is essential to understand their biological role and function. Lipids can exist in multiple isomeric forms which cannot always be separated using classical analytical lipid methods. A slight difference in chemical composition and molecular conformation contribute to profound differences in their physicochemical properties and biological functions. The SELECT SERIES Cyclic ion mobility has a unique multi-pass capability, that increases ion mobility resolution to meet a given challenge. Here we demonstrate the complete separation of GalCer and GlcCer isomers that require ion mobility resolution of  $290 \Omega/\Delta\Omega$  (twenty passes) only possible with the multi-pass capability of the SELECT SERIES Cyclic IMS.

### Benefits

- Complete separation of previously indistinguishable lipid isomers using SELECT SERIES Cyclic IMS
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- The SELECT SERIES Cyclic IMS provides unique multi-pass cyclic ion mobility capability, to scale ion mobility resolution to meet a given challenge.
  - Increase throughput with rapid millisecond analysis time
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## Introduction

Lipids form a class of biological molecules with many important roles and functions such as energy storage, cellular signaling, and the pathophysiology of a broad spectrum of diseases including cancer, neurodegenerative diseases, infections, diabetes, etc.<sup>1,2</sup> Lipidomics has been employed for the analysis of lipids of all types, to detect and identify thousands of lipids across the eight common lipid classes. These classes comprise of the fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, polyketides, sterol lipids, and prenol lipids. Lipidomics is commonly performed using liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), or supercritical fluid chromatography-mass spectrometry (SFC-MS), which the chromatography system providing separation of lipids either into classes (HILIC, SFC) or based upon their lipophilicity (reversed-phase LC, GC), with databases and compound libraries being employed to identify lipids of interest, based on accurate mass, fragment ion patterns, chromatographic retention time, and more recently collision cross section (CCS). While these databases are extremely valuable, they are often challenged with the unequivocal identification of lipid isomers. These isomers can be constitutional, or stereo isomers such as diastereomers, enantiomers, cis/trans isomers, or rotamers.

The Waters SELECT SERIES Cyclic IMS employs high resolution ion mobility (IM) separation prior to the MS analyzer to provide an orthogonal mechanism of analyte separation. The IM separation is based upon the charge and shape of the molecule allowing the potential to separate isomers, also with appropriate calibration the CCS of the molecule can be measured. GalCer and GlcCer have a slight difference in their chemical configuration and molecular conformation (namely the hydroxyl at C-4 of the sugar) that contribute to profound differences in their physicochemical properties and biological functions.<sup>3</sup> Because of their high structural similarity, separation, and identification of GalCer and GlcCer are difficult using classical analytical lipid methods. Here we demonstrate the use of multi-pass cyclic ion mobility MS for the complete separation of the GalCer and GlcCer isomers, Figure 1.

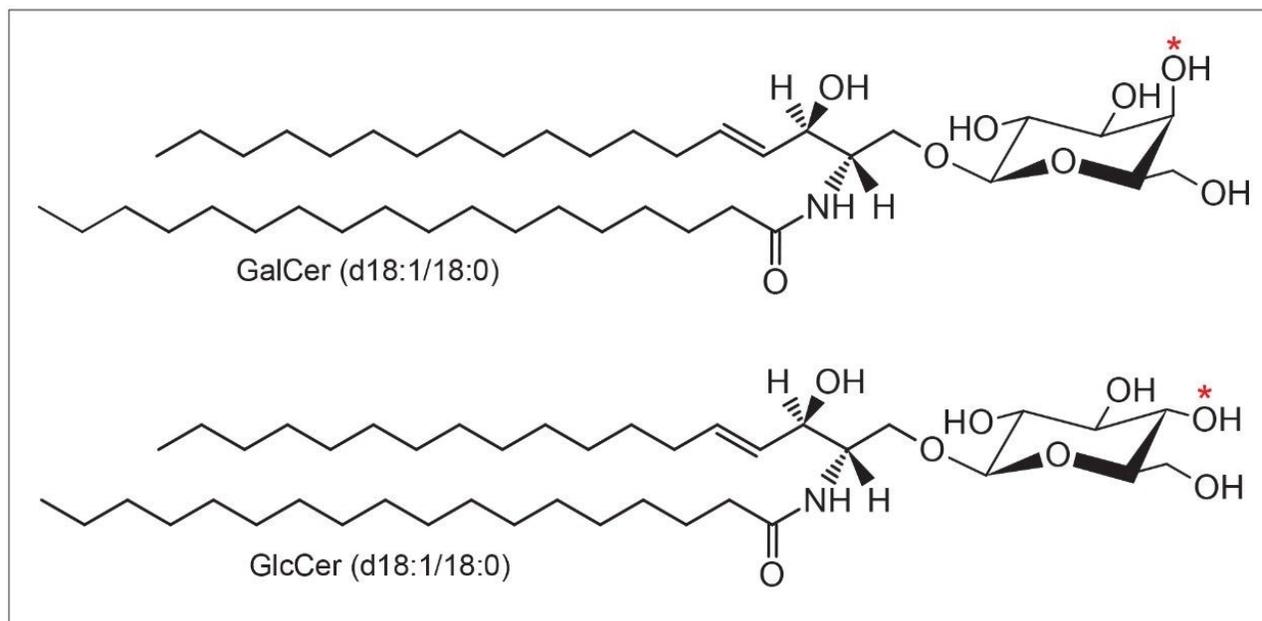


Figure 1. The chemical structure of analysed GalCer and GlcCer isomers. The difference in the position of the hydroxyl group at the C-4 position is indicated by red asterisk.

## Experimental

### Sample Preparation

GalCer d18:1/18:0 (p/n: 860844) and GlcCer d18:1/18:0 (p/n: 860547) standards were obtained from Avanti Polar Lipids. A stock solution of 1 mg/mL of each standard was prepared in chloroform/methanol/water (80/20/2). A final concentration of 1 ng/ $\mu$ L of individual and equimolar mixtures was prepared and infused at 5  $\mu$ L/min into the ESI source of the SELECT SERIES Cyclic IMS.

### MS Conditions

The deprotonated ion at  $m/z$  726.54  $[M-H]^-$ ; was 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.

MS system:	SELECT SERIES Cyclic IMS
Ionization mode:	Electrospray Negative Ion
Acquisition range:	50–800 <i>m/z</i>
Capillary voltage:	2 kV
Cone voltage:	30 V
Trap collision energy:	6 V
Transfer collision energy:	4 V
Cone voltage:	30 V
TW static height:	30 V
TW velocity:	375 m/s
Infusion flow rate:	5 $\mu$ L/min

## Data Management

MS software:	MassLynx™ 4.2
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## Results and Discussion

GalCer and GlcCer consist, respectively, of d-galactose and d-glucose residue linked by a  $\beta$ 1-1'-glycosidic bond to ceramide composed of d-erythro-sphingosine and long-chain fatty acid (Figure 1). These two compounds

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represent very similar structures since d-galactose is an epimer (different configurations of atoms around one of several asymmetric carbon atoms present) of d-glucose and the two sugars differ only in the configuration at C-4 position.<sup>3</sup> Therefore, it is difficult to separate GalCer and GlcCer isomers using classical analytical lipid methods. The SELECT SERIES Cyclic IMS employs a novel cyclic ion mobility separation device allowing multiple passes to be performed with an ion mobility resolution more than  $400 \Omega/\Delta\Omega$  in either MS or MS<sup>n</sup> mode. An equimolar mixture of the two ceramides was infused into the mass spectrometer at a flow rate of 5  $\mu\text{L}/\text{min}$ . An initial single pass of the ion mobility cell, with a resolution of approximately  $65 \Omega/\Delta\Omega$ , resulted in an Arrival Time Distribution (ATD) of 22.57 msec with no separation of the ceramide isomers,  $m/z$  726.54 [M-H]<sup>-</sup>; Figure 2A. Increasing the number of passes of the IMS cell to five (IMS resolution around  $145 \Omega/\Delta\Omega$ ), produced marginal separation of the two deprotonated species, with ATD of 61.91 and 62.96 msec, Figure 2B. Increasing the number of passes to ten (IMS resolution around  $205 \Omega/\Delta\Omega$ ) resulted in separation of the two lipids with a 15% valley and ATD of 111.47 and 113.58 msec, Figure 2C. Complete resolution of the deprotonated GalCer and GluCer was achieved by increasing the number of passes of the IM cell to twenty passes (IMS resolution around  $290 \Omega/\Delta\Omega$ ), Figure 2D with ATD of 210.53 and 214.49 msec.

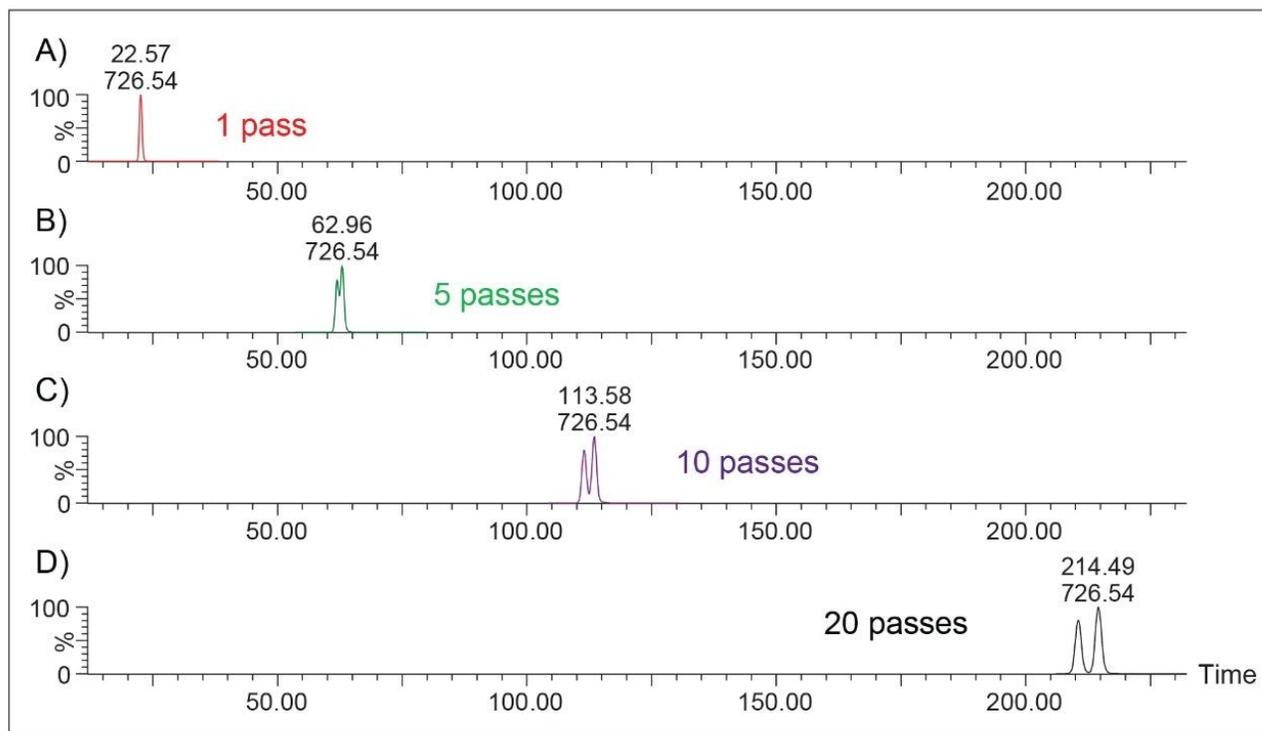


Figure 2. Arrival Time Distribution (ATD) for the separation of GalCer (d18:1/18:0) and GlcCer (d18:1/18:0) m/z 726.5440 mixtures using 1(A), 5(B), 10(C), and 20(D) passes of the ion mobility device.

The data displayed in Figure 3 show separation obtained following the infusion of either GalCer (Figure 3A), GluCer (Figure 3B) or the equimolar mixture of the two ceramides (Figure 3C) using twenty 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. This resulted in IMS resolution of  $290 \Omega/\Delta\Omega$ . 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 SELECT SERIES Cyclic IMS for the determination of stereoisomer impurities.

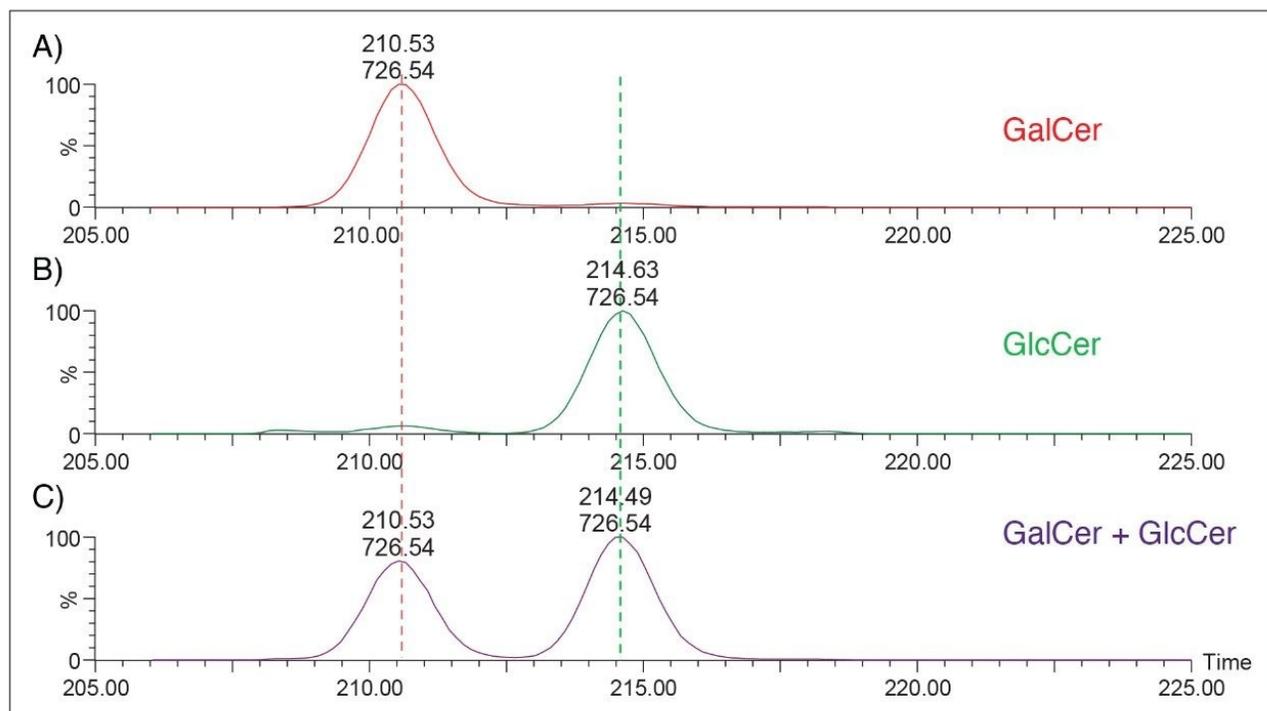


Figure 3. Arrival Time Distribution (ATD) for the separation of individual GalCer (A), GlcCer (B) or the equimolar mixture of the two ceramides (C) using 20 passes of the ion mobility device.

## Conclusion

With its unique multi-pass cyclic ion mobility capability, it is possible to scale ion mobility resolution to meet a given challenge. Here we show that the lipid isomers GalCer (d18:1/18:0) and GlcCer (d18:1/18:0) were base line resolved using twenty passes of the IM cell, with IMS resolution of  $290 \Omega/\Delta\Omega$ . Moreover, ten passes of the ceramides afforded sufficient resolution to give 80% separation of the lipids, providing clear evidence of two separate species. The method also shows the potential of the SELECT SERIES Cyclic IMS for the determination of stereoisomer impurities.

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## References

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