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Application Note

Separation of Ganglioside Isomers Using the SELECT SERIES™ Cyclic™ IMS

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Abstract

Understanding the role and function of lipids in biological processes is critical to the development of new biomarkers of health and disease state. The complex nature of the ganglioside chemistry due to structural variation in both their oligosaccharidic chain and ceramide moiety can make the molecular characterization a challenging task. A slight difference in chemical composition and molecular conformation contribute to profound differences in their physicochemical properties and biological functions. The SELECT SERIES Cyclic IMS instrument has a unique multi-pass capability, that increases ion mobility resolution. Here we demonstrate the complete separation of GD1a (d18:1/18:0) and GD1b (d18:1/18:0) ganglioside isomers that require ion mobility resolution of 145 Ω/ΔΩ (five 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
- Scale the ion mobility resolution to maximize peak capacity and address all your analytical challenges

- Increase throughput with rapid millisecond analysis time

Introduction

Gangliosides are glycosphingolipids composed of ceramide linked by a glycosidic bond to an oligosaccharide chain containing hexose, N-acetylneuraminic acid (NANA, acidic sugar known also as sialic acid) and N-acetylgalactosamine (GalNAc) units. Their structural diversity results from variation in the composition and sequence of the sugar residues (Figure 1). They were first isolated from the ganglion cells of the brain and to date there are many known gangliosides which differ mainly in the position and number of sugar residues. To completely describe these lipids and understand their biological role and function it is necessary to characterize their isomerism. Ganglioside analysis has been achieved using liquid chromatography and tandem mass spectrometry by combining the various glycan head groups, long chain bases, and the experimentally determined fatty acyls.[1]

Although it is possible to resolve ganglioside isomers using liquid chromatography this can often require extensive method development and long analysis times. Unlike liquid chromatography, ion mobility separates analytes based on their shape and charge. The greater the molecular similarity between analytes the greater the separation challenge. The Waters SELECT SERIES Cyclic IMS employs high resolution ion mobility (IM) separation prior to the Tof-MS analyzer to provide an orthogonal mechanism of analyte separation.[2,3] GD1a (d18:1/18:0) and GD1b (d18:1/18:0) have a slight difference in their chemical configuration (based on the NANA sequence as shown in Figure 1) that contribute to profound differences in their physicochemical properties and biological functions. Because of their high structural similarity, separation, and identification of GD1a, GD1b, and other ganglioside isomers is difficult using classical analytical lipid methods. Here we demonstrate the use of multi-pass SELECT SERIES Cyclic Ion Mobility MS that provides scalable IM resolution for the complete separation of the GD1a and GD1b isomers, Figure 1.

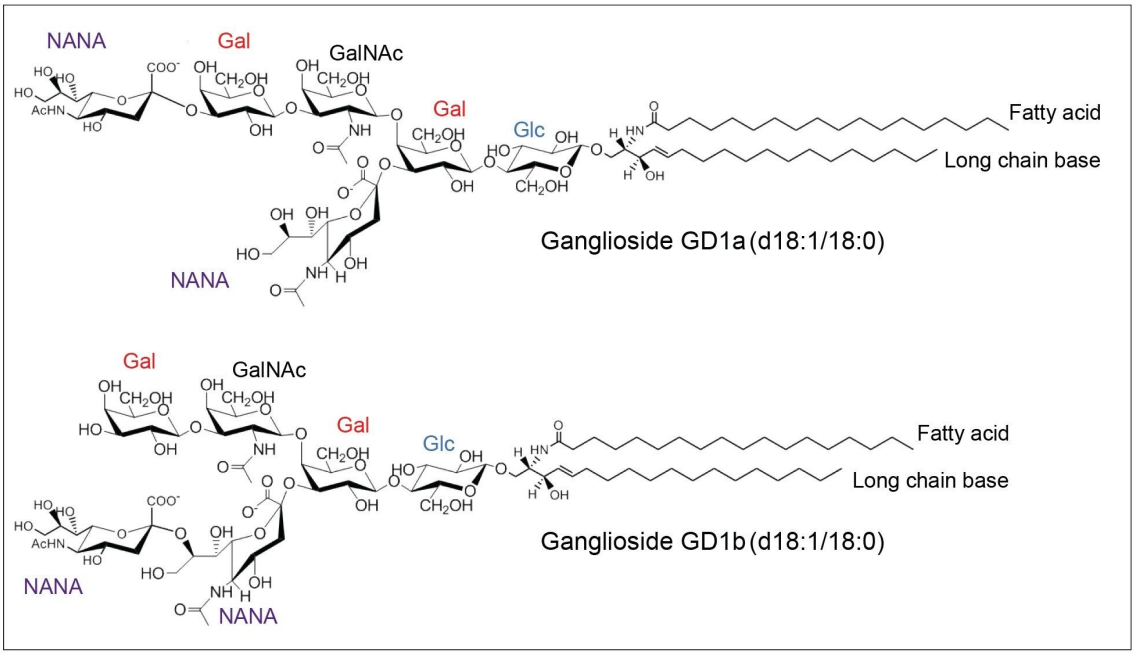

Figure 1. Structure of ganglioside GD1a (d18:1/18:0) and GD1b (d18:1/18:0). It is composed of ceramide linked with a glycosidic bond to an oligosaccharide chain containing glucose (Glc), galactose (Gal), N-acetylneuraminic acid (NANA), and N-acetylgalactosamine (GalNAc) units.

Experimental

Sample Description

GD1a (d18:1/18:0) (p/n: 860055) and GD1b (d18:1/18:0) (p/n: 860056) ganglioside standards of porcine brain were obtained from Avanti Polar Lipids as diammonium salt powder. A stock solution was prepared individually at a concentration of 1 mg/mL in chloroform/methanol (1/2). These standards were then diluted either as pure individual standards or as a 1:1 mix in chloroform/methanol (1/2) at a concentration of 1 ng/mL and infused at 5 µL/min into the ESI source of the SELECT SERIES Cyclic IMS.

Method Conditions

MS Conditions

The doubly charged deprotonated ion at *m/z* 917.4875 $[M-2H]^{-2}$; 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:	ESI-
Acquisition range:	50–2000 *m/z*
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 µL/min

Data Management

MS software:	MassLynx™ 4.2

Results and Discussion

The standard ganglioside lipid mixture was infused into the SELECT SERIES Cyclic IMS System at a flow rate of 5 µL/minute and monitored in negative ion ESI mode. The mixture was subjected to either one, two, three, four, or five passes of the cyclic ion mobility cell, Figure 2. The data displayed in Figure 2A show that with one pass of the IMS cell (a mobility resolution of 65 $\Omega/\Delta\Omega$) the ganglioside isomers are unresolved, being detected with an arrival time distribution (ATD) centered on 18.10 msec. A close inspection of the apex of the peak shows a non-gaussian profile suggesting the presence of two species. When the number of passes of the IMS cell is increased to two (IMS resolution approximately 90 $\Omega/\Delta\Omega$) there is a significant increase in the peak width and a clear shoulder appears at the front of the peak with an arrival time of 23.63 msec with the major peak having an arrival time of 24.14 msec. As the number of passes of the IMS cell is increased to three (IMS resolution approximately 110 $\Omega/\Delta\Omega$) it is evident that there are two species, being resolved with an 80% valley. With four passes (IMS resolution approximately 130 $\Omega/\Delta\Omega$) there are two clear peaks resolved to almost baseline and with five passes (IMS resolution approximately 145 $\Omega/\Delta\Omega$) 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. The multi-pass scalable nature of the SELECT SERIES Cyclic IMS System provides increased IMS resolution to meet a given challenge.

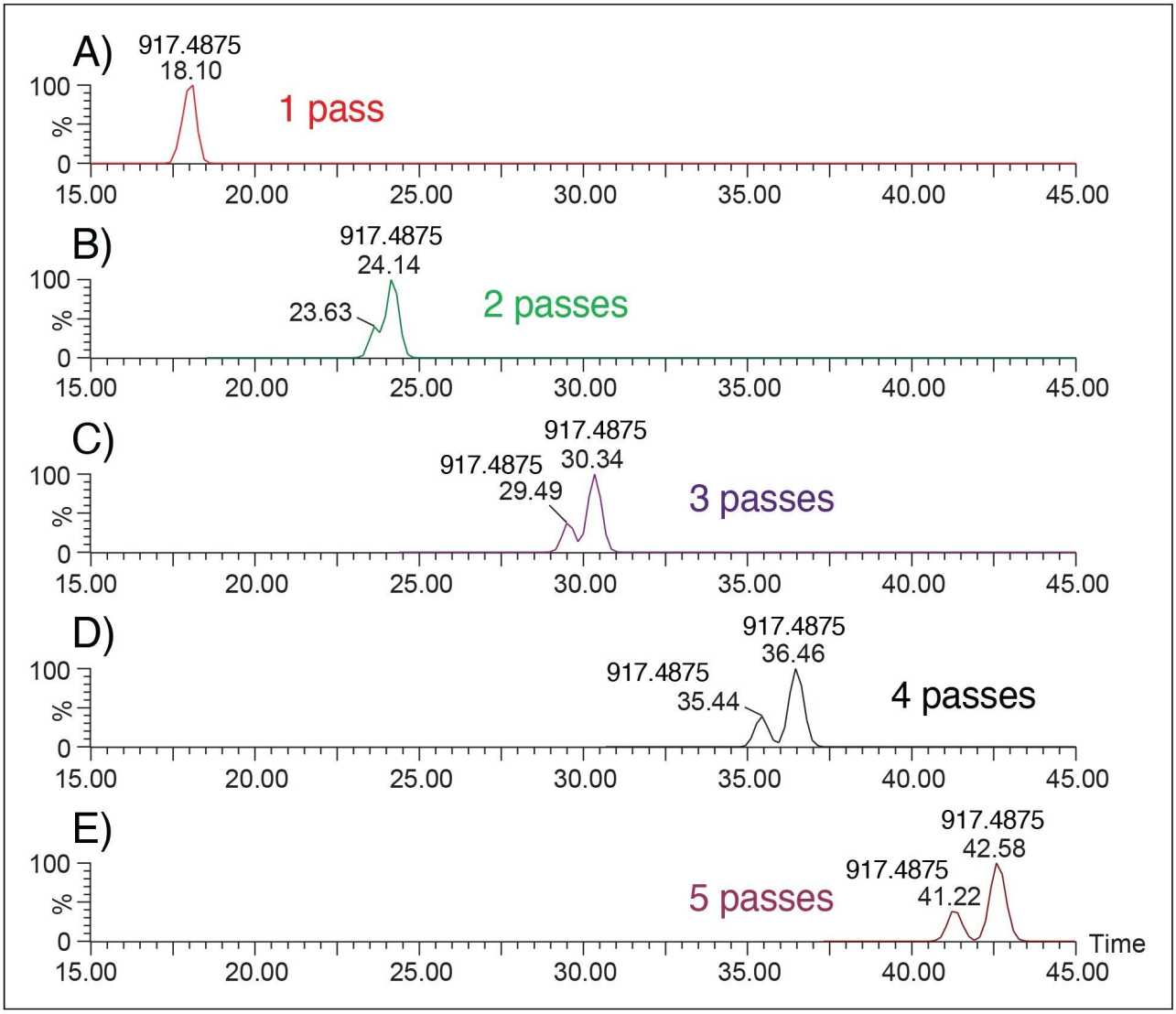

Figure 2. Arrival time distribution for the separation of GD1a (d18:1/18:0) and GD1b (d18:1/18:0) at m/z 917.488 $[M-2H]^{-2}$ mixtures using (A) 1 pass, (B) 2 passes, (C) 3 passes, (D) 4 passes, and (E) 5 passes of the ion mobility device.

To determine the arrival order of the GD1a (d18:1/18:0) and GD1b (d18:1/18:0) species, the individual gangliosides and their equimolar mixture were introduced into the SELECT SERIES Cyclic IMS System separately and subjected to five passes of the ion mobility cell in negative ion ESI mode. The data obtained are shown in Figure 3, where the ganglioside lipid GD1a (d18:1/18:0) was detected with an ATD centered on 42.67 msec (Figure 3A) with that of GD1b (d18:1/18:0) centered on 41.39 msec (Figure 3B). The data on these individual standards reflect

from the mixture showing baseline resolution of the ganglioside lipid isomers with IMS resolution of 145 Ω/ΔΩ (five passes).

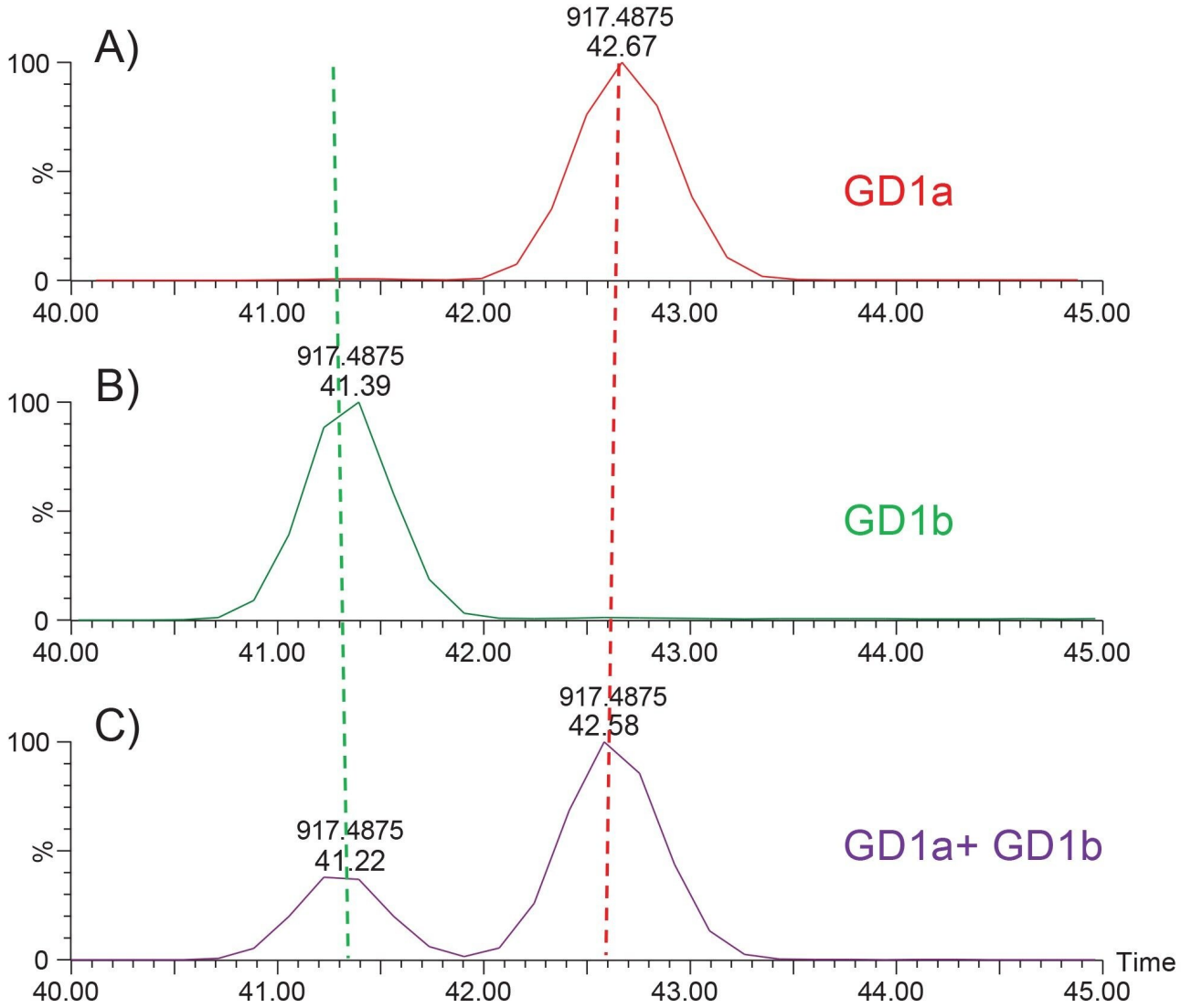

Figure 3. Arrival time distribution for the separation of individual (A) GD1a (d18:1/18:0), (B) GD1b (d18:1/18:0), or (C) the equimolar mixture of the two ganglioside isomers at m/z 917.488 $[M-2H]^{-2}$ using five passes of the ion mobility device.

Conclusion

The correct identification of lipids is critical in understanding their biological role and importance. With its unique multi-pass cyclic IM capability, it is possible to scale ion mobility resolution to meet a given challenge. The two ganglioside isomers GD1a (d18:1/18:0) and GD1b (d18:1/18:0) were successfully resolved using five passes of the cIMS device of the SELECT SERIES Cyclic IMS, with ion mobility resolution of 145 $\Omega/\Delta\Omega$. Although it required five passes of the IMS cell to completely resolve the two lipid isomers the two species were clearly visible after three passes and 80% resolved after four passes of the IMS cell. This data illustrates the power of the SELECT SERIES Cyclic IMS in the detection and identification of lipid isomers in lipidomics studies.

References

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2. Giles K, Ujma J, Wildgoose J, Pringle S, Richardson K, Langridge D, Green M. A Cyclic Ion Mobility-Mass Spectrometry System. *Anal Chem*. 2019; 91: 8564–8573.
3. Ujma J, Ropartz D, Giles K, Richardson K, Langridge D, Wildgoose J, Green G, Pringle S. Cyclic Ion Mobility Mass Spectrometry Distinguishes Anomers and Open-Ring Forms of Pentasaccharides. *J Am Soc Mass Spectrom*. 2019; 30: 1028–1037.

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MassLynx MS Software <https://www.waters.com/513662>

720007592, April 2022