Flexibility of HRIM-MS Analysis for Targeted Lipid Profiling: Choose Your Own Adventure!

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Abstract

- Lipids are challenging to analyze due to the presence of numerous isomeric species, many of which cannot be distinguished via traditional LC-MS approaches.
- The introduction of HRIM provides the analytical separation power to begin to unravel very structurally similar lipid species, including isomers.
- Flow injection and liquid chromatography are complementary front-end sample introduction methods for HRIM-MS that provide high throughput analysis and deeper sample characterization, respectively.

Methods

- Data acquisition was carried out on a commercial equivalent high resolution ion mobility (HRIM) platform, MOBIE™ (MOBILion Systems) coupled to a 6545XT QTOF (Agilent Technologies) with a 1290 Infinity II Autosampler (Agilent Technologies) for sample introduction.
- The sample analyzed in these experiments was a total ganglioside extract from Avanti Polar lipids diluted to approximately ~100 ug/mL
- Source settings and HRIM method parameters conserved between LC and FIA workflows

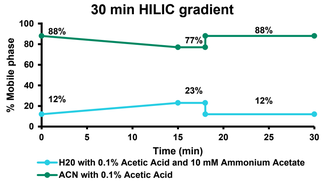


Figure 1. Optimized gradient used in LC-HRIM-MS workflow with an Ascentis Si column.

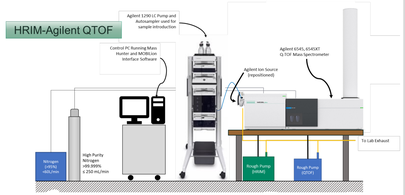


Figure 2. Experimental system configuration and set-up.

FIA-HRIM-MS	LC-HRIM-MS
• 2 min high-throughput analysis	• 30 min HILIC gradient separation
• Quantitation of most abundant species in sample	• Higher sensitivity quantitation of low abundance species
• Isomers separated via high resolution ion mobility	• Multidimensional isomer separation via LC and HRIM
• Ability to convert arrival time to collision cross section (CCS)	• Ability to convert arrival time to collision cross section (CCS)
• Fast, reproducible sample profiling	• Deep sample characterization

Table 1. Qualitative comparison of FIA-HRIM-MS and LC-HRIM-MS workflows that users should consider when deciding whether a given workflow meets their analytical needs.

Results – HRIM resolves isomeric lipid species and methods are complementary between FIA and LC workflows

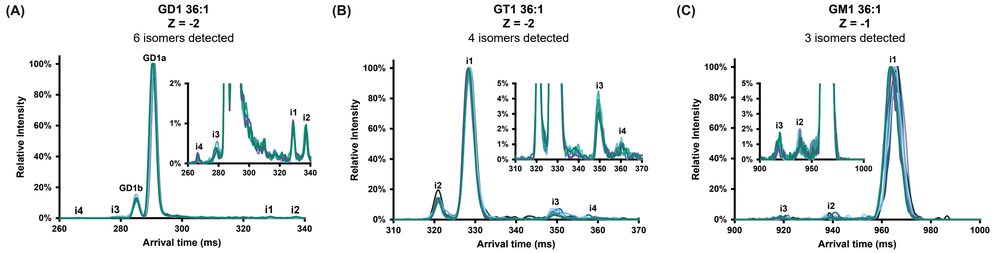


Figure 3. FIA-HRIM-MS and LC-HRIM-MS extracted ion mobiligrams of select gangliosides in porcine ganglioside extract. Overlaid extracted ion mobiligrams of six FIA-HRIM-MS replicate injections and six LC-HRIM-MS replicate injections for the gangliosides (A) GD1 d36:1, (B) GT1 d36:1, and (C) GM1 d36:1. Insets are magnifications of the six LC-HRIM-MS replicate injections alone.

Results – LC-HRIM-MS reduces ion suppression for improved quantitation of low abundance species

Addition of LC to workflow enabled:

- The quantitation of nearly 6 times as many isomeric species as FIA alone
- Lower peak area %RSD per species, on average
- The detection of nearly 4.5 times as many high-quality features in an untargeted feature finding assessment

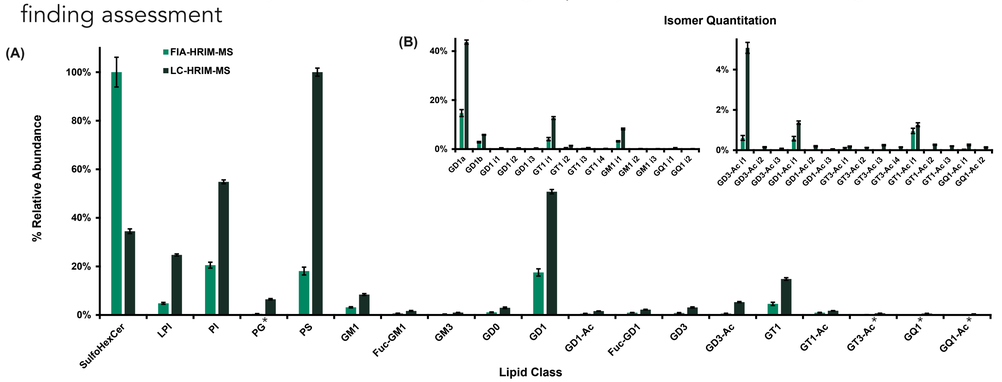
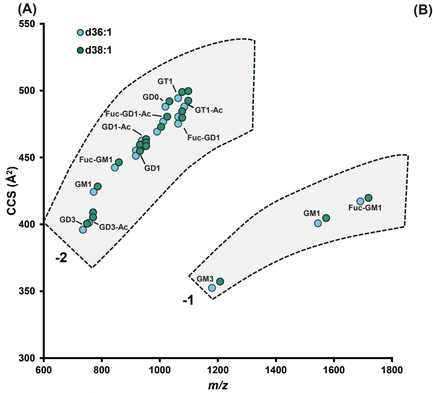


Figure 4. FIA-HRIM-MS and LC-HRIM-MS detection of the most abundant lipid species in porcine ganglioside extract. (A) In the LC dataset, the % abundance of the most abundant gangliosides, sulfatides, and glycerophospholipids in each class is plotted relative to the most abundant species, PS. The FIA dataset was handled similarly, plotted relative to a sulfatide. Only species with a relative standard deviation (RSD) < 20% in the LC-HRIM-MS datasets are included. The peak areas were summed for species that had multiple detected isomeric forms. *Species in the FIA-HRIM-MS dataset with a %RSD>20% are noted. (B) The % abundance of the major isomers of GD1, GT1, GM1, GD3-Ac, GD1-Ac, GT3-Ac, GT1-Ac, GQ1-Ac, GQ1.

Results – Ganglioside CCS in Conformational Space

CCS values for gangliosides were determined as follows:

- MOBILion TuneMix data was acquired at identical traveling wave settings as the lipid data.
- TuneMix data for non-surfing ions was used to generate a third-order polynomial function for CCS calculation.
- Ganglioside arrival times were extracted from the FIA-HRIM-MS dataset and converted to CCS using the polynomial function.



(B)

Lipid	CCS (Å²)	%RSD	Lipid	CCS (Å²)	%RSD
GD1a d36:1	455.3	0.02%	GD3 d38:1	400.5	0.01%
GD1b d36:1	451.3	0.02%	GD1-Ac d36:1 i1	462.6	0.10%
GD1a d38:1	459.6	0.01%	GD1-Ac d36:1 i2	459.7	0.06%
GD1b d38:1	454.9	0.01%	GD1-Ac d36:1 i3	457.8	0.08%
GT1 d36:1 i1	480.5	0.02%	GD1-Ac d38:1 i1	463.8	0.03%
GT1 d36:1 i2	475.3	0.02%	GD1-Ac d38:1 i2	461.3	0.05%
GT1 d36:1 i3	494.4	0.06%	GD1-Ac d38:1 i3	458.6	0.15%
GT1 d38:1 i1	484.6	0.04%	Fuc-GM1 d36:1	442.5	0.02%
GT1 d38:1 i2	479.7	0.02%	Fuc-GM1 d36:1	417.3	0.03%
GT1 d38:1 i3	499.0	0.16%	Fuc-GM1 d38:1	446.4	0.01%
GM1 d36:1	424.3	0.02%	Fuc-GM1 d38:1	419.8	0.06%
GM1 d36:1	400.8	0.02%	GD0 d36:1	488.1	0.02%
GM1 d38:1	428.4	0.03%	GD0 d38:1	492.0	0.02%
GM1 d38:1	404.8	0.01%	Fuc-GD1-Ac d36:1	476.9	0.10%
GM3 d36:1	352.6	0.03%	Fuc-GD1-Ac d38:1	480.6	0.02%
GM3 d38:1	357.3	0.06%	GT1-Ac d36:1	488.0	0.01%
GD3-Ac d36:1	401.2	0.06%	GT1-Ac d38:1	499.7	0.03%
GD3-Ac d38:1 i1	408.9	0.04%	GT1-Ac d38:1	492.4	0.10%
GD3-Ac d38:1 i2	405.3	0.16%	Fuc-GD1 d36:1	469.3	0.03%
GD3 d36:1	396.0	0.04%	Fuc-GD1 d38:1	472.9	0.02%

Figure 5. Ganglioside conformational space plot. (A) The CCS-calibrated FIA-HRIM-MS data for the d36:1 and d38:1 species from 12 ganglioside classes are plotted against m/z. (B) RSD values were calculated for the CCS values across three replicates.

Conclusions

- FIA-HRIM-MS provides high-throughput (≤2 min) analysis of the most abundant lipids in a ganglioside extract.
- Coupling HRIM-MS with LC enables deeper characterization of the sample by reducing ion suppression.
- Both workflows utilize HRIM for highly reproducible gas phase separation.
- Most exciting results:
  - CCS values of 40 gangliosides from 12 different classes were determined using FIA-HRIM-MS, all with %RSD <0.2%
  - Adding LC to the workflow enabled the quantitation of an additional 24 low abundance isomers not detected via FIA