Sensitive bioanalysis of galactosyl sphingosine (GalSPH) and glucosyl sphingosine (GluSPH) in cerebral spinal fluid

Kean Woodmansey[1], Jack Steed[1], Jessica Smith[1], Freddy Oostebring[2] and Jianru Stahl-Zeng[3]
[1]SCIEX, UK; [2]Ardena Bioanalysis Assen, The Netherlands; [3]SCIEX, Germany

ABSTRACT

Sensitivity remains a critical challenge in the development of analytical methods to quantify biomarkers in complex matrices, such as cerebral spinal fluid (CSF). As is common with biomarker analysis, several assays are performed on a single sample when quantifying biomarkers in matrix. Since this results in sample volume limitations, sensitive analytical techniques are crucial for this analysis. Here, a highly sensitive method was developed for GalSPH and GluSPH where hydrophilic interaction chromatography (HILIC) was applied for baseline separation of the isomers for accurate and sensitive quantification using a high-end triple quadrupole mass spectrometer.

Standards for GalSPH and GluSPH and their deuterated internal standards, GluSPH-D5 and GalSPH-D5, were obtained from Avanti Polar Lipids. Standards were prepared in surrogate CSF across a range of concentrations suitable for analyzing endogenous levels of the CSF samples, with the deuterated internal standards held at a constant concentration. Sample preparation was performed using a methanol extraction followed by solid phase extraction. Isomer separation of GalSPH and GluSPH was achieved using HILIC. The samples were then analyzed on both the QTRAP 6500+ system and the SCIEX 7500 system. MRM analysis was performed using electrospray ionization in positive polarity. In summary, a highly sensitive and accurate HILIC-MS/MS method was developed for the monitoring and quantification of GalSPH and GluSPH in surrogate CSF.

INTRODUCTION

GalSPH and GluSPH are useful biomarkers for several diseases such as Krabbe and Gaucher disease as well as being a potential marker for carriers of heterozygous mutated GBA1 gene, which increases the risk of Parkinson's.[1,2] Therefore, it is important to be able to accurately monitor these compounds within the brain.

CSF is analyzed for GalSPH and GluSPH due to being the closest matrix available to monitor changes in the brain. However, the volume available is extremely limited as typically several biomarker assays need to be performed on a single sample. Therefore, it is important to have as much sensitivity as possible to ensure that lower volumes of CSF can be used.

Currently, this analysis is performed by Ardena Bioanalysis Assen using the SCIEX Triple Quad 6500+ system but, due to the increased sensitivity available with the SCIEX 7500 system, a comparison study to evaluate impact was performed. The objective was to assess whether the increase in sensitivity allows for lower CSF volumes to be analyzed.

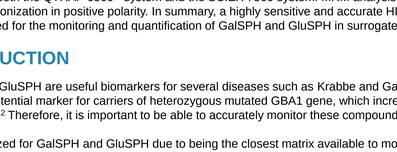

Figure 1. Extracted ion chromatograms (XIC) of GluSPH and GalSPH. Using HILIC chromatography, good separation, and peak shape of the two isomers was achieved. Both compounds are analyzed using the same MRM transition and so chromatographic separation is paramount.

MATERIALS AND METHODS

Sample Preparation:
Standards for GalSPH and GluSPH and their deuterated internal standards, GluSPH-D5 and GalSPH-D5, were obtained from Avanti Polar Lipids. Extracted standards and CSF samples were prepared by Ardena Bioanalysis.

Standards were prepared in surrogate CSF across a range of concentrations suitable for analyzing endogenous levels of the CSF samples, with the deuterated internal standards held add a constant concentration. The samples were then split to be analyzed on both the SCIEX Triple Quad 6500+ system and SCIEX 7500 system.

LC-MS analysis:
Samples were analyzed using both the QTRAP 6500+ system and the SCIEX 7500 system. Separation of Gal- and Glu-SPH was achieved using HILIC chromatography. MRM analysis was performed using electrospray ionization in positive polarity.

Data processing:
Datasets were processed using both SCIEX OS software and Analyst software.

RESULTS

To compare the sensitivities between the two systems, a set of standards and CSF samples were prepared by Ardena Bioanalysis Assen before being divided and analyzed on both MS systems. Figure 2 highlights the calibration curves obtained for GluSPH and GalSPH on the SCIEX 7500 system across a concentration range between 0.001 – 1.000 pmol/mL.

Both provided an r value >0.99, with accuracies between 80 – 120% at each level (Table 1), providing good linearity across the 3 orders of dynamic range studied. A similar concentration curve was generated on the QTRAP 6500+ system (data not shown).

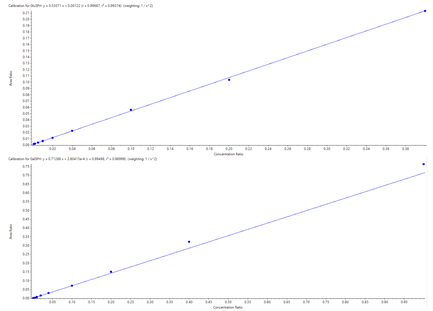

Figure 2. Calibration curves using the SCIEX 7500 system. Calibration curves for both GluSPH (top) and GalSPH (bottom) were generated in surrogate CSF. Both have an r value >0.99 and use a $1/x^2$ weighting, with a range between 0.001 – 1.000 pmol/mL. Results were obtained using internal standard correction with the deuterated forms of the analytes (GluSPH-D5 and GalSPH-D5).

The %RSD and calculated concentrations for the bottom four surrogate CSF standards are also detailed in Table 2 highlighting that the %RSD values are below acceptable criteria of 10%.

The sensitivity difference between the two systems was next assessed by using peak-to-peak S/N with similar noise regions, across the concentration curves.

A summary is shown in Table 3 for GluSPH and Table 4 for GalSPH. The S/N increase was found to be between 2.4 and 6.7x, with improvements seen across the concentration range analyzed.

Table 1. The % accuracy values for GalSPH and GluSPH on the SCIEX 7500 system.

Concentration (pmol/mL)	% Accuracy (GluSPH)	% Accuracy (GalSPH)
0.001	*101.23*	*103.21*
0.002	*96.08*	*99.03*
0.005	*103.61*	*86.66*
0.010	*99.92*	*97.04*
0.020	*100.19*	*110.23*
0.040	*101.06*	*105.15*
0.100	*103.81*	*100.55*
0.200	*97.13*	*106.42*
0.400	*100.49*	*112.73*
1.000	*94.81*	*107.08*

- The accuracy values calculated from the calibration curve generated in surrogate CSF were within 80-120% for all concentrations analyzed

Table 2. The %RSD and average calculated concentrations for GalSPH and GluSPH on the SCIEX 7500 system.

Concentration (pmol/mL)	%RSD of area (GluSPH)	Average calculated concentration (GluSPH, pmol/mL)	%RSD of area (GalSPH)	Average calculated concentration (GalSPH, pmol/mL)
0.0010	*8.75*	*0.0010*	*4.07*	*0.0010*
0.0020	*8.97*	*0.0019*	*1.48*	*0.0020*
0.0050	*8.08*	*0.0052*	*3.14*	*0.0043*
0.0100	*4.13*	*0.0100*	*1.73*	*0.0097*

- The peak area %RSD was calculated for the bottom 4 concentrations (n=3) and was below acceptable criteria of 10% at each level assessed
- The average calculated concentration (pmol/mL) was derived based on the calibration curve analyzed to show similarity to the theoretical values

Table 3. Signal/Noise improvement for GluSPH.

Concentration (pmol/mL)	SCIEX 6500+ system - S/N (GluSPH)	SCIEX 7500 system - S/N (GluSPH)	S/N improvement
1.000	*6233*	*21850*	*3.5x*
0.020	*119*	*284*	*2.4x*
0.001	*10*	*65*	*6.5x*

- The peak-to-peak S/N for GluSPH was measured at selected concentrations across the calibration curves for both the SCIEX 7500 system and the QTRAP 6500+ system
- The peak-to-peak S/N improvement is between 2.4 and 6.5x

Table 4. Signal/Noise improvement for GalSPH.

Concentration (pmol/mL)	SCIEX 6500+ system - S/N (GalSPH)	SCIEX 7500 system – S/N (GalSPH)	S/N improvement
1.000	*6283*	*33324*	*5.3x*
0.020	*122*	*388*	*3.2x*
0.001	*6*	*40*	*6.7x*

- The peak-to-peak S/N for GalSPH was measured at selected concentrations across the calibration curves for both the SCIEX 7500 system and the QTRAP 6500+ system
- As can be seen the peak-to-peak S/N improvement is between 3.2 and 6.7x

CONCLUSIONS

- Sensitivity for two glycosphingolipids, GalSPH and GluSPH were evaluated in CSF
- Two MS systems were evaluated, the QTRAP 6500+ system was compared to the SCIEX 7500 system
- Improvements in S/N of between 2.4 and 6.7x were achieved on the SCIEX 7500 system, as compared to the QTRAP 6500+ system
- Good accuracy and linearity were observed across the range evaluated on the SCIEX 7500 system
- Improved S/N at similar concentrations of the standards in matrix suggests that lower volumes of CSF could be used in this assay on the SCIEX 7500 system, this hypothesis will be evaluated next
- Based on the results, it is concluded that the objective to increase the sensitivity is met. Based on the improved S/N seen on the SCIEX 7500 system when compared to similar analysis on the SCIEX Triple Quad 6500+ System it may be possible to reduce the required assay volume of CSF for this method when using the SCIEX 7500 system.

REFERENCES

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2. Matthew Surface et al. (2021). Plasma Glucosylsphingosine in GBA1 Mutation Carriers with and without Parkinson's Disease. *Mov Disord*. **10**: 1-6.

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