Research into Lysosomal Storage Metabolism using Plasma Lipid Characterization by LC-MS/MS

Sibylle Heidelberger[1]; Daniel Blake[1]; Jean-Baptiste Vincendet[2]; Rachel Webster[3]; Karen Smith[3]; Martin Roch[3]
[1]SCIEX, Warrington, UK; [2]SCIEX, Darmstadt, Germany; [3]Queen Elizabeth Hospital Birmingham, Birmingham UK

ABSTRACT

Research into plasma sphingolipids and determining the concentrations of such is of growing importance in the clinical research laboratory, particularly within groups researching Lysosomal Storage Metabolism. Current methods of analysis primarily involve either enzyme activity procedures or derivatization of compounds prior to analysis. Direct analysis of these groups can be complex due to extensive structural homogeneity between individual compounds. We present here a method for a direct multi compound screen approach to this analysis, employing modern advances in column technology to produce a rapid and sensitive LC-MS/MS method for these compounds

INTRODUCTION

The plasma lipids lysosphingomyelin (SPC), Glucosylsphingosine (Glu-SPH), Psychosine (Gal-SPH) and Globotriasosylsphingosine (Lyso Gb3) were analysed. Research indicates that levels of these lipids are elevated in the lysosomal storage conditions Niemann-Pick C, Gaucher, Krabbe and Fabry respectively. C17-SPC was added as an internal standard.

The enzyme assays currently employed in research into these conditions, regardless of analytical endpoint, require a lengthy incubation process prior to analysis. Despite recent advances in end point analysis to incorporate advances in sample preparation, full (sample to result) analysis times can be lengthy (approx 16h) In addition, some modern advances in assay technology incorporate artificial substrates which can prove costly.

HILIC (**H**ydrophilic **I**nteraction **LI**quid **C**hromatography) is a variant of normal phase liquid chromatography that partly overlaps with other chromatographic applications such as ion chromatography and reversed phase liquid chromatography. HILIC uses hydrophilic stationary phases with reversed-phase type eluents. Effectively HILIC offers a system where analytes elute in order of decreasing hydrophobicity, or increasing polarity. As traditional reversed phase technologies are known to separate lipids based on their carbon chain lengths and degree of saturation, lipids with small functional group differences (such as Gal-SPH and Glu-SPH) will often co-chromatograph. HILIC separation can overcome these differences and provide a separation mechanism for almost identical compounds.

MATERIALS AND METHODS

Sample Preparation:
Two sample preparation approaches were compared – one method was a modified SPE approach as described in Welford et al[1] and a second method was a simplified protein precipitation/direct injection approach utilizing 100µL of plasma and 500µL of 90% Acetonitrile in water.

HPLC Conditions:
HPLC separation was provided by a Shimadzu Prominence XR UHPLC system equipped with Phenomenex Kinetex HILIC 50x2.1mm HPLC column, maintained at 50°C. A gradient of water and acetonitrile (both containing 0.1% formic acid) was used at a flow rate of 500µL/min. The injection volume was set to 3µL.

MS/MS Conditions:
A SCIEX Triple Quad™ LC-MS/MS system with IonDrive technology operating under low mass mode was used. Two MRM transitions per compound were analysed. MRM transitions were optimised using individual standards and direct infusion. Source and gas conditions were optimised by flow injection of the lowest sensitivity compound (LysoGB3). The total run time for all compounds, including column reequilibration time, was 10 minutes.

RESULTS

HILIC Separation of isobaric compounds:
Mixed standards of all compounds were analysed using a standard C18 column (Phenomenex Kinetex C18 50x2.1mm) and a HILIC column (Phenomenex Kinetex HILIC, 50x2.1mm). Results are shown in Fig 1 and 2.

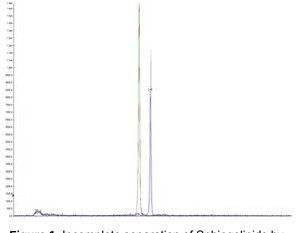

Figure 1. Incomplete separation of Sphingolipids by Reversed Phase LC-MS/MS

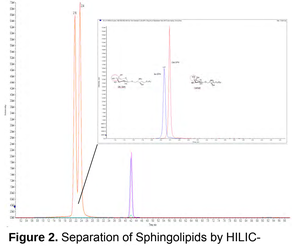

Figure 2. Separation of Sphingolipids by HILIC-MS/MS (Glu-and Gal-SPH individual compound analyses shown in inset)

Comparison of SPE and Protein Precipitation:
Pooled control plasma was spiked at required LOQ levels (5nmol/L for LysoGB3 and SPC, and 0.5nmol/L for Glu- and Gal-SPH) and extracted by the two approaches already described. Results are shown in Fig 3 and 4 and Table 1

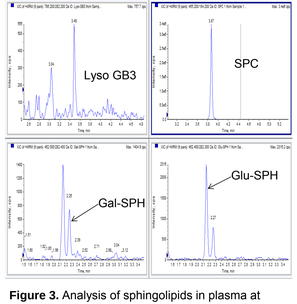

Figure 3. Analysis of sphingolipids in plasma at LOQ levels using Solid Phase Extraction

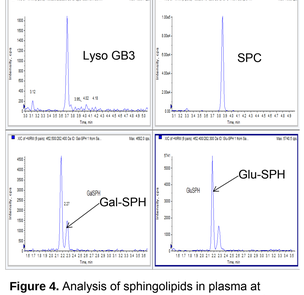

Figure 4. Analysis of sphingolipids in plasma at LOQ levels using Protein Precipitation and direct injection

Compound	S/N (SPE)	S/N (PP)
SPC	448	2489
Glu-SPH	48	46
Gal-SPH	13	49
LysoGB3	49	191

Table 1. Signal – Noise values (1SD) of all analytes in extracts by Solid Phase Extraction and Protein Precipitation

Calibration curves in matrix

Fig 5 shows all standard curves in matrix extracted using protein precipitation, concentration ranges 0.5 – 10nmol/L for Glu-and Gal-SPH and 5-100nmol/L for SPC and LysoGB3

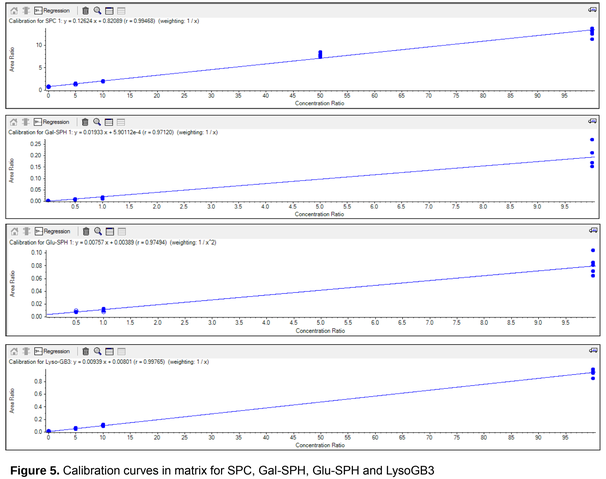

Figure 5. Calibration curves in matrix for SPC, Gal-SPH, Glu-SPH and LysoGB3

Comparison with enzymatic methods.

Pooled spiked plasma samples previously analysed using traditional methods were anonymized and separated into control and positive groups and randomised therein. Samples were analysed using the proposed method in triplicate.

Figure 6 shows a plot of concentration for the control vs positive groups

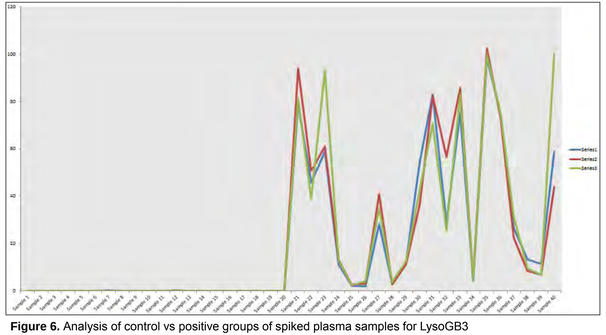

Figure 6. Analysis of control vs positive groups of spiked plasma samples for LysoGB3

CONCLUSIONS

- We have presented here proof-of-concept results for the direct analysis of a series of sphingolipids in plasma using a simple protein precipitation and HILIC chromatography coupled to MS/MS
- Research into these compounds as biomarkers of lysosomal storage metabolism is ongoing
- The proposed method offers advantages over enzyme activity applications as incubation is unnecessary
- The proposed method shows potential throughput and cost improvements on SPE methodologies and is rapid and simple, and also amenable to automation
- Initial comparisons with existing non-MS methodologies shows potential

TRADEMARKS/LICENSING

AB Sciex is doing business as SCIEX.

Document number: RUO-MKT-10-4026-A