Tandem Mass Spectrometry Quantification of Lyso-Gb3 in Plasma for Fabry Disease Patients.

Justyna Spiewak 1, Ivan Doykov 1, Derek Burke 2, Simon Heales 2, Kevin Mills 1, Wendy Heywood 1

1Biological Mass Spectrometry Centre, UCL Great Ormond Street Institute of Child Health,
2Chemical Pathology Enzyme Laboratory, Great Ormond Street Hospital for Children

INTRODUCTION

Fabry disease is an inherited lysosomal storage disorder caused by the deficiency of the α-galactosidase A (α-GAL A) (1, 2). The enzyme is responsible for the breakdown of globotriaosylceramide (Gb3), which together with its decacetylated derivative globotriaosylphosphoglycerine (Lyso-Gb3) accumulates in Fabry tissues affecting primarily the heart and kidneys (1, 3, 4). Plasma Lyso-Gb3 has a higher diagnostic sensitivity compared to Gb3 and reflects better the disease severity (5). New improved treatments are emerging for Fabry disease in the form of chaperone therapy which has resulted in increased demand for testing for monitoring of patients who are changing treatment regimens. To accommodate an increased demand modifications to an existing LC-MS/MS method are required to reduce run times and improve sensitivity and reproducibility to meet Good Clinical Laboratory Practice standards. In this work we describe the modification of existing methods for the analysis of plasma Lyso-Gb3 (3), in particular the choice of two internal standards currently used clinically, to create a quicker, more robust and accurate test for Fabry disease.

MATERIALS & METHODS

We compared the use of dimethyl psychosine internal standard (IS) with N-Glycinated Lyso-Gb3. N-Glycinated Lyso-Gb3 is an analogue of Lyso-Gb3 and is better IS to control for extraction and LC-MS/MS analysis but also co-elutes closer with native Lyso-Gb3. The free amine group gives this product a more similar physical characteristic to the natural Lyso-Gb3 while the glycine adds an additional 57 units to the molecule making it easy to detect by MS. The N-Glycinated Lyso-Gb3 IS, generates two potential transitions: 843.639→264.426 and 843.639→339.446. We have validated both transitions for the assay.

COMMERCIAL AVAILABLE, CONTROL PLASMA POOLED SAMPLES WERE SPUN WITH LOW (1 ng/ml), MEDIUM (10 ng/ml) AND HIGH (80 ng/ml) LYSO-GB3 REFERENCE STANDARD CONCENTRATION TO VALIDATE THE NEW METHOD.

Plasma samples to establish new control and Fabry patient ranges, 22 controls (11 female and 11 male), and 26 Fabry (13 female and 13 male) were provided by Chemical Pathology Enzyme Laboratory, Great Ormond Street Hospital for Children. Samples were classified according to sex.

IS TESTING RESULTS

Our findings demonstrated that the N-Glycinated Lyso-Gb3 IS was a more reproducible IS compared to dimethyl psychosine (better inter- and intra-batch variation). While both tested IS transitions produced similar results, 264 m/z daughter ion from the IS showed both better accuracy and precision CVs compared to the 339 m/z IS transition. All presented validation results are based on 264 m/z daughter ion from the IS.

REFERENCES AND ACKNOWLEDGMENTS


VALIDATION RESULTS

The calibration curve analyses demonstrated that the assay was linear up until a value of 300 ng/ml r²=0.99.

Limit Of Detection (LOD) of 0.146 ng/ml was established based on 3.1 Signal-to-Noise ratio using water calibration curve and confirmed with plasma based calibration curve.

For establishing matrix effect water and plasma based serial dilution calibration lines were compared. There was 28.90% inhibition effect observed.

To test the extraction recovery, Lyso-Gb3 was spiked into control plasma before and after extraction procedure before drying.

CONCLUSIONS & DISCUSSION

We confirm that the N-glycinated Lyso-Gb3 internal standard improves assay reproducibility and accuracy compared to the dimethyl psychosine IS. Additionally, out of two daughter ion transitions evaluated, 264 m/z IS provides both better specificity and accuracy of the assay.

Finally, the method run time was reduced from 10 min to 5 min and created a precise, accurate and highly sensitive LC-MS/MS method to reliably quantify plasma levels of Lyso-Gb3, with 0.146 ng/ml LOD and 0.5 ng/ml LOQ. This optimised method has increased the high throughput capability of the assay by approximately 50% and at the same time improved accuracy and reproducibility of the test.