Accurate Quantitation of Plasma Globotriaosylsphingosine (lyso-Gb3) in Healthy Individuals and Fabry Patients by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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Introduction

Fabry disease is a lysosomal storage disorder caused by mutations in the gene (GLA) that encodes α-galactosidase A (α-Gal A), and is characterized by pathological accumulation of globotriaosylceramide (GL-3) and its deacylated form, globotriaosylsphingosine (lyso-Gb3). Lyso-Gb3 is an important indicator of Fabry disease, and warrants further evaluation as a marker of disease severity and progression. An LC-MS/MS method was developed and used to measure plasma lyso-Gb3 levels in healthy individuals and Fabry patients enrolled in the ATTRACT Phase 3 clinical study (AT1001-012).

Normal human plasma contains significant endogenous levels of lyso-Gb3, which causes high background interference and negatively impacts the quantitative accuracy of the method at low lyso-Gb3 levels. A novel, stable isotope-labeled internal standard (Sil-IS), 13C2-lyso-Gb3, was synthesized and enabled accurate quantification of lyso-Gb3 in plasma from both healthy individuals and Fabry patients using solvent calibration standards.

Sample Preparation

- Plasma Volume: 50 μL
- Internal Standard: Sil-IS, 13C2-lyso-Gb3
- Extraction: Solid Phase (Waters Oasis MCH)

LC-MS/MS Conditions

- LC System: Shimadzu LC-20AD Pumps
- LC Column: Halo HILIC, 4.6 x 75 mm (Advanced Materials Technology)
- MP A: 5 mM ammonium formate in acetonitrile/water: 5/95 + 0.5% formic acid
- MP B: 5 mM ammonium formate in acetonitrile/water: 95/5 + 0.5% formic acid
- Flowrate: 0.550 mL/min
- Elution: Gradient
- Autosampler System: Shimadzu SIL-30AC
- Injection volume: 5-10 μL
- Mass Spectrometer: 4000 QTRAP, AB Sciex
- Ionization: Positive Ion Electrospray
- MRM transitions: m/z 787 → 282 (lyso-Gb3) and m/z 793 → 282 (13C2-lyso-Gb3)
- Regression Type: Linear, 1/x2 weighting
- Solvent Calibration Standards Assay Range: 0.200 - 200 ng/mL (0.254 – 254 nM)

1. Lyso-Gb3 LC-MS/MS Experimental Method

Solvent Double Blank

Plasma Double Blank

Plasma QC (12.6 ng/mL)

A = lyso-Gb3, B = 13C2-lyso-Gb3 (IS)

2. Lyso-Gb3 LC-MS/MS Method Validation Results

Previously published LC-MS/MS methods have reported endogenous lyso-Gb3, plasma levels from healthy individuals by extrapolation below the lowest limit of quantitation (LLOQ) of the assay range. A method was analytically validated (non-GLP) at Amicus Therapeutics, using both solvent and plasma calibration standards and quality control (QC) samples. The slopes for the solvent and plasma calibration curves matched well in all validation runs. The Sil-IS corrects for plasma matrix effects and enables the use of solvent standards and accurate quantitation in plasma from 0.200 - 200 ng/mL. The endogenous lyso-Gb3 concentration of the pooled plasma used to prepare plasma standards and QCs was determined in the three core runs to be 0.590 ng/mL and was added to the nominal spike-in concentrations of lyso-Gb3 for the purpose of run accuracy acceptance criteria.

- Plasma lyso-Gb3 levels were measured in Fabry patients enrolled in the ATTRACT (AT1001-012) Phase 3 clinical study
- All patients received enzyme replacement therapy (ERT), Fabrazyme, agalsidase beta; Genzyme, Cambridge, MA or Replagal, agalsidase alfa; Shire, Cambridge, MA) for at least 12 months prior to entering the study
- Subjects were randomized to either continue with ERT or switch to oral migalastat treatment (150 mg QOD)
- Plasma samples were collected at months 0 (baseline) 6, 12, and 18, and lyso-Gb3 levels were measured
- Of the 60 subjects in study AT1001-012, 56 had amenable mutations, and 4 had non-ameenable mutations, based on the GLP HEA assay, the assay used to identify α-Gal A mutations responsive to migalastat treatment (i.e. amenable)
- In subjects with amenable mutations, the plasma lyso-Gb3 levels remained low and stable for up to 18 months following the switch from ERT to migalastat, comparable to that seen with subjects who remained on ERT
- In two male subjects with non-amenable mutations, plasma lyso-Gb3 levels increased following the switch from ERT to migalastat, as compared to two subjects (one male and one female) who remained on ERT

3. Normal Plasma Reference Range

Plasma lyso-Gb3 levels from 46 healthy individuals were measured to establish a normal human plasma reference range. Plasma was obtained from a diverse group of individuals, with regards to gender, age and ethnicity. Each individual plasma lot was analyzed in duplicate and the mean of the two values reported.

4. ATTRACT Phase 3 Clinical Study (AT1001-012)

An LC-MS/MS method was analytically validated (non-GLP) at Amicus Therapeutics for the quantitation of lyso-Gb3 in human plasma. A novel stable isotope-labeled internal standard, 13C2-lyso-Gb3, was synthesized and enabled the use of solvent calibration standards and the accurate quantitation of plasma lyso-Gb3 levels in the concentration range 0.200 – 200 ng/mL.

The method was used to measure lyso-Gb3 levels in plasma from 46 healthy individuals to establish a normal human plasma reference range.

Using this validated assay, plasma lyso-Gb3 levels were measured in Fabry subjects enrolled in the ATTRACT Phase 3 clinical study (AT1001-012). In subjects with amenable mutations, lyso-Gb3 levels remained low and stable for up to 18 months in both males and females following their switch from ERT (Fabrazyme or Replagal) to oral migalastat.

Conclusions

Plasma lyso-Gb3 levels from 46 healthy individuals were measured to establish a normal human plasma reference range. Plasma was obtained from a diverse group of individuals, with regards to gender, age and ethnicity. Each individual plasma lot was analyzed in duplicate and the mean of the two values reported.

Amenable

Non-Amenable

Data points are median values, error bars represent the range
Blue dotted line represents upper range of the normal reference range
Dotted and solid lines (green for males and magenta for females) represent the baseline and median values, respectively, for a cohort of classic males and females after 1 year of ERT (Fabrazyme or Replagal) treatment (van Breenen, Rombach et al. 2011)