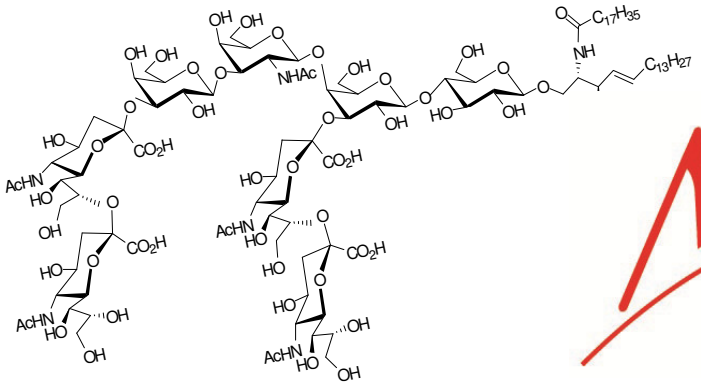


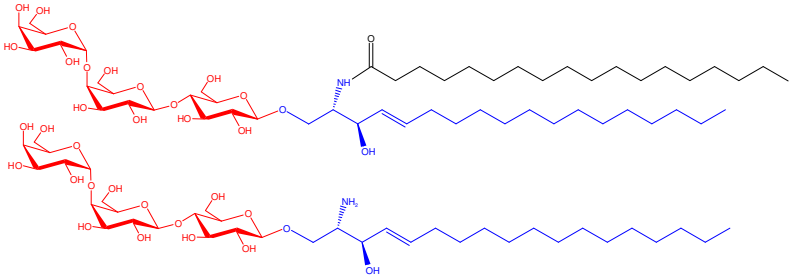
MATREYA LLC

Lipids and Biochemicals

NEWSLETTER FOR GLYCO/SPHINGOLIPID RESEARCH

JUNE 2017

Biomarker Standards for Fabry Disease Diagnosis and Monitoring



Globotriaosylceramide
(Gb_3, CTH) catalog #1067

Globotriaosylsphingosine
(*lyso*-Gb_3, *lyso*-CTH) catalog #1520

Lysosomal storage disorders are a set of more than 70 inherited conditions that result in the accumulation of various lipids in cells due to an inability to enzymatically degrade them. Fabry disease (FD) is one such disorder that is characterized by a deficiency of the enzyme α-galactosidase A, resulting in an accumulation of globotriaosylceramide (Gb_3), globotriaosylsphingosine (*lyso*-Gb_3), galabiaosylceramide (Ga_2), and blood group B glycolipids. This debilitating disease is an X-linked chromosomal disorder and early diagnosis is critical as progression will lead to multiorgan dysfunction and early death. To aid in timely detection and treatment monitoring several disease biomarkers have been identified, including Gb3 and *lyso*-Gb3.

The use of *lyso*-Gb_3 for the determination of Fabry disease has now been well established, resulting in the development of reproducible and highly sensitive methods that require extremely small plasma, dried blood spot, or urine samples.[1,2] *lyso*-Gb_3 has been demonstrated to be an effective biomarker for FD in symptomatic patients, showing higher diagnostic sensitivity than Gb_3.[1] However, this lipid has not always been found to be a good candidate biomarker for asymptomatic females.[3] Heterozygous females can also manifest symptoms of FD making early detection of critical importance in these cases as well. Urinary Gb_3 (but not plasma Gb_3) has been reported to be elevated in both symptomatic and asymptomatic males and females even though it's sensitivity is lower than *lyso*-Gb_3. Although levels of Gb_3 do not necessarily correlate with disease severity, and though false positives do occur using this biomarker, nevertheless Gb_3 has been recognized as a useful diagnostic marker and may also indicate the formation of antibodies during enzyme replacement therapy.[3] Other possible biomarkers for FD include galabiaosylceramide (Ga_2)[4] and blood group B glycolipids.

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Currently FD is most often treated by enzyme replacement therapy (ERT) and the levels of Gb_3 and *lyso*-Gb_3 have also been found to be useful in monitoring the levels of replacement enzymes needed as well as disease progression. ERT has shown a reduction not only in plasma Gb_3 but also in plasma *lyso*-Gb_3. Because FD is a result of deficient enzyme activity, ERT is able to successfully halt the disease progress in many patients. This is especially evident in patients that begin treatment early in the disease progression as untreated

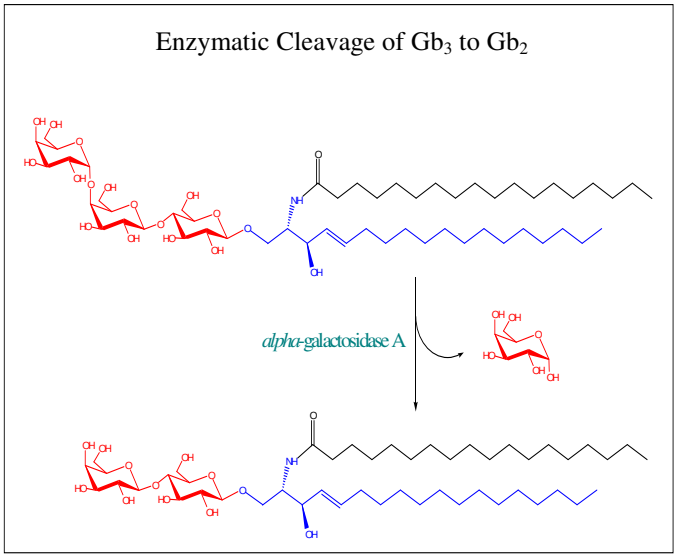
patients can quickly develop irreversible organ damage.[5]

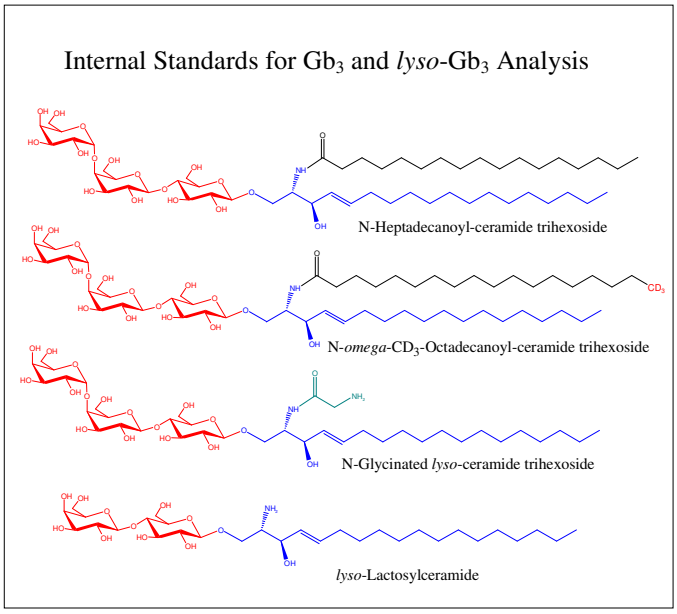
Internal standards for Gb_3 and *lyso*-Gb_3 are critical for the effective quantitation of these FD biomarkers. Glycinated *lyso*-Gb_3 (cat. #1530) was explored as an internal standard and the physical and chemical properties were found to be almost identical to that of natural *lyso*-Gb_3 in terms of extraction, stability, and sensitivity, making it an excellent internal standard for clinical work.[6] This internal standard contains a glycine molecule attached to the amine of *lyso*-Gb_3, preserving the key primary amine functionality. Another commonly used internal standard for *lyso*-Gb_3 analysis is *lyso*-lactosylsphingosine (*lyso*-Gb_2, cat. #1517) which lacks the terminal galactose of *lyso*-Gb3 but does contain a primary amine.[6,2] For Gb_3 analysis the most useful internal standard is a stable isotope labeled standard (such as deuterated octadecanoyl-Gb_3, cat. #1537) or a well-defined Gb_3 containing an unusual fatty acid (such as heptadecanoyl-Gb_3, cat. #1523). Armed with these internal standards, diagnosis and monitoring of FD can go forward steadily.

An example of the ongoing development of sensitive and efficient analyses of *lyso*-Gb_3 in FD is seen in the work by J. Lukas and coworkers[2]: three 3.2 mm dried blood spot samples were extracted with DMSO:water 1:1 in the presence of *lyso*-Gb_2 (as an internal standard) in ethanol with agitation and sonication. After particle filtration by centrifugation the sample was ready for analysis. Similarly, 25 µL of plasma was extracted in ethanol in the presence of *lyso*-Gb_2 (again as an internal standard). After protein precipitation the supernatant was filtrated by centrifugation and analyzed. Analysis was performed by UPLC/triple quadrupole mass spectrometer in MRM mode, monitoring the mass transitions of both *lyso*-Gb_3 and *lyso*-Gb_2.

Another method has been evaluated for the analysis of *lyso*-Gb_3 from dried blood spots by B. Johnson and colleagues.[7] This HPLC-MS/MS method yielded reproducible results in patients with Fabry disease, although the method was found to be unsuitable for newborn screening and late onset females.

A method that takes advantage of detectable *lyso*-Gb_3 in urine is reported by H. Gold and coworkers and includes the use of a stable isotope labeled *lyso*-Gb_3 (not yet commercially





Product Name	Catalog #	Amount	Purity
Ceramide trihexoside (Globotriaosylceramide, Gb_3)	1067	1 mg	98+%
lyso-Ceramide trihexoside (Globotriaosylsphingosine, *lyso*-Gb_3)	1520	1 mg	98+%
N-Glycinated *lyso*-ceramide trihexoside	1530	1 mg	98+%
N-Heptadecanoyl-ceramide trihexoside	1523	1 mg	98+%
N-*omega*-CD_3-Octadecanoyl-ceramide trihexoside	1537	500 µg	98+%
lyso-Lactosylceramide, synthetic	2088	1 mg	98+%
lyso-Lactosylceramide, bovine buttermilk	1517	1 mg	98+%

Please visit www.matreya.com for a full list of ceramide trihexosides and other lipid standards

available).[1] Urine was extracted in methanol/chloroform along with the internal standard. The sample was centrifuged and the supernatant diluted with chloroform/water to make an upper and lower layer. The upper methanol/water phase was collected, evaporated, and partitioned with butanol/water. The upper butanol phase was collected, evaporated, and re-dissolved in methanol for analysis by UPLC-ESI-MS/MS.

It will be interesting to see what the future holds for enhanced early diagnosis, treatment, and monitoring of FD. As methods become more refined we hope to see such techniques as solid phase microextraction being developed for even more efficient analysis. Equipped with an expanding array of standards, researchers can continue to delve deeper into the role of accumulated lipids in this disease's devastating pathogenesis. The recent and ongoing push to make newborn screening for α-galactosidase A more prevalent will undoubtedly have tremendous benefits for the generations ahead.

References:
1. H. Gold et al., Clinical Chemistry (2013) 59:3 547-556
2. J. Lukas et al. Human Mutation (2016) 37(1) 43-51
3. E. Paschke et al., Am J. Kidney Dis. (2011) 57:5 673-681
4. M. Boutin and C. Auray-Blais J. Am. Soc. Mass Spectrom. (2015) 26:499-510
5. R. Hopkin et al. Molecular Genetics and Metabolism (2016) http://dx.doi.org/10.1016/j.ymgme.2016.06.007
6. R. Krüger et al., J. Chromatogr. B (2012) 1:883-884 128-135
7. B. Johnson et al., Clinical Chemistry (2013) 33:274-278

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