Ceramide Trihexosides and Sulfatides Quantitation in Urine by LC-MS/MS

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Introduction: The 3-O-sulfo- galactosylceramides, a sulfatide substrate for arylsufatase A, are excreted in increased amounts in urine of metachromatic leukodystrophy (MLD) patients. However, sulfatiduria may also be increased in multiple sulfatase deficiency (MSD), and sphingolipid activator deficiency (Sap-B). Alpha-galactosidase deficiency, X-linked lysosomal storage disorder Fabry, leads to the accumulation of glycosphingolipids, mainly ceramide trihexosides in tissues, and are excreted in urine. Demonstration of abnormal urinary excretion of ceramide trihexosides and sulfatides is a useful urine screening test to identify patients affected with Fabry disease, MLD, MSD, Sap-B, and some cases of mucolipidosis II (MLII). We describe a highly sensitive and specific method as an alternative to thin layer chromatography (TLC).

Methods: Urine specimens are extracted with 2:1 chloroform: MeOH. After evaporation, the dry residue is reconstituted in 10 mM ammonium formate in MeOH for ceramide trihexosides (CT) analysis and MeOH for sulfatides (S) analysis. LC-MS/MS is performed using a mobile phase composed of 10 mM ammonium formate in 90:10 MeOH: H_2O + 0.05% formic acid using a short C8 column (50 mm x 2.1 mm, 3.5 µ) to separate the ceramide trihexosides and sulfatides from the bulk of the specimen matrix. The MS/MS is operated in the multiple reaction monitoring (MRM) positive mode to follow the ceramide trihexosides and MRM negative mode to follow the sulfatides.

Results: Intra- and inter-assay precision were assessed using urine samples (N=3) of varying concentrations (S = 39, 310 and 3332 ng/mL; CT = 68, 847 and 6702 ng/mL). Intra-assay precision CVs were 4.9, 4.4 and 11.0% for S and 4.6, 10.3 and 7.0% for CT, respectively (N=20). Inter-assay precision CVs were 9.5, 12.9 and 9.9% for S and 15.0, 19.7 and 17.1% for

CT, respectively in the same specimens (N=20). Serial dilution of three urine specimens demonstrated the method response was linear from 4 ng/mL to 4000 ng/mL for S (R^2 = 0.9963) and 3 ng/mL to 10000 ng/mL for CT (R^2 = 0.9999). Clinical specificity was 98% and 90% for S and CT, respectively (0-18M, N=80) and 96% and 90% for S and CT, respectively (>18M, N=175). Clinical sensitivity was 100% for ceramide trihexosides (Fabry: N=25), and sulfatidurias (MLD: N=8, MSD: N=1, MLII: N=7, and Sap-B: N=1). Differences between the LC-MS/MS approach over thin layer chromatography include markedly reduced sample volume requirements (0.15 mL vs. 30 mL) and shorter sample preparation time (30 min vs. 6 hours). The former has significant implications for urine collection in pediatric patients.

Conclusion: We describe a LC-MS/MS method for routine determination of CT and S, which avoids laborious and time-consuming TLC separation and improves upon the existing method with respect to sample volume, sample preparation and reduces the potential for false positive and false negative diagnosis that exists with our current thin layer chromatography method.