A Method for the Analysis of Lysosomal Storage Disorders using LC/MS/MS with Automated On-Line Sample Clean-up

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Introduction

Lysosomal Storage Disorders (LSD) are a heterogeneous group of over 40 inherited genetic disorders, each centered on a total or partial defect of a specific enzymatic activity.

Gelb's group has demonstrated that many lysosomal enzymes are still active in rehydrated dried blood spots (DBS), the same spots used for "classical" neonatal screening[1].

The approach presented in this discussion enables the simultaneous screening of five different LSD disorders (Fabry, Pompe, Niemann-Pick, Krabbe, Gaucher) in a single LC/MS/MS run.

With the proposed protocol, 200 samples can be measured in less than 14 hours and sample manipulation is limited to the incubation activation, with no further modification before tandem mass spectrometer measurement.

Experimental Method

The approach includes the incubation procedure as documented by Gelb's group1, but skips sample treatment between the end of incubation and the mass spectrometric measurement by using an automated on-line sample extraction and clean-up process prior to mass-spectrometry[2].

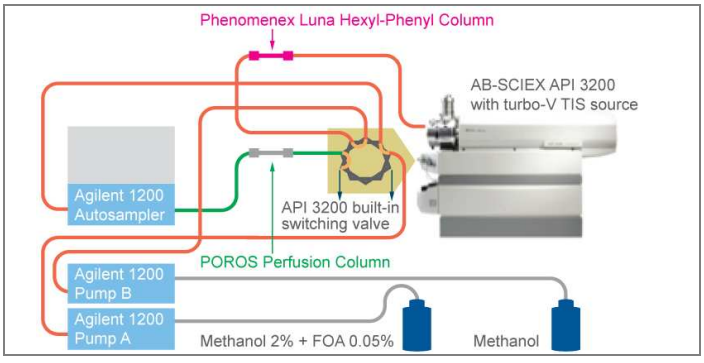

Figure 1. Schematic of the plumbing for the Lysosomal Storage Disorders screening

Five dried blood spots are added to 20 µL of the specific assay cocktail for each of the above disorders, described in references 1 and 2, and incubated at 37℃ for 24 hours.

Each mixture is quenched by adding 60 µL of methanol containing 0.1% formic acid, and these are combined to create a single sample. After centrifugation, the mixture is analyzed on the system configuration depicted in Figure 1, which includes:

- ABI SCIEX API 3200™ LC/MS/MS System with the ESI probe on the TurboV™ ion source
- 2 Isocratic Pumps
- Autosampler
- POROS® R1/20 2 x 30 mm Perfusion Column (Applied Biosystems)
- Luna® Hexyl-Phenyl 2 x 50 mm Column (Phenomenex)

The on-line sample clean-up and measurement process is as follows:

1. The sample mixture is injected onto the POROS® perfusion column and is flushed for 1 minute with solvent A (aqueous solution containing 2% methanol and 0.05% formic acid). All buffers and matrix components are washed out, while the incubation reaction products are retained.

2. After 1 minute the perfusion column is placed on-line with the hexyl-phenyl analytical column. The retained analytes are eluted from the perfusion column with solvent B (methanol), and chromatographically separated on the analytical column.
3. At 2.5 minutes, after chromatographic separation is complete, the perfusion column is automatically taken off-line from the phenyl column. It is then equilibrated for 1 minute with solvent A to prepare the column for the next injection. The run is completed in 3.5 minutes.
4. The measurement is performed in Multiple Reaction Monitoring (positive ion) mode by monitoring the incubation products and their respective internal standards, included in the assay cocktail for each of the five disorders[2].

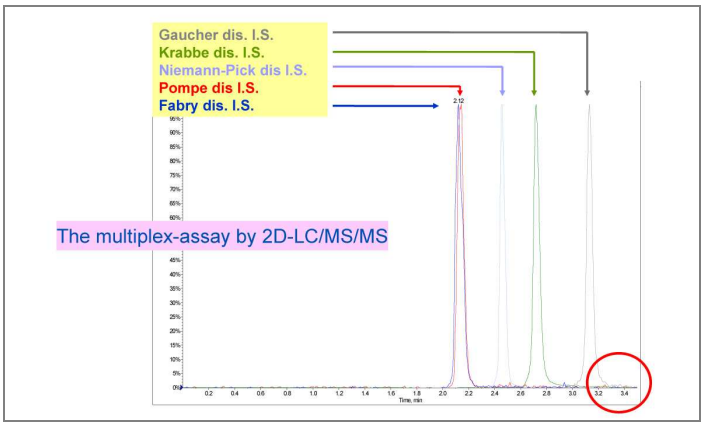

Figure 2. XICs for the internal standards for each disorder explored (normalized scale)

Results

Figure 2 shows the normalized Extracted Ion Chromatograms (XIC) for the internal standards associated with the five disorders.

LODs for each disorder ranged from 0.04 to 0.30 µmol/h/L (the concentration is expressed in terms of enzymatic activity). The interday precision, measured in normal patients, ranged from 5% (for the acid α-galactosidase A deficiency –GLA-) to 15% (for the acid sphingomyelinase deficiency –ASM).

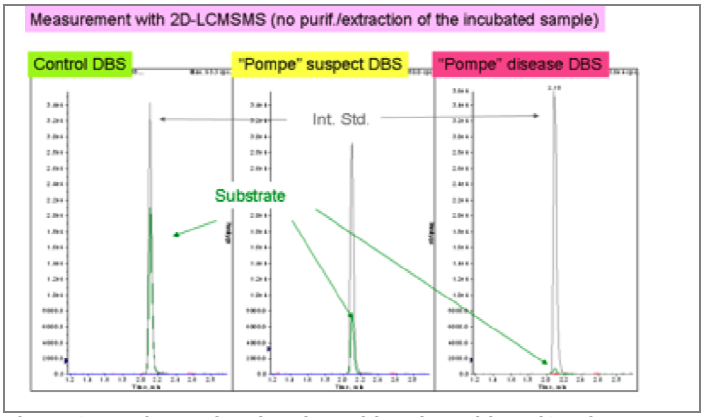

Figure 3. Tracings related to the Acid α-glucosidase (GAA) deficiency (Pompe disease). Left panel: a control sample (no deficiency in the enzymatic activity). Right panel: a manifested deficiency (almost no enzymatic activity). Center panel: a sample with a reduced enzymatic activity.

Conclusions

Figure 3 shows the comparison of the acid α-glucosidase (GAA) deficiency (Pompe disease) with: 1) a control sample (left panel: no deficiency in the enzymatic activity), 2) a manifested deficiency (right panel: almost no enzymatic activity), and 3) a sample with reduced enzymatic activity (center panel). The grey trace references the internal standard signal and the green trace represents the substrate product generated by the specific enzyme.

With the proposed protocol, 200 samples can be measured in less than 14 hours and sample manipulation is limited to the incubation activation; no further treatment is necessary before the tandem mass spectrometric measurement.

References

1. Yijun Li, C. Ronald Scott, Nestor A. Chamoles, Ahmad Ghavami, B. Mario Pinto, Frantisek Turecek, and Michael H. Gelb, 2004, Clinical Chemistry, 50:10, p. 1785–1796.
2. Giancarlo la Marca, Bruno Casetta, Sabrina Malvagia, Renzo Guerrini and Enrico Zammarchi, 2009, Analytical Chemistry, 81:15, p. 6113-6121.

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