Technical brief | 003537

Clinical research

# Measurement of enzyme activities in dried blood spots by targeted high-resolution mass spectrometry for six lysosomal diseases


## Authors

Franklin Ducatez,[1] Abdellah Tebani,[1] Soumeya Bekri,[1] Jingshu Guo,[2] Kerry Hassell[2]

[1]Normandie Univ, UNIROUEN, AIMS - Systems Medicine Lab, CHU Rouen, Referral Center for Lysosomal Diseases, Filière G2M, Department of Metabolic Biochemistry, 76000 Rouen, France

[2]Thermo Fisher Scientific, San Jose, CA, United States



## Keywords

Lysosomal diseases, sphingolipidoses, mucopolysaccharidoses, enzyme activity, inborn errors of metabolism, flow injection analysis – high-resolution mass spectrometry, Orbitrap Exploris mass spectrometer, TraceFinder software, dried blood spots


## Introduction

Lysosomal disorders (LD) are a group of inherited genetic disorders characterized by the dysfunction, deficiency, or absence of specific lysosomal enzymes that are responsible for breaking down various cell components, such as lipids, polysaccharides, and proteins inside the lysosomes.[1] Depending on the specific enzyme affected and the extent of substrate accumulation, LD leads to lysosomal dysfunction and cellular damage with a broad variety of disease severities and widespread symptoms. Thus, early diagnosis and treatment of LD are essential for infants before irreversible tissue damage may occur.

The detection of LD in newborns is usually performed by measuring the activities of the affected enzymes from the dried blood spots (DBS) of infants' heel pricks via flow injection analysis – triple quadrupole tandem mass spectrometry (FIA-QqQ-MS/MS), followed by DNA sequencing of the encoding gene of the subject enzyme, or measurement of the disease-specific biomarkers from the same DBS spots.[2,3] Although triple quadrupole (QqQ) MS provides robustness and sensitivity when quantitating analytes in selected reaction monitoring (SRM) mode, high-resolution MS, such as Thermo Scientific™ Orbitrap™ mass spectrometers, can separate nominal mass isobaric compounds with similar SRM transitions in the absence of a liquid chromatography separation, improving the detection accuracy.[4] In this technical brief, we developed a flow injection analysis – high-resolution accurate mass (FIA-HRAM) method on an Orbitrap mass spectrometer to verify the measurement of enzyme activities on DBS using a Revvity NeoLSD™ MSMS kit: acid-β-glucocerebrosidase (ABG) for Gaucher disease, acid-sphingomyelinase (ASM) for Niemann-Pick A/B disease, acid-α-glucosidase (GAA) for Pompe disease, β-galactocerebrosidase (GALC) for Krabbe disease, α-galactosidase A (GLA) for Fabry disease, and α-L-iduronidase (IDUA)

for Mucopolysaccharidosis Type I (MSP I). The results were compatible with those obtained from a QqQ MS intended by the kit manufacturer, but with higher levels of confidence due to the low ppm mass accuracy. Orbitrap MS has great potential in facilitating comprehensive metabolite profiling necessitated in the routine public health laboratories screening for inborn errors of metabolism (IEM).

## Experimental

### Sample preparation

The NeoLSD MSMS kit (ref no. 3093-001U) was purchased from Revvity (Waltham, MA). Sample preparation, handling, and storage followed the kit instructions to ensure reliable and reproducible measurement results.

In brief, 6.6 mL NeoLSD assay buffer was used to reconstitute one vial of NeoLSD substrates and internal standards (IS) to make the incubation cocktail. One punch of 3.2 mm of control DBS sample was placed in the provided U-bottom microplate, followed by 30 µL of the incubation cocktail, and sealed by the provided aluminum foil microplate cover. The enzyme reaction took place during the overnight incubation (18 ± 2 hr) at 37 °C with agitation, and was quenched by the addition of 100 µL of the quench solution (methanol:NeoLSD extraction solution 1:1). All liquid was transferred to a deep-well plate, followed by 400 µL of the NeoLSD extraction solution and 200 µL of water. After mixing and centrifugation, 50 µL of the organic top layer was transferred into a new U-bottom microplate and dried. The extract was reconstituted with 100 µL provided flow solvent and 10 µL was injected for the FIA-HRAM analysis. For the inter-day and intra-day imprecision measurements, each control level was prepared 3 times over 8 days.

### Liquid chromatography and mass spectrometry

A Thermo Scientific™ Vanquish™ Flex UHPLC system was conditioned with the provided mobile phase for at least 10 minutes prior to sample injection. Each sample was injected twice at an injection volume of 5 µL. The samples were delivered to the MS via a constant flow rate of 0.29 mL/min with the provided mobile phase. The sample needle was washed before and after each injection using the provided needle wash solution.

The analyte quantitation was achieved using the Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer coupled to a Thermo Scientific™ OptaMax™ NG ion source with a heated electrospray ionization probe in the positive mode. The MS source parameters and scan event properties are listed in Table 1. The theoretical *m/z* values of the analytes and their IS precursor ions are shown in Table 2.

**Table 1. Orbitrap Exploris 120 mass spectrometer settings**

| Ion source properties | |
|---|---|
| Ion source type | H-ESI (OptaMax NG ion source) |
| HESI probe position | Center - 1.0 - L/M (x - y - z) |
| **Global parameters** | |
| Spray voltage (V) | +4,750 |
| Sheath gas (Arb) | 35 |
| Aux gas (Arb) | 10 |
| Sweep gas (Arb) | 0 |
| Ion transfer tube temp. (°C) | 325 |
| Vaporizer temp. (°C) | 275 |
| Expected LC peak width (s) | 6 |
| Lock mass correction | Thermo Scientific™ EASY-IC™ system, Run Start |
| **tSIM scan properties** | |
| Multiplex | 6 |
| Isolation window (m/z) | 2 |
| Resolution | 30,000 |
| RF lens (%) | 70 |
| AGC | Standard |
| Max. injection time (ms) | Auto |
| Microscans | 1 |
| Data type | Profile |
| Targeted mass list | See Table 2 |
| **Data-dependent properties** | |
| Intensity threshold | 1.0 e3 |
| Dynamic exclusion | Auto |
| Targeted mass list | See Table 2 |
| Mass tolerance (ppm) | 10 |
| Ignore charge state | True |
| Apex detection (%) | 30 |
| Number of dependent scans | 4 |
| **ddMS2 scan properties** | |
| Resolution | 30,000 |
| Isolation window (*m/z*) | 2 |
| Collision energy type | Absolute |
| HCD collision energies (V) | 15, 20, 30 |
| AGC | Standard |
| Max injection time (ms) | Auto |
| Microscans | 1 |
| Data type | Profile |

**Table 2. The theoretical *m/z* values of the analytes and IS precursor ions**

| Targeted enzymes | Formula | Precursor (*m/z*) | IS | IS concentration (µM) | Formula | Precursor (*m/z*) |
|---|---|---|---|---|---|---|
| GLA | $C_{27}H_{37}N_3O_5$ | 484.2806 | GLA-IS | 24 | $[^2H_5]$-$C_{27}H_{32}N_3O_5$ | 489.3120 |
| ABG | $C_{23}H_{45}NO_3$ | 384.3472 | ABG-IS | 20 | $[^2H_7]$-$C_{23}H_{38}NO_3$ | 391.3912 |
| ASM | $C_{24}H_{47}NO_3$ | 398.3629 | ASM-IS | 15 | $[^2H_7]$-$C_{24}H_{38}NO_3$ | 405.4068 |
| GALC | $C_{25}H_{49}NO_3$ | 412.3785 | GALC-IS | 10 | $[^2H_5]$-$C_{25}H_{44}NO_3$ | 417.4099 |
| IDUA | $C_{24}H_{31}N_3O_4$ | 426.2387 | IDUA-IS | 15 | $[^2H_5]$-$C_{24}H_{26}N_3O_4$ | 431.2701 |
| GAA | $C_{28}H_{39}N_3O_5$ | 498.2963 | GAA-IS | 24 | $[^2H_5]$-$C_{28}H_{39}N_3O_5$ | 503.3276 |

## Data analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software (ver 5.1 SP3 Clinical). The mass tolerance for the analyte quantitation was set to 5 ppm. The enzyme activity was calculated using the equation below in Microsoft™ Excel™.

$$\text{enzyme activity } \left(\frac{\mu M}{hr}\right) = \frac{\text{product peak area}}{\text{IS peak area}} \times \frac{\text{IS concentration } (\mu M) \times \text{IS vol } (\mu L)}{3.1\ \mu L \times \text{incubation time (hr)}} \times RRF$$

- IS concentrations are listed in Table 2
- IS volume: 30 µL
- Incubation time: 18 hr
- 3.1 µL is an estimate of the average volume of liquid blood in a 3.2 mm punch of the DBS disc
- RRF (relative response factor) is assumed to be 1 for all calculations

The enzyme activities from 87 DBS samples from Rouen University Hospital, Department of Metabolic Biochemistry, were measured using the Orbitrap Exploris 120 MS and a QqQ MS system with a validated FIA-QqQ-MS/MS method. The Spearman correlation plots between the measurements were generated in R (version 4.0.0) using data exported from TraceFinder software.

## Results and discussion

The measurement of enzyme activities related to six monitored lysosomal diseases using the Revvity NeoLSD MSMS kit were verified on a Vanquish Flex UHPLC coupled to an Orbitrap Exploris 120 MS using a FIA-HRAM method. The inter- and intra-day imprecision measurements compared to the targeted values specified by the kit manufacturer are listed in Table 3. All the measured values were within the target range listed by NeoLSD – Control Dried Blood Spot (DBS) specification sheet, except for the slightly higher QC2 values obtained for IDUA inter-day imprecision. The observed discrepancy was likely caused by slight differences in ionization efficiency among MS instrumentations.

**Table 3. Inter- and intra-day imprecision results compared to the targeted values specified by the kit manufacturer (intra-day N = 3, inter-day N = 8)**

| Targeted enzymes | QC levels | Inter-day | | | Intra-day | | |
|---|---|---|---|---|---|---|---|
| | | Target activities | Measured activities | %RSD | Target activities | Measured activities | %RSD |
| GLA | QC-1 | 2.66 (1.76 - 3.56)[a] | 3.19 | 7.47 | 2.89 (0.93 - 4.85)[b] | 3.12 | 6.20 |
| | QC-2 | 4.51 (2.98 - 6.04)[a] | 5.24 | 3.92 | 3.82 (1.22 - 6.42)[c] | 4.08 | 7.40 |
| ABG | QC-1 | 2.72 (1.99 - 3.45)[a] | 3.04 | 12.23 | 2.94 (1.36 - 4.52)[b] | 2.21 | 8.60 |
| | QC-2 | 5.11 (3.73 - 6.49)[a] | 4.04 | 10.20 | 4.18 (1.92 - 6.44)[c] | 4.38 | 9.80 |
| ASM | QC-1 | 1.48 (1.08 - 1.88)[a] | 2.19 | 9.48 | 1.67 (0.77 - 2.57)[b] | 1.42 | 16.10 |
| | QC-2 | 2.80 (2.04 - 3.56)[a] | 2.77 | 12.72 | 2.34 (1.08 - 3.60)[c] | 2.13 | 12.60 |
| GALC | QC-1 | 1.51 (0.91 - 2.11)[a] | 2.03 | 4.11 | 1.38 (0.28 - 2.48)[b] | 2.03 | 7.30 |
| | QC-2 | 2.52 (1.66 - 3.38)[a] | 3.30 | 7.16 | 1.84 (0.60 - 3.08)[c] | 2.86 | 9.30 |
| IDUA | QC-1 | 3.50 (2.34 - 4.66)[a] | 4.51 | 9.33 | 3.71 (1.27 - 6.15)[b] | 3.29 | 10.10 |
| | QC-2 | 5.68 (4.09 - 7.27)[a] | 7.58* | 3.47 | 4.91 (2.31 - 7.51)[c] | 4.33 | 10.40 |
| GAA | QC-1 | 2.92 (2.10 - 3.74)[a] | 3.39 | 11.48 | 3.52 (1.54 - 5.50)[b] | 3.71 | 13.40 |
| | QC-2 | 4.78 (3.44 - 6.12)[a] | 5.20 | 7.33 | 4.64 (2.04 - 7.24)[c] | 4.33 | 14.80 |

a: lot number 752447
b: lot number C1692909
c: lot number C2692909
*: IDUA activity was slightly higher than the values specified by the kit manufacturer.

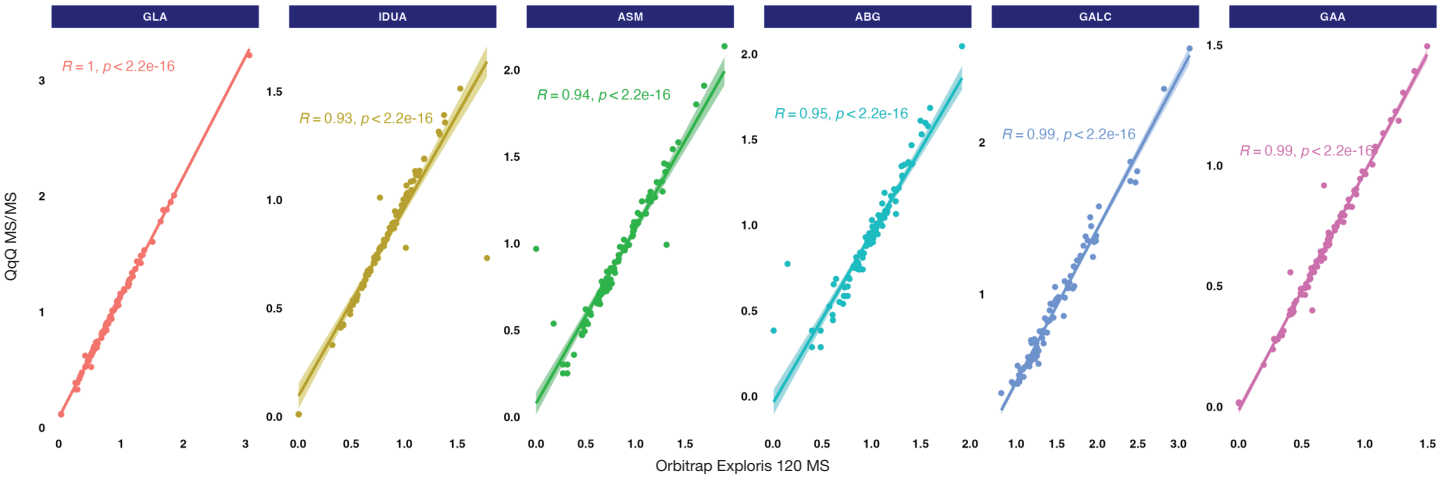


**Figure 1. Correlation of the enzyme activities from 87 DBS samples measured on the reported FIA-HRAM method using an Orbitrap Exploris 120 MS and a validated FIA-QqQ-MS/MS method**

All the measured levels were deemed acceptable since the %RSD of all compounds was below 15%, indicating the method was robust and reproducible.

The same 87 DBS samples were measured both with the reported FIA-HRAM method and a validated FIA-QqQ-MS/MS method. The Spearman correlation analysis results are shown in Figure 1. The correlation coefficient (R) is over 0.93 for all enzyme activities, indicating a very strong positive relationship between the two methods under consideration. The *p*-value associated with this correlation is substantially below the conventional threshold of 0.05, suggesting that the observed correlation is statistically significant.

## Conclusions

We developed a Vanquish Flex UHPLC and Orbitrap Exploris 120 MS-based FIA-HRAM method to verify the measurement of enzyme activities on DBS using a Revvity NeoLSD MSMS kit for six LDs. The method demonstrated good accuracy and precision. The measurement results are highly comparable to those obtained by a validated FIA-QqQ-MS/MS method. The Orbitrap MS provides high mass accuracy detection over the *m/z* ranges of common metabolites and offers full-$MS^2$ fragmentation spectra for enhanced analyte identification confidence. This technical brief highlights the versatility of using the Orbitrap MS to meet the needs for routine primary screening and the potential to discover novel biomarkers for treatable metabolic disorders in public health laboratories.

### References


1. Sun, A. Lysosomal storage diseases overview. *Ann. Transl. Med.*, **2018**, 476.
2. Gelb, M. H.; Lukacs, Z.; et.al. Newborn screening for lysosomal storage disorders: methodologies for measurement of enzymatic activities in dried blood spots. *Int. J. Neonatal Screen.* **2019**, 1.
3. Ducatez, F.; Plichet, T.; et. al. Quantitation of lysosphingolipids in dried blood spots and plasma using high-resolution mass spectrometry for sphingolipidoses. Thermo Fisher Scientific Technical Note 003143, **2024**.
4. Guo, J.; Song, Y.; Hassell, K. Enhanced disease detection in newborns: quantitation of amino acids and acylcarnitines in dried blood spots by FIA-Orbitrap mass spectrometry. Thermo Fisher Scientific Technical Note 002655, **2024**.


Learn more at **thermofisher.com/clinicalresearchapps**