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Comparison of FIA and on-column LC-MS/MS method to measure simultaneously ABG, ASM, GAA, GALC, GLA and IDUA activities

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Introduction

Lysosome is a membrane-enclosed organelle and contains hydrolytic enzymes. They can digest virtually all kinds of biomolecules. Some techniques such as fluorometric and spectrophotometric assays have been developed to measure these enzyme activities.
Multiplex flow injection analysis using tandem mass spectrometry (FIA-MS/MS) method has been developed that simultaneously measures the activities of the enzymes, alphaglucocerebrosidase (ABG), sphingomyelinase (ASM), alpha-glucosidase (GAA), galactocerebrosidase (GALC), alpha-galactosidase A (GLA) and alpha-iduronidase (IDUA). The use of mass spectrometric techniques has exhibited advantages over the other techniques in the simultaneous quantification of several targets. However, FIA-MS/MS method can exhibit background noise levels that lead to inaccurate data interpretation.
Here we compared and evaluated FIA LC-MS/MS method and on-column LC-MS/MS method to simultaneously measure these enzyme activities.

Methods and Materials

6-plex NeoLSD reagent (PerkinElmer Inc.) was used for this study. This kit contains assay buffer, substrates for 6 enzymes and internal standards. 3.2 mm diameter disks were punched from the DBSs and placed in a 96-well plate. Assays were run according to the procedure of the kit with overnight incubation (16-20 hours).

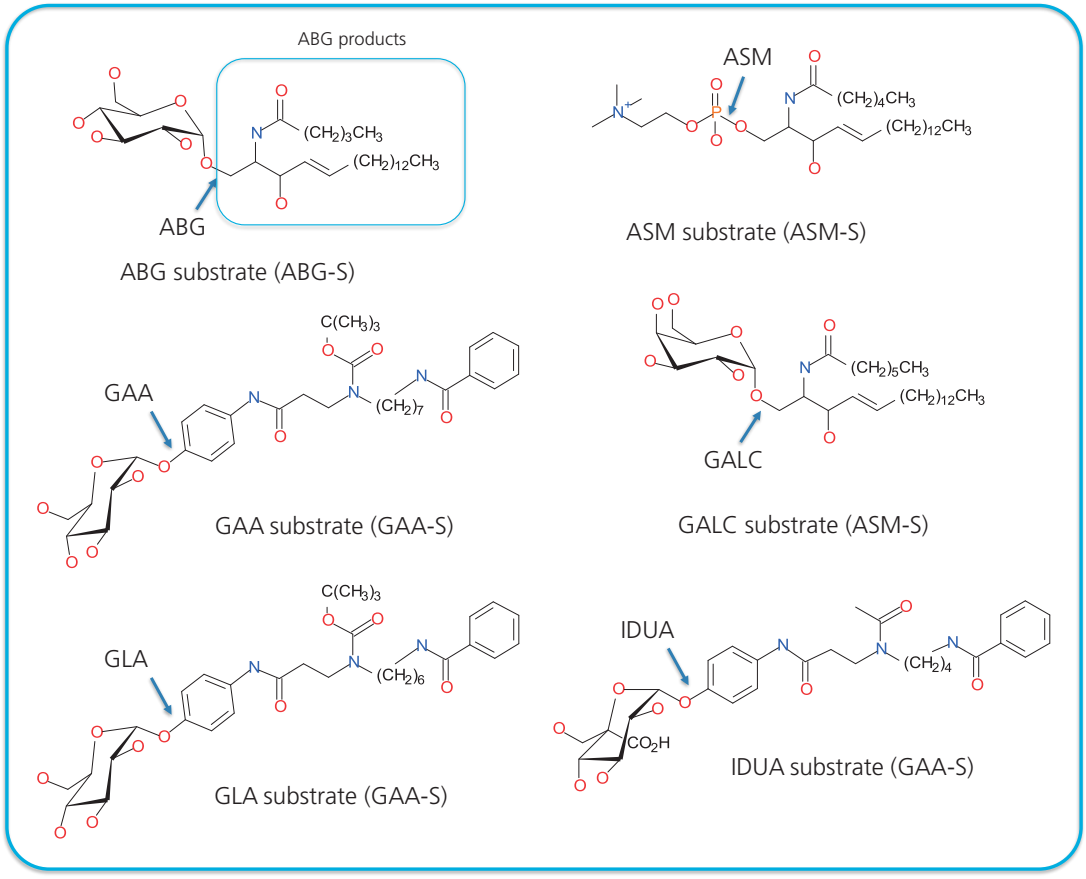

Figure 1 6 substrates for 6 enzymes; IS are deuterated forms of enzyme products

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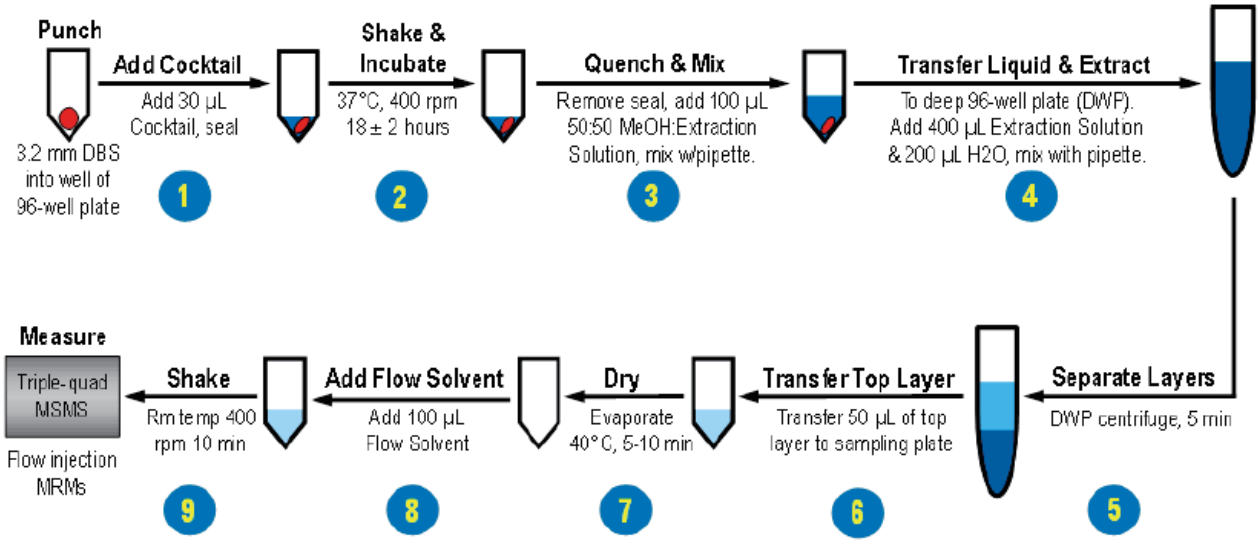

Figure 2 6-plex NeoLSD reagent workflow

Flow injection analysis (FIA) and on-column analysis were achieved using LC-MS/MS system; UHPLC was coupled to triple quadrupole mass spectrometer (Nexera with LCMS-8050, Shimadzu Corporation, Kyoto, Japan). The mobile phase consisted of (A) water + 0.1 % formic acid and (B) Acetonitrile + 0.1 % formic acid. LC-MS/MS with electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode.

Analytical Conditions

HPLC conditions (Nexera MP system)	
Flow Injection Analysis	
Mobile phase	: A: water + 0.1 % formic acid B: Acetonitrile + 0.1 % formic acid
Isocratic Flow	: B 84 %
Flow rate	: 0.3 mL/min
Injection volume	: 10 µL
On Column Analysis	
Mobile phase	: A: water + 0.1 % formic acid B: Acetonitrile + 0.1 % formic acid
Flow rate	: 0.2 mL/min
Injection volume	: 1 µL
Column	: GL Sciences MonoTower C18 100 mm (3.0 um x 50 mm x 2 pieces)
Time program	: 0 min. B 75 % > 2 min. B 75% > 3 min. B 90 % > 6 min. B 100 % 10 min. B 100 % > 10.1 min. B 75 %
MS conditions (LCMS-8050)	
Ionization ESI, Positive MRM mode	

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Table 3 MRM Transition

Compound	Precursor ion m/z	Product ion m/z	Compound	Precursor ion m/z	Product ion m/z
ABG-IS	391.4	271.3	GALC-IS	417.4	264.3
ABG-P*	384.3	264.3	GALC-P	412.4	264.3
ABG-S	546.4	264.2	GALC-S	574.5	264.25
ASM-IS	405.4	264.3	GLA-IS	489.3	389.3
ASM-P	398.4	264.3	GLA-P	489.3	384.2
ASM-S	563.4	184.1	GLA-S	646.4	546.25
GAA-IS	503.3	403.3	IDUA-IS	431.3	322.2
GAA-P	498.3	398.2	IDUA-P	426.2	317.2
GAA-S	660.4	560.25	IDUA-S	602.3	317.2

* P means product

Result

In Japan, when newborn screening of lysosomal storage disease using LC-MS/MS is implemented, it is necessary to perform validation of the analysis method. The FIA method has been developed as the analysis method of 6-plex using tandem mass spectrometer. We evaluated both analysis method of FIA and on-column.

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Results of FIA method

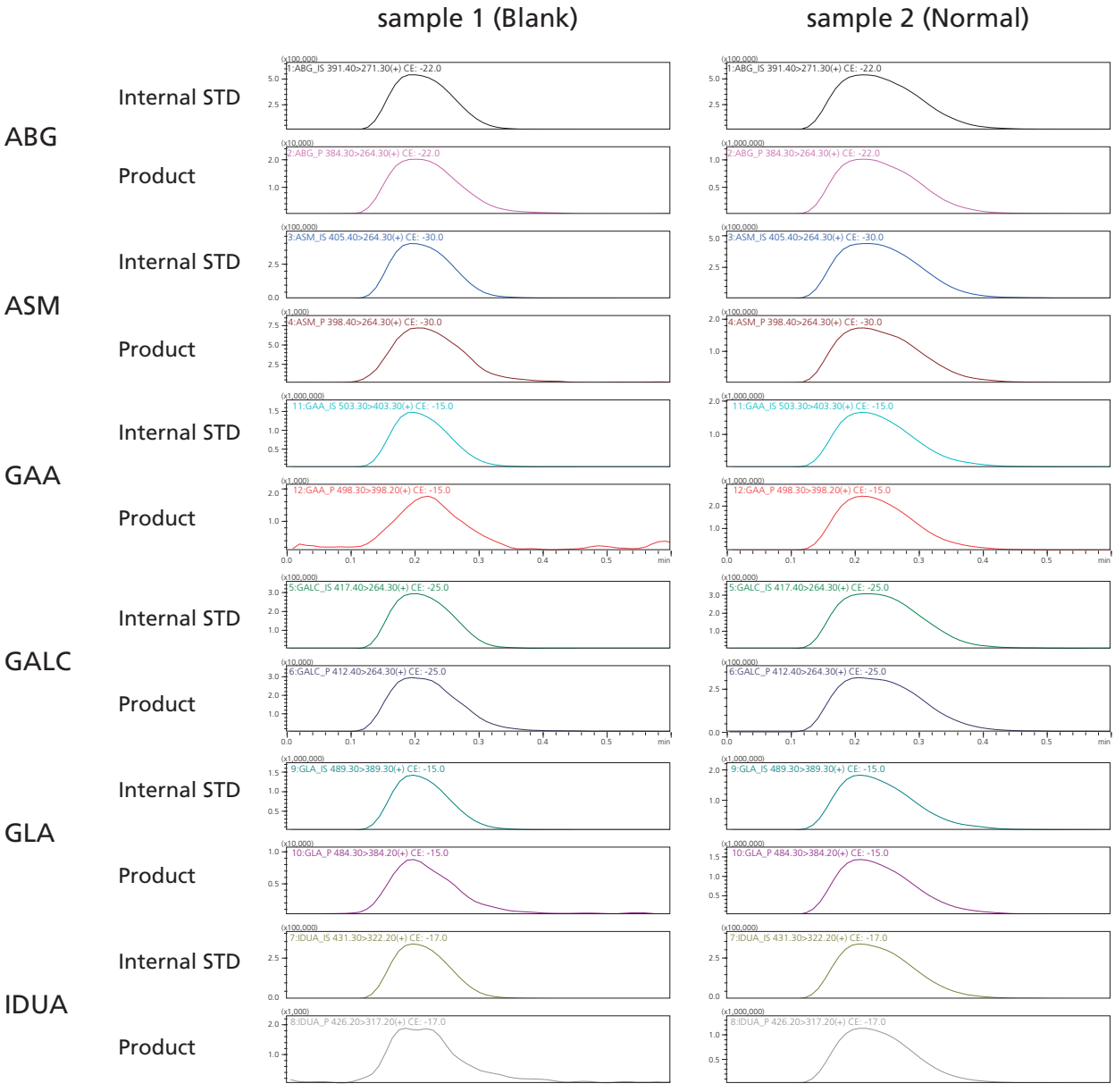

Figure 3 Results of FIA method; MRM chromatograms of each target compound
Sample #1 (Blank): sample containing no blood; Sample #2 (Normal): Sample containing enzymes with all activities

All peaks were clearly detected for enzyme decomposition products in Sample #1 and repeatability of measurement was good (data not shown). These results were indicated to be able to distinguish sample #1 and sample #2 in all enzyme activity data. However, all data in sample #1, especially ABG, GALC, GLA, showed significant peaks in their product chromatograms.

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Result of on-column method

Then, an analysis using on-column separation was used to confirm the reason of the peak in product MRMs of the blank sample. In this analysis, 3 MRMs, Internal STD, Product and Substrate, were monitored. The result are shown in Figure 4.

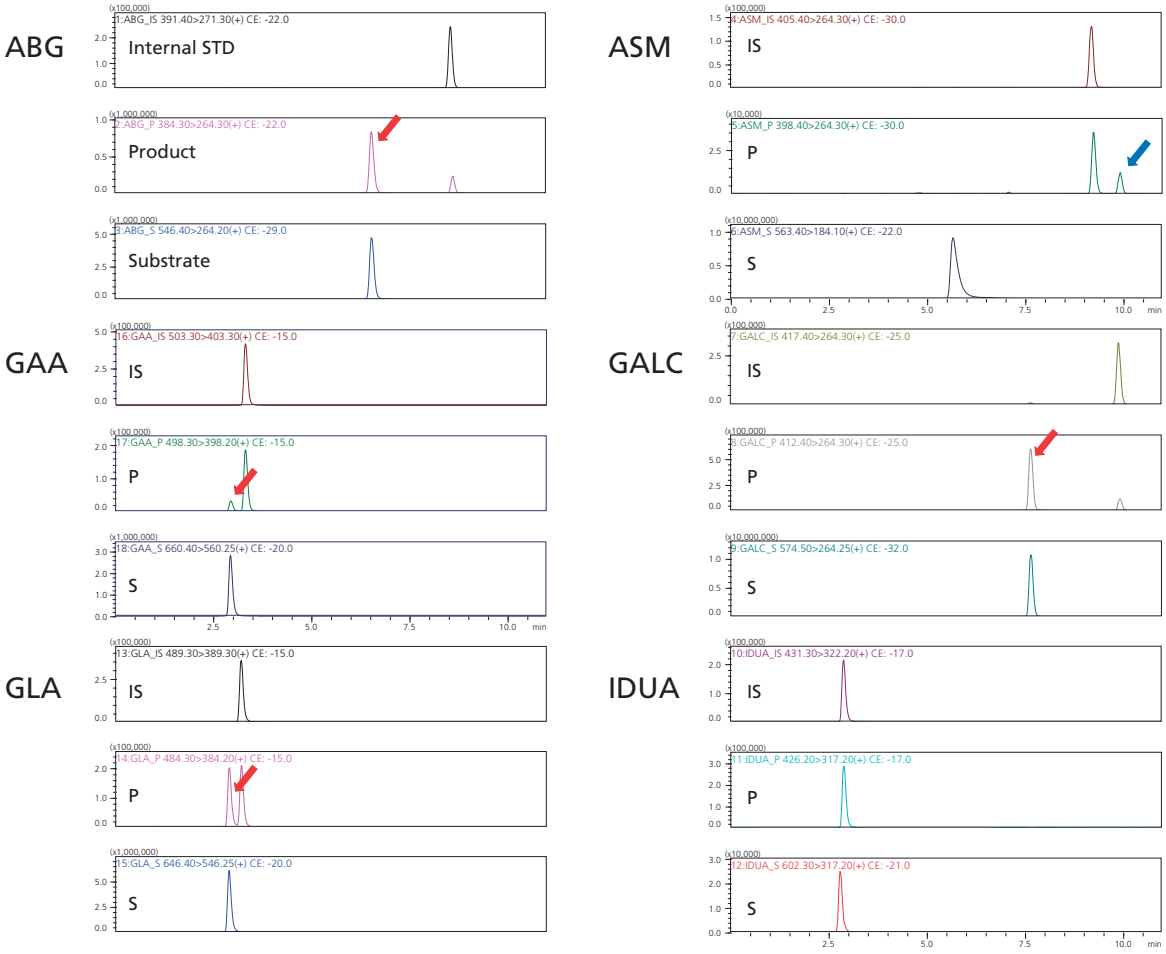

Figure 4 Results of on-column method; MRM chromatograms of each target compound
Sample #2 (Normal) was analyzed.

In MRM chromatograms of ABG, GAA, GALC and GLA products, interfering peaks at the same retention time as those of substrates were found (red arrow in Figure 4). This peaks were probably produced by in-source fragmentation of the substrates. Since these peaks could not be chromatographically separated from enzymatic reaction products in FIA method, they were creating a peak detected even without enzyme activity.
In the case of ASM, the interfering peak observed (blue arrow) is probably due to in-source fragmentation of GALC IS or GALC product.

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Conclusions

Measurement of six enzymatic activities in DBS (6-plex assay) by FIA and on column method was conducted by LCMS-8050 system.
FIA method had higher throughput than with column separation, but it is expected to be less precise and accurate.

Acknowledgement

Poster presenters thank Dr. Osamu Ohara and Dr. Kazuhiro Sato (Kazusa DNA Research Institute) for measurements and data analysis.

Disclaimer: Shimadzu LCMS-8050 and Nexera MP system are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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