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MP 083

A rapid LC-MS/MS method to measure simultaneously IDUA, IDS, NAGLU, GALNS and ASRB enzymes activities in dried blood spots

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Overview

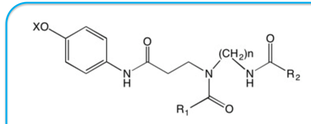
A novel and rapid LC-MS/MS method was developed to measure simultaneously the activities of 5 lysosomal enzymes simultaneously for newborn screening.

1. Introduction

Mucopolysaccharidoses (MPSs) is a group in lysosomal storage disorders (LSDs) caused by a deficiency of lysosomal hydrolases responsible for the catabolism of glycosaminoglycans (GAGs). Some techniques such as fluorometric and mass spectrometric assays have been developed to measure these enzyme activities for the purpose of newborn screening. The use of mass spectrometric techniques has exhibited advantages over the other techniques in the ability to multiplex several enzymes in one assay. In this study, we will present a novel method using tandem mass spectrometry that is capable to measure simultaneously the activities of the five MPS enzymes IDUA (MPS I), IDS (MPS II), NAGLU (MPS IIIB), GALNS (MPS IVA) and ARSB (MPS VI) in a short time scale. In the presentation, this developed method is detailed.

2. Methods and Materials

Cocktail of 5 enzyme substrates, and internal standards and quality control (QC) dried-blood sample (DBS) were purchased from PerkinElmer Inc. A disc (3 mm) was punched from each DBS sample and placed into a 96-well plate. Assay cocktail was added to each well, and the whole plate was shaken at 37ºC for 16 hours. Thereafter, quenching of the enzyme assay was followed by liquid-liquid extraction for purification.



Compound	X	R1	R2	n
MPS-I Substrate	α-Iduronosyl	Methyl	Phenyl	6
MPS-I Product	H	Methyl	Phenyl	6
MPS-II-Substrate	α-Iduronosyl-2-sulfate	n-Butyl	Phenyl	6
MPS-II Product	α-Iduronosy	n-Butyl	Phenyl	6
MPS-IIIB-Substrate	α-N-Acetyl-glucosyl	n-Butyl	Ethyl	6
MPS-IIIB Product	H	n-Butyl	Ethyl	6
MPS-IVA-Substrate	β-N-Acetyl-galactosyl-6-sulfate	n-Penty	Phenyl	6
MPS-IVA Product	β-N-Acetyl-galactosyl	n-Penty	Phenyl	6
MPS-VI-Substrate	β-N-Acetyl-galactosyl-4-sulfate	n-Butyl	Phenyl	5
MPS-VI Product	β-N-Acetyl-galactosyl	n-Butyl	Phenyl	5

Figure 1 5 substrates for 5 enzymes; IS are deuterated forms of enzyme products

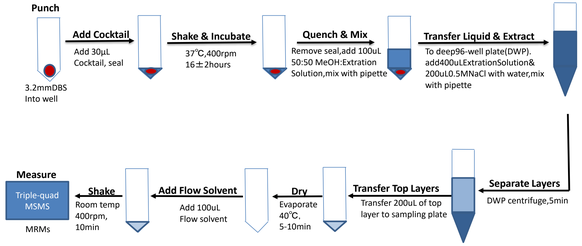


Figure 2 5-plex NeoLSD reagent workflow

On-column analysis was performed using an LC-MS/MS system consisting of UHPLC with a triple quadrupole mass spectrometer (Nexera™ with LCMS-8050, Shimadzu Corporation, Kyoto, Japan). The mobile phases used were (A) 0.1 % formic acid in water and (B) 0.1 % formic acid in acetonitrile. LC-MS/MS with electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode.

Analytical Conditions

HPLC conditions (Nexera™ MP system)

On Column Analysis

Mobile phase A : Water + 0.1 % formic acid
Mobile phase B : Acetonitrile + 0.1 % formic acid
Flow rate : 0.4 mL/min

- 6 minutes method
Injection volume : 1 µL
Column : Phenomenex Kinetex XB-C18 150 mm x 2.1 mm, 1.7 µm
Time program : 0 min. B 30% > 0.5 min. B 30 % > 3.5 min. B 100 % > 5.0 min. B 100 % > 5.01 min. B 30 %> 6.0 min. B 30 %

- 2.5 minutes method
Injection volume : 0.2 µL
Column : Phenomenex Kinetex XB-C18 30 mm x 2.1 mm, 1.7 µm
Time program : 0 min. B 30% > 0.1 min. B 30 % > 1.3 min. B 100 % > 1.5 min. B 100 % > 1.51 min. B 30 %> 2.5 min. B 30 %

MS conditions (LCMS-8050)

Ionization ESI, Positive MRM mode

Table 1 MRM Transitions

Compound	Precursor ion *m/z*	Product ion *m/z*	Compound	Precursor ion *m/z*	Product ion *m/z*
MPSI-IS	431.3	322.3	MPSIIIB-P	420.3	311.4
MPSI-P*	426.3	317.3	MPSIVA-IS	690.4	378.2
MPSII-IS	649.4	364.4	MPSIVA-P	685.4	373.2
MPSII-P	644.4	359.4	MPSVI-IS	662.4	350.4
MPSIIIB-IS	423.3	314.4	MPSVI-P	657.4	345.4

* P means product

3. Results and Discussions

This presentation is the result of the project of AMED (Japan Agency for Medical Research and Development). In this study, we developed a method for simultaneous analysis of five enzyme activities (5-plex) as MPS-I, MPS-II, MPS-IIIB, MPS-IVA, and MPS-VI using LC-MS/MS. The method requires up to 6 minutes per sample on this LCMS-8050 with an Nexera MP HPLC system.

In the flow injection method, MRM product peaks derived from in-source breakdown of substrates are observed, especially, IDUA (alpha-L-iduronidase) for MPS-I and IDS (Iduronate-2-sulfatase) for MPS-II. As UHPLC led to the full separation of enzymatic product and substrate peaks, this in-source breakdown was of no concern. Although this method requires 6 minutes cycle now, the method has the potential to be shortened as Liu Y et al. reported [Liu Y et al., Clin Chem. 2017, 63(6):1118-1126]. We evaluated the possibility to shorten the analysis cycle to 2.5 minutes to make it more suitable for newborn screening.

3-1. On-column analysis

In each MRM chromatogram of products, an interfering peak from each of substrate at different retention time was observed (red arrow in Figure 3). Those peaks were considered as the substrates caused by in-source decay from the each product.
In flow injection analysis (FIA), the signal is detected even without enzyme activity since substrate peaks could not be chromatographically separated from enzymatic reaction products. On column analysis showed that the product and IS were detected at the same retention time and products were separated from each substrate for all of MPS. The result from the control dried-spot (high activity) with 6min and blank with 2.5 min method are shown in Figure 3.

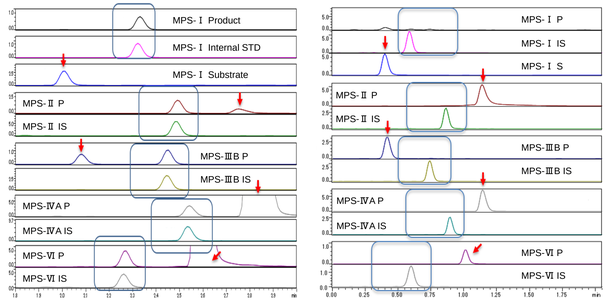


Figure 3 MRM chromatograms of each target compound with on-column analysis. Left: High-activity QC, 6 minutes method; Right: Blank, 2.5 minutes method

3-2. Results of enzyme activity evaluation

The enzyme activities for DBS from 312 healthy newborns and quality control (QC) samples are shown below. The vertical axis shows enzyme activity, and the horizontal axis shows sample groups. Enzyme activity at the base level considered to be equivalent to the patient could be distinguished from enzyme activity at the normal level.

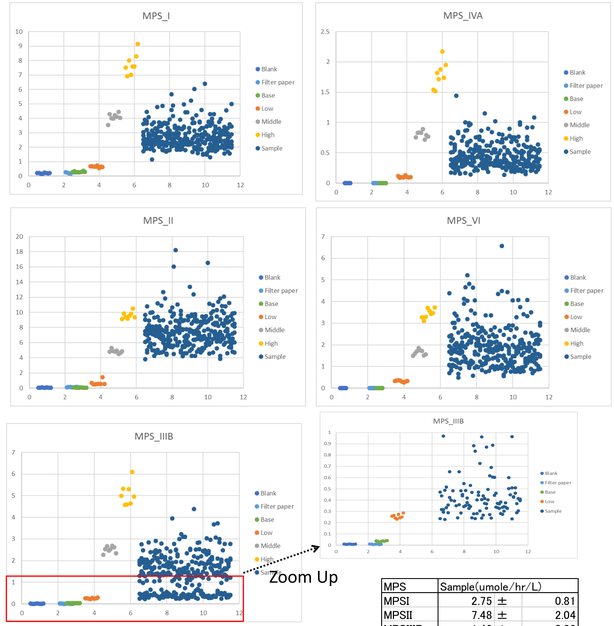


Figure 4 Results of enzyme activity with 6 min method

MPS	Sample(umole/hr/L)	
MPSI	2.75 ±	0.81
MPSII	7.48 ±	2.04
MPSIIIB	1.42 ±	0.83
MPSIVA	0.45 ±	0.21
MPSVI	1.89 ±	0.91

n= 312

4. Conclusions

- ✓ Measurement of five enzyme activities in DBS (5-plex assay) by the on column method was conducted by LCMS-8050 system.
- ✓ Our method had throughput capability within 6 minutes analytical cycle and also suggested that method could be shortened to 2.5 minutes, which is suitable for NBS.