SHIMADZU
Excellence in Science

PO-CON1803E

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

ASMS 2018 WP 132

Misa Tanaka[1], Hironori Kobayashi[2], Tsubasa Ooguni[2], Jun Watanabe[3], Manami Kobayashi[1], Junichi Masuda[1], Michael H. Gelb[4]
[1] Shimadzu Corporation, Kanagawa, Japan,
[2] Department of Pediatrics, Shimane University Faculty of Medicine, Shimane, Japan,
[3] Shimadzu Corporation, Kyoto, Japan,
[4] Depts. of Chemistry and Biochemistry, Univ. of Washington Seattle, WA

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

Overview

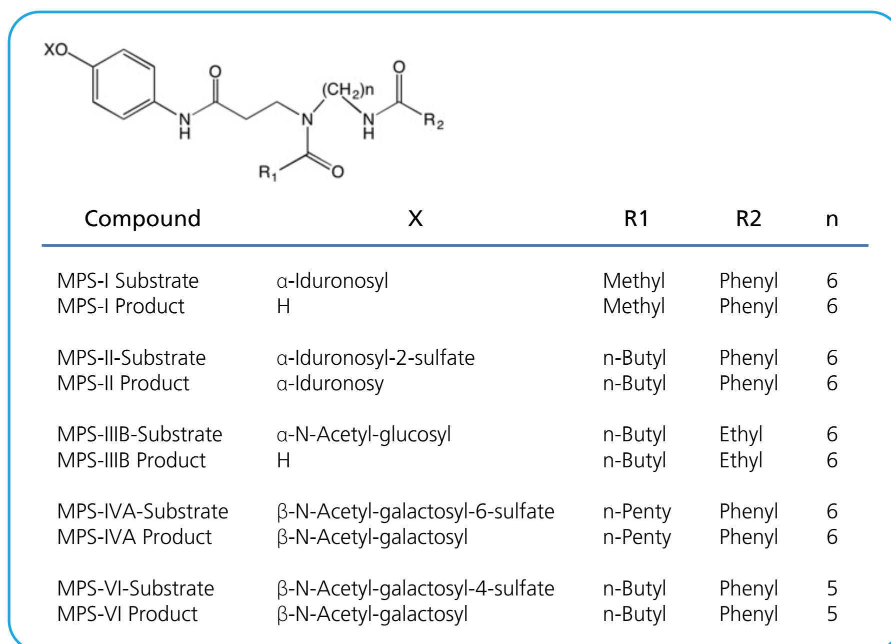
A novel and rapid LC-MS/MS method was developed to measure the activities of 5 lysosomal enzymes simultaneously for newborn screening.

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 of simultaneous measurement of 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 using LC-MS/MS is demonstrated for measuring these enzyme activities in a few minutes cycle.

Methods and Materials

Cocktail of substrates of 5 enzymes 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 37°C 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

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

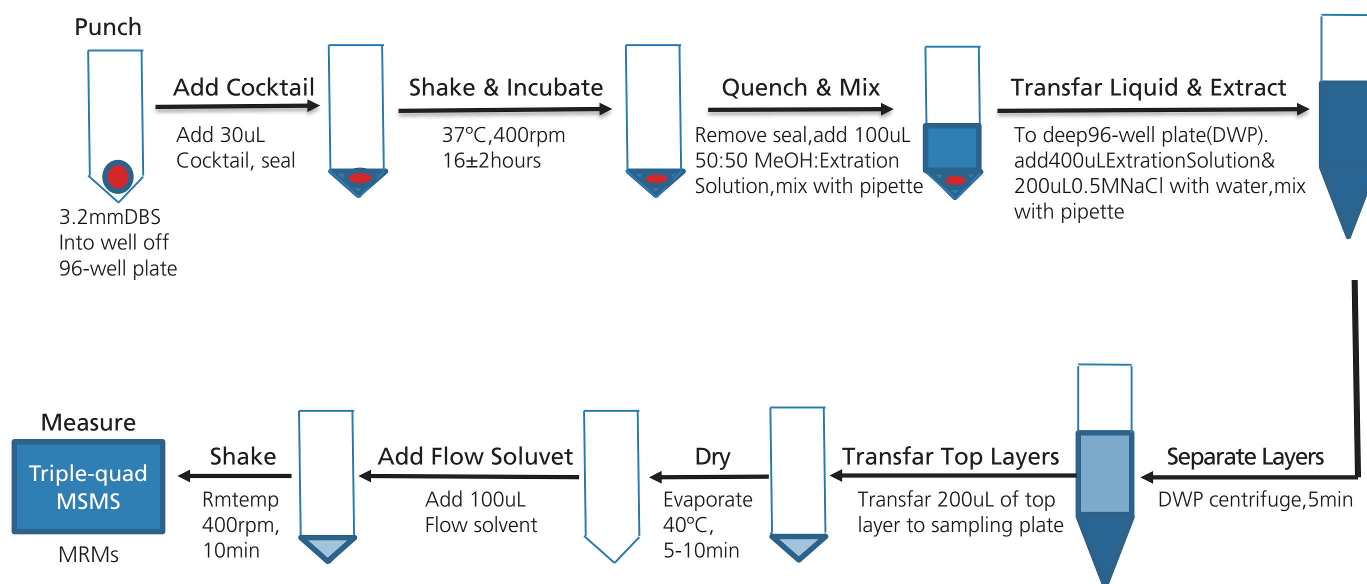


Figure 2 5-plex NeoLSD reagent workflow

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
Injection volume	: 1 µL
Column	: Penomenex Kinetex XB-C18 150 mm x 2.1 mm, 1.7 µm
Time program	: 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 %
MS conditions (LCMS-8050)	
Ionization ESI, Positive MRM mode	

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

Table 1 MRM Transition

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

* P means product

Results and Discussions

In this study, we developed a method for simultaneously 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.
For 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 UPLC led to the full separation of enzymatic product and substrate peaks, this in-source breakdown was of no concern since only the product peak was integrated.
Although this method requires 6 minutes cycle now, the method has the potential for the shorten analytical cycle as Liu Y et al. reported.[1] We are now trying to shorten analytical cycle around 2 minutes suitable for newborn screening.

(1) Liu Y et al., Clin Chem. 2017, 63(6):1118-1126

On-column analysis

In each MRM chromatogram of product, 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.
For a flow injection analysis (FIA), the signal is detected even without enzyme activity since the peaks of the substrates 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 with high activity are shown in Figure 3.

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

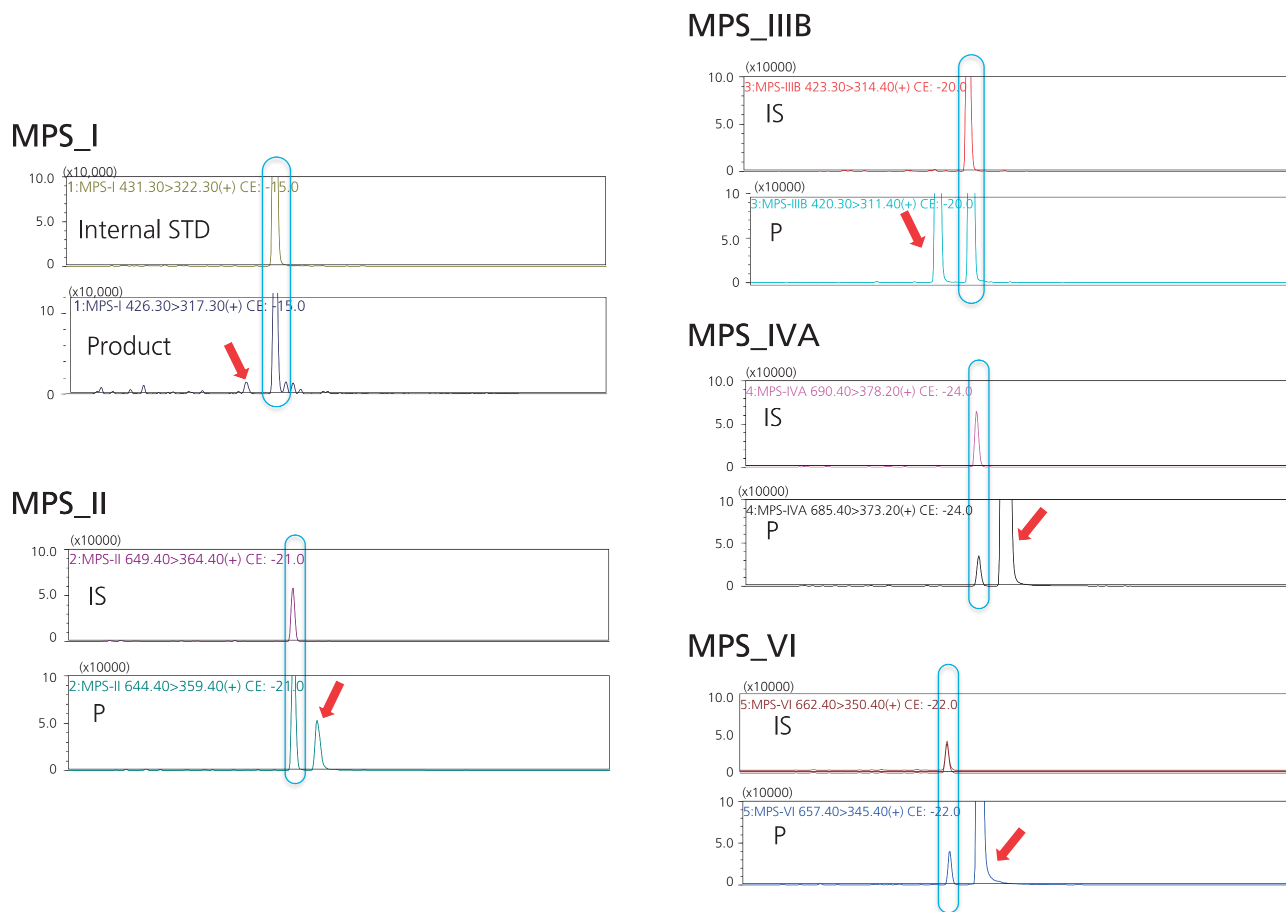


Figure 3 Results of on column method; MRM chromatograms of each target compound

Results of enzyme activity evaluation

The enzyme activities for DBS from 312 healthy newborns and quality control (QC) samples are shown below.

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

* Sample:312

Figure 4 Results of enzyme activity

Conclusions

- Measurement of five enzyme activities in DBS (5-plex assay) by the on column method was conducted by LCMS-8050 system.
- Challenging: Our method had throughput capability within 6 minutes analytical cycle, we are trying to be shorten the analytical time within 2 minutes suitable for NBS.

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

First Edition: June, 2018

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/

For Research Use Only. Not for use in diagnostic procedures.
This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Company names, products/service names and logos used in this publication are trademarks and trade names of Shimadzu Corporation, its subsidiaries or its affiliates, whether or not they are used with trademark symbol "TM" or "®".
Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services, whether or not they are used with trademark symbol "TM" or "®".
Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2018