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Application News

Liquid Chromatograph-Mass Spectrometer LCMS-8045

High Throughput Testing Method with Newborn's DBS for Hemoglobinopathies by LCMS-8045 with ZenTech Targeted MS/MS Hemo Device

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User Benefits

- End-to-end solution provided by Shimadzu and ZenTech
- High throughput, sensitive & robust methodology for hemoglobinopathies using newborn's dried blood spots (DBS)
- Analysed in MRM-mode targeting the unique peptides of hemoglobin variants.

■ Introduction

Hemoglobin

Hemoglobin (Hb) (Fig. 1) is a tetramer composed of two α-globin and two non-α-globin chains working in conjunction with heme to transport oxygen in the blood. Normal adult hemoglobin (HbA) is designated αA2βA. Hemoglobin Variant is derived from gene abnormalities affecting the α-globin genes (HBA1 or HBA2) or β-globin (HBB) structural genes (exons). More than a thousand hemoglobin variants have been identified relative to changes in the globin chains. Qualitative changes correspond to amino acid substitutions resulting in hemoglobinopathies. [1,2,3]

Hemoglobinopathies

Hemoglobinopathies are genetic defects that result in one of the chains of the hemoglobin molecule. These are usually single-gene disorders, such as sickle cell disease, and they are usually inherited as autosomal codominant traits. To date, over 1000 hemoglobinopathies have been found but fortunately, only a few have clinical significance. Although there is a formal system for naming hemoglobinopathies, most hemoglobinopathies are known by a letter or identifier from the location where the hemoglobin variant was found or a combination of these. [4] It is important to be able to structurally identify and quantitate these variants in order to detect abnormal hemoglobin quickly and precisely. The conventional technologies have reported false (positive/negative) results as they are very sensitive to pH and a minute deviation impact the results.

This application highlights high throughput, sensitive and reproducible method for hemoglobinopathies using Shimadzu LCMS-8045 (Fig. 2) with Targeted MS/MS Hemo device (ZenTech s.a., Avenue du Pré-Aily 10, 4031 Angleur, Belgium) (Fig. 3).

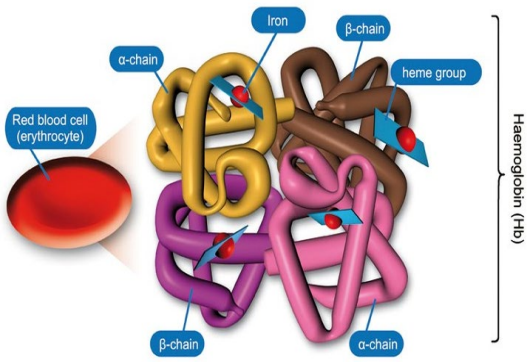

Fig. 1 Structure of Normal Hemoglobin

Liquid chromatography mass spectrometer (LC-MS/MS) methods with high throughput, high sensitivity and specificity have been developed for analysis of Hb variants. The application is capable of analysing α-thalassemia, β-thalassemia and Hb variants like HbS, HbC, HbE, HbO^{Arab}, HbD^{Punjab} having fast turnaround time required for large numbers of samples. The techniques have produced accurate analysis that would have been missed by the traditional methods.

Fig. 2 Nexera™ X3 with LCMS-8045

Fig. 3 Targeted MS/MS Hemo device

■ Experimental

Sample preparation

The LCMS-8045 triple quadrupole mass spectrometer was coupled to a Nexera X3 UHPLC system (Fig. 2). The detection of normal and variant Hb was performed using the commercially available Targeted MS/MS Hemo device kit. Prior to the LC-MS/MS analysis, the Hb is extracted and denatured from dried blood spots samples before being digested by trypsin. The flow of sample preparation is described (Fig. 4). Controls are provided in the device to ensure the proper course of extraction, denaturation and digestion steps as well as the correct functioning of the mass spectrometer for all peptides from normal and variant Hb. The samples were analysed in MRM-mode. Analytical conditions are listed in Table1. The optimized MRM transitions are listed in Table 2.

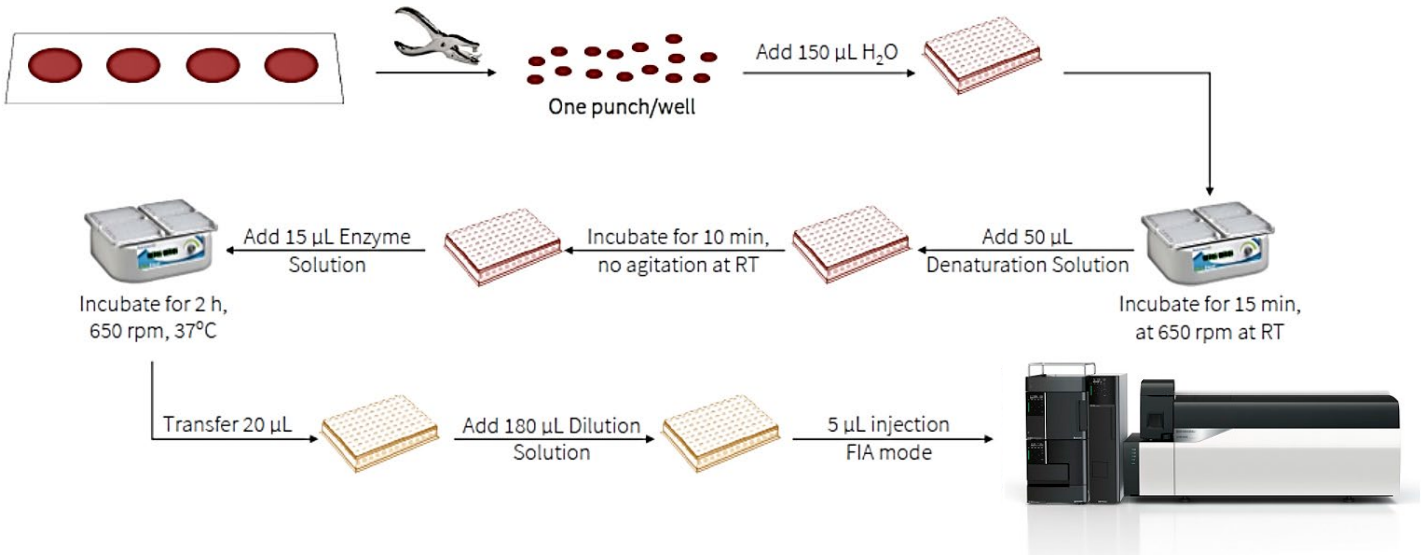

Fig. 4 Flow of sample preparation

Table 1 Analytical Conditions

Mass Spectrometer	: LCMS-8045
Ionisation	: Electrospray Ionization (ESI), positive
Interface Voltage	: 3.5 kV
Desolvation Line	: 200 ℃
Heating Gas	: 15 L/min
Interface Temp.	: 400 ℃
Nebulizing Gas	: 3 L/min
Drying Gas	: 5 L/min
Heat Block	: 200 ℃
CID	: 270 kPa
UHPLC	: Nexera X3
Pump A	: 0.1% Formic acid in Water
Pump B	: 0.1% Formic acid in Acetonitrile
Column Oven	: RT
Injection Volume	: 5 µL
Analytical column	: None
Gradient	: Isocratic 50% A / 50% B
Flow program	: 0.0 min 0.4 mL/min
	0.5 min 0.4 mL/min
	0.6 min 0.6 mL/min
	0.9 min 0.6 mL/min
	1.0 min 0.4 mL/min
Autosampler temperature	: 5 ℃

Table 2 MRM Transitions

Target compound	Parent ion (m/z)	Product ion 1 (m/z)	Product ion 2 (m/z)
α-peptide 1	536.5	446.2	680.3
α-peptide 2	627.0	233.3	261.2
β-peptide 1	477.0	237.2	502.2
β-peptide 2	658.0	313.3	758.3
β-peptide 3	690.0	378.3	501.3
β-peptide S	462.0	237.2	472.1
β-peptide C	694.5	237.1	244.2
β-peptide E	459.0	214.2	360.2
β-peptide O^{Arab}	625.6	249.0	501.1
β-peptide D^{Punjab}	689.6	377.1	276.1
γ-peptide 1	550.0	251.3	634.2
δ-peptide 1	480.3	390.3	688.4

Table 3 Sample Status with the Targeted MS/MS Hemo Device

Expected Phenotype	Result Obtained	Number of Samples
Normal	Normal	508
HbS Carrier	HbS Carrier	5
HbE Carrier	HbE Carrier	1
HbD^{Punjab} Carrier	HbD^{Punjab} Carrier	1

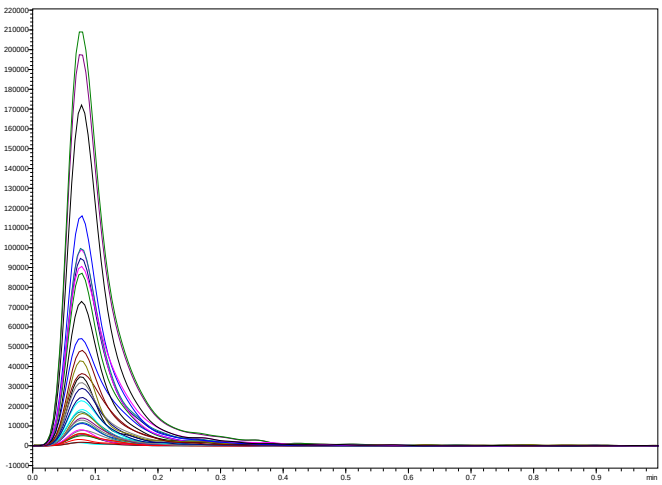

Fig. 5 Example of Chromatogram

■ Results

Using the Targeted MS/MS Hemo device from ZenTech, the newborn screening of hemoglobinopathies can be performed quite easily and very fast, in less than one minute, showing a good peak shape (Fig. 5).
All the dried blood spots samples analysed came from routine newborn screening labs. The status of these DBS are known, and their clinical status has been successfully corelated and assigned (Table 3).

■ Conclusion

Similar application note is available on LCMS-8050 by Shimadzu Europe but this application note was developed to showcase that Targeted MS/MS Hemo device from ZenTech can be very well reproduced on Shimadzu LCMS-8045 for testing newborn DBS of hemoglobinopathies.
Needless to say, that ease of sample preparation, rugged technology, ambiguous outcome and overall performance, will appeal to laboratories.

■ References

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