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Application News

Liquid Chromatograph Nexera X3 - Mass Spectrometer LCMS-8050

Hemoglobinopathies Newborn Screening by LCMS-8050 using ZenTech Targeted MS/MS Hemo device

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User Benefits

- Full solution provided by Shimadzu and ZenTech
- Sensitive and robust methodology for routine analysis
- Multiplexed analysis for the detection of all types of hemoglobinopathies

■ Introduction

Hemoglobin (Hb) is a tetrameric protein in red blood cells which transports oxygen in the blood. It is composed of four subunits, called globins (α, β, γ or δ). The normal adult form (HbA) is designated as $\alpha_2\beta_2$ and the foetal form (HbF) $\alpha_2\gamma_2$.[1,2]

Haemoglobinopathy is a kind of genetic defect that affects either the rate of synthesis (quantitative change, i.e. thalassaemia) or the structure of a globin (qualitative change, i.e. globin variants). More than 1,000 mutations have been identified but the clinically significant ones include α- and β-thalassaemias, sickle cell disease (SCD) and HbC disease.[3,4] In 2008, the World Health Organization established that at least 5.2% of the world's population carry a significant haemoglobinopathy-causing gene and that there are annually over 332,000 affected conceptions or births, mainly with SCD (82.8%) and thalassaemias (16.9%).[5] These diseases are prevalent in malaria-endemic regions (the Mediterranean Basin, Asia, and sub-Saharan Africa) but become increasingly prevalent in nonendemic regions such as Europe, America or Australia.[4]

Thalassaemias

In α-thalassaemias, aggregation of Hb Bart's (γ_4) and HbH (β_4) are found due to the lack of α globin. The minor α-thalassaemias (deletion of one or two out of four α genes) are respectively harmless or well tolerated. The symptoms of the intermediate form (three α genes deleted), also called HbH disease, include anaemia, splenomegaly and jaundice. The major form (deletion of the 4 α genes) is associated to Hb Bart's hydrops fetalis, leading in vast majority to the death in utero or during the first days of life.[1,6]

In β-thalassaemias, the production of HbF and HbA_2 ($\alpha_2\delta_2$) is increased. The β-thalassaemia trait (mutation of one out of two β genes) is asymptomatic while the homozygous form is associated with severe anaemia, bones deformation, splenomegaly and leads to death within 3 to 4 years if left untreated.[1,4]

Globin variants

The globin variants consist of one amino acid mutation in the protein sequence. Those of clinical importance are mainly found in β globin and are named according to their final protein sequence: HbS, HbE, HbC, and in a lesser extent, HbO^{Arab} and $HbD^{Punjab/Los\ Angeles}$.

Homozygosity HbS/S results in SCD, associated with severe life-threatening vaso-occlusive crises, overwhelming sepsis, splenic sequestration, aplastic crises, stroke, priapism, pulmonary hypertension, proliferative retinopathy and chronic organ damage, such as a vascular necrosis of the hips and shoulders.[1,5]

Compounds heterozygosity – i.e. HbS/E, HbS/C, HbS/O^{Arab}, HbS/D^{Punjab} and HbS/β-thalassaemia – is commonly associated with a less severe phenotype of SCD while the carriers of sickle cell trait (one sickle cell gene and one normal gene) are asymptomatic.[7,8]

The HbC trait is also phenotypically normal whereas homozygosity HbC/C gives rise to HbC disease (moderate haemolytic anaemia and mild splenomegaly). However, the compound heterozygosity HbC/β-thalassaemia may worsen the condition.[1]

Homozygosity HbE/E, called HbE disease, typically does not cause any symptoms. The HbE trait is also asymptomatic but the compound heterozygosity HbE/β-thalassaemia may lead to severe anaemia.[9]

Fig. 1 LCMS-8050 coupled to a Nexera UHPLC system

■ Materials and Methods

The LCMS-8050 triple quadrupole mass spectrometer was coupled to a Nexera X3 UHPC system (Figure 1). The detection of normal and variant Hb was performed using the commercially available Targeted MS/MS Hemo device (ZenTech s.a., Avenue du Pré-Aily 10, 4031 Angleur, Belgium). Prior to the LC-MS/MS analysis, the Hb is extracted and denatured from dried blood spots samples before being digested by trypsin. 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 Table 1. The optimized MRM transitions are listed in Table 2.

Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8050
Ionization	: Electrospray Ionization (ESI), positive
Interface Voltage	: 3.5 kV
Desolvation Line	: 200 °C
Heating Gas	: 15 L/min
Interface Temp.	: 400 °C
Nebulizing Gas	: 3 L/min
Drying Gas	: 5 L/min
Heat Block	: 200 °C
Dwell-/Pause-time	: 10 / 3 msec
CID	: 270 kPa

Table 1 Analytical conditions (continued)

UHPLC	: Nexera X3	
Pump A	: H_2O + 0.1% formic acid	
Pump B	: Acetonitrile + 0.1% formic acid	
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
Cooler temperature	: 10 °C	

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	237.3	472.1
β -peptide C	694.5	237.2	244.3
β -peptide E	459.0	214.0	360.4
β -peptide O^{Arab}	625.6	249.3	501.2
β -peptide D^{Punjab}	689.6	276.2	377.2
γ -peptide 1	550.0	251.1	634.2
δ -peptide 1	480.3	390.3	688.2

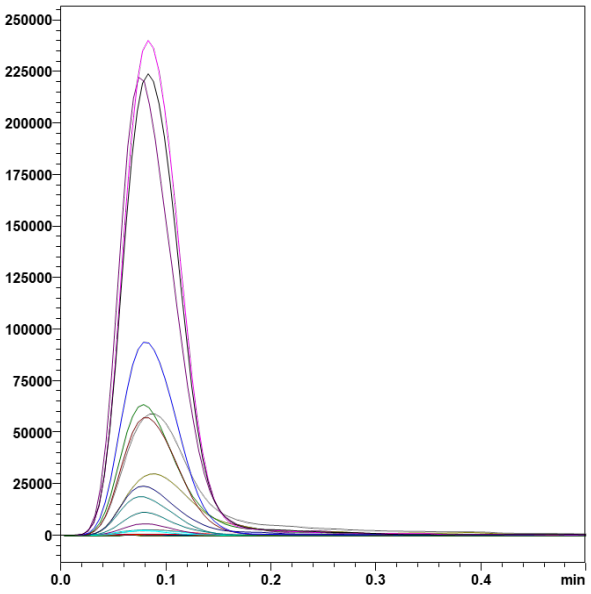

Fig. 2 Exemplary chromatogram

■ 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 (Figure 2).

Among all the samples analysed, the dried blood spots came from routine newborn screening or the Newborn Sickle Screening Surveys of UK National External Quality Assessment (UK NEQAS). Their clinical status has been successfully assigned (Table 3).

Table 3 Sample assignment with the Targeted MS/MS Hemo device

Expected Phenotype	Origin		Number of samples
	Routine	UK NEQAS	
No Pathology Detected*	X	X	79
Sickle Cell Disease	X		1
HbC Carrier	X	X	5
HbS Carrier	X	X	10
HbD^{Punjab} Carrier	X		1

*the α-thalassaemia silent or trait cannot be excluded, as well as for the β-thalassaemia trait

■ Conclusions

The application of the Targeted MS/MS Hemo device from ZenTech proved to be easy to implement and showed a trueness of 100% for all the samples analysed. This method could therefore be a technique of choice for newborn screening for hemoglobinopathies.

■ References

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