Rapid Analysis for the Diagnosis of MSUD

Aurélie Lolia and Sally Bee
Biochrom Ltd, Cambridge, UK

Using the Biochrom 30 Amino Acid Analyser, the levels of the branched amino acids, leucine, isoleucine, and valine can be analysed for screening of the metabolic disorder MSUD.

Maple Syrup Urine Disease (MSUD) is an inherited metabolic disorder that, if untreated, causes mental retardation, physical disabilities and death. The disorder affects the way the body metabolizes (processes) certain components of protein. These components are the three branched-chain amino acids leucine, isoleucine, and valine. Testing for MSUD is included in many of the newborn screening programes. Each infant should be tested within 24 hours of birth and the test results available by two to three days of age, highlighting the importance for rapid turnaround of analysis.

A shortened program developed on the Biochrom 30 Amino Analyser allows the separation and quantification of the branched chain amino acids valine, isoleucine, and leucine using norleucine as the internal standard. In addition L-alloisoleucine, which has been identified as the most specific and most sensitive diagnostic marker for all forms of MSUD when present in plasma above the cut-off value of 5 µmol/L (1), can be well resolved and quantified.

The extended program also enables the determination of phenylalanine, tyrosine and methionine thus allowing the simultaneous screening for other common metabolic diseases PKU (Phenylketonuria), tyronisis and homocystinuria.

The separation is achieved using a 20 cm x 4.6 mm physiological high performance column using predominantly buffer CII. The program enables between 17 and 22 analyses to be performed a day depending on the amino acids required.

The Biochrom 30 Amino acid analyser is proven to be a useful tool for clinical diagnosis and monitoring of aminoacidopathies.

References:

1) Schadewelt et al., (1999) Significance of L-Alloisoleucine in Plasma for Diagnosis of Maple Syrup Urine Disease, ***Clinical Chemistry.*** **1999;45:1734-1740.**

Applications notes 43, 58, and 60

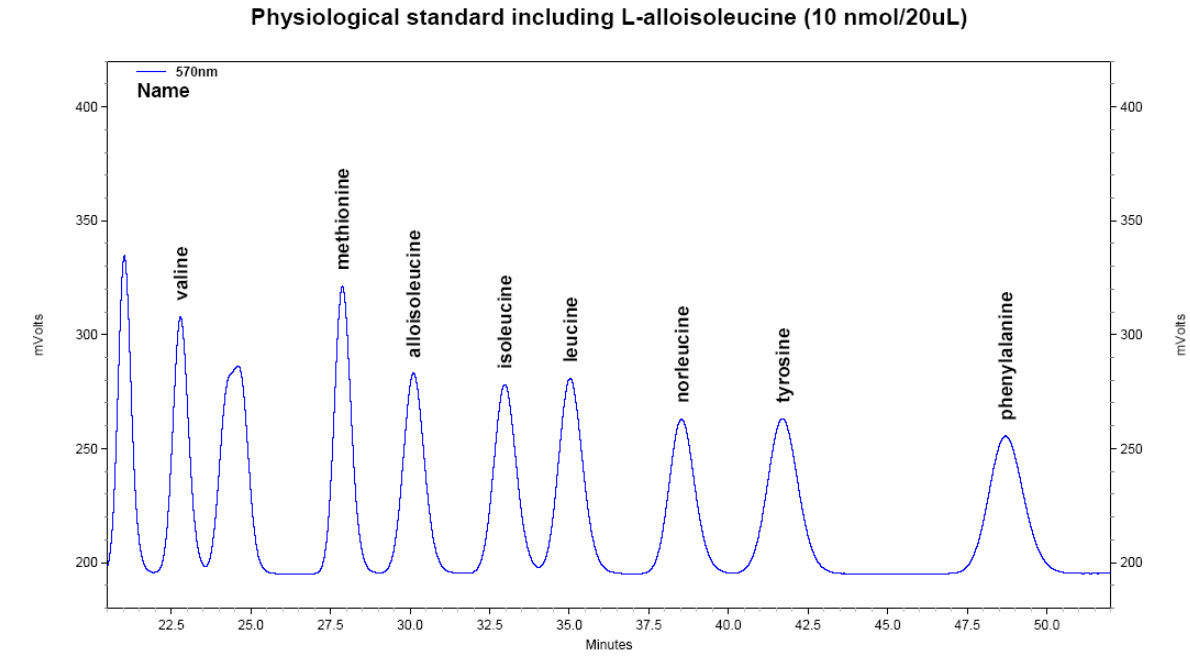

Figure 1. MSUD screening

BioSys

Sample: Physiological Fluid std — Amount Loaded: 10 nmol

Column Type: Peek — Column Number: U-1085 — Resin Batch: 07216

Bed Length (mm): 200 — Diameter (mm): 4.6 — Instrument Serial Number: 88352

	Buffer	Nin
Flow Rate (ml/h):	30	20

	Buffer	**Molarity**	**pH**	**Batch No.**
Buffer 1 -	Lithium Citrate A	0.20	2.80	11900
Buffer 3 -	Lithium Citrate Buffer CII	0.50	3.15	11570
Buffer 6 -	Lithium hydroxide Solution	0.30		
Reagent	Ninhydrin			11884
	Ultrosolve			11839

Title: **MSUD**

Nin Flow Rate: 20.0 ml/h

No.	**Time**	**Temp**	**Buffer**	**Pump**	**Nin**	**Rec**	**Commands**
1	01:00	35°C	3	30.0ml/h	ON	OFF	
2	00:00	35°C	3	30.0ml/h	ON	OFF	Reset
3	01:00	35°C	3	30.0ml/h	ON	OFF	Load
4	17:00	35°C	3	30.0ml/h	ON	ON	
5	30:00	70°C	3	30.0ml/h	ON	ON	
6	05:00	70°C	6	30.0ml/h	ON	ON	
7	02:00	80°C	3	30.0ml/h	ON	ON	
8	15:00	70°C	1	30.0ml/h	ON	ON	
9	10:00	70°C	3	30.0ml/h	OFF	OFF	
10	02:00	65°C	3	30.0ml/h	ON	OFF	