Application Note

Date: No. 430011H-E

Mass screening for inborn error of amino acid metabolism using semi-micro HPLC (3)

This report concerns the simultaneous analysis of the anomalous metabolic amino acids of three illnesses targeted by the Ministry for Health and Welfare (homocystinuria, maple syrup urine disease, phenylketonuria): methionine (Met), leucine (Leu), phenylalanine (Phe), valine (Val), tyrosine (Tyr), isoleucine (Ile).
The time for analysis of a single specimen is 20 minutes. Detection employs OPA post column derivatization. Also, the use of a micro-plate enables easy simultaneous pre-processing of multiple samples (Fig. 1).
Below (Fig. 2) the standard sample chromatogram is shown.

Dried blood filter paper 3 mm diameter (x 1 sheet)
- put in micro-plate well
- add 10 ml EtOH
- leave 1, 2 minutes
- add 100 ml mobile phase
- sonicate for 5 minutes

placed directly in the auto-sampler and analyzed

Fig. 1 pre-processing procedure

Conditions:

Column:	AMINOMETAPAK (2.1mm dia. x 125mmL)
Eluent:	H2O/CH3CN(94/6) +25mM NaClO4 (pH2.2) +5mM CH3(CH2)4SO4Na
Reagent:	0.2M Potassium Hydroxide 0.2M Boric Acid 0.3% OPA(W/V)/0.06% Brij-35 0.2% Mercaptoethanol
Wave length:	Ex.345nm Em.455nm (Gain x100)
Flow rate:	0.2ml/min(Eluent) 0.1ml/min(Reagent)
Column Temperature:	35 degree celsius
Sample:	Amino Acid Std. Soln. Type H (Wako Pure Chem) 5pmol/ul
Injection volume:	10ul

Keywords: 1. Amino Acid, 2. Dried blood on filter paper, 3. ODS, 4. FL, 5. OPA/post-column derivatization

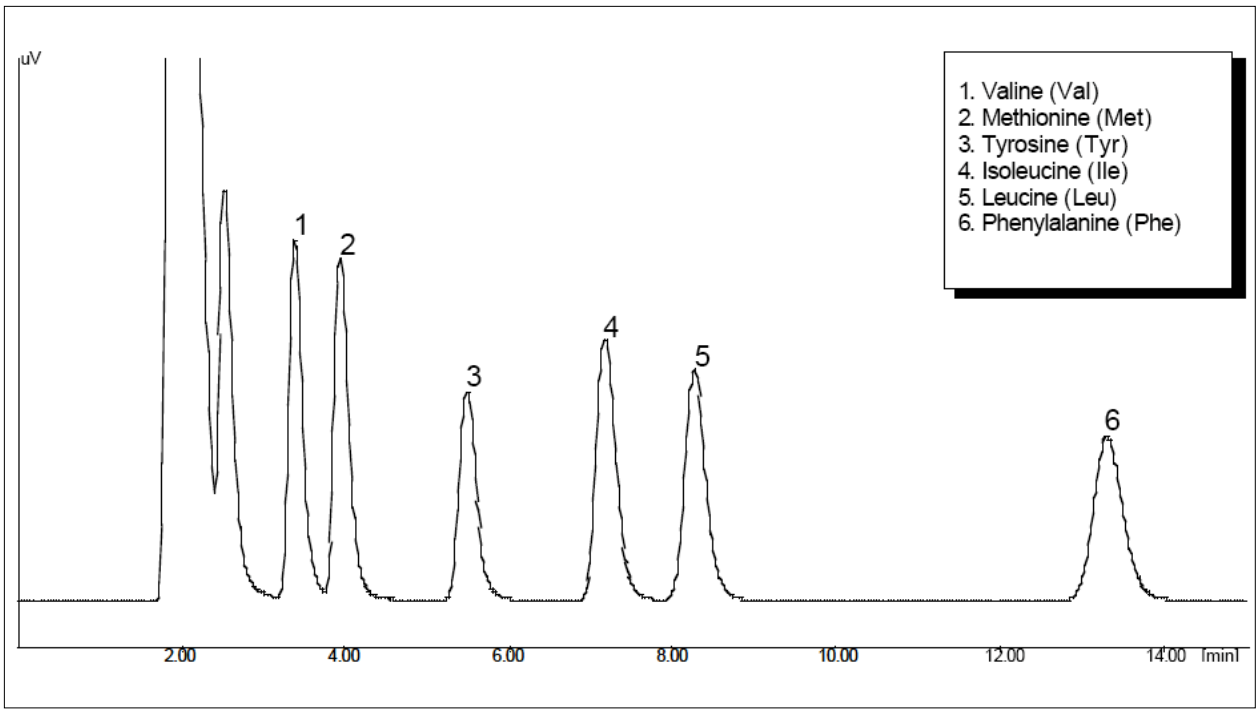

Fig. 2 Chromatogram of standard sample

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