Application Note

Date: No. 430009H-E

Mass screening for inborn error of amino acid metabolism (1)

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 15 minutes. Detection employs OPA post column derivatization. The use of a micro-plate enables easy simultaneous pre-processing of multiple samples (Fig. 1).
The chromatograms of a standard sample (Fig. 2), a sample from healthy newborn baby (Fig. 3) and a sample from phenylketonuria patient (Fig. 4) are shown below.

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
Eluent:	H2O/CH3CN(940/60) +25mM $NaClO_4$ (pH2.2) +5mM $CH_3(CH_2)_4SO_4Na$
Reagen:	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:	1.0ml/min(Eluent) 0.3ml/min(Reagent)
Column Temperature:	35 degree celsius
Sample:	Amino Acid Std. Soln. Type H (Wako Pure Chem) 5pmol/ul Dried blood on filter paper
Injection volume:	10ul

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

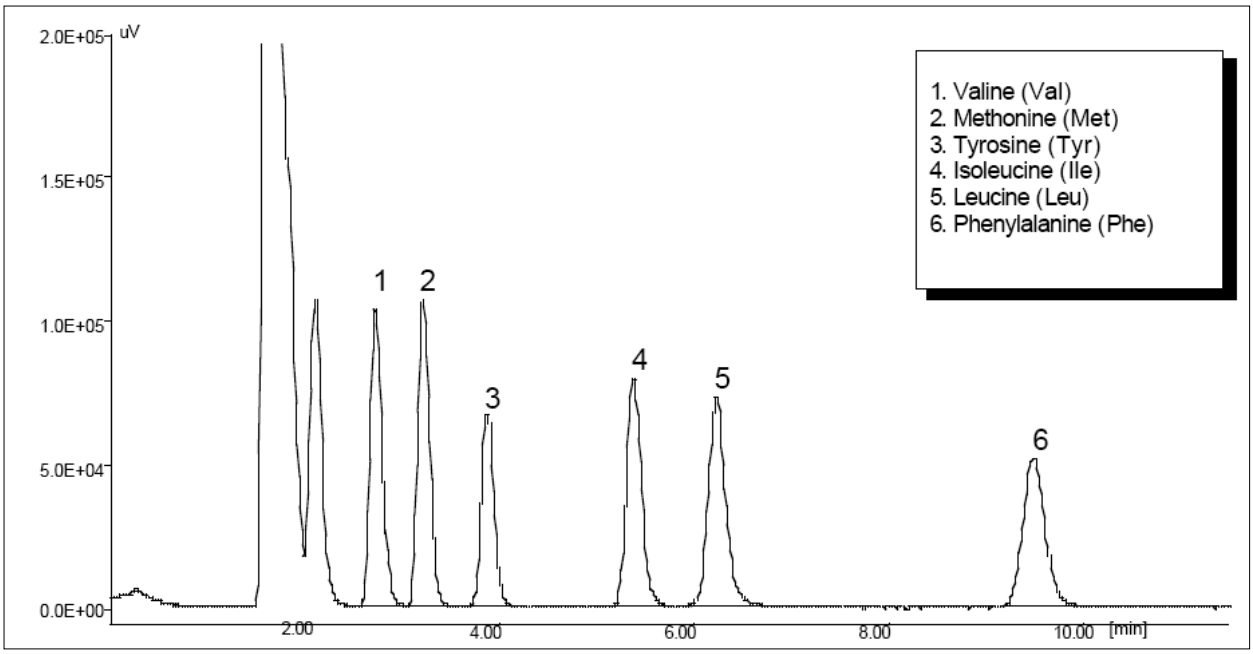

Fig. 2 Chromatogram of standard sample

JASCO INTERNATIONAL CO., LTD.
4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512