Dried blood spot analysis on the Biochrom 30 Amino Acid Analyser

Dried blood spots are commonly used when performing neonatal screening. Although plasma and urine samples are usually the preferred sample type for amino acid analysis, when these aren't available it may be necessary to use dried blood spots.

This application note describes a protocol for sample preparation of dried blood spots for analysis on the Biochrom 30 Amino Acid Analyser. The sample preparation protocol and chromatograms were kindly provided by Sheffield Children's Hospital, UK.

Introduction

When newborn babies are about a week old a blood sample is taken from their heel, referred to as the heel prick test. Blood from the baby's heel is dropped onto a Guthrie card and stored as a series of blood spots.

A number of tests are then carried out on these blood spots for the purposes of newborn screening.

Typically the Biochrom 30 Amino Acid Analyser is used for the amino acid analysis of plasma and urine. However it can also be used for analysis of other types of samples such as dried blood spots following suitable sample preparation.

Sample preparation

Trichloroacetic acid (TCA) and 5-sulphosalicylic acid (SSA) are used both to extract the amino acids from the filter paper and to precipitate protein at the same time.

The sample preparation protocol is as follows:

1- Cut a 6 mm spot into quarters and place it into a microtube

2- Add 100 µL of 5% TCA

3- Add 100 µL of a solution containing 20 µmol/L Norleucine (internal standard) in SSA

This solution is a 10 times dilution of a 200 µmol/L Norleucine stock solution in SSA which is prepared as follows:
- Dilute 100 mL of a 2000 µmol/L Norleucine stock solution to 500 mL with 25% SSA
- Transfer this solution into a 1L volumetric flask and add about 400 mL of buffer 6 (Lithium hydroxide 80-2038-20) to adjust the pH to 2.20
- Make up to 1L with distilled water

(the reagent is prepared in large amounts to avoid batch to batch variations but quantities can be reduced if required).

4- Mix for 5 minutes then spin down the precipitate.

5- Take off the supernatant

6- Spin again before injecting 50 µL of sample onto the analyser

A calibration standard is prepared at a concentration of 200 µmol/L using the Sigma AAS18 Amino Acid Standard. The calibration standard is diluted 10 times before injection.

Experimental conditions

The samples can be analysed on the Biochrom 30 Amino Acid Analyser using the MSUD short program for the determination of branched chain amino acids (see application note B30-3 Rapid analysis for the diagnosis of MSUD).

A control sample was prepared using blood from a volunteer. The sample was spiked with L-Allo-isoleucine and spot onto a Guthrie card paper.

Examples of chromatograms of a standard and a control sample are displayed below.

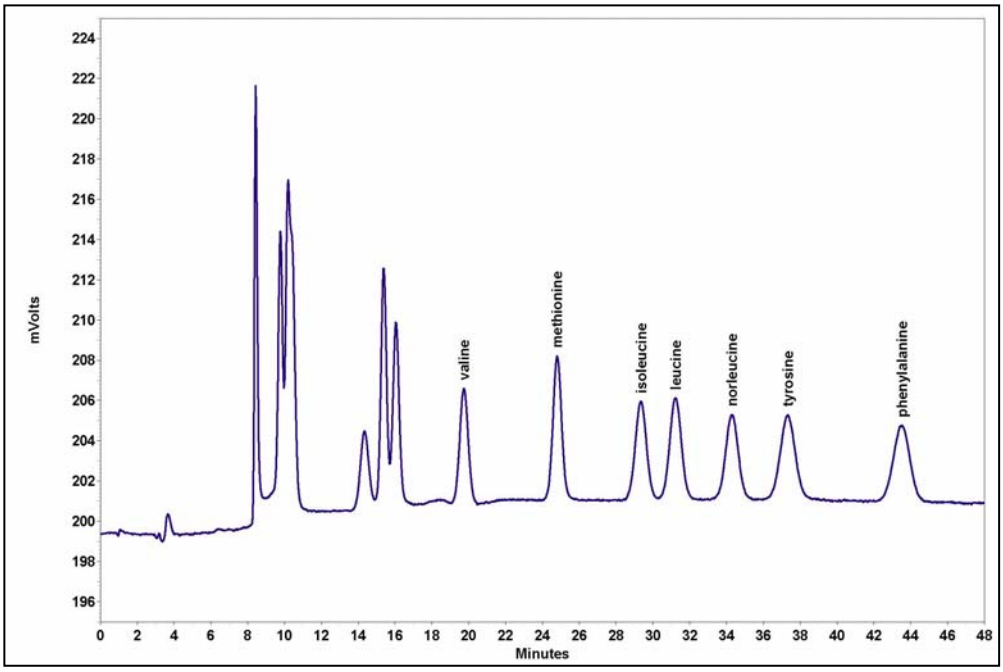

Figure 1: Calibration standard 20 µmol/L (prepared using Sigma AAS18)

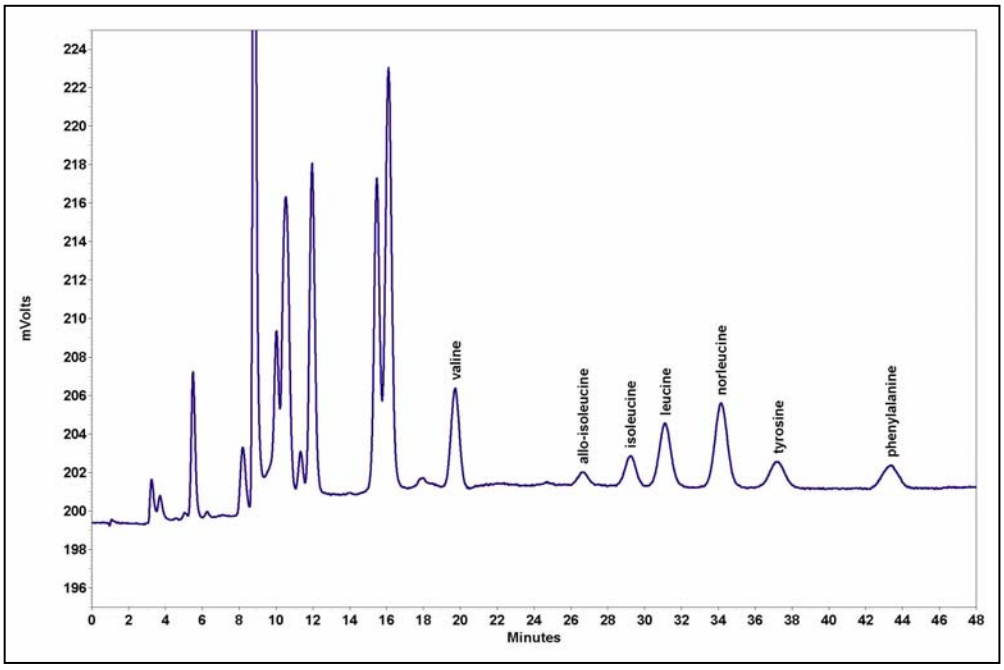

Figure 2: Control sample (spiked with Allo-isoleucine)