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SIM-GC-MS AMINO ACID BLOOD TEST

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Abstract
A rapid method for amino acids blood test by gas chromatography/mass spectrometry (GC/MS) was developed for metabolic diseases fingerprint test. Trifluoroacetyl butyl ester amino acid derivatives blood samples were used. Diagnosis of neonatal phenylkenuria (PKU) and maple syrup urine disease (MSUD) was performed by blood samples analysis for five amino acids. Simultaneous screening for neonatal PKU and MSUD will contribute to a rapid diagnosis. By following a proper diet, children could have a normal life.
Keywords: blood test, diagnosis, amino acid, SIM-GC-MS

1. Introduction

Phenylketonuria (PKU) is a metabolic disease usually caused by phenylalanine hydroxylase deficiency. Maple syrup urine disease (MSUD), other metabolic disease, could be diagnosis by some branched amino acids determination in blood [1]. Newborn amino acids determination by gas chromatography–mass spectrometry is a useful method for diagnosis of inborn errors in metabolism [2]. A rapid method by some amino acid profiling determination was adapted. Volatile derivatives of amino acids are analyzed in very small volumes of plasma or whole blood by using filter paper blood specimens and GC-MS technique. PKU relies on the amino acid profiling by mass spectrometry detection of Phe. Neonatal screening for phenylketonuria and other aminoacidemia is low cost method suited in our country [3].

2. Method and samples

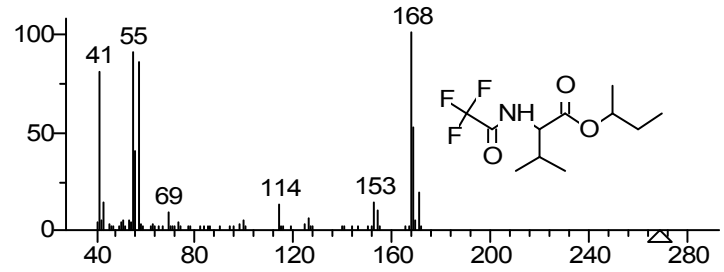
Amino acids in blood samples were derivatized as trifluoroacetyl butyl ester derivative. The derivatization method was described [1]. The method is useful for diagnosis of metabolic diseases as PKU by determination of phenylalanine (Phe) in blood and maple syrup urine disease (MSUD) by determination of valine (Val), leucine (Leu) and proline (Pro) in blood. The analyses of the five amino acids in blood samples by GC-MS is useful in the diagnosis of the both diseases.

Apparatus: A Trace DSQ ThermoFinnigan quadruple mass spectrometer coupled with a Trace GC was used. The capillary column Rtx-5MS was of 30 m length x 0.25 mm, 0.25µm film thickness, by using a temperature program from 50°C (3 min), then increased to 100°C, at 6 °C/min, then 4 °C/min to 200 °C and then increased to 300°C, at 20 °C/min, in the selected ion monitoring mode (SIM).The ions used were: m/z 168 for valine, m/z 182 for leucine and isoleucine, m/z 166 for proline, m/z 203 for tyrosine and m/z 91 for phenylalanine.

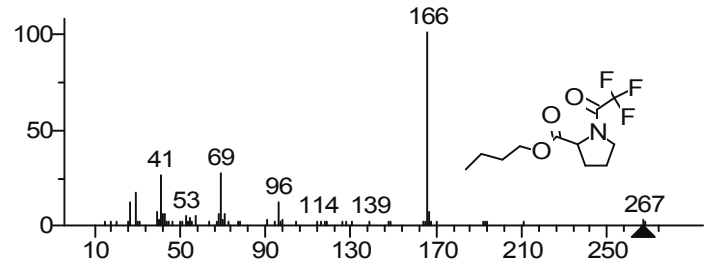
Written informed consents were obtain from each subject parent prior to this study.

3. Results and Discussions

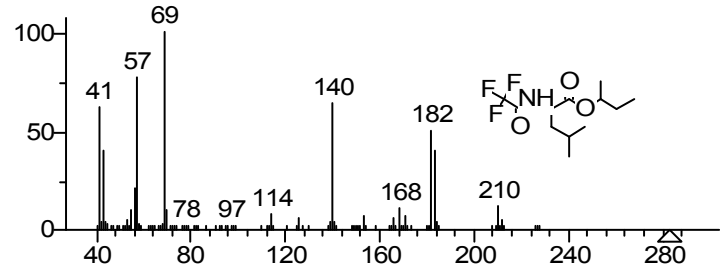
The mass spectra of the amino acids used for profiling determination are presented in fig. 1.



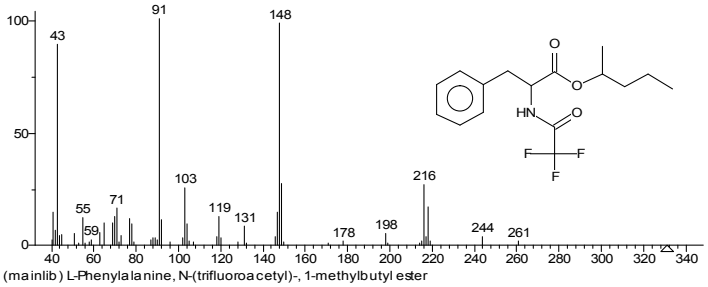
(mainlib) L-Valine, N-(trifluoroacetyl)-, 1-methylpropyl ester



(mainlib) L-Proline, 1-(trifluoroacetyl)-, butyl ester



(mainlib) L-Leucine, N-(trifluoroacetyl)-, 1-methylpropyl este



(mainlib) L-Phenylalanine, N-(trifluoroacetyl)-, 1-methylbutyl ester

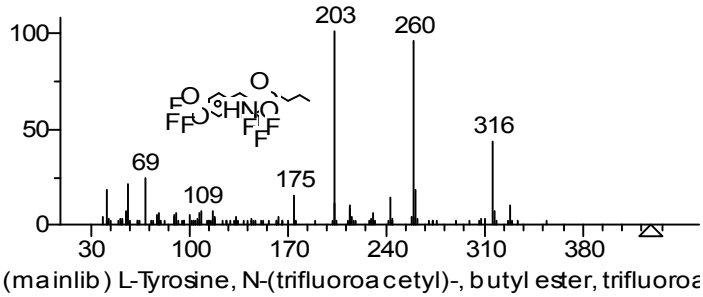


Fig. 1. The mass spectra of the five amino acids tested for metabolic diseases

The base peaks in the mass spectra of the five important amino acids tested in the two metabolic disease are: m/z 168 for Val, m/z 182 (the third important peak) for Leu, m/z 166 for Pro, m/z 91 for Phe and m/z 203 for Tyr, as shown in fig. 2.

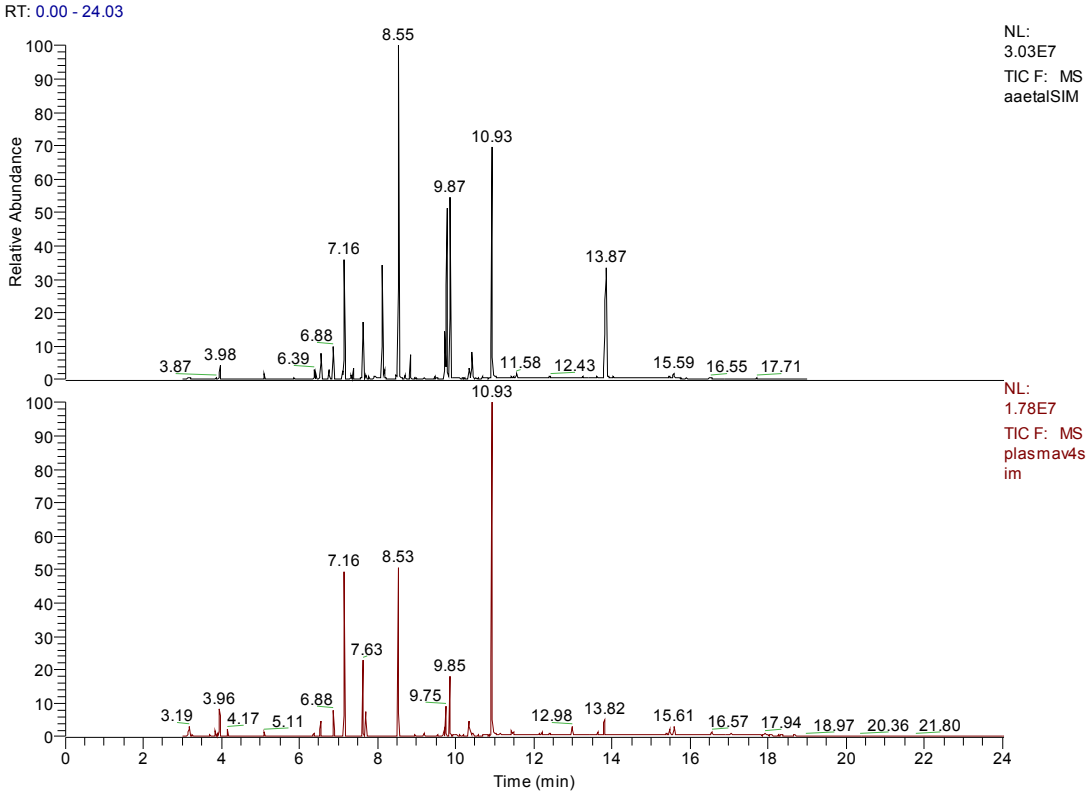


Fig.2 Amino acid screening in the SIM-GC-MS mode for a standard mixture of amino acids and a blood sample (Val: 7.16 min; 7.63: Leu; 8.53 Pro; 10.35: Tyr; 10.93: Phe).

The results were calculated by using as internal standard 10 µg/ml ^{15}N-Gly. By comparing the results obtained for different blood samples, the amino acid concentration obtained showed significant differences in comparison with control.

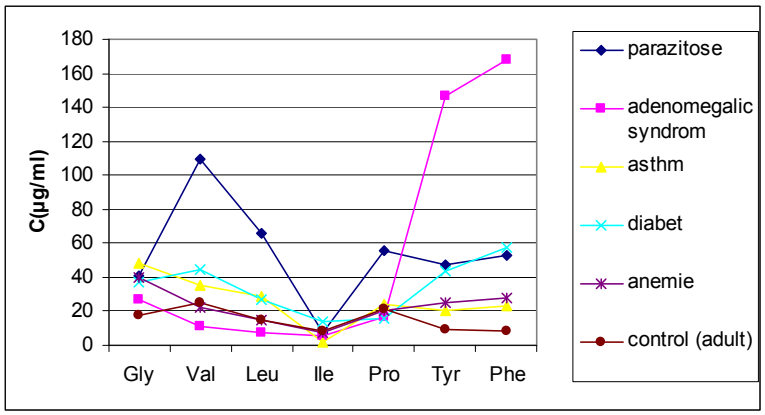


Fig.3 Plasma amino acid concentrations in different diseases

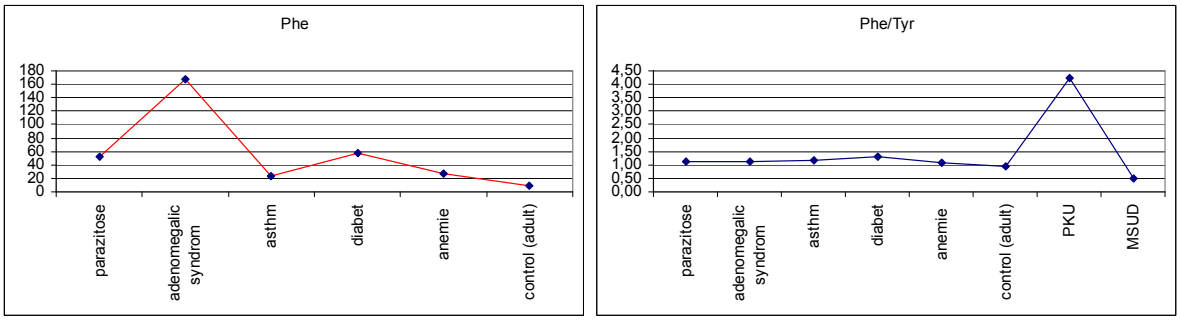


Fig. 4 Phenylalanine concentrations in different plasma samples and Phe/Tyr ratio

PKU diagnosis could be tested by calculating the ratio Phe/Tyr, as shown in fig. 4. Diagnosis of MUSD disease could be obtained by calculating the ratio between aliphatic and aromatic amino acids in blood samples.

4. Conclusions

The GC/MS methods are very useful for PKU and MSUD diseases diagnosis The methods used are suitable for metabolic dysfunction diseases diagnosis, either by quantization or screening of some AA. Screening of plasma amino acids is the first step in diagnosis of metabolic diseases. Significant differences were found for the amino acids studied. Diagnosis in the first three months of newborn saves lives (MSUD) or normal intellectual development (PKU).

References

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