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Simple and rapid analytical method for detection of amino acids in blood using blood spot on filter paper, fast-GC/MS and isotope dilution technique

Shuichi Kawana*, Katsuhiro Nakagawa, Yuki Hasegawa, Seiji Yamaguchi

Department of Pediatrics, Shimane University School of Medicine, 89-1 En-ya-cho, Izumo, Shimane 693-8501, Japan

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ABSTRACT

A simple and rapid method for quantitative analysis of amino acids, including valine (Val), leucine (Leu), isoleucine (Ile), methionine (Met) and phenylalanine (Phe), in whole blood has been developed using GC/MS. In this method, whole blood was collected using a filter paper technique, and a 1/8 in. blood spot punch was used for sample preparation. Amino acids were extracted from the sample, and the extracts were purified using cation-exchange resins. The isotope dilution method using $^{2}H_{8}$-Val, $^{2}H_{3}$-Leu, $^{2}H_{3}$-Met and $^{2}H_{5}$-Phe as internal standards was applied. Following propyl chloroformate derivatization, the derivatives were analyzed using fast-GC/MS. The extraction recoveries using these techniques ranged from 69.8% to 87.9%, and analysis time for each sample was approximately 26 min. Calibration curves at concentrations from 0.0 to 1666.7 μmol/l for Val, Leu, Ile and Phe and from 0.0 to 333.3 μmol/l for Met showed good linearity with regression coefficients = 1. The method detection limits for Val, Leu, Ile, Met and Phe were 24.2, 16.7, 8.7, 1.5 and 12.9 μmol/l, respectively. This method was applied to blood spot samples obtained from patients with phenylketonuria (PKU), maple syrup urine disease (MSUD), hypermethionine and neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), and the analysis results showed that the concentrations of amino acids that characterize these diseases were increased. These results indicate that this method provides a simple and rapid procedure for precise determination of amino acids in whole blood.

1. Introduction

Free amino acids in whole blood reflect the state of amino acid metabolism; consequently, amino acids in blood or serum are monitored in patients with inborn errors of amino acid metabolism [1–4]. The filter paper technique is widely used for sampling whole blood for analysis of amino acids, acylcarnitines and fatty acids due to its superior features in sampling, transportation and sample retention compared with serum samples [1,5,6].

Tandem mass spectrometry (MS/MS) [1,3], high performance liquid chromatography (HPLC) [2], liquid chromatography mass spectrometry (LC/MS) [7], gas chromatography (GC) [8] and gas chromatograph mass spectrometry (GC/MS) [5,9] methods have been reported for quantitative analysis of amino acids in whole blood using the filter paper technique. In the aforementioned methods, MS/MS is commonly used for screening of inborn errors of metabolism because of the short analysis time [3]. Conversely, HPLC, LC/MS, GC and GC/MS methods have been applied to quantitative analysis due to their performance in chromatographic separation. In particular, the GC/MS method offers exceptional chromatographic separation, detailed mass spectral analysis and low ion suppression.

For these reasons, GC/MS methods for the analysis of amino acids in blood have been developed. In sample preparation process, amino acids are extracted using cation-exchange resins [10–12] after proteins are removed from blood samples using sulphosalicylic acid [10,13,14] and picric acid [12,14] treatments. Extracted amino acids are derivatized for GC/MS analysis. If organic solvent treatment is used for the removal of proteins, the sample goes directly to derivatization following the drying procedure [5,8,15–17]. For the derivatization procedure, trimethylsilylation [4,18,19], tert-butyldimethylsilylation [20,21], esterifcation-acylation [5,16], and alkyl chloroformation [8,9,22–25] have been reported and those methods were summarized by Knapp [26] and Blau and Halket [27]. For quantitative calculation, the absolute calibration method is widely used but the isotope dilution method was used to improve the accuracy [10,17,28].

In this study, we have developed a method for analysis of amino acids in blood using the filter paper technique and GC/MS. For easy and simple sample preparation, a commercially available kit for analysis of amino acids was used; after sample purification

* Corresponding author. Tel.: +81 75 823 1410; fax: +81 75 841 9326.
E-mail addresses: s_kawana@shimadzu.co.jp (S. Kawana), nk@shimadzu.co.jp (K. Nakagawa), yukirin@med.shimane-u.ac.jp (Y. Hasegawa), seijiyam@med.shimane-u.ac.jp (S. Yamaguchi).

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on cation-exchange resins and propyl chloroformate derivatization were performed, the treated samples were analyzed using the fast-GC/MS method to shorten the analysis time. To improve the accuracy of the results, the isotope dilution technique was also applied for quantitative analysis.

For the validation of this method, phenylalanine, leucine, isoleucine, valine and methionine were chosen as target amino acids in consideration of current Japanese neonatal mass screening, in which phenylalanine, leucine, and methionine are measured for detection of phenylketonuria (PKU), maple syrup urine disease (MSUD), and homocystinuria (HCY), respectively.

This method was successfully applied to the analysis of amino acids in blood spot samples obtained from patients suffering from PKU, MSUD, hypermethioninemia and even neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD).

2. Experimental

2.1. Chemicals

$^2H_8$-valine ($^2H_8$-Val), $^2H_3$-leuicine ($^2H_3$-Leu), $^2H_3$-methionine ($^2H_3$-Met) and $^2H_5$-phenylalanine ($^2H_5$-Phe) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA) to be used as internal standards. A mixture of the d-labeled amino acids was prepared at a concentration of 1 μmol/l in methanol. The solution mixtures of valine (Val), leucine (Leu), isoleucine (Ile), methionine (Met) and phenylalanine (Phe) were prepared at concentrations of 0.5, 1, 5, 10 and 50 μmol/l by diluting a stock solution of the amino acid mixture (200 μmol/l; EZ:faast™) with 0.05 N HCl solution.

2.2. Preparation

Whole blood samples were absorbed on a filter paper (ADVANTEC PKU, Tokyo, Japan). After drying at room temperature, one 1/8 in. punch from each blood spot was transferred into a 1.5 ml-vial, and 100 μl each of the internal standard mixture and 0.05 N HCl solution was added to the vial. The mixture was vortexed for 10 min, allowed to stand for 40 min and then vortexed a second time for an additional 10 min. The obtained supernatant was prepared following the procedure described in the Phenomenex EZ:faast™ amino acid analysis kit for GC/MS (Phenomenex Inc., CA, US) with the exception of the addition of the internal standard mixture to the solution instead of novaline.

2.3. GC/MS measurement

The blood sample analysis was performed on a gas chromatograph coupled to a quadrupole mass spectrometer (GCMS-QP2010 Plus, Shimadzu, Kyoto, Japan) equipped with an automatic injection system (AOC-20i + s) and a split/splitless injection port. The analytical conditions are shown in Table 1. A short capillary column (10 m × 0.25 mm I.D.) was used. The data acquisition interval was set to 0.2 s to collect more than eight data points for each of the observed GC peaks along the GC peaks [15,29].

2.4. Method validation

2.4.1. Recovery of preparation

A control experiment was performed to evaluate the extraction recovery of the amino acids from a blood spot sample; the amounts of amino acids recovered were assumed to be equal to those of the labeled amino acids. Whole blood from healthy control, which contained amino acids within the normal concentration ranges, was spotted onto filter paper, and the blood spot (1/8 in.) was punched. Internal standard (0.1 nmol each of $^2H_8$-Val, $^2H_3$-Leu, $^2H_3$-Met and $^2H_5$-Phe) was added directly to each punch, and they were dried as spiked samples. The punches were treated following the preparation procedure shown in Section 2.2, with the exception that the internal standard mixture was added. For the blank test, five blank samples that did not contain a blood spot punch were prepared in 1.5 ml-vials following the preparation procedure (Section 2.2). The extraction recovery was calculated by dividing the peak areas of spiked samples by those of blank samples for $^2H_8$-Val, $^2H_3$-Leu, $^2H_3$-Met and $^2H_5$-Phe.

Table 1
Analytical conditions for GC/MS.

Gas Chromatography	
Injection volume	1.0 μl
Injection mode	Split (1:15)
Injection temp.	280 °C
Column oven	110 °C→(30 °C/min)→320 °C (0 min)
Carrier gas	He
Flow control mode	70.2 cm/s (linear velocity)
Total Flow	21.8 ml/min
Column Flow	1.18 ml/min
Purge Flow	3.0 ml/min
Mass spectrometry	
Interface temp.	280 °C
Ion box temp.	200 °C
Ionization voltage	70 eV
Emission current	150 μA
Data acquisition rate	0.2 s
Monitor ion *(m/z)*	
1.15–2.09 min	72,74,80,86,89, 116, 124, 130, 133, 158, 166, 172, 175
2.09–2.76 min	61,64, 190, 193
2.76–3.76 min	120, 126, 148, 154, 190, 196

2.4.2. Calibration curve

Blank and standard mixtures of Val, Leu, Ile, Met and Phe (0.5, 1, 5, 10 and 50 nmol/l) were analyzed and used to construct calibration curves according to a least-squares linear regression equation. Because one blood spot punch corresponds to 3 μl of whole blood, the concentrations of the standard mixtures (0.0, 0.5, 1, 5, 10 and 50 nmol/l in analysis) were converted to those of amino acids in whole blood (0.0, 16.7, 33.3, 166.7, 333.3 and 1666.7 μmol/l). The concentrations were varied from 0.0 to 1666.7 μmol/l for Val, Leu, Ile and Phe and from 0.0 to 333.3 μmol/l for Met. A concentration of 33.0 μmol/l of $^2H_8$-Val, $^2H_3$-Leu, $^2H_3$-Met and $^2H_5$-Phe was added as an internal standard for corresponding non-labeled amino acids, and $^2H_3$-Leu was substituted for isotope-labeled Ile.

2.4.3. Method detection limit

The method detection limits were determined by analyzing five blood punches from a healthy control, which contained amino acids within the normal concentration ranges, and multiplying the standard deviation of the mean by the appropriate Student's *t*-value for the 99% confidence level using the appropriate degrees of freedom.

2.5. Healthy control and patient sample analysis

Blood spot samples were obtained from 33 normal controls and analyzed by the methods outlined in Sections 2.2 and 2.3. The mean values and the standard deviations obtained from 33 normal controls were calculated. The cut-off value was defined as the mean plus three standard deviations.

Blood spot samples were obtained from 5 patients with disorders diagnosed by the MS/MS method and clinical symptoms for PKU, MSUD and hypermethionine NICCD. To validate the new method, each patient's sample was analyzed to determine Val, Leu, Ile, Met and Phe concentrations, which were compared with the cut-off values.

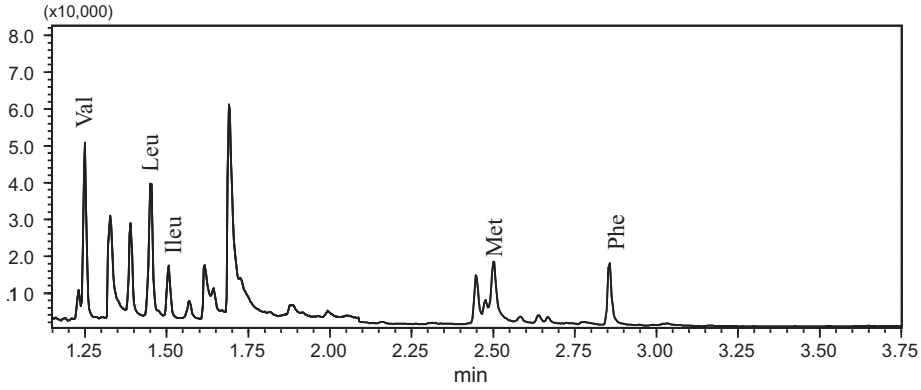

Fig. 1. Total ion chromatogram of a blood spot sample. A total ion chromatogram of a blood spot sample obtained from a healthy control is shown. Val = Valine, Leu = Leucine, Ile = Isoluecine, Met = Methionine and Phe = Phenylalanine.

3. Results

3.1. GC/MS measurement

Fig. 1 shows the total ion chromatogram of a blood spot sample obtained from a healthy control. Retention times of Val, Leu, Ile, Met and Phe were 1.25, 1.46, 1.51, 2.50 and 2.86 min, respectively. All target amino acids were separated by selective ion monitoring (*m/z* 116, 172, 130, 190 and 190 for Val, Leu, Ile, Met and Phe, respectively) without overlapping component peaks (Fig. 2).

3.2. Method validation

3.2.1. Recovery of preparation

The extraction recoveries varied from 69.8% to 87.9%, as shown in Table 2. The repeatabilities for blank samples and control samples were <4.4% and 14.1% (RSD%, $n = 5$), respectively.

Table 2
Recovery and repeatability.

	Blank (n = 5)		Punch of blood (n = 5)		
Compound	Mean[a]	%RSD	Mean[b]	%RSD	Recovery[c]
$^2H_8$-Valine	3575	4.4%	2496	12.3%	69.8%
$^2H_3$-Leucine	6449	2.9%	4515	13.5%	70.0%
$^2H_3$-Methionine	615	2.5%	541	13.4%	87.9%
$^2H_5$-Phenylalanine	2096	3.6%	1655	14.1%	79.0%

[a] The internal standard mixture (0.1 nmol) was added directly to each punch of the blood spot sample obtained from a healthy control. The dried punch was treated following the preparation procedure shown in Section 2.2. The means of the indicated peak areas are shown.

[b] For the blank test, five blank samples that did not contain a blood spot punch were prepared in the 1.5 ml vial were prepared following the preparation procedure (Section 2.2). The means of the indicated peak areas are shown.

[c] Recovery = peak area (punch of blood)/peak area (blank).

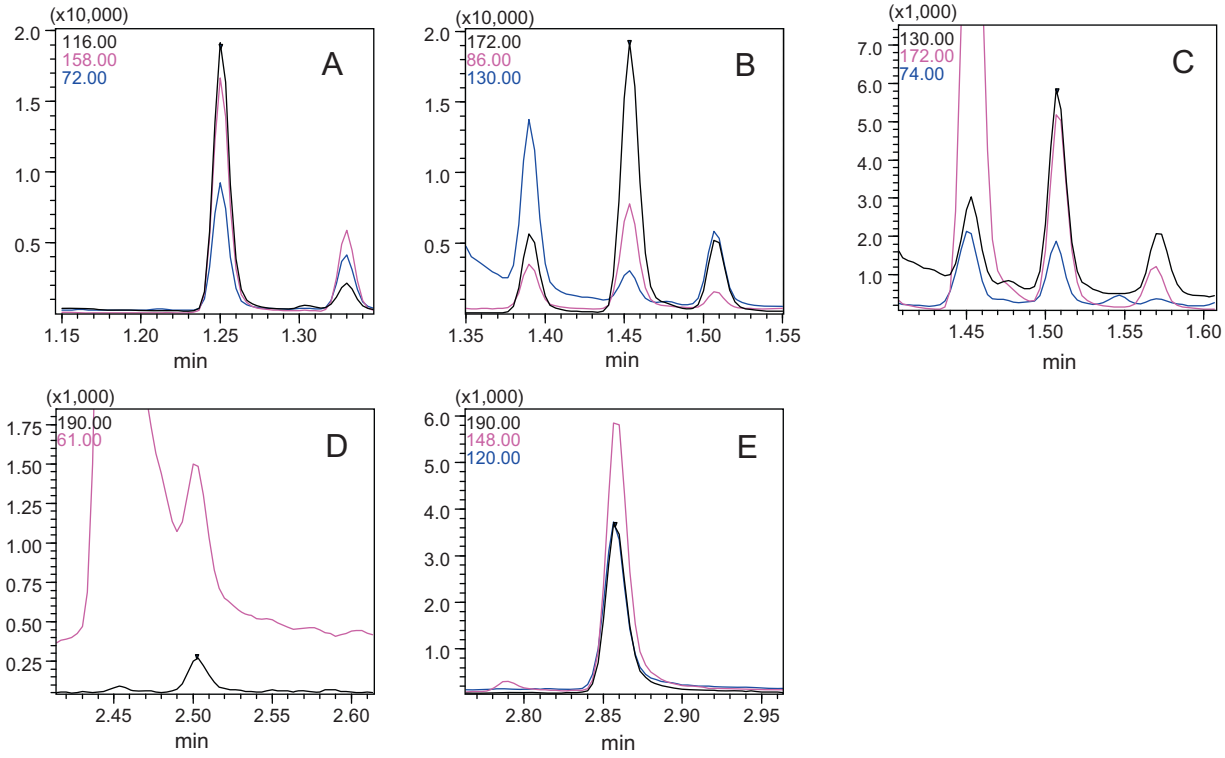

Fig. 2. Selected ion monitoring of a blood spot sample. Selected ion monitoring of amino acids in a blood spot sample obtained from a healthy control is shown. All target amino acids were separated by selected ion monitoring without overlapping of component peaks. A: Valine, *m/z* 116, 129.7 μmol/l; B: Leucine, *m/z* 172, 104.7 μmol/l; C: Isoleucine, *m/z* 130, 41.3 μmol/l; D: Methionine, *m/z* 190, 13.3 μmol/l and E: Phenylalanine, *m/z* 190, 63.0 μmol/l.

Table 3
Calibration curves.

Compound	Equations	R^2 [a]
Valine	$y = 1.41x + 2.60 \times 10^{-2}$	1.000
Leucine	$y = 1.08x + 0.10 \times 10^{-1}$	1.000
Isoluecine	$y = 0.73x + 2.76 \times 10^{-2}$	1.000
Methionine	$y = 0.91x + 4.62 \times 10^{-1}$	0.999
Phenylalanine	$y = 1.12x + 5.17 \times 10^{-2}$	1.000

The concentrations varied from 0.0 to 1666.7 μmol/l for Val, Leu, Ile and Phe and from 0.0 to 333.3 μmol/l for Met. A concentration of 33.0 μmol/l of $^2H_8$-Val, $^2H_3$-Leu, $^2H_3$-Met and $^2H_5$-Phe was added as an internal standard for corresponding non-labeled amino acids; $^2H_3$-Leu was also substituted for isotope labeled Ile. The concentrations of 0.0, 0.5, 1, 5, 10 and 50 nmol/l were converted to 0.0, 16.7, 33.3, 166.7, 333.3 and 1666.7 μmol/l of amino acids in whole blood.

[a] Correlation coefficient.

3.2.2. Calibration curve

The correlation coefficients for Val, Leu, Ile and Phe were 1.000 at concentrations from 0.0 to 1666.7 μmol/l, and the correlation coefficient for Met was 0.999 at concentrations from 0.0 to 333.3 μmol/l as shown in Table 3.

3.2.3. Method detection limit

Results obtained from 5 blood spot samples of a healthy control are shown in Table 4. The repeatabilities for the method (%RSD, $n = 5$) were <5.0% for the evaluated amino acids. The method detection limits ranged from 1.5 (Met) to 24.2 μmol/l (Val).

3.3. Healthy control and patient sample analysis

Table 5 shows the analytical results obtained for blood spot samples from 5 patients suffering from PKU, MSUD and hypermethionine NICCD and from 33 healthy controls. The concentrations of Val, Leu, Ile, Leu + Ile, Met and Phe in the 33 healthy controls were lower than the cut-off values.

4. Discussion

Methods using GC or GC/MS for analysis of amino acids in whole blood, which used blood spot samples on filter papers, have been reported [5,9]. In these previous reports, a sample punch 8 mm in diameter was used; in this study, we used a punch of 1/8 in., which allowed for more sample punches to be taken from the same spot if re-analysis or other biochemical tests are required.

Amino acids were extracted from punches of blood spots with a mixture of methanol, which was used as the solvent for the internal standard, and 0.05 N HCl (1:1, v/v). During the solvent extraction, most of protein could be removed similar as organic solvent treatment [4,5,8,16]. Cation-exchange resins were used to extract amino acids from the solvent. Adsorption efficiency of aliphatic amino acids, such as Phe, on ion exchange resins was not decreased by the non-polar extraction solvent, which was approximately 50% methanol. The extraction process showed excellent recovery and repeatability (79.0% and 14.1% %RSD) for $^2H_5$-Phe. For the other amino acids, the recoveries were more than 69.8%, and the repeatability was <13.5% (%RSD, $n = 5$) without internal standard correction. These results indicate that not only non-aliphatic amino acids, such as Val, Leu, Ile and Met, but also aliphatic amino acids, such as Phe, can be quantitatively extracted using this method.

Amino acids are usually analyzed by GC/MS after derivatization of the amine and carboxylic functional groups. Various derivatization methods were evaluated as described in Section 1. In those methods, residual water in the sample does not interfere with propyl chloroformation and this derivatization method may be highly preferable in biological samples [9,24,25,30]. Additionally, derivatization time was shortened by using propyl chloroformate. Trimethylsilylation, which is commonly used in derivatization for GC/MS analysis, requires solvent dehydration via heating and longer derivatization reaction time (30–60 min). Conversely, propyl chloroformate is unaffected by water, and the reaction is complete within 1 min at room temperature. For these reasons, propyl chroloformation was applied to this study.

Amine and carboxylic functional groups are converted to carboxylicpropyl and propylester, respectively, by the propyl chloroformation derivatization, and the mass number of the molecular ion is increased by 128 u. In Leu, Met and Phe, mass spectra of the derivatives showed molecular ions at m/z 259, 277 and 293, respectively; however, a similar molecular ion was not detected in mass spectra of Ile. In addition, mass spectra of derivatives of Val, Leu, Ile, Met and Phe showed specific ions formed by loss of a C_3H_7COO fragment (87 u) from the molecular ion at m/z 158, 172, 172, 190 and 206, corresponding to the loss of this fragment, i.e. Val: 245 – 87, Leu: 259 – 87, Ile: 259 – 87, Met: 277 – 87 and Phe: 293 – 87 (data not shown). These results demonstrate that the evaluated amino acids were completely derivatized by this method.

A high linear velocity of 70.2 cm/s, which was generated by a short capillary column (10 m × 0.25 mm I.D.), and a fast oven temperature program of 30 °C/min were used to shorten GC/MS analysis time (Table 1). The GC/MS analysis cycle time was approximately 10 min; the Phe had the longest retention time (2.86 min). Under these conditions, all target amino acids were separated and selectively detected (Fig. 2). During 50 sample analyses, interference due to peak overlap did not occur. These results suggest that the solvent extraction and purification on the cation ion-exchange resins were appropriate to selectively separate the amino acids from sample contaminants, and these pre-analysis steps resulted in good chromatographic separation. This method reduces GC/MS analysis time by one-third to one-sixth compared to the conventional method (30–60 min) while retaining good chromatographic separation [9,20,31].

Table 4
Repeatability and method detection limits.

Compound	Concentrations for five blood punches[b] [μmol/l]							
	1	2	3	4	5	Mean	%RSD	MDLs[c]
Valine	129.7	145.7	140.7	135.3	139.3	138.1	4.3%	24.2
Leucine	104.7	114.0	113.7	108.0	113.0	110.7	3.7%	16.7
Isoluecine	41.3	45.3	44.7	40.7	44.7	43.3	5.0%	8.7
Leu + Ile[a]	146.0	159.3	158.3	148.7	157.7	154.0	4.0%	24.9
Methionine	13.3	12.7	12.3	12.7	13.0	12.8	3.0%	1.5
Phenylalanine	63.0	70.7	70.0	66.7	69.7	68.0	4.7%	12.9

[a] Total values of Leu (Leucine) and Ile (Isoluecine).

[b] The method detection limits were determined by analyzing five punches of the same blood spot from a healthy control, which contained amino acids within the normal concentration ranges.

[c] MDLs (Method detection limits) were determined by multiplying the standard deviation of the mean by the appropriate Student's t-value for the 99% confidence level using the appropriate degrees of freedom.

Table 5
Analytical results obtained for blood spot samples from 5 patients.

		Val		Leu		Ile		Leu + Ile		Met		Phe	
Disease		[μmol/l]	Ratio[e]	[μmol/l]	Ratio[e]	[μmol/l]	Ratio[e]	[μmol/l]	Ratio[e]	[μmol/l]	Ratio[e]	[μmol/l]	Ratio[e]
PKU[a]	1	110.7	0.40	69.8	0.34	40.6	0.36	110.3	0.36	9.4	0.17	***619.0***	***6.01***
	2	*189.3*	0.68	124.3	0.61	66.5	0_190	190.8	0.62	8.7	0.16	***201.6***	***1.96***
MSUD[b]	1	***360.3***	***1.29***	***2646.6***	***13.07***	***141.4***	***1.27***	***2788.0***	***9.08***	4.6	0.08	40.4	*0.39*
	2	***297.0***	***1.06***	***1017.4***	***5.02***	***257.0***	***2.30***	***1274.4***	***4.15***	6.6	0.12	43.7	*0.42*
NICDD[c]	1	178.1	0.64	91.6	0.45	49.9	0.45	141.5	0.46	***300.8***	***5.39***	***168.0***	***1.63***
Control[d]													
Mean		166.0	0.59	105.5	0.52	59.9	0.54	165.3	0.54	25.9	0.46	60.3	0.59
Cut-off		279.7	1.00	202.5	1.00	111.5	1.00	307.0	1.00	55.8	1.00	102.9	1.00

[a] Phenylketonuria.
[b] Maple syrup urine disease.
[c] Hypermethionine and neonatal intrahepatic cholestasis caused by citrin deficiency.
[d] 33 healthy controls.
[e] Ratio to cut-off value.

From these results, total analysis time was 80 min, which included a 60-min extraction time, a 10-min purification and derivatization time and a 10-min fast-GC/MS analysis time that included column cool-down. Although the extraction time was relatively long, it could be easily shortened by processing more samples in a batch. If 10 samples were processed as one batch, the analysis time for each sample would be only 6 min extraction time. In the reported results, total analysis time per sample with this method was 26 min and could be dramatically shortened compared to the conventional method.

The evaluated method was applied to amino acid analysis in a blood spot punch, and the method detection limits were determined (Table 3). The MDLs of Val, Leu, Ile, Leu + Ile, Met and Phe were lowered by factors of 5.96, 8.23, 14.95, 5.42, 5.97 and 16.20, respectively, compared with cut-off values (Table 5). The maximum concentrations were up to 11.56, 12.13, 12.82, 12.33, 37.2 and 7.98 times higher compared to cut-off values. These results show that this method can be applied to amino acids in whole blood at concentrations ranging from 0.18 (Leu + Ile) to 7.98 (Phe) of cut-off values, which should be sufficient for a biochemical test for inborn errors of amino acid metabolism [3,32].

Deng and Deng [5] reported that amino acids in blood were measured using the blood filter paper technique similar to our method. Amino acids were derivatized by n-buthanol and trifluoroacetic acid. The repeatability was lower than 5%, which was similar to our results, but the detection limits were lower than ours. The supposed reason is that the diameter of the punch (8 mm) was larger than ours (1/8 in.). However, the linearity of calibration curves ranged from 0.988 to 0.998, which were not good compared to ours. As those results, isotope dilution method is superior to non-isotope method for a quantitative calculation.

The method developed in this study was applied to five blood spot samples obtained from patients with inborn errors of amino acid metabolism, including PKU, MSUD and hypermethionine NICCD (Table 5). PKU is characterized by an increasing concentration of phenylalanine in the blood. Our results showed that the concentration of Phe was 1.96 and 6.01 times higher than the cut-off value. In maple syrup urine disease (MSUD), Leu, Ile, and Val accumulate in the blood. Our results showed the concentration of Leu was 13.07 and 5.02 times higher and that of Ile was 1.27 and 2.03 times higher than the cut-off values. In hypermethionine NICCD, phenylalanine, galactose, methionine or threonine increase in the blood. In this study, samples from a hypermethionine NICCD patient exhibited a concentration of Met that was 5.39 times higher than the cut-off value. These results show that this method can be applied to the chemical diagnosis of inborn errors of amino acid metabolism through the determination of the concentrations of the amino acids that are characteristically higher when these diseases are present.

The MS/MS method is superior to other methods in analysis time (only 2 min) and less expensive due to the application of flow injection as a method of sample introduction in MS/MS. For these reasons, the MS/MS method is widely applied to neonatal screening for inborn errors of amino acid, organic acid and fatty acid metabolism [1,5,6]. However, the GC/MS method has several aspects that are superior to the MS/MS method. In the MS/MS method, Leu and Ile are detected at the same m/z value without chromatographic separation and cannot be separated and determined individually. Ion-suppression effects due to co-eluting matrix components are not negligible in the MS/MS method, which prevents precise determination of analytes [33–35]. GC/MS can be used to avoid possible matrix effects that are detected by the MS/MS because the GC/MS can separate target compounds from the sample matrix with high chromatographic resolution. Electron ionization (EI)-GC/MS is also more resistant to ion-suppression than electrospray ionization-MS/MS. The characteristic mass spectral pattern obtained by EI can provide the mass numbers in the target compound, which do not overlap with other substances, so target compounds can be detected selectively. These advantages indicate that the GC/MS method is more appropriate for analyses in which lower analytical errors are required, such as for therapy monitoring and for specific patient diagnosis (e.g. moderate hyper-excretions or not an acute episode). The GC/MS method is necessary as a back-up method for MS/MS, especially as a precise quantitative method. In clinical laboratories, GC/MS is already widely used for various analyses, such as for organic acids in urine and for very long chain fatty acids in plasma that are indicative of an inborn error of metabolism [19,36–38]; thus, this method of amino acid analysis using GC/MS would be useful for those laboratories[9].

5. Conclusion

This new method enables simple, rapid and precise analysis for determination of amino acids in whole blood using GC/MS. It was successfully applied to 5 patients with 3 types of amino acid disorders, providing similar concentration levels to those reported using other methods.

Our study demonstrated the feasibility of routine biochemical test of amino acids using this method. Therefore, further studies to expand other amino acids should be meaningful in order to apply this method to routine biochemical tests for inborn errors of amino acid metabolism.

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