Journal of Chromatography, 579 (1992) 215–224
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6459

Measurement of free amino acid levels in ultrafiltrates of blood plasma by high-performance liquid chromatography with automatic pre-column derivatization

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(First received April 9th, 1992; revised manuscript received May 20th, 1992)

ABSTRACT

Although there are many techniques available for the analysis of amino acids, deproteinization is still one of the major problems in the analysis of amino acids in physiological fluids. The method used to prepare the plasma and to remove the plasma protein has a marked effect on the final results. The most widely used method of deproteinization is precipitation with 5-sulphosalicylic acid followed by centrifugation to remove the precipitated protein. We have not had success in using this deproteinization agent for the analysis of plasma amino acids by a high-performance liquid chromatographic method with automatic pre-column *o*-phthaldialdehyde–3-mercaptopropionic acid and 9-fluorenylmethyl chloroformate derivatization because of the adverse effect of the sulphosalicylic acid supernatant on the quantitation and separation. Ultrafiltration was used as an alternative method for the preparation of plasma samples in this experiment. The results were satisfactory for the analysis of plasma amino acids in 1500 samples during a period of four years. Some factors that might influence the results of the ultrafiltration were investigated.

INTRODUCTION

The analysis of amino acids is considered to be one of the most important applications in the biomedical and biochemical fields. Many diseases are associated with disorders in amino acid metabolism. Profiling of plasma amino acids is of great importance today and has found broad application in clinical practice. The analysis of amino acids also offers the possibility of genetic prevention in both pre-marital and prenatal stages for those diseases resulting from inborn errors of metabolism. The plasma amino acid pattern has also been used to follow the course of prolonged dietary treatment [1].

For nearly 30 years, amino acid determinations have been carried out mainly by means of ion-exchange chromatography with post-column derivatization [1,2]. In recent years, methods employing pre-column derivatization combined with reversed-phase high-performance liquid chromatography (HPLC) have been recognized as a powerful method. Publications have demonstrated the usefulness of this technique for the determination of amino acids in physiological fluids [3–17].

Although there are many techniques available for the analysis of amino acids, and the sample can be analysed quickly, accurately and sensitive-

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0378-4347/92/$05.00

ly, deproteinization is still one of the major problems in the analysis of amino acids in physiological fluids [2]. The whole plasma contains soluble peptides and proteins, which should be removed from the sample because they will clog the chromatographic column, increase back-pressure on the instruments and interfere with the separation. The method used to prepare the plasma and to remove the plasma protein has a marked effect on the final results [1,2]. One collaborative trial on the determination of free amino acids in blood plasma showed that the precision was much poorer than that of protein hydrolysate. It was concluded that this was due mainly to the deproteinization procedure [18].

The most widely used method of deproteinization is precipitation with 5-sulphosalicylic acid (SSA) followed by centrifugation to remove the precipitated protein [1]. We have not had success in using SSA as the deproteinization agent for the analysis of plasma amino acids by HPLC with automatic pre-column *o*-phthaldialdehyde (OPA)–3-mercaptopropionic acid (3-MPA) and 9-fluorenylmethyl chloroformate (FMOC-Cl) derivatization [19], because of the adverse effect of SSA supernatant on the quantitation and separation. A similar problem of SSA interference with amino acid quantitation has been reported by others [9,11]. The masking of early-eluting peaks in the chromatogram by SSA has also been reported in the pre-column derivatization with phenyl isothiocyanate [15].

Ultrafiltration has the advantages of achieving a protein-free sample without the addition of chemical agents, thus keeping the sample close to the physiological state. It is an attractive alternative to equilibrium dialysis because of the ease and speed with which it can be accomplished. Another potential benefit of ultrafiltration is that platelets and leukocytes are removed from the plasma and thus the contamination of amino acids from these blood components can be eliminated [20]. So ultrafiltration was used for the preparation of the plasma in this study. Satisfactory results were achieved for the analysis of plasma amino acids by the automatic pre-column OPA–3-MPA and FMOC-Cl derivatization and reversed-phase HPLC method. Some factors that might have an influence on the final results of the ultrafiltration were investigated.

EXPERIMENTAL

Chromatographic conditions

A Hewlett-Packard 1090M series high-performance liquid chromatographic system was used. This system consists of a DR-5 solvent delivery system, an auto-injector and autosampler, a build-in 1040A photodiode-array UV detector and an HP 1046A fluorescence detector. Data were processed by an HP 79994A analytical workstation. Automatic pre-column derivatization with OPA–3-MPA and FMOC-Cl was performed by an injector programme (Table I). The separation of amino acids both in a standard solution containing 29 amino acids and in plasma was done by gradient elution according to a chromatographic timetable. Mobile phases were 0.010 or 0.015 *M* sodium acetate buffer (pH 6.8) and methanol. The flow-rate was 0.3 ml/min and the stop time was 30 min after the injection. Two HP Hypersil-ODS 5-μm columns (100 mm × 2.1 mm I.D.) were put in series for the separation, preceded by a guard column (20 mm × 2.1 mm I.D.). The column temperature was set at 40°C. For the detection of amino acid derivatives, the photodiode-array detector was set at three sam-

TABLE I

INJECTION PROGRAMME

Line	Function	Amount	Reagent
1	Draw	0.0 μl from vial 4	Water
2	Draw	2.5 μl from vial 5	OPA
3	Draw	0.0 μl from vial 4	
4	Draw	2.5 μl from vial X	Sample
5	Mix	5.0 μl cycles 2	
6	Draw	0.0 μl from vial 4	
7	Draw	1.0 μl from vial 8	FMOC-Cl
8	Mix	6.0 μl cycles 2	
9	Wait	2.5 min	
10	Inject		

ple wavelengths of 338, 266 and 230 nm with bandwidths of 10, 4 and 4 nm, respectively. The reference wavelength was 550 nm, with a bandwidth of 100 nm. The initial wavelengths for the fluorescence detector were 230 and 450 nm for excitation and emission, respectively, and 20 min after the injection, they were changed to 260 and 315 nm, respectively, for the determination of secondary amino acids. This method has been described in detail previously [19].

Reagents

Water and methanol were HPLC grade (Curtin Matheson Scientific, Houston, TX, USA). Chemicals used were analytical grade, including sodium acetate, glacial acetic acid, boric acid, sodium hydroxide, *o*-phthaldialdehyde, 3-mercaptopropionic acid, 9-fluorenylmethyl chloroformate and 5-sulphosalicylic acid (all from Sigma, St. Louis, MO, USA).

Ultrafiltration

A 1-ml sample of blood was taken by venipuncture and put into a vacutainer tube containing heparin. The blood was centrifuged at 1500 *g* for 10 min at 10°C. Heparinized plasma samples were ultrafiltered by using the Centrifree system (Amicon, Beverly, MA, USA). A 0.2-ml volume of plasma was put in the sample reservoir, then the device was placed in a centrifuge with a 45° fixed-angle rotor. About 60 μl of ultrafiltrates were collected after centrifugation at 750 *g* for 15 min. The ultrafiltrates were stored at −80°C until analysis. MPS-1 micropartition systems with YM30 or YC05 membrane were also tested for a comparison of the effect of different membranes on ultrafiltration.

RESULTS AND DISCUSSION

Reversed-phase HPLC is a powerful method for assaying physiological amino acid concentrations in biological fluids. In comparison with the post-column derivatization, which has been used for nearly 30 years in most amino acid analyses, pre-column derivatization has the advantages of shorter analysis time, greater sensitivity and the more versatility of the equipment [3–17]. Among the methods of pre-column derivatization, OPA has become the most popular because the procedure is relatively easy and the reaction occurs rapidly at room temperature [14]. One disadvantage of OPA derivatization has been the lack of stability of the adduct when 2-mercaptoethanol (2-ME) is used as sulfhydryl reagent [5,8]. Consequently, time variances between the reaction and injection during a manual procedure may cause significant errors in quantitation. The other disadvantage is that OPA reacts only with primary amines. Secondary amino acids (imino acids) are not detected [14]. We have reported an automatic pre-column derivatization procedure that eliminates most of the errors due to the variation in reaction time and in volumes of sample or reagents [19]. In addition, 2-ME has been replaced by 3-MPA with considerable improvement in stability. FMOC-Cl is also incorporated, as a second reagent for the derivatization of secondary amino acids. Photodiode-array and programmable fluorescence detectors are used for the detection. Therefore, both primary and secondary amino acids can be detected simultaneously [19].

Chromatograms of a standard mixture of amino acids and a representative plasma sample are shown in Figs. 1 and 2. These chromatograms showed a satisfactory separation of 29 primary and secondary amino acids. The reproducibility of the peak areas of this method was tested by eight consecutive injections of 29 standard amino acids mixture. The results are listed in Table II. The coefficients of variation (C.V.) for peak areas ranged from 0.78 to 2.84%, with a mean ± S.D. of 1.73 ± 0.67%. These results are very close to the reproducibility reported by Furst *et al.* [14]. The high precision of this method would allow analysis without an internal standard for quantitation.

When this method was used for the analysis of plasma samples deproteinized by SSA, several major problems were encountered. First, the yield of the derivatization was low in the SSA supernatant. This is probably because the strongly acidic nature of SSA inhibits the formation of OPA–amino acid derivatives, which require an

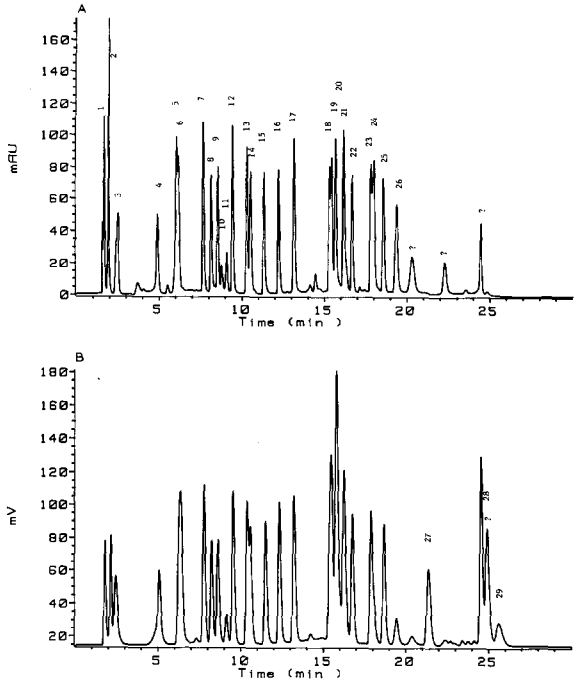

Fig. 1. Typical chromatograms showing the separation of 29 derivatized standard amino acids (500 μM): (A) UV detection at 338 nm; (B) fluorescence detection. Peaks: 1 = O-phospho-L-serine; 2 = aspartic acid; 3 = glutamic acid; 4 = glutathione (reduced); 5 = asparagine; 6 = serine; 7 = glutamine; 8 = glycine; 9 = threonine; 10 = histidine; 11 = cystine; 12 = citrulline; 13 = taurine; 14 = alanine; 15 = arginine; 16 = tyrosine; 17 = α-amino-*n*-butyric acid; 18 = methionine; 19 = valine; 20 = norvaline; 21 = tryptophan; 22 = phenylalanine; 23 = isoleucine; 24 = ornithine; 25 = leucine; 26 = lysine; 27 = hydroxyproline; 28 = sarcosine; 29 = proline.

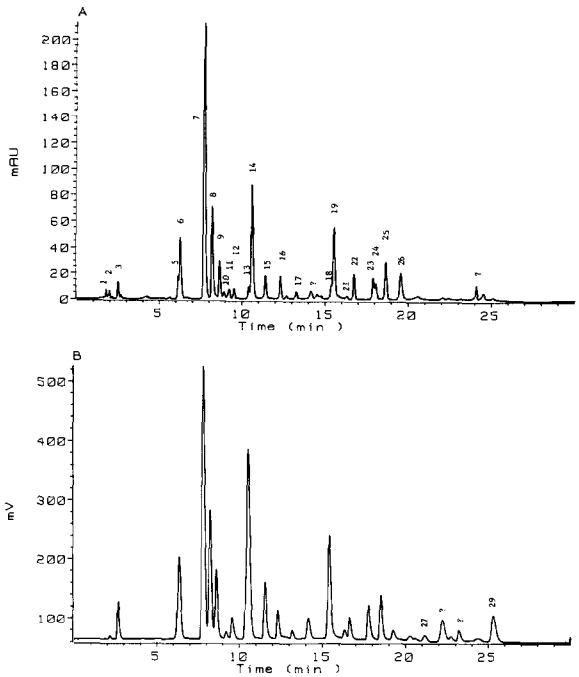

Fig. 2. Chromatograms of a representative plasma sample: (A) UV detection at 338 nm; (B) fluorescence detection. Peak numbers as in Fig. 1.

alkaline pH. A similar problem has been reported when trichloroacetic acid was used to precipitate proteins from biological samples [6]. Secondly, the first three amino acid peaks of the chromatogram [O-phospho-L-serine (OPS), Asp and Glu] were superimposed on the large SSA peak. The SSA peaks were higher than 4000, 2000 and 300 mA.U. at 230, 260 and 338 nm, respectively. The problem of SSA interference with amino acids determination was also reported by others [9,11]. The third problem was the adverse effects of the SSA sample on the separation of other amino acids. When ethanol or methanol was used for the deproteinization, the sample was diluted and some of the less concentrated plasma amino acids (OPS, Asp, Glu, AABA and Trp) became undetectable by the UV detector at 338 nm. Other problems were the high level of organic solvent in the injected material, which resulted in broad peaks in the early part of the chromatogram, and the increased volatility of the sample, which made it difficult to keep intact.

Among the other techniques that have been used in the deproteinization of plasma, equilibrium dialysis and ultrafiltration are the most likely candidates for adoption in a clinical laboratory. Ultrafiltration appears to be more appropriate than dialysis because it can be carried out rapidly. Although ultrafiltration methods are not widely applied in the ion-exchange chromatography of amino acids (for unknown reasons they decrease the retention time during chromatographic separation and this consequently leads to distorted separations of critical pairs of amino acids) [1,2], ultrafiltration has been chosen for the preparation of protein-free samples in the analysis of amino acids by HPLC with pre-column de-

TABLE II

REPRODUCIBILITY OF PEAK AREAS FOR STANDARD AMINO ACIDS

Peak areas obtained from UV detection at 338 or 266 nm; amino acid concentration at 250 μM (n = 8). Abbreviations: OPS = O-phospho-L-serine; Asp = aspartic acid; Glu = glutamic acid; GSH = glutathione (reduced); Asn = asparagine; Ser = serine; Gln = glutamine; Gly = glycine; Thr = threonine; His = histidine; Cys = cystine; Cit = citrulline; Tau = taurine; Ala = alanine; Arg = arginine; Tyr = tyrosine; AABA = α-amino-n-butyric acid; Met = methionine; Val = valine; N-Val = norvaline; Trp = tryptophan; Phe = phenylalanine; Ile = isoleucine; Orn = ornithine; Leu = leucine; Lys = lysine; Hyp = hydroxyproline; Sar = sarcosine; Pro = proline.

Amino acid	Mean	S.D.	C.V. (%)
OPS	527.6	5.466	1.03
Asp	528.1	4.134	0.78
Glu	545.4	15.49	2.84
GSH	532.4	13.69	2.57
Asn	581.9	16.58	2.84
Ser	694.3	8.961	1.29
Gln	676.1	8.986	1.32
Gly	498.8	9.966	1.99
Thr	536.4	10.78	2.00
His	106.0	1.591	1.50
Cys	170.8	3.943	2.30
Cit	637.7	4.881	0.76
Tau	582.4	5.758	0.98
Ala	589.0	5.216	0.88
Arg	554.4	7.371	1.32
Tyr	553.7	8.140	1.47
AABA	707.5	13.78	1.94
Met	548.4	10.52	1.91
Val	660.3	7.686	1.16
N-Val	829.4	12.16	1.46
Trp	638.4	16.49	2.58
Phe	535.1	11.55	2.15
Ile	646.1	18.51	2.86
Orn	540.6	15.80	2.92
Leu	545.1	10.01	1.83
Lys	587.8	9.541	1.62
Hyp	1469	28.91	1.91
Sar	2427	20.85	0.85
Pro	645.9	8.856	1.37

rivatization [10,11,17,20]. The results indicate that ultrafiltration of plasma may replace chemical deproteinization in the HPLC analysis of free amino acids [15].

TABLE III

COMPARISON OF ULTRAFILTRATION BY DIFFERENT ROTORS

Peak areas obtained from UV detection at 338 or 266 nm. Amino acid abbreviations as in Table II.

Amino acid	Fixed-angle rotor (F)	Swinging-bucket rotor (S)	S/F (%)
OPS	20.73	18.62	89.82
Asp	252.08	181.97	72.19
Glu	151.41	92.45	61.06
Asn	253.04	175.47	69.36
Ser	759.11	526.41	69.31
Gln	2351	1628	69.25
Gly	627.71	372.35	59.32
Thr	188.95	126.05	66.72
His	73.04	50.77	69.51
Cit	149.09	101.56	69.47
Tau	203.73	141.23	69.36
Ala	1833	1271	69.38
Arg	601.73	409.36	68.03
Tyr	338.41	241.52	71.37
AABA	119.76	75.04	62.66
Met	132.52	89.41	67.48
Val	1192	804.76	67.44
Trp	34.77	14.84	42.68
Phe	210.83	138.16	65.53
Ile	440.12	300.98	68.39
Orn	410.72	288.52	70.25
Leu	772.58	559.15	72.37
Lys	1071	709.98	66.29
Hyp	69.39	41.56	59.89
Pro	644.77	445.36	69.07

In our study, the commercially available Centrifree system was used. During ultrafiltration, the sample is deproteinized by filtration of plasma through an ultrafiltration membrane, protein is retained by the membrane while ultrafiltrates with free amino acid pass through and are collected in the filtrater cup. We investigated several factors, such as the rotor, the membrane and the time of the ultrafiltration, that may influence the final results of the ultrafiltration.

First, we compared the effect of different rotors on the ultrafiltration. Two aliquots of the same specimen were loaded into two Centrifree systems, one put into a centrifuge with fixed-an-

gle rotor and another into a centrifuge with swinging-bucket rotor. Both were centrifuged at 750 *g* for 15 min. The results are presented in Table III. The levels of amino acids in ultrafiltrates obtained with the fixed-angle rotor were higher than those obtained with swinging-bucket rotor. This may be due to the different polarization control between fixed-angle and swinging-bucket rotors. The use of a fixed-angle rotor provides polarization control. The angle counteracts the build-up of retained protein at the membrane surface, because this dense layer slides outward and accumulates at the edge of membrane. In a swinging-bucket rotor, the polarization layer is compacted over the entire membrane surface, restricting the passage of solute and solvents through the membrane. Our experiment suggests that the polarization occurring during the ultrafiltration may be one of the important factors that influence the recovery rate of amino acids. This might be the reason why some other authors could not achieve satisfactory results by ultrafiltration [21], because it is difficult to control polarization when ultrafiltration is performed under nitrogen pressure or a syringe is used as the driving force for the ultrafiltration.

Table IV presents a comparison of ultrafiltration by different membranes. Two MPS-1 micropartition systems with different membranes were tested for the treatment of the same sample. One was YC05 membrane and another was YM30 membrane, with nominal molecular mass cut-offs of 500 and 30 000, respectively. The results showed that the recovery was markedly lower when the YC05 membrane was used. Thus the Centrifree system with a YMT membrane was used for further experiments.

The time of ultrafiltration is another factor that might have an influence on the recoveries of amino acids, and data on this effect are listed in Table V. The results indicated that the ultrafiltration of an amino acid standard mixture and plasma for 5, 15 and 30 min produced similar amino acid concentrations. Ultrafiltration for 15 min was chosen for the rest of our studies.

The recovery and the reproducibility were tested with ten aliquots of amino acid standards.

TABLE IV

COMPARISON OF ULTRAFILTRATION BY DIFFERENT MEMBRANES

Peak areas obtained from UV detection at 338 or 266 nm. Amino acid abbreviations as in Table II.

Amino acid	YC05	YM30	YC05/YM30 (%)
OPS	10.38	17.31	59.97
Asp	10.58	17.87	59.21
Glu	68.51	192.73	35.55
Asn	22.96	47.82	48.01
Ser	96.73	144.05	67.15
Gln	577.19	1034.23	55.81
Gly	174.52	276.13	63.20
Thr	49.31	99.11	49.75
His	21.06	64.76	32.52
Cys	34.68	77.51	44.74
Cit	24.57	46.25	53.12
Tau	68.08	77.81	87.50
Ala	226.93	416.84	54.44
Arg	68.31	104.32	65.48
Tyr	54.16	86.18	62.85
AABA	10.76	24.33	44.23
Met	10.19	26.16	38.95
Val	136.77	290.12	47.14
Trp	8.38	31.14	26.91
Phe	43.52	59.02	73.74
Ile	33.43	72.31	46.23
Orn	44.96	52.62	85.44
Leu	68.42	114.42	59.80
Lys	34.15	88.61	38.54
Hyp	34.31	47.25	72.61
Pro	150.71	253.89	59.36

Five were analysed by HPLC before ultrafiltration, and five aliquots after ultrafiltration (Table VI). The results showed that the recoveries for all of the standard amino acids were excellent, ranging from 95 to 102%. It should be mentioned that when standard amino acids were added to the plasma, Trp and GSH (a peptide) showed poor recoveries (81 and 32%, respectively) and all other amino acids showed the same recoveries [19]. The reason is unknown.

During practical applications, analysis of physiological samples with different amino acid concentrations is required. The relationship of amino acid concentrations in the ultrafiltrates

TABLE V

INFLUENCE OF ULTRAFILTRATION TIME ON PEAK AREAS OF STANDARD AND PLASMA AMINO ACIDS

Peak areas obtained from UV detection at 338 or 266 nm. Amino acid abbreviations as in Table II.

Amino acid	Standard amino acids			Plasma amino acids		
	5 min	15 min	30 min	5 min	15 min	30 min
OPS	209.79	209.16	193.26	11.71	11.57	12.68
Asp	327.65	330.39	318.22	18.68	17.96	21.59
Glu	343.31	360.87	350.26	40.65	41.35	44.02
GSH	98.79	102.01	89.86	–	–	–
Asn	415.61	444.43	440.19	51.68	52.18	52.54
Ser	623.42	666.65	660.04	103.38	104.38	105.08
Gln	986.52	1022	1011	533.17	527.15	536.48
Gly	530.93	531.74	533.57	222.96	222.81	229.14
Thr	414.45	433.15	430.35	136.73	136.03	139.52
His	101.56	112.80	112.09	23.55	22.51	23.08
Cys	108.07	109.29	104.61	86.94	87.81	86.03
Cit	394.99	411.26	404.38	22.06	21.21	20.68
Tau	318.99	340.35	336.93	51.21	51.22	52.22
Ala	703.67	734.85	721.43	382.38	382.05	389.22
Arg	307.21	327.76	329.89	86.72	83.31	84.51
Tyr	294.93	328.56	305.55	63.56	59.41	61.15
AABA	350.01	385.05	374.12	20.74	18.11	18.75
Met	253.11	268.72	267.71	24.57	24.33	25.86
Val	512.41	532.58	520.61	221.18	219.03	232.78
Trp	385.52	395.76	392.73	25.89	26.34	24.93
Phe	286.81	301.81	294.72	47.71	46.68	47.62
Ile	317.27	328.48	333.65	60.32	58.73	59.88
Orn	485.86	504.11	499.15	56.53	55.44	55.34
Leu	423.03	440.04	450.75	105.37	104.66	107.65
Lys	504.86	554.25	573.61	138.89	141.75	143.59
Hyp	679.32	664.41	676.87	29.17	27.29	28.97
Pro	258.49	265.65	275.41	116.94	107.90	109.34

was investigated. A linear relationship between the concentration and the peak areas of each standard amino acid was determined by analysing the standard amino acid mixture at concentrations ranging from 31.25 to 500 μM ($n = 5$, by serial dilution). These concentrations covered the normal range of most plasma amino acids. For plasma, the original plasma sample and plasma samples diluted by HPLC water to 75, 50 and 25% ($n = 4$) of the plasma were analysed. The linear regression analysis showed satisfactory coefficients of correlation (>0.99) between the concentration and peak areas of each amino acid from both UV and fluorescent signals, and in both standard amino acids and plasma samples (Table VII).

This method has been successfully used for four years for the preparation of plasma, serum and cerebrospinal fluid for a total 1500 samples. Precautions should be taken when this method is used for some samples with higher protein concentration, such as blood cell lysates or tissue extracts. It has been reported that for the lipemia samples, the time required to filter sufficient sample was very variable and occasionally no filtrate could be obtained, presumably because the membrane pores got blocked [16]. We did not observe this problem, possibly because different ultrafil-

TABLE VI

RECOVERIES OF STANDARD AMINO ACIDS BY ULTRAFILTRATION

Peak areas obtained from UV detection at 338 or 266 nm. Amino acids abbreviations as in Table II. $n = 5$.

Amino acid	Before UF (mean ± S.D.)	After UF (mean ± S.D.)	Recovery (mean ± S.D.) (%)
OPS	471.16 ± 6.69	452.31 ± 5.58	96 ± 6.3
Asp	551.51 ± 4.39	529.44 ± 6.22	96 ± 3.2
Glu	548.12 ± 11.68	531.67 ± 9.74	97 ± 3.9
GSH	544.35 ± 12.84	517.13 ± 11.67	95 ± 3.6
Asn	562.22 ± 10.27	560.08 ± 9.83	100 ± 3.1
Ser	679.37 ± 9.25	672.57 ± 10.41	99 ± 1.6
Gln	772.93 ± 7.53	762.20 ± 6.94	99 ± 1.6
Gly	518.21 ± 10.24	513.02 ± 9.36	99 ± 2.3
Thr	503.26 ± 8.69	494.19 ± 7.28	98 ± 2.1
His	154.05 ± 1.72	157.13 ± 1.68	102 ± 5.5
Cys	171.17 ± 3.25	162.61 ± 2.51	95 ± 2.2
Cit	638.12 ± 5.98	625.35 ± 7.37	98 ± 3.5
Tau	576.18 ± 6.27	570.41 ± 8.24	99 ± 4.3
Ala	617.22 ± 7.38	611.04 ± 6.15	99 ± 1.9
Arg	559.57 ± 6.22	553.97 ± 5.58	99 ± 2.0
Tyr	546.50 ± 7.89	535.57 ± 6.48	98 ± 1.3
AABA	693.52 ± 10.14	686.58 ± 11.26	99 ± 1.8
Met	501.93 ± 11.13	491.89 ± 9.28	98 ± 3.4
Val	668.21 ± 7.39	654.84 ± 8.19	98 ± 2.6
N-Val	835.02 ± 13.87	826.66 ± 10.54	99 ± 1.6
Trp	668.65 ± 14.36	648.59 ± 11.28	97 ± 3.1
Phe	551.18 ± 9.92	540.15 ± 8.43	98 ± 2.6
Ile	626.08 ± 17.32	613.55 ± 15.24	98 ± 1.7
Orn	663.89 ± 16.15	643.94 ± 14.32	97 ± 2.5
Leu	572.55 ± 9.23	566.82 ± 10.07	99 ± 2.8
Lys	603.80 ± 10.15	585.68 ± 9.64	97 ± 4.2
Hyp	1328.06 ± 31.36	1341.76 ± 29.85	101 ± 2.5
Sar	2758.56 ± 22.47	2702.82 ± 20.13	98 ± 3.1
Pro	506.27 ± 6.97	490.54 ± 6.35	97 ± 3.2

tration devices were used. Another problem with our method is that Trp (an amino acid with higher molecular mass) and the peptide GSH show relatively poor recoveries. Changing the membrane in the ultrafiltration system to a membrane with a higher molecular mass cut-off might help to solve this difficulty.

ACKNOWLEDGEMENT

This study was supported by a grant from the Research Committee of Texas Scottish Rite Hospital for Children.

TABLE VII

CORRELATION COEFFICIENTS OF PEAK AREAS OF STANDARD AND PLASMA AMINO ACIDS

Amino acid abbreviations as in Table II.

Amino acid	UV 338 or 266 nm		Fluorescence	
	Standard[a]	Plasma[b]	Standard[a]	Plasma[b]
OPS	0.998	0.999	0.999	0.998
Asp	0.999	0.999	0.999	0.999
Glu	1.000	0.995	0.999	0.998
GSH	0.997	–	0.999	–
Asn	0.998	1.000	0.999	0.999
Ser	0.998	1.000	0.999	0.999
Gln	1.000	0.999	1.000	0.999
Gly	0.999	0.995	0.999	0.997
Thr	1.000	0.999	0.999	1.000
His	0.998	0.994	0.999	0.996
Cys	0.998	0.995	0.998	0.996
Cit	1.000	0.993	0.999	0.999
Tau	0.999	0.997	0.999	0.999
Ala	0.999	1.000	0.999	0.999
Arg	1.000	0.996	1.000	0.999
Tyr	0.999	0.999	0.999	0.999
AABA	0.999	1.000	0.999	0.998
Met	0.998	1.000	0.999	0.999
Val	0.998	1.000	0.999	0.999
N-Val	0.999	–	0.999	–
Trp	1.000	0.994	0.999	0.995
Phe	0.999	0.998	0.999	0.999
Ile	0.999	0.999	1.000	0.998
Orn	0.999	0.998	1.000	0.998
Leu	1.000	0.998	1.000	1.000
Lys	0.997	0.998	0.991	0.991
Hyp	0.997	0.996	0.999	0.998
Sar	0.999	–	0.997	–
Pro	0.998	0.997	0.999	0.998

[a] Standard amino acids, concentration range 31.25–500 μM (n = 5).

[b] Plasma amino acids, original plasma sample and plasma diluted by HPLC water to 75, 50 and 25% of the plasma (n = 4).

REFERENCES

1 Z. Deyl, J. Hyanek and M. Horakova, *J. Chromatogr.*, 379 (1986) 177.
2 A. P. Williams, *J. Chromatogr.*, 373 (1986) 175.
3 P. Lindroth and K. Mopper, *Anal. Chem.*, 51 (1979) 1667.
4 D. C. Turnell and J. D. H. Cooper, *Clin. Chem.*, 28 (1982) 527.
5 M. O. Fleury and D. V. Ashley, *Anal. Biochem.*, 133 (1983) 330.
6 S. S. O. Hung and T. W. Moon, *J. Chromatogr.*, 269 (1983) 114.
7 B. N. Jones and J. P. Gilligan, *J. Chromatogr.*, 266 (1983) 471.
8 H. Liu, Z. M. Liu, W. N. Zhu, Y. H. Li, X. S. Jiang and S. D. Wang, *Chin. J. Chromatogr.*, 1 (1984) 83.
9 H. Godel, T. Graser, P. Foldi, P. Pfaender and P. Furst, *J. Chromatogr.*, 297 (1984) 49.
10 G. Blundell and W. G. Brydon, *Clin. Chim. Acta*, 170 (1987) 79.
11 R. Schuster, *J. Chromatogr.*, 431 (1988) 271.
12 G. A. Qureshi and A. R. Qureshi, *J. Chromatogr.*, 491 (1989) 281.
13 D. T. Blankenship, M. A. Krivanek, B. L. Ackermannn and A. D. Cardin, *Anal. Biochem.*, 178 (1989) 227.
14 P. Furst, L. Pollack, T. A. Graser, H. Godel and P. Stehle, *J. Chromatogr.*, 499 (1990) 557.

15 B. L. Rosenlund, *J. Chromatogr.*, 529 (1990) 258.
16 J. F. Davey and R. S. Ersser, *J. Chromatogr.*, 528 (1990) 9.
17 A. S. Feste, *J. Chromatogr.*, 574 (1992) 23.
18 A. P. Williams, D. Hewitt, J. E. Cockburn, D. A. Harris, R. A. Moore and M. G. Davies, *J. Sci. Food Agric.*, 31 (1980) 474.
19 H. G. Worthen and H. Liu, *J. Liq. Chromatogr.*, 15 (1992) in press.
20 P. R. Borum, *Amicon Applications*, Publication No. 596.
21 J. G. Cummings, *Applications Data*, Beckman Instruments Inc., 4SP66877-280-8B.