Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Suppl 2, 2012 ISSN - 0974-2441

Research Article

SIMULTANEOUS IDENTIFICATION AND DETERMINATION OF TOTAL CONTENT OF AMINOACIDS IN FOOD SUPPLEMENTS – TABLETS BY GAS CHROMATOGRAPHY

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Received: 20 December 2011, Revised and Accepted: 4 February 2012

ABSTRACT

The aim of current study is simultaneously identification and determination of total content of 17 aminoacids in food supplements – tablets. Gas chromatographic method with flame ionization detector (GC – FID) was applied. Acid and alkaline (for analysis of Triptophan) hydrolysates were purified by cation – exchange solid – phase extraction, followed by the derivatization of aminoacids with ethylchloroformate. The dissolved in isooctane derivatives were analysed by GC – FID.

The identity is proved by the fact, that all data for the retention time (t_R) of aminoacids in tablets correspond to the values of t_R of the respective aminoacid in reference solution. Reference standard solution contained 200 nmol/l of each of aminoacids: L – Alanine, L – Glycine, L – Valine, L – Leucine, L – Isoleucine, L – Threonine, L – Serine, L – Proline, L – Aspartate, L – Methionine, L – Glutamate, L – Phenylalanine, L – Cystine, L – Lysine, L – Histidine, L – Tyrosine and L – Triptophan. Internal standard Norvaline was used. The best results for the recovery are obtained for the following aminoacids: 1) Amino 1000 tabl.: L – Glycine – 100 %; L – Triptophan – 100 %; L – Threonine – 109.52 %; 2) Amino 2300 tabl.: L – Isoleucine – 96.69 %; L – Proline – 96.81 %; L – Tyrosine – 99.81 %; L – Leucine – 101.67 %; L – Cystine – 102.51 %; L – Triptophan – 106.67 %.

The applied GC – FID method is appropriate for quality control with great accuracy for aminoacids L – Cystine, L – Glycine, L – Isoleucine, L – Leucine, L – Proline, L – Threonine, L – Triptophan and L – Tyrosine in food additives – tablets.

Keywords: GC, aminoacids, food supplements, tablets, determination.

INTRODUCTION

Food supplements like aminoacids combination products [1] and antioxidant vitamins for prevention of Parkinson's disease are very often applied [2]. Aminoacids possess important functions in human body. L – Aspartic acid increases the absorption of mineral supplements, enhances the wound healing, lowers the blood pressure, reduces the fatigue, protects the liver by removing excess ammonia and other toxins from the bloodstream and is important for brain, function of RNA, DNA and production of immunoglobulin and anttibodies [3]. L – Phenylalanine helps brain in the production of norepinephrine, promotes alertness and vitality, elevates mood, decreases pain, aids memory and learning and is used for treatment of arthritis, vitiligo [4], migraines, Parkinson's disease, depression and schizophrenia [5]. L – Tyrosine have shown application in Parkinson's disease, Alzheimer's disease and arthritis [6], improves cognitive and physical performance [7], is used in headaches, narcolepsy, anxiety and depression [8]. Oxidative damage caused by a disturbance of the balance between the antioxidant defense mechanisms of the human organism and the level of reactive oxygen species (ROS) has been associated with many pathological disorders such as atherosclerosis, diabetes and cancer [9]. L – Arginine, L – Cysteine, L – Glutamic acid, L – Glycine, L – Histidine, L – Lysine, L – Phenylalanine, L – Proline, L – Tryptophan, L – Tyrosine at 1 mM possesse protective role against oxidative damage produced by 1 mM H_2O_2 solution in in – vitro cataract model [10]. L – Tryptophan has shown effectiveness for treatment of depression [11].

For the simultaneous determination of aminoacids in cerebral spinal fluid are developed the following methods: 1) capillary zone electrophoresis (CZE) with pre – capillary derivatization of aminoacids using phenylisothiocyanate and separation of phenylthiocarbamyl – derivatives by CZE with a detection at λ = 254 nm [12]; 2) HPLC, with precolumn derivatization with different reagents, such as: 1) o – phthalaldehyde, used predominantly for analysis of L – Aspartate and L – Glutamate [13]; 2) phenylisothiocyanate [14]; 3) dabsyl chloride (in physiologic fluid and tissue extracts) for convertion of primary and secondary amines to their colored derivatives [15]: gradient RP – HPLC on LiChrosphere 100 RP – C_{18} column (250/2 mm i. d./5 µm particle size/50 °C); mobile phase A:9 mmol/l lithium phosphate : 40 ml/l dimethylformamide : 3 g/l guanidine thiocyanate : 2 g/l potassium perchlorate, with pH = 6.5, adjusted with 0.33 mol/l phosphoric acid; mobile phase B: 800 ml/l acetonitrile in water; flow rate = 200 µl/min [16].

Analytical methods, that have been reported for the determination of some aminoacids are:1) L – Ornithine and L – Aspartic acid in human plasma: capillary electrophoresis: with UV – detection at λ = 200 nm, on an uncoated silica capillary and buffer solution composed with 10 mM sodium tetraborate and 1 M sodium hydroxide (pH = 10.0) [17]; 2) L – Phenylalanine: fluorescence method in presence of combination of the cucurbit[7]uril and palmatine hydrochloride [18]; 3) L – Tyrosine: vis – spectrophotometry, based on absorption at λ = 750 nm of blue derivative, obtained after reaction with Folin – Ciocalteu reagent [19]; 4) L – Histidine: a) vis – spectrophotometry at λ = 405 nm, as a result of the formation of yellow colour from reaction between L – Histidine and diazotized sulfanilic acid [20]; b) isocratic HPLC in tissues [21];5) L – Tryptophan: chemiluminescence method, based on the reaction of galangin – potassium permanganate with L – Tryptophan in polyphosphoric acid media [22].

The aim of current study is to apply gas chromatographic method with flame ionization detector (GC – FID) for simultaneously identification and determination of total content of 17 aminoacids in food suppelements – tablets.

MATERIALS

I) Food additives: Amino 1000 tabl. (Bio – form Essential, Bulgaria, 100 tabl.); Amino 2300 tabl. (BioTech – USA, 325 tabl.).

II) Reference sertified standard solution with 200 nmol/l of standard substance of each of the following aminoacids: L – Alanine, L – Glycine, L – Valine, L – Leucine, L – Isoleucine, L – Threonine, L – Serine, L – Proline, L – Aspartate, L – Methionine, L – Glutamate, L – Phenylalanine, L – Cystine, L – Lysine, L – Histidine, L – Tyrosine, L – Triptophan.

III) Internal standard: Norvaline solution.

IV) Reagents with analytical grade quality: 6N HCl, 4% thioglycolic acid, Na_2CO_3, ethylchloroformate, isooctane, distilled water.

METHODS

Gas chromatography (GC)

I) Chromatogtaphic conditions

Analytical column – 10 m x 0.25 mm EZ: Faast; temperature program of analytical column – 0.3 min. at 110°C, 27°C/min. from 110 °C to

320°C, 5 min. at 320°C; temperature of injector (split – splittless in split regime) – 250°C; temperture of flame – ionization detector – 320°C.

II) Preparation of solutions of food additives Amino 1000 tabl., and Amino 2300 tabl. for the determination of aminoacids in acid hydrolysate.

To an accurately weighed quantity (50 mg) of powdered tablets Amino 1000 tabl. (average weight: 1.4 g) and Amino 2300 tabl. (average weight: 3.85 g) were added 10 ml 6N HCl, containing 4% thioglycolic acid. Solutions were incubated for 72 h at 110°C in hermetically closed glass container. An aliquot part of hydrolysates were neutralyzed by adding of Na_2CO_3 to pH: 2.5 ÷ 5.0. To 100 µl of the neutralized hydrolysed sample were added 100 µl of solution of internal standard Norvaline. The samples were purified by cation – exchange solid – phase extraction. The aminoacids in purified solutions were derivatized with ethylchloroformate. The derivatizing reagent was removed by scavenge with nitrogen. The derivatives of aminoacids were dissolved in aliquot part of isooctane and were analysed by gas chromatography with flame – ionization detector.

III) Preparation of solutions of food additives Amino 1000 tabl., and Amino 2300 tabl. for the determination of aminoacid L – Thyptophan in alkaline hydrolysate.

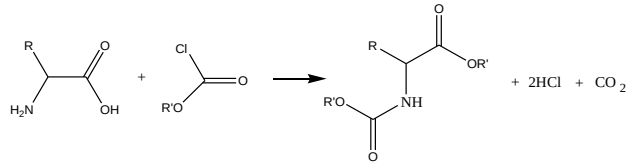
Aminoacid L – Triptophan in acid solutions is destructed as a result of acid hydrolysis. Because of it's unstabillity in acid solutons, for the determination of L – Thyptophan the samples were prepared by adding of 10 ml 1N KOH to an accurately weighed quantity (50 mg) of powdered tablets Amino 1000 tabl. (average weight: 1.4 g) and Amino 2300 tabl. (average weight: 3.85 g). The solutions were incubated for 48 h at 110°C in hermetically closed glass container. After the alkaline hydrolysis, the hydrolysate was neutralized by adding of Na_2CO_3 to pH: 2.5 ÷ 5.0. 100 µl of solution of internal standard Norvaline was added to 100 µl of the neutralized hydrolysate. The samples were purified by cation – exchange solid – phase extraction. The aminoacids in purified solutions were derivatized with ethylchloroformate. The derivatizing reagent was removed by scavenge with nitrogen. The derivatives of aminoacids were dissolved in aliquot part of isooctane and were analysed by gas chromatography with flame – ionization detector.

RESULTS AND DISCUSSION

I) Mechanism of derivatization reaction of aminoacids.

The derivatization of aminoacids with ethylchloroformate is described by the following reaction:

R' = C_2H_5



II) Selectivity of GC – FID method.

For the calibration of gas chromatograph was used a sertified standard solution of aminoacids.

Placebo solutions, containing all labeled in tablets supplements, without the active substances were prepared in the same manner like sertified standard solution. The selectivity of the applied method is confirmed by the fact that on chromatograms with placebo preparations are not exist peaks with retention time (t_R), corresponding to t_R of the respective aminoacid in sertified standard solution.

The identity of the examined animoacids is proved by the fact, that all of the data for the retention time (t_R) of aminoacids in tablets correspond to the values of t_R of the respective aminoacid in reference solution. On Table 1. are pointed out the values for t_R and area under the curve (AUC) for each aminoacid in the investigated products.

Table 1: Retetion time (t_R) and area under the curve (AUC) for aminoacids in Amino 1000 tabl. and Amino 2300 tabl.

N :	Aminoacid	Reference standard		Amino 1000 tabl.		Amino 2300 tabl.	
		t_R	Area [uV sec.]	t_R	Area [uV sec.]	t_R	Area [uV sec.]
Internal standard	Norvalin	2.864	13063.52	2.917	10229.64	2.864	15154.68
1.	L – Alanin	2.331	12585.93	2.393	15263.59	2.330	23961.03
2.	L – Glycine	2.469	10216.19	2.528	6109.75	2.466	9398.04
3.	L – Valine	2.707	11255.75	2.765	20869.95	2.711	32236.71
4.	L – Leucine	2.964	14558.44	3.024	62541.41	2.975	83037.22
5.	L – Isoleucine	3.036	10286.64	3.091	24489.09	3.044	38463.33
6.	L – Threonine	3.310	8168.72	3.353	5189.00	3.313	9967.72
7.	L – Serine	3.368	7000.09	3.410	1867.32	3.370	4095.46
8.	L – Proline	3.456	11979.44	3.500	21229.73	3.457	34907.26
9.	L – Aspartate	4.225	9514.03	4.257	18795.00	4.228	27858.18
10.	L – Methionine	4.280	11798.77	4.310	5515.56	4.279	9672.44
11.	L – Glutamate	4.661	5314.38	4.692	18960.29	4.669	32788.17
12.	L – Phenylalanine	4.729	19208.57	4.758	22693.96	4.732	36398.36
13.	L – Cystine	4.999	5649.46	5.069	1211.33	4.999	3228.09
14.	L – Lysine	6.270	13946.90	6.297	50723.04	6.320	280125.91
15.	L – Histidine	6.521	13822.86	6.531	10273.91	6.528	17914.69
16.	L – Tyrosine	6.871	21001.30	6.879	19066.32	6.879	29969.46
17.	L – Triptophan	7.300	18983.87	7.319	707.72	7.318	1339.95

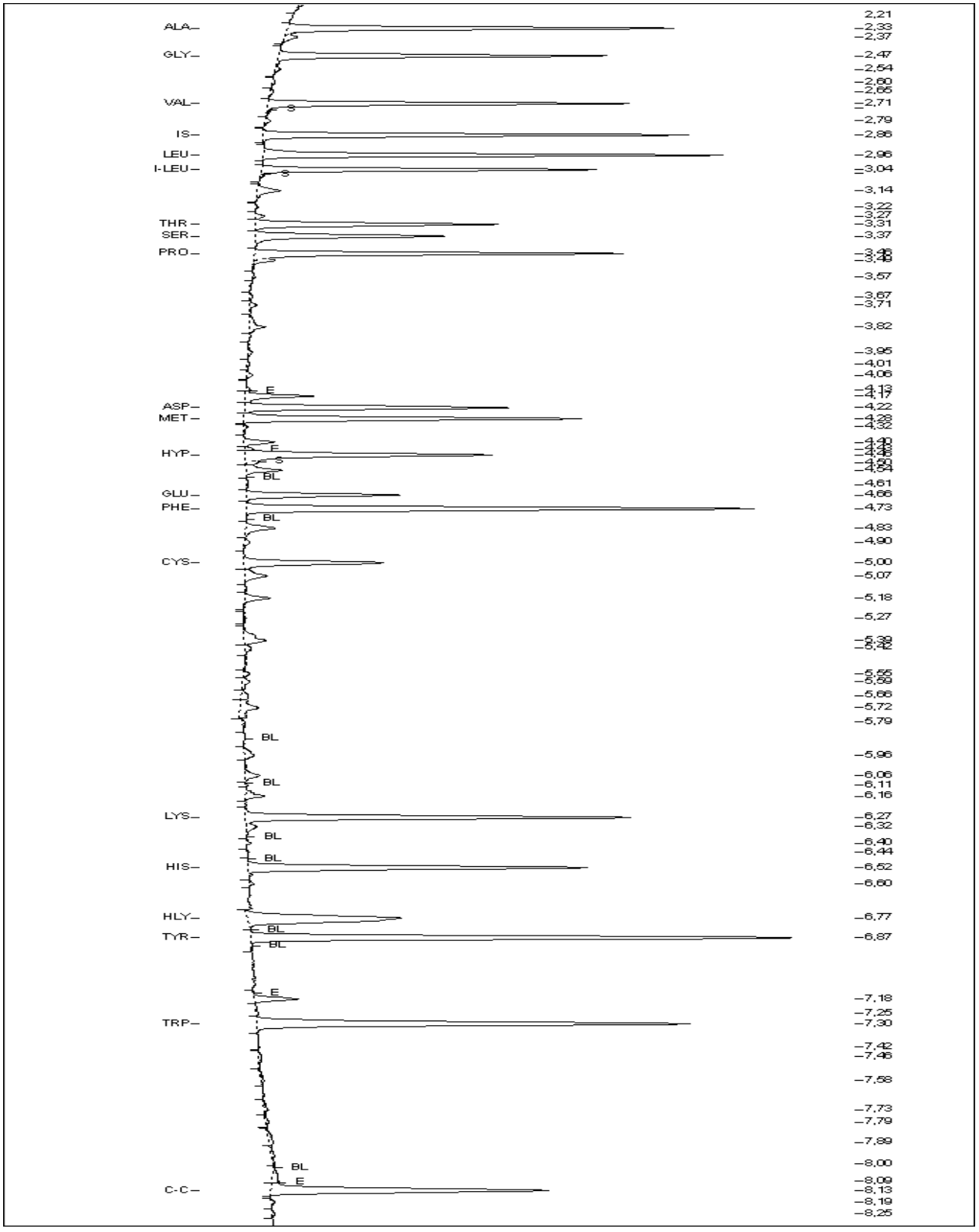


Fig. 1: Chromatogram of reference standard solution of aminoacids, used for the calibration before the analysis of acid hydrolysed sample of Amino 1000 tabl.

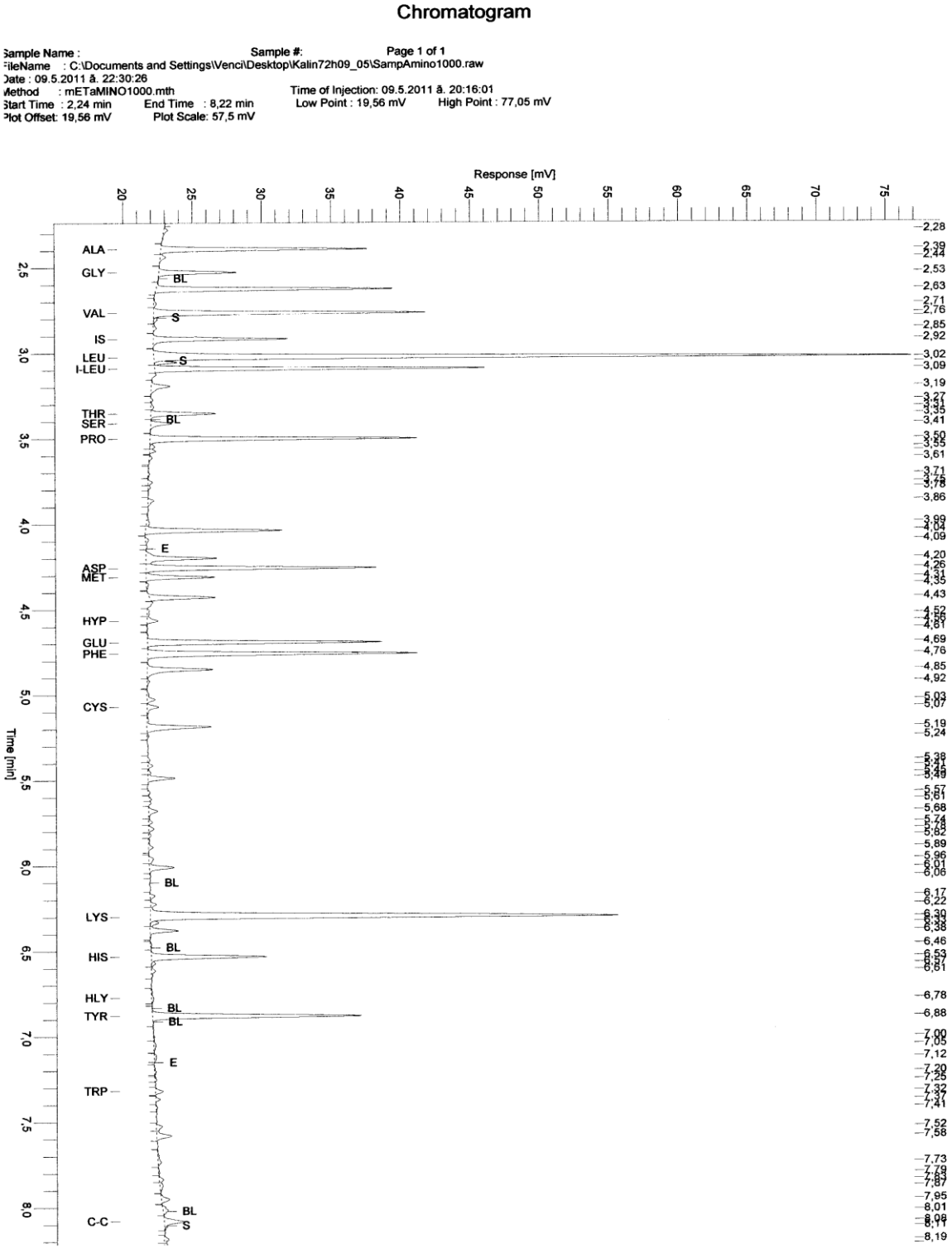


Fig. 2 : Chromatogram of sample of Amino 1000 tabl., hydrolysed with 6N HCl for 72 h at 110°C.

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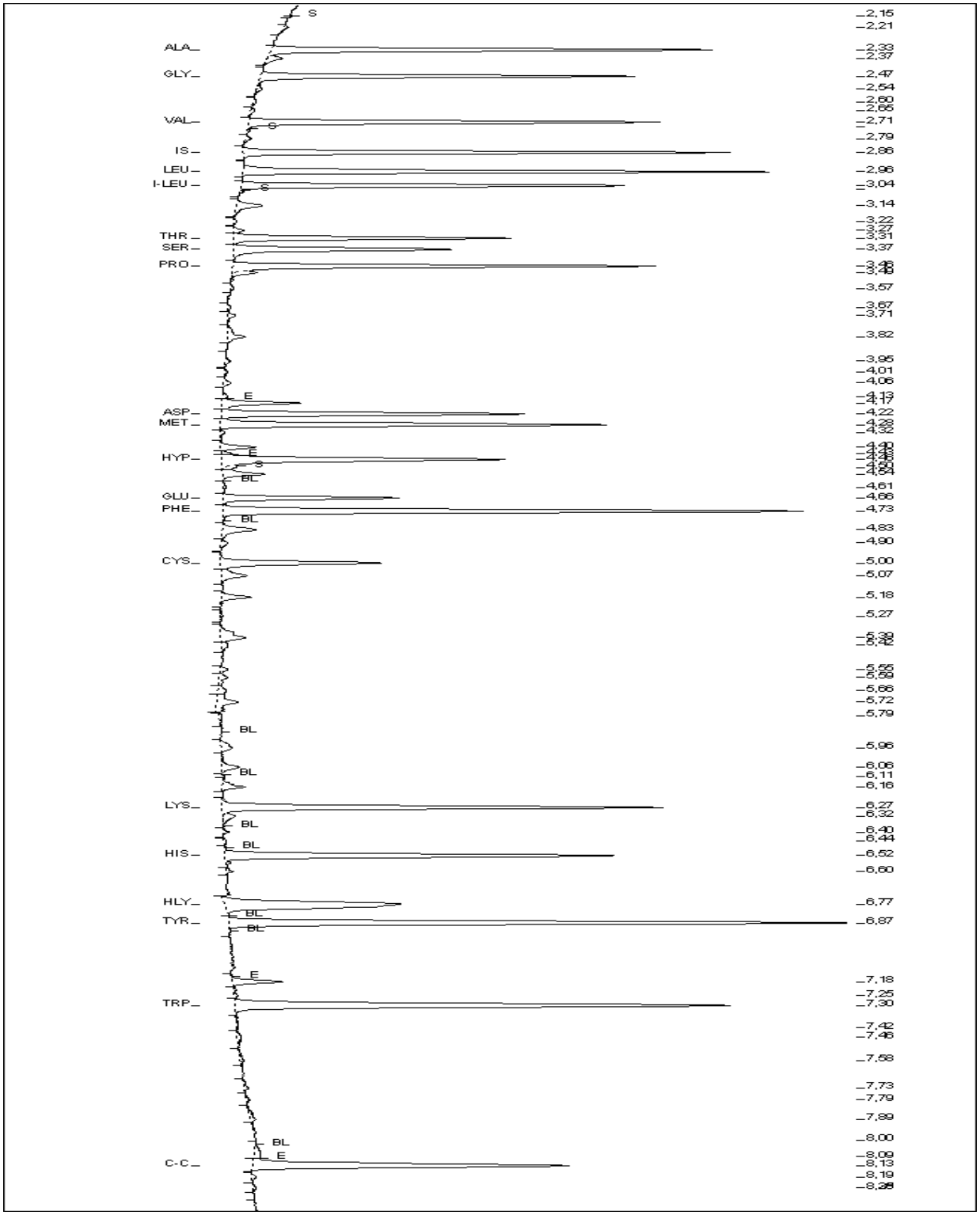

Fig. 3: Chromatogram of reference standard solution of aminoacids, used for the calibration before the analysis of acid hydrolysed sample of Amino 2300 tabl.

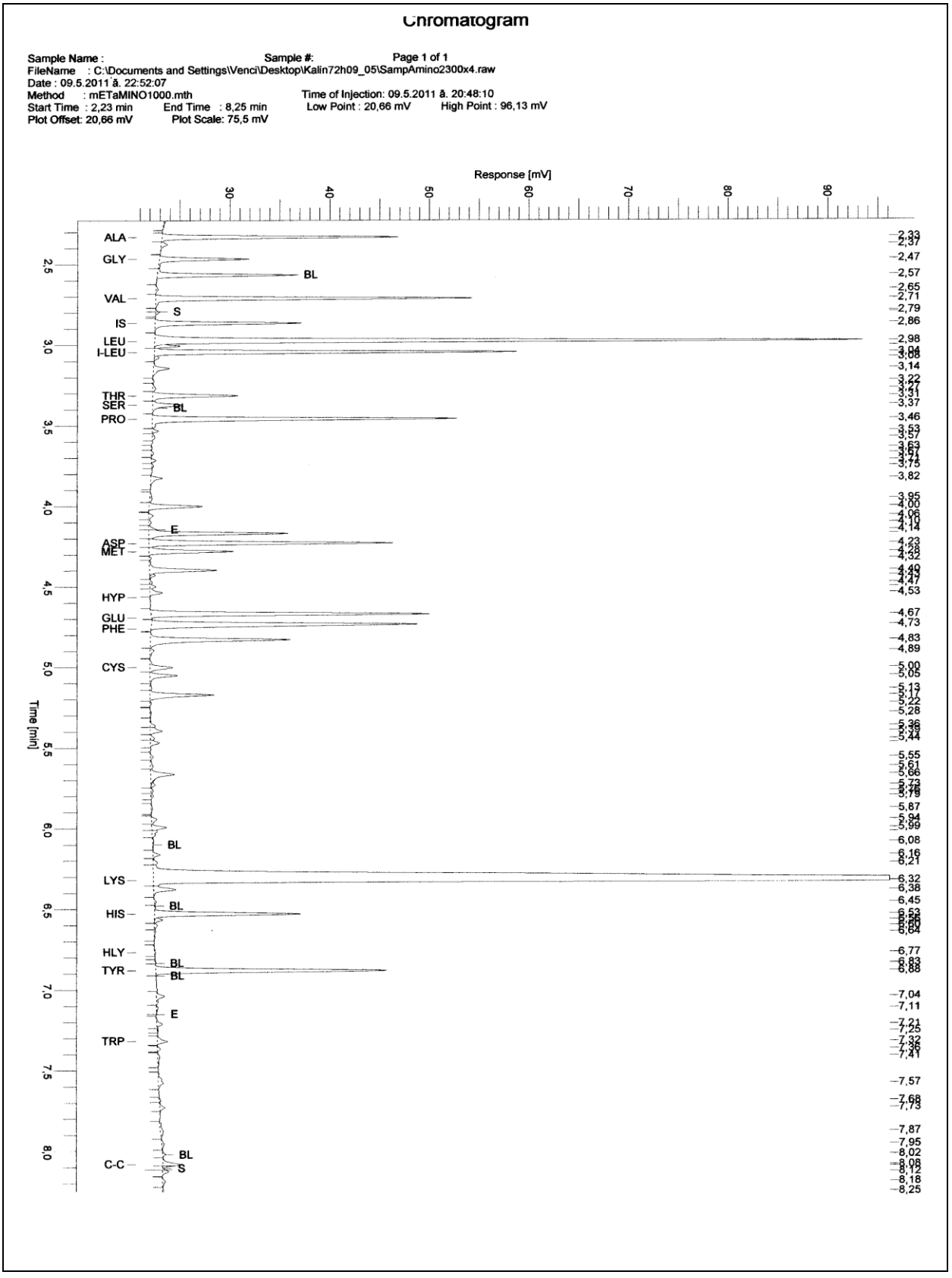

Fig. 4: Chromatogram of sample of Amino 2300 tabl., hydrolysed with 6N HCl for 72 h at 110°C.

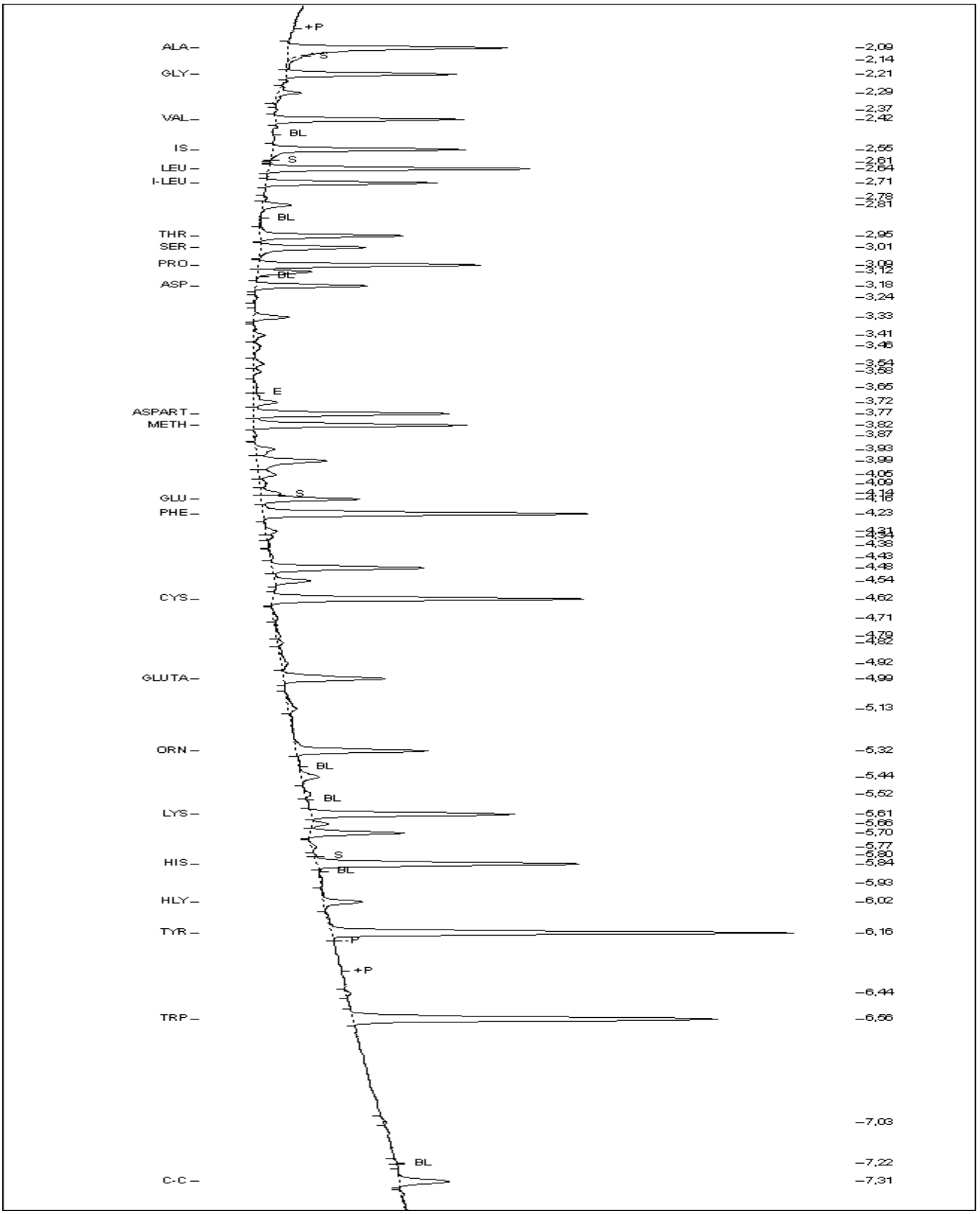

Fig. 5: Chromatogram of reference standard solution of aminoacids, used for the calibration before the analysis of alkaline hydrolysed sample of Amino 1000 tabl.

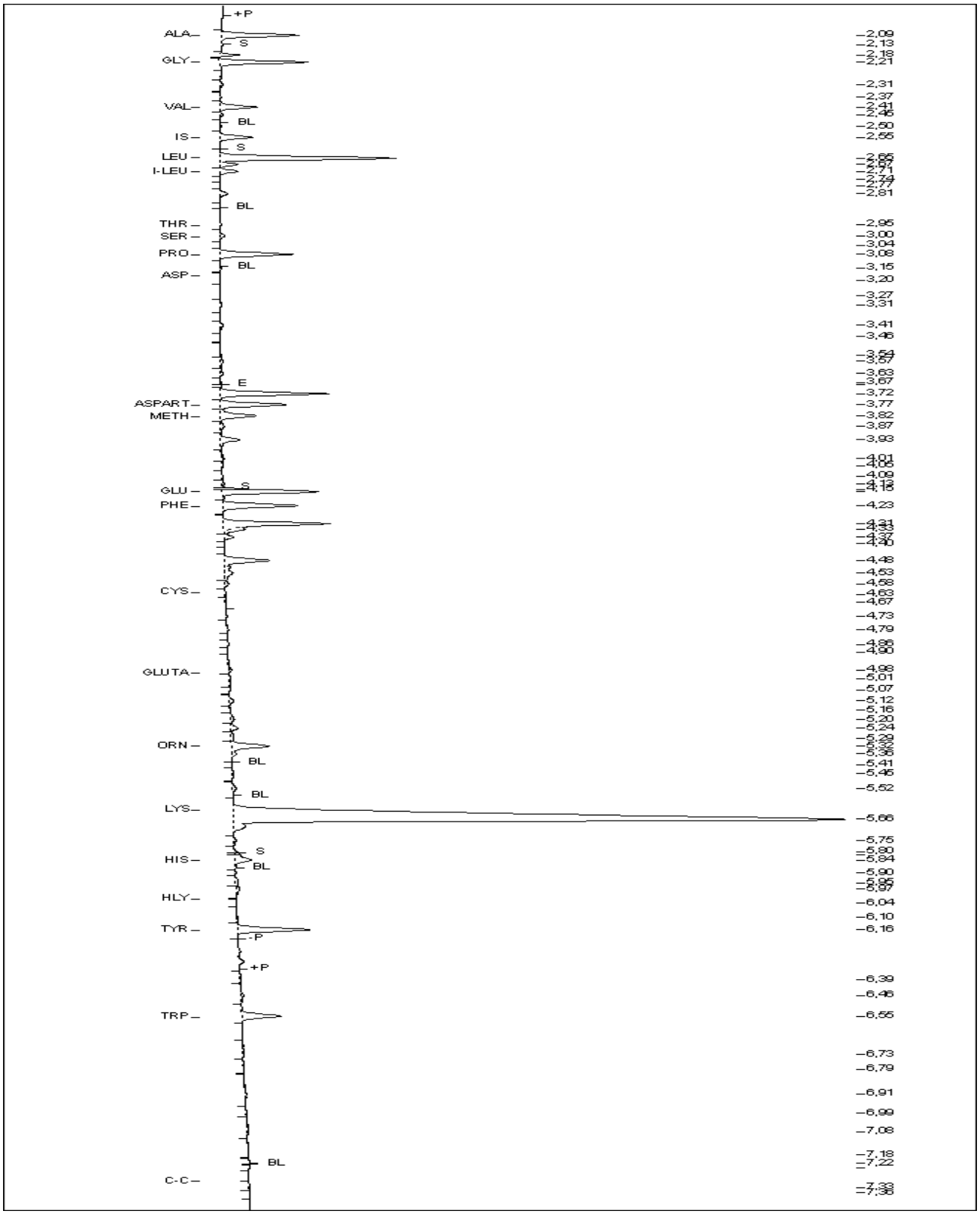

Fig. 6: Chromatogram of sample of Amino 1000 tabl., hydrolysed with 1N KOH for 48 h at 110°C.

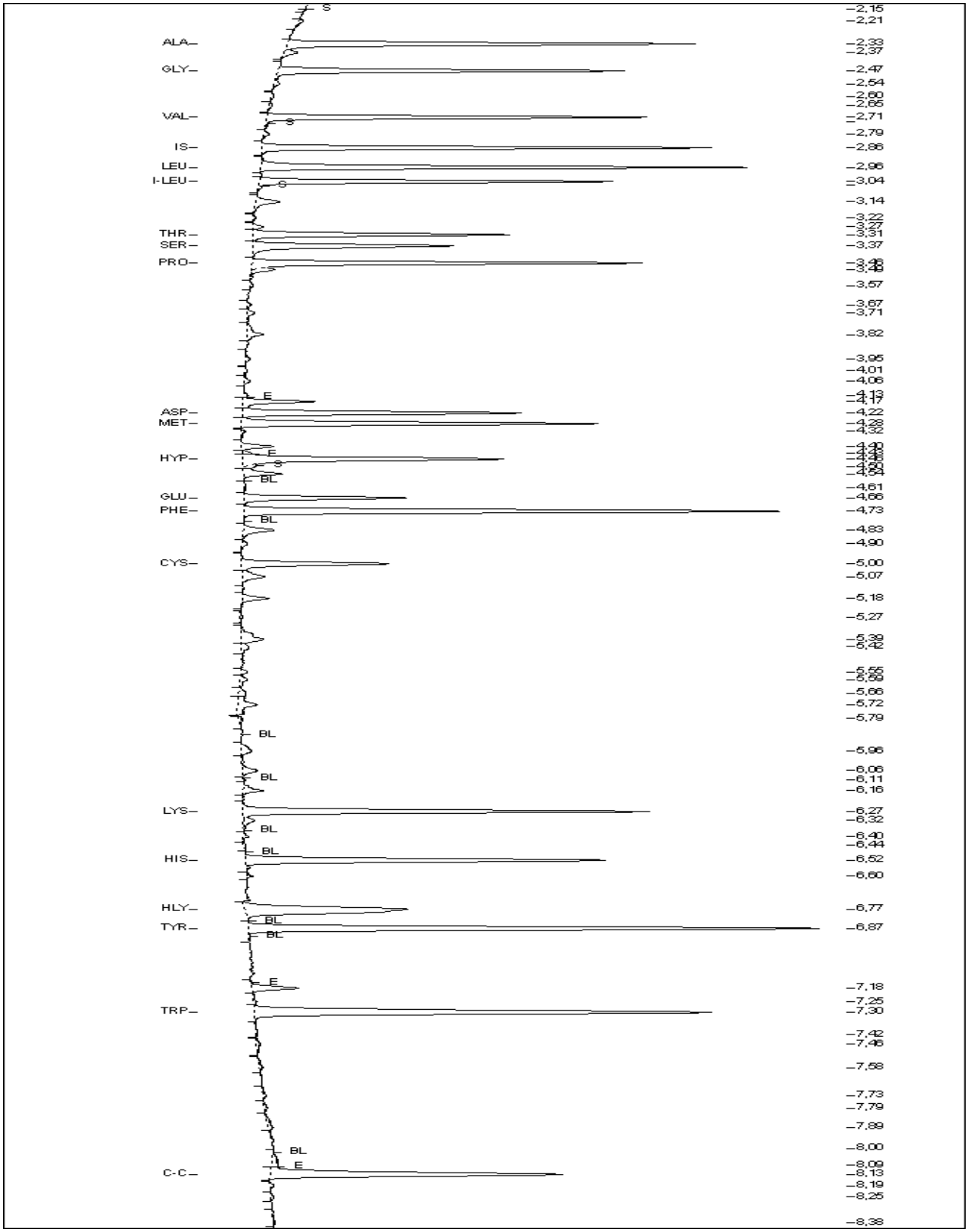

Fig. 7 : Chromatogram of reference standard solution of aminoacids, used for the calibration before the analysis of alkaline hydrolysed sample of Amino 2300 tabl.

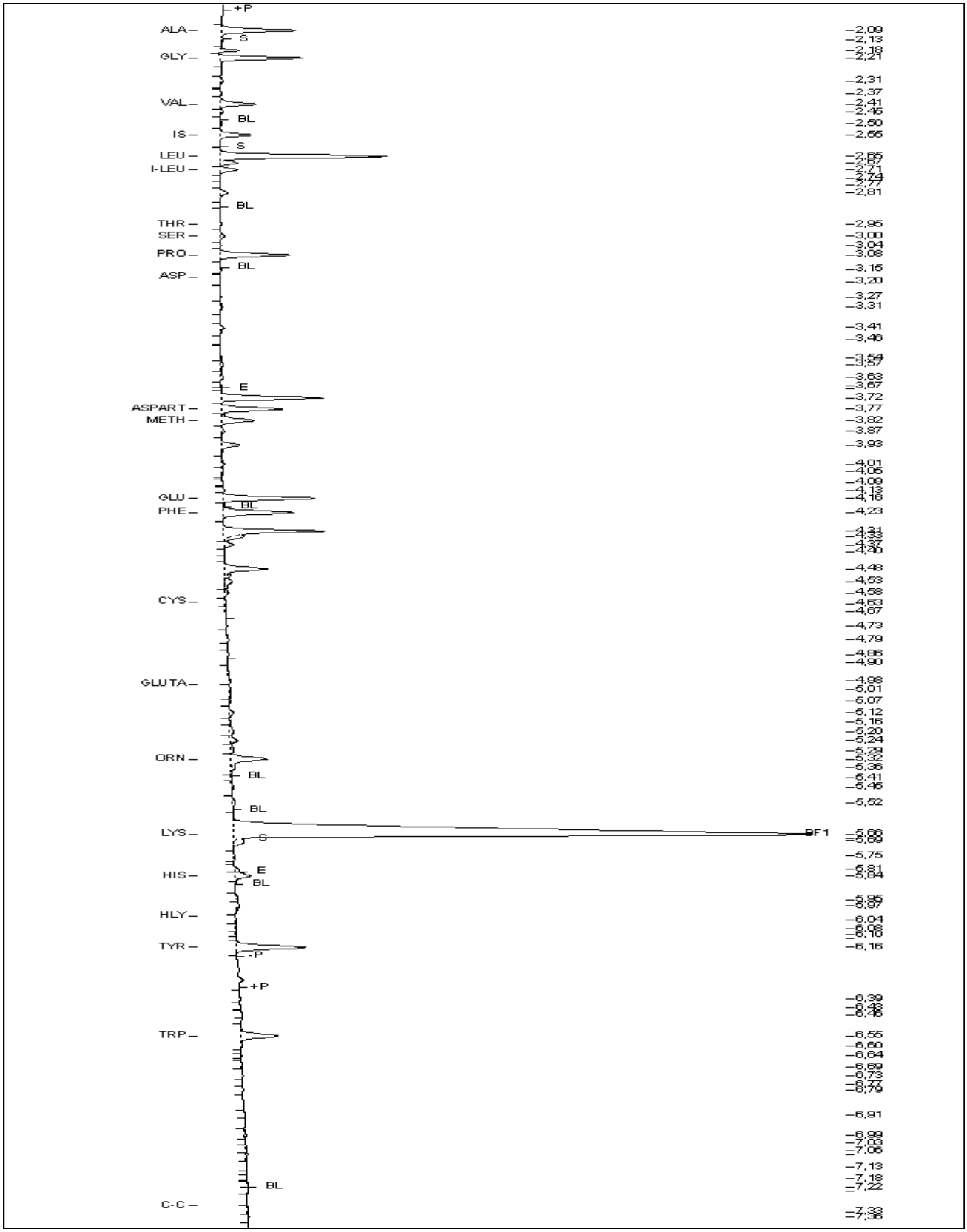

Fig. 8: Chromatogram of sample of Amino 2300 tabl., hydrolysed with 1N KOH for 48 h at 110°C.

III) Determination of total content of aminoacids in food supplements Amino 1000 tabl. and Amino 2300 tabl.

For the simultaneous determination of the total content of different aminoacids in the examined food supplements Amino 1000 tabl. and Amino 2300 tabl. is applied method of internal standard, by using the respective experimentally obtained data for the area inder the curve as analytical parameter.

On Table 2. (Amino 1000 tabl.) and Table 3. (Amino 2300 tabl.) are summarized the results for labeled quantity and the obtained total content of aminoacids of Amino 1000 tabl. and Amino 2300 tabl., after acid hydrolysis in 6N HCl for 72 h at 110 ^{o}C. For aminoacid L – Triptophan in Amino 1000 tabl. and Amino 2300 tabl., the obtained results are after alkaline hydrolysis in 1N KOH for 48 h at 110 ^{o}C.

Table 2: Labeled quantity and total content of aminoacids, after hydrolysis in Amino 1000 tabl.

N :	Aminoacid	Labeled content of aminoacids in Amino 1000 tabl. [mg/tabl.]	Total content of aminoacids in Amino 1000 tabl. [nmol/ml]	[nmol/100g]	[mg/tabl.]
1.	L – Alanin	16.8	309.7428	12.3897	34
2.	L – Glycine	14.0	152.7440	6.1098	14
3.	L – Valine	241	473.5618	18.9425	68
4.	L – Leucine	452	1097.1919	43.8877	176
5.	L – Isoleucine	231	608.0350	24.3214	99
6.	L – Threonine	21.0	162.2404	6.4896	23
7.	L – Serine	16.8	68.1310	2.7252	9
8.	L – Proline	18.2	452.6242	18.1050	57
9.	L – Aspartate	21.0	504.5536	20.1821	64
10.	L – Methionine	15.4	119.3939	4.7758	22
11.	L – Glutamate	32.2	911.2168	36.4487	173
12.	L – Phenylalanine	14.0	301.7483	12.0699	63
13.	L – Cystine	14.0	54.7629	2.1905	21
14.	L – Lysine	30.8	928.8748	37.1550	225
15.	L – Histidine	14.0	189.8310	7.5932	40
16.	L – Tyrosine	16.8	231.8731	9.2749	50
17.	L – Triptophan	14.0	9.5215	0.3809	14

Table 3: Labeled quantity and total content of aminoacids, after hydrolysis in Amino 2300 tabl.

N :	Aminoacid	Labeled content of aminoacids in Amino 2300 tabl. [mg/tabl.]	Total content of aminoacids in Amino 2300 tabl. [nmol/ml]	[nmol/100g]	[mg/tabl.]
1.	L – Alanine	85.5	328.2188	16.7392	47
2.	L – Glycine	32.4	158.5958	8.0884	21
3.	L – Valine	101.7	493.7641	25.1820	87
4.	L – Leucine	191.8	983.3346	50.1501	195
5.	L – Isoleucine	108.6	644.6389	32.8766	105
6.	L – Threonine	113.2	210.3706	10.7289	66
7.	L – Serine	83.2	100.8655	5.1441	55
8.	L – Proline	97.1	502.3687	25.6208	94
9.	L – Aspartate	187.2	504.8139	25.7455	102
10.	L – Methionine	37.0	141.3327	7.2080	33
11.	L – Glutamate	298.1	1063.6715	54.2472	201
12.	L – Phenylalanine	60.1	326.6855	16.6610	56
13.	L – Cystine	43.9	98.5105	5.0240	45
14.	L – Lysine	685.2	3462.7315	176.5993	570
15.	L – Histidine	34.7	223.4366	11.3953	47
16.	L – Tyrosine	53.1	246.0231	12.5472	53
17.	L – Triptophan	30.0	12.1688	0.6206	32

The best results for the recovery (used for the estimation of analytical parameter accuracy) are obtained for the following aminoacids: 1) Amino 1000 tabl.: L – Glycine – 100 %; L – Triptophan – 100 %; L – Threonin – 109.52 %; 2) Amino 2300 tabl.: L – Isoleucin – 96.69 %;L – Prolin – 96.81 %; L – Tyrosine – 99.81 %; L – Leucin – 101.67%; L – Cystine – 102.51 %; L – Triptophan – 106.67 %.

In the examined tablets, the obtained content of S – aminoacids and of hydroxyl – aminoacids are lower than the labeled content of the respective free aminoacid, because of high sensitive to trases of oxygen.

CONCLUSION

The applied GC – FID method is appropriate for quality control with great accuracy for aminoacids L – Cystine, L – Glycine, L – Isoleucine, L – Leucine, L – Proline, L – Threonine, L – Triptophan and L – Tyrosine in food additives – tablets.

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