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Application of the 6-aminoquinolyl-*N*-hydroxysccinimidyl carbamate (AQC) reagent to the RP-HPLC determination of amino acids in infant foods

Lourdes Bosch, Amparo Alegría, Rosaura Farré *

Nutrition and Food Chemistry, Faculty of Pharmacy, University of Valencia, Avda. Vicente Andrés Estellés s/n, 46100 Burjassot, Valencia, Spain

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Abstract

The validation of a pre-column derivatization procedure with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) to the determination of the amino acid content by RP-HPLC with fluorescence detection (λ excitation 250 nm, λ emission 395 nm) in milk-cereal based infant foods was carried out. The analytical parameters: linearity (0.0025–0.2 mM), precision of the method (0.2–3.5% variation coefficients), accuracy (derivatization: 86–106% average recovery and method: 88.3–118.2% average recovery) and the limits of detection (0.016–0.367 μM) and quantification (0.044–1.073 μM) were determined. Glutamic acid, proline and leucine were the most abundant amino acid whereas the lowest contents corresponded to tyrosine and cysteine.

Keywords: Infant foods; Amino acids; RP-HPLC; AQC

1. Introduction

Infant foods have to provide nutrients in sufficient amounts to permit optimal development and growth and to prevent diseases. From the 5th/6th month an exclusively milk based diet is gradually widened with the introduction of other foods. Cereals in the form of paps prepared with milk are usually one of the first foods added in the diversification of the infants diet.

Nowadays, milk and cereal based ready-to-eat infant foods are available on the market. Their nutritional value depends on the content and quality of the nutrients that they contain [1,2]. With respect to the protein fraction the quality depends mainly on the amino acid profile, the determination of the amino acid content in the evaluation of the nutritional quality of baby food products is therefore of some interest.

The reference method for determining amino acid contents in protein hydrolysates is a cation exchange chromatography which includes a post-column ninhydrin derivatization [3]. The method is often cited for its accuracy and precision, but reverse-phase high performance chromatographic methods (RP-HPLC) comprising pre-column derivatization have often been used because they are faster, more sensitive and less costly for the analysis of amino acids [4,5].

The most frequently used reagents in pre-column derivatization are: phenylisothiocyanate (PITC), which yields derivates detectable by UV, and *o*-phthaldialdehyde (OPA), dimethylaminonaphthalensulphonyl chloride (Dansyl-Cl) and 9-fluorenylmethyl-chloroformate (FMOC), which yield fluorescent derivatives. However, all of these reagents have disadvantages or drawbacks: PITC needs a long derivatization time (20 min) and it is necessary to remove any excess reagent by drying. OPA and its analogs fail to react with secondary amino acids and the derivatives are often unstable. FMOC has been reported to yield multiple derivatives and significant interference due to reagent is observed unless the reagent is extracted prior to chromatographic analysis or the molar excess of reagent carefully limited. Dansyl-Cl needs a long derivatization time (30 min) and derivatives are often unstable. FMOC and PITC exhibit decreased derivatization efficiency in the presence of common buffer salts or detergents [4–7].

The use of 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC), which reacts with primary and secondary amino acids to yield fluorescent derivates (λ excitation and emission

* Corresponding author. Tel.: +34 96 3544950; fax: +34 96 3544954.
E-mail address: rosaura.farre@uv.es (R. Farré).

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at 250 and 395 nm, respectively), allowing amino acid detection at under-picomolar levels, overcomes many of the drawbacks associated with the rest of derivatising reagents. The advantages are numerous: derivatives are formed in a matter of seconds, the excess of reagent is destroyed without interfering with the analysis; salts and detergents do not interfere with the reaction; good results are obtained with small sample sizes (in the ng range); the derivatized amino acids are stable at room temperature for 1 week; and the results obtained coincide well with those of the reference method. In addition, the technique is reproducible and lineal over a broad range of contents (from 2.5 to 200 μM) [4].

However, up to now the application of the AQC method has focused mainly on the analysis of physiological samples, its use in the analysis of foods having been limited to: grains [8], agricultural products and feeds [5], cheese [9], grape juices and wine [10], pickled garlic [11] and tiger nut and orgeat [12].

The aim of this study was to apply the AQC method that has been successfully used in the determination of the amino acid profile of tiger nuts and orgeats to milk-cereal based infant foods as well as the analysis of sulfur amino acids.

2. Experimental

2.1. Reagents and standards

HPLC-grade acetonitrile was obtained from J.T. Baker (Deventer, Holland); high-purity water was supplied by the Milli-Q plus system from Millipore Corp. (Bedford, MA, U.S.A.); hydrochloric acid (sp gr = 37%) was purchased from Merck (Darmstadt, Germany); L-α-amino-*n*-butyric acid was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and used as an internal standard, AccQ.Tag eluent A concentrate (acetate–phosphate aqueous buffer). One hundred and forty millimolars sodium acetate trihydrate with 17 mM triethylamine pH 5.05 with phosphoric acid containing 1 mg/l de disodium ethylenediamine tetraacetic acid (EDTA) and AccQ. Fluor reagent kit containing borate buffer, reagent powder (AQC) and reagent diluent (acetonitrile) were obtained from Millipore Corp. (Milford, MA, U.S.A.); amino acid hydrolysate standard mixture (2.5 mM) was from Pierce Chemical Co. (Rockford, IL, U.S.A.). A standard solution added with internal standard of 0.1 mM was prepared (stable 1 month −20 °C).

L-Cysteic acid monohydrate and L-methionine sulfone were obtained from Sigma Chemical Co. Standard solutions of L-cysteic acid and L-methionine sulfone 2.5 mM in hydrochloric acid 0.1 M were prepared. A solution containing both standards added to internal standard of 0.1 mM was prepared.

Performic acid was prepared by mixing formic acid 98–100% (Merck) and hydrogen peroxide 30% (Fluka, Buchs, Switzerland) v/v at a ratio 9:1. The solution was allowed to stand for 1 h at room temperature in a fume hood and then stored at 0 °C for 1 h. Hydrobromic acid (47%) was purchased from Merck.

2.2. Samples

A liquid milk-cereal based infant food containing 88% skimmed milk and 8.8% hydrolyzed eight cereals flour (wheat, corn, rice, oat, barley, rye, sorghum and millet) marketed by Hero España S.A. was used in the set up and evaluation of the method. The sample packed in a 250 ml tetrapack was kept at room temperature until analysis.

2.3. Apparatus

The HPLC system (Waters Corporation, Milford, MA, U.S.A.) consisted of a 600E quaternary pump, an in-line degasser 4-channel, a model 7725 manual injection valve (Rheodyne, Cotati, CA, U.S.A.) equipped with a 5 μl sample loop and a 474 scanning fluorescence detector (Waters Corporation, Milford). Data were collected and analyzed with the Millennium 32 Chromatography Manager Single System v 3.2 (Waters Corporation, Milford). The column temperature was set with a model 7970 column block-heater manufactured by Jones Chromatography LTD (Hengoed, Wales, UK).

A block heater Stuart Scientific SHT200D (20–199.9 °C) (Bibby Sterilin, Stone, Staffordshire, UK) was used in the derivatization step.

A vacuum system consisting of a Speed Vac Plus AR SC110 centrifuge (Savant Instruments. Inc, Farmingdale, NY), a RCT60 trap (Jouan, St Herblain, France) and a RD-9 vacuum oil pump (Telstar, Terrassa, Spain) were used in the sample preparation step in the determination of methionine and cysteine.

All solvents and samples were filtered using a Millipore (Milford) system with 0.20 μm membrane filters (47 and 13 mm, respectively). A UT 6060 air-circulation drying oven (Heraus, Hanau, Germany) was used in the sample hydrolysis.

2.4. Sample preparation

Acid hydrolysis was used for all amino acids except cysteine (Cys) and methionine (Met) for which performic acid oxidation followed by acid hydrolysis was used.

2.4.1. Acid hydrolysis

0.666 g of infant food was weighed into a 10 ml Pyrex glass tube fitted with teflon-lined screw caps. Five milliliters of HCl 6N was added (5.06 mg protein/ml HCl) and mixed. The tube was flushed with nitrogen for 1 min to remove air. Hydrolysis was then carried out at 110 °C for 23 h. After letting the tubes cool at room temperature, the content was filtered through wet filter paper and collected into a 250 ml volumetric flask. The internal standard (10 ml of 2.5 mM L-α-amino-*n*-butyric acid in HCl 0.1 M) was added and diluted with water to 250 ml. The solution was filtered with 0.20 μm filter.

2.4.2. Performic acid oxidation

An amount of 0.666 g sample was weighed in a centrifuge tube. After adding 2 ml performic acid, samples were kept in an ice bath for 16 h at 0 °C. Then 0.3 ml of hydrobromic acid was added to remove excess performic acid. A vacuum system was used to remove the bromine formed during the reaction. Oxidized sample was transferred to a 10 ml Pyrex glass tube fitted with teflon-lined screw cap. The acid hydrolysis procedure using 6N HCl was then performed.

Table 1
Selected gradient elution

Time (min)	% A	% B	% C
0.00	100.0	0.0	0.0
0.50	99.0	1.0	0.0
18.00	94.0	6.0	0.0
19.00	91.0	9.0	0.0
29.50	83.0	17.0	0.0
38.00	83.0	17.0	0.0
38.01	0.0	60.0	40.0
55.00	0.0	60.0	40.0
55.01	100.0	0.0	0.0

A, AccQ.Tag eluent A; B, acetonitrile; C, Milli-Q water.

2.4.3. Derivatization

Ten microliters of filtered hydrolysated sample or standard were transferred to a 1.5 ml amber glass vial with teflon-lined septum, 70 μl of borate buffer were added, because the optimal pH range for derivatization is 8.2–9.7, and the solution was briefly vortexed. Then, 20 μl of reconstituted AccQ.Fluor reagent (3 mg/ml in acetonitrile) was added and the mixture was immediately vortexed for several seconds. The vial was closed and left to stand for one minute at room temperature. It was then heated in a heating block at 55 °C, for 10 min. Heating converts a minor side product of tyrosine to a major mono-derivatized compound. Derivatives were stable at room temperature for up to 1 week [13].

Before being used sample vials and pyrex tubes were pyrolyzed at 450 °C for 3–4 h.

2.5. Cromatographic conditions

Chromatographic separation was carried out in a Waters AccQ.Tag amino acid analysis Nova-Pak™ column (3.9 mm × 150 mm, 4 μm) fitted with a Nova-Pak™ C_{18} Sentry™ Guard column (3.9 mm × 20 mm, 4 μm). The column was thermostatted at 37 °C and the flow rate was 1.0 ml/min. The injection volume was 5 μl. Mobile phase A consisted of AccQ.Tag eluent A (100 ml AccQ.Tag A concentrate + 1 L Milli-Q water). Mobile phases B and C were acetonitrile and Milli-Q water, respectively. Gradient conditions shown in Table 1, were selected in a previous study [12]. Before beginning the gradient, the column was equilibrated in 100% A for 10 min.

After the last analysis of the day, the column was washed for 30 min with 100% C and then conditioned for 15–20 min at B:C (60:40). The column then had to be stored for more than 72 h, it was kept in 100% B; in this case it had to be conditioned with B:C (60:40) for 5 min before equilibrating in 100% A.

Detection was carried out by fluorescence (λ excitation 250 nm and λ emission 395 nm).

3. Results and discussion

3.1. Qualitative and quantitative analysis

Qualitative and quantitative analysis were carried out on the basis of retention times and internal standard method, respectively. Amino acid contents were estimated as follows: $C_i = (A_i/A_s) \times C_s \times F$, where C_i, amino acid content in mM; A_i, amino acid area in sample; A_s, internal standard area; C_s, concentration of internal standard (0.01 mM); F, response factor [14].

Response factors were determined by injecting derivatized standards with an internal standard (0.01 mM) several times on different days, the values obtained are reported in Table 2.

Chromatograms corresponding to amino acids from the analyzed infant food, obtained when acid hydrolysis had been applied without (a) and with (b) a previous performic oxidation, are shown in Fig. 1.

3.2. Analytical parameters

3.2.1. Detection and quantification limits (LOD and LOQ)

Detection and quantification limits were determined according to Knoll [15]. The amino acids standard (0.01 mM) was

Table 2
Response factors (F), detection (LOD) and quantification (LOQ) limits

Amino acid	F	S.D.	CV (%)	LOD in assay (μM)	LOQ in assay (μM)
Aspartic acid	2.98	0.02	0.84	0.066	0.194
Serine	2.30	0.03	1.35	0.053	0.156
Glutamic acid	2.64	0.01	0.56	0.057	0.168
Glycine	2.56	0.05	1.81	0.046	0.134
Histidine	1.63	0.02	1.03	0.026	0.078
Arginine	1.52	0.04	2.37	0.107	0.313
Threonine	1.46	0.04	2.67	0.108	0.315
Alanine	1.57	0.03	1.63	0.123	0.359
Proline	3.33	0.095	2.87	0.183	0.535
Tyrosine	1.61	0.01	0.79	0.031	0.093
Valine	0.92	0.01	0.60	0.018	0.054
Lysine	1.99	0.03	1.78	0.367	1.073
Isoleucine	0.80	0.01	1.61	0.181	0.529
Leucine	0.85	0.01	1.05	0.016	0.047
Phenylalanine	0.69	0.01	1.04	0.016	0.044
Cysteic acid	2.99	0.20	6.64	0.052	0.151
Methionine sulfone	0.91	0.06	6.47	0.087	0.253

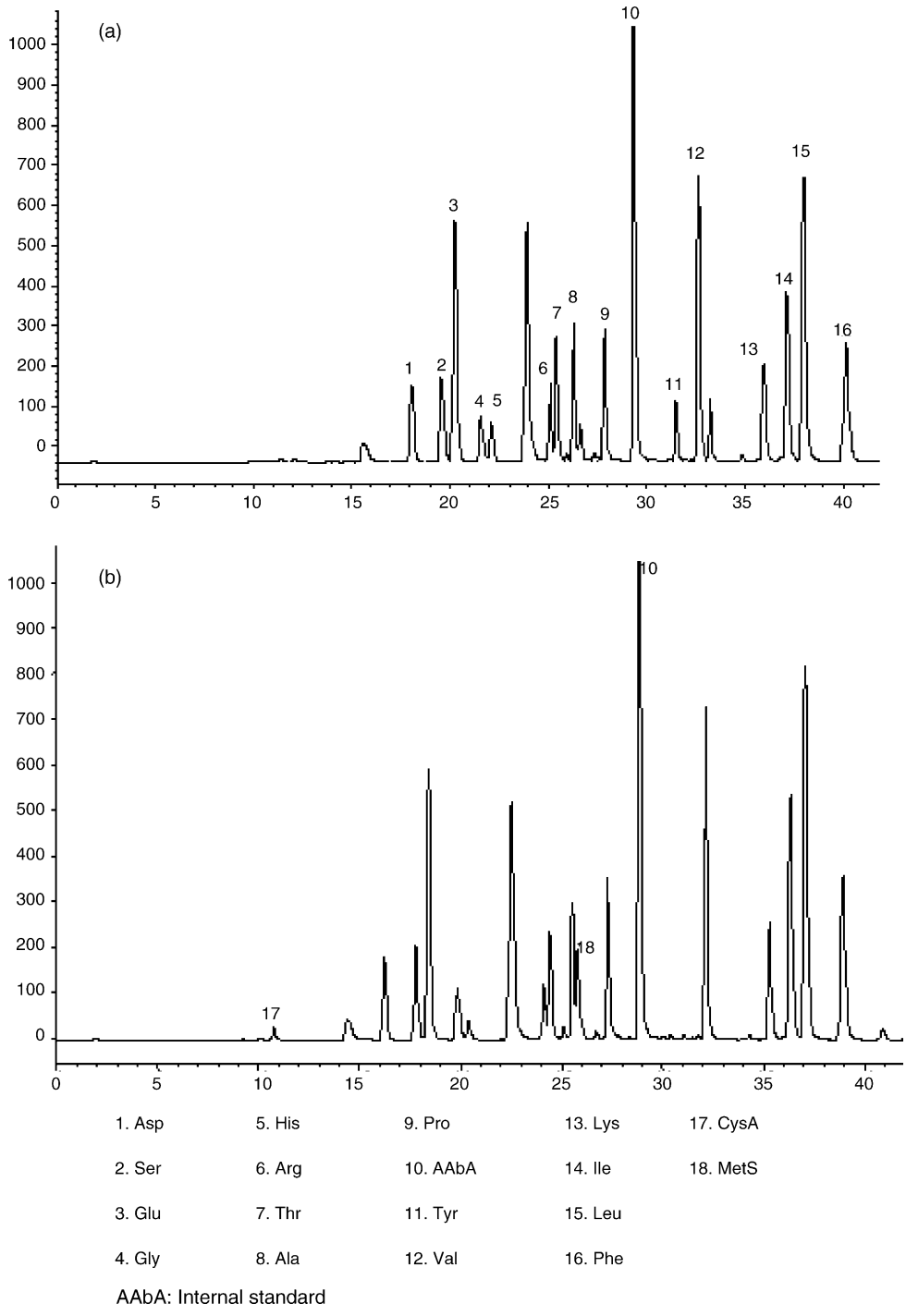

Fig. 1. Chromatograrns corresponding to the amino acids of the analyzed infant food obtained without (a) and with (b) a prior performic acid oxidation.

injected and the peak height (h_s) and width at the half peak height (W_h) were measured. A line segment was marked on the chart equal to a multiple of W_h. Then three blanks were injected and the height of the largest noise fluctuation (h_n) in the pre-selected chart segment was measured. The following formulas were applied: $LOD = 1.9718 \times h_n \times C_s/h_s$ and $LOQ = 5.7550 \times h_n \times C_s/h_s$, where 1.9718 and 5.7550 are the constants used in the calculation of LOD and LOQ when $W_h = 10$ is taken, C_s, amino acid standards concentration (0.01 mM); h_s, peak height of amino acid standards; h_n, height of the largest noise fluctuation observed in the noise measurement range.

LOD and LOQ values corresponding to the different amino acids are reported in Table 2. LOD values were in the range 0.016–0.367 μM (0.79–20.14 mg/100 g sample) and LOQ values in the range between 0.044 and 1.073 μM (2.31–58.89 mg/100 g sample).

AQC provides lower LOD values than other derivatising agents used in UV or fluorescence detection, such as PITC, utilized in the amino acid determination in infant

Table 3
Matrix interference study: addition's method

Amino acid	Confidence interval of slope (aqueous standard)[a]	r	Confidence interval of slope (added matrix)[a]	r
Aspartic acid	25.47–36.00	0.992	27.05–31.03	0.999
Serine	31.98–45.38	0.992	35.12–39.95	0.999
Glutamic acid	25.42–40.44	0.987	29.44–37.13	0.996
Glycine	29.27–40.39	0.993	32.32–34.83	1.000
Histidine	50.03–65.56	0.995	49.96–56.57	0.999
Arginine	37.15–59.79	0.986	42.01–51.47	0.997
Threonine	57.77–72.72	0.997	53.38–64.82	0.997
Alanine	51.67–64.66	0.997	49.87–56.35	0.999
Proline	27.29–31.37	0.999	22.78–28.30	0.997
Tyrosine	49.02–64.49	0.995	51.39–55.13	1.000
Valine	49.76–96.20	0.975	88.84–95.77	1.000
Lysine	43.44–55.89	0.996	41.46–49.25	0.998
Isoleucine	105.12–131.80	0.997	102.23–115.76	0.999
Leucine	100.99–124.20	0.997	93.47–111.12	0.998
Phenylalanine	121.19–152.48	0.997	119.93–133.90	0.999
Cysteic acid	38.43–39.94	1.000	35.41–40.37	0.999
Methionine sulfone	36.75–99.98	0.949	30.12–98.42	0.934

r, correlation coefficient.
[a] Overlapping of the confidence intervals of the slope (95%) indicates the lack of statistically significant differences ($p < 0.05$) between the slopes of both standard sets.

formulas (9–190.4 mg/100 g) [16,17] and in mixed feed, mozzarella cheese, meat, bone meal and soy flour (1 pmol) [18,19] and *o*-phthalaldehyde-3-mercaptopropionic acid (OPA-3-MPA) and FMOC used in soya-bean cattle-cake hydrolysates (0.279–1.368 μM) [20].

The AQC method has been used to determine amino acid content in different foods. LOD values obtained in our work are in the range reported by other authors using AQC with UV detection (0.06–0.29 pmol) [21] and fluorescence detection (0.04–0.32 pmol, except for Cys 0.8 pmol) [4] (lower than 0.05 pmol, except Cys lower than 0.6 pmol) [10]. In the case of Arg, Thr, Ala, Pro and Lys the LOD values obtained (see Table 2) were higher, but adequate for the determination of the amino acid content in infant foods. Some reports do not include LOD values and/or the validation is incomplete [9,11].

3.2.2. Linearity

Linearity was tested by the analysis of standards containing 0.0025, 0.005, 0.03, 0.05, 0.1 and 0.2 mM each amino acid added with 0.1 mM of internal standard derivatized according to the procedure described before. The set of standards includes the amino acid content of the analyzed infant food. Linearity data were calculated by examining the correlation coefficient of linear regression line for the response versus concentration of amino acid.

The results (regression equations and correlation coefficients) obtained are reported in Table 3. Correlation coefficients were higher than 0.992 with the exception of glutamic acid (0.987), arginine (0.986), valine (0.975) and methionine sulfone (0.949), giving values that were also acceptable. The range of amino acid content giving a linear answer permitted us to measure lower content than other derivatizing agents such as PITC (lowest measurable content 0.02 mM) [18,19] and OPA-3-MPA-FMOC (lowest measurable content 0.003 mM) [20].

The linear ranges for amino acid content obtained were 10 times lower than that reported for AQC by Liu with UV detection UV (0.025–0.5 mM) [21] and Cohen and Michaud with fluorescence detection (0.025–0.2 mM) [4].

3.2.3. Study of interferences due to the matrix effect

The matrix interference study was carried out by the standard additions method applied to the hydrolysate of the sample. Two sets of aqueous standards in the range 0.00025–0.02 mM for each amino acid were prepared, and to one of them matrix was added in a proportion of 5%. Amino acids of both sets were analyzed and the regression equations calculated. A "*t*-test" was applied to compare the slopes of the regression equations corresponding to the added matrix with those of aqueous standards; differences between them indicated matrix interferences. The results obtained are reported in Table 3. No significant differences were found between the confidence interval of the slope of aqueous standards with and without matrix added. It was concluded that amino acid determination in milk-cereal based infant food was free from matrix interferences.

3.2.4. Precision

The results of instrumental, derivatization procedure and method precision, expressed as coefficients of variation, are reported in Table 4.

Instrumental precision was calculated from three injections of one derivatized standard (0.01 mM). Average coefficients of variation in the range from 0.04 to 1.28% were obtained. The precision of the derivatization procedure was checked from injections of a standard that had been derivatized eight times over 3 days (interday 0.56–2.87%) and three times on 1 day (intraday 0.20–2.71%). The precision of the method was finally estimated by applying the whole procedure to three aliquots of the infant food, variation coefficients in the range of 0.24–3.49% (except for MetS 6.74%) were obtained.

Table 4
Precision values expressed in relative standard deviation/variation coefficient

Amino acid	Instrumental[a]	Derivatization procedure[a]		Method ($n = 3$)[b]
		Intraday ($n = 3$)	Interday ($n = 8$)	
Aspartic acid	0.7	0.8	0.8	1.6
Serine	0.6	1.0	1.4	3.1
Glutamic acid	0.4	0.5	0.6	0.2
Glycine	0.2	0.5	1.8	3.3
Histidine	0.7	0.2	1.0	2.3
Arginine	0.2	0.2	2.4	1.8
Threonine	0.8	0.6	2.7	3.0
Alanine	0.6	0.3	1.6	2.4
Proline	0.9	1.5	2.9	1.0
Tyrosine	0.8	1.0	0.8	3.5
Valine	0.7	0.5	0.6	2.8
Lysine	0.8	2.7	1.8	1.2
Isoleucine	0.7	0.5	1.6	1.9
Leucine	0.9	0.4	1.1	1.9
Phenylalanine	0.8	0.4	1.0	1.9
Cysteic acid	0.7	1.2	2.3	3.4
Methionine sulfone	0.04	1.3	1.8	6.7

[a] Estimated with standards.
[b] Estimated with infant food.

The precision values reported in the literature are similar to those obtained here, regardless of the derivatising agent used [5,7,8,11,16–20,22,23].

3.2.5. Accuracy

Free amino acids are oxidized in the acid hydrolysis, so recovery assays to estimate the accuracy can only be done from the derivatization step on. Known amounts of standards, once and twice the expected average amino acid content, were added to the hydrolyzed sample. Amino acids were measured in the spiked and the unspiked samples and recoveries were calculated. The assay was carried out in triplicate. The average recovery values of amino acids ranged from 85.95 to 105.86% (see Table 5).

A reference material similar to the studied sample was not available, so the accuracy of the method was evaluated by recovery assays adding a protein (casein) to the milk-cereal based infant food before hydrolysis. Casein was selected because it is the main protein of cow milk, which is the major component (88%) of the analyzed sample. An amount of casein corresponding to the casein present in the amount of sample taken was added. The assay was carried out in triplicate. The average recoveries ranged from 88.30 to 118.16% (see Table 6), except for cystein were 55.3% was obtained. This is because the added amount of cysteine coming from casein was four times lower than the amount of cysteine coming from the sample, where cysteine is provided by milk and cereals, and the present and found contents were very similar. The small difference between the amount present and added can be the responsible for the low recovery value.

Table 5
Accuracy of the derivatization procedure of amino acids from infant food

Amino acid	Present (mg/100 g) ($n = 3$)	Added (mg/100 g)	Found (mg/100 g) ($n = 3$)	Recovery (%)
Aspartic acid	254 ± 4	597	860 ± 9	101.5 ± 1.5
Serine	193 ± 6	470	673 ± 6	102.1 ± 1.3
Glutamic acid	835 ± 2	662	1536 ± 15	105.9 ± 2.3
Glycine	94 ± 3	339	432 ± 6	99.7 ± 1.8
Histidine	99 ± 2	723	831 ± 18	101.3 ± 2.5
Arginine	147 ± 3	796	966 ± 17	102.9 ± 2.2
Threonine	150 ± 4	561	685 ± 13	95.3 ± 2.3
Alanine	126 ± 3	410	517 ± 1	95.3 ± 0.3
Proline	340 ± 3	481	843 ± 7	104.5 ± 1.5
Tyrosine	128 ± 4	825	934 ± 15	97.8 ± 1.7
Valine	206 ± 6	478	617 ± 50	86.0 ± 10.4
Lysine	241 ± 3	662	914 ± 16	101.5 ± 2.4
Isoleucine	167 ± 3	555	696 ± 40	95.4 ± 7.2
Leucine	318 ± 6	569	832 ± 53	90.2 ± 9.3
Phenylalanine	171 ± 3	717	884 ± 37	99.4 ± 5.2
Cysteic acid	51 ± 2	122	173 ± 7	99.7 ± 5.5
Methionine sulfone	80 ± 5	131	211 ± 19	99.7 ± 14.3

Table 6
Accuracy of the method estimated by recovery assays of amino acids from infant food added of casein

Amino acid	Present (mM) ($n = 3$)	Added (mM)	Found (mM) ($n = 3$)	Recovery (%)
Aspartic acid	0.0051 ± 0.0001	0.0056	0.0111 ± 0.0006	109.0 ± 10.1
Serine	0.0049 ± 0.0002	0.0047	0.0100 ± 0.0006	110.1 ± 12.7
Glutamic acid	0.0151 ± 0.0001	0.0151	0.0322 ± 0.0013	112.6 ± 8.3
Glycine	0.0033 ± 0.0001	0.0023	0.0060 ± 0.0003	118.1 ± 13.6
Histidine	0.0017 ± 0.0000	0.0017	0.0037 ± 0.0002	115.5 ± 12.3
Arginine	0.0023 ± 0.0000	0.0035	0.0065 ± 0.0010	107.1 ± 17.4
Threonine	0.0033 ± 0.0001	0.0042	0.0070 ± 0.0003	88.5 ± 7.0
Alanine	0.0038 ± 0.0001	0.0036	0.0075 ± 0.0003	102.8 ± 9.5
Proline	0.0079 ± 0.0001	0.0099	0.0175 ± 0.0006	97.0 ± 6.3
Tyrosine	0.0019 ± 0.0001	0.0017	0.0038 ± 0.0002	108.0 ± 6.8
Valine	0.0047 ± 0.0001	0.0061	0.0100 ± 0.0001	88.3 ± 1.8
Lysine	0.0044 ± 0.0001	0.0056	0.0106 ± 0.0005	110.9 ± 9.7
Isoleucine	0.0034 ± 0.0001	0.0040	0.0078 ± 0.0003	110.2 ± 8.5
Leucine	0.0065 ± 0.0001	0.0075	0.0132 ± 0.0001	89.5 ± 1.9
Phenylalanine	0.0028 ± 0.0001	0.0028	0.0060 ± 0.0003	115.9 ± 9.7
Cysteic acid	0.0008 ± 0.0000	0.0002	0.0009 ± 0.0000	55.3 ± 2.2
Methionine sulfone	0.0012 ± 0.0001	0.0022	0.0033 ± 0.0001	100.4 ± 6.8

3.3. Application of the method to determination of amino acid in infant foods

The amino acid contents (g/100 g infant food and mg/g protein) of the analyzed samples are reported in Table 7. Values are the mean of four determinations.

Glutamic acid (0.949 g/100 g), proline (0.385 g/100 g) and leucine (0.362 g/100 g) were the most abundant amino acids. To methionine (0.108 g/100 g), histidine (0.102 g/100 g), glycine (0.099 g/100 g), tyrosine (0.064 g/100 g) and cysteine (0.046 g/100 g) corresponded the lowest contents.

Proteins in the analyzed infant food came mainly from skimmed milk (88%) and in a smaller amount from cereals (8.8%), this means the amino acid profile has to be similar to those of milk. The most abundant amino acids in cow milk (mg/g protein) are glutamic acid (228–234.3), leucine (97.1–104) and proline (94.4–108.6); while glycine (22–22.9), tryptophan (14–15) and cystine (8–9.6) are the minor [24–26].

Table 7
Amino acid contents in the analyzed infant food

	Infant food	
	g/100g	mg/g protein
Aspartic acid	0.275 ± 0.003	80.94 ± 0.99
Serine	0.207 ± 0.005	60.80 ± 1.49
Glutamic acid	0.949 ± 0.015	279.19 ± 4.43
Glycine	0.099 ± 0.002	29.19 ± 0.53
Histidine	0.102 ± 0.001	30.12 ± 0.20
Arginine	0.137 ± 0.009	40.34 ± 2.52
Threonine	0.153 ± 0.003	44.88 ± 1.01
Alanine	0.130 ± 0.001	38.32 ± 0.36
Proline	0.385 ± 0.002	113.20 ± 0.65
Tyrosine	0.064 ± 0.001	20.02 ± 2.32
Valine	0.232 ± 0.002	68.14 ± 0.66
Lysine	0.255 ± 0.009	75.10 ± 2.77
Isoleucine	0.191 ± 0.003	56.16 ± 0.87
Leucine	0.362 ± 0.006	106.34 ± 1.73
Phenylalanine	0.191 ± 0.002	56.21 ± 0.52
Cysteic acid	0.046 ± 0.004	13.48 ± 1.05
Methionine sulfone	0.108 ± 0.014	31.62 ± 3.98

4. Conclusions

The method is reproducible and accurate enough to allow the determination of amino acid content in infant foods, including methionine and cysteine. LOD and LOQ obtained with AQC are better than those provided by other amino acid derivatizing agents.

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