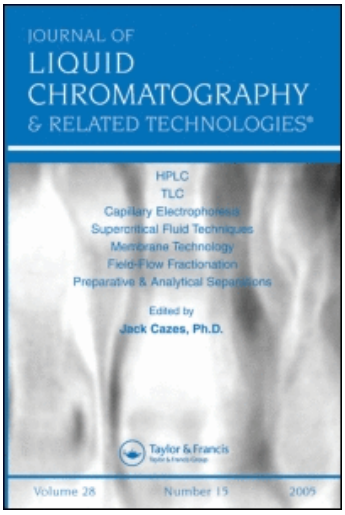

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Development of An HPLC-UV Method for Determination of Taurine in Infant Formulae and Breast Milk

I. M. P. L. V. O. Ferreira[a]; M. V. Nunes[a]; E. Mendes[a]; F. Remião[b]; M. A. Ferreira[a]
[a] CEQUP, [b] Laboratório de Bromatologia Laboratório de Toxicologia Faculdade de Farmácia, Universidade do Porto Rua Aníbal Cunha, Porto, Portugal

To cite this Article Ferreira, I. M. P. L. V. O. , Nunes, M. V. , Mendes, E. , Remião, F. and Ferreira, M. A.(1997) 'Development of An HPLC-UV Method for Determination of Taurine in Infant Formulae and Breast Milk', Journal of Liquid Chromatography & Related Technologies, 20: 8, 1269 — 1278
To link to this Article: DOI: 10.1080/10826079708010975
URL: http://dx.doi.org/10.1080/10826079708010975

J. LIQ. CHROM. & REL. TECHNOL., 20(8), 1269–1278 (1997)

DEVELOPMENT OF AN HPLC-UV METHOD FOR DETERMINATION OF TAURINE IN INFANT FORMULAE AND BREAST MILK

I. M. P. L. V. O. Ferreira,[†] M. V. Nunes,[†] E. Mendes,[†]
F. Remião,[‡] M. A. Ferreira[†]

[†]CEQUP
[‡]Laboratório de Bromatologia
Laboratório de Toxicologia
Faculdade de Farmácia, Universidade do Porto
Rua Aníbal Cunha 164
4050 Porto, Portugal

ABSTRACT

A rapid and accurate high performance liquid chromatographic (HPLC) procedure was proposed for routine and selective determination of taurine in infant formulas and breast milk. The sample preparation was simple, involving protein removal and filtration operations. Afterwards, taurine was derivatised with o-phthalaldehyde/2-mercaptoethanol prior to injection onto a reversed phase column C_{18} ($S_{10}ODS_2$). Isocratic elution was carried out using 0.05 M phosphate buffer pH 5.3/ methanol (60:40) mixture. The effluent was monitored by a UV detector at 350 nm. A linear relationship was found between peak area and a concentration range of 1-70 μg/mL. The detection limit was 0.3 μg/mL. Effective separation and quantification was achieved in under six minutes. No interference of other amino acids was observed.

Extensive validation of the proposed method was carried out both by the standard additions method and by comparison with a fluorimetric method of known accuracy. The precision was better than 1.4%.

INTRODUCTION

Taurine, 2-aminoethanesulphonic acid, is a sulphur-containing β-amino acid that is not incorporated into proteins. However, it takes part in biochemical reactions of major importance, such as conjugation with bile acids to form bile salts essential for fat absorption, cell membrane stabilization, antioxidation, detoxification, osmoregulation, neuromodulation, and brain and retinal development.[1]

Considerable evidence has accumulated that neonates and infants, who have a very limited ability to synthesize taurine, are especially prone to develop taurine deficiency and that they depend on an external taurine supply.[2]

Taurine concentrations in milk are very variable, depending on species. While breast milk is an excellent source of taurine in the developing infant, cow's milk has a very low taurine content. Nowadays the supplementation of infant formulae with taurine in concentrations similar to those found in human milk is therefore recommended.[3,4]

Many methods for measuring taurine in several matrices viz. biological samples, such as tissue homogenates, urine, and serum have been published, including gas chromatography[5] and HPLC analysis of diferent amino acid derivatives.[6-12] One of the most sensitive and commonly used derivatising agent techniques is the use of o-phthaldehyde / mercaptoethanol, which, originates a fluorescent adduct that enables the fluorimetric determination of taurine.[6,11]

Other HPLC fluorimetric determinations in similar matrices include derivatising reaction with thiamine[8] and fluorescamine.[9]

It is also possible to use UV absorbance detection at 350 nm with different derivatisation techniques, including o-phthaldehyde / mercaptoethanol[7] and dinitrofluorobenzene.[10,12] Interestingly, research work developed by Chen at al.[12] is distinguished from the rest since one of the matrices used was human milk.

Some of these methods require time-consuming pre-treatment of the samples, in order to eliminate interfering substances or complicated derivatising treatment of the sample before assay.

This paper describes a rapid and accurate method for the determination of taurine in infant formulae and breast milk. Although, the sample matrix is of complex nature, the pre-treatment applied is simple and the method is suitable for rapid routine assays of a large number of samples and uses a UV detector which is much more common in control laboratories than the fluorimetric detector.

MATERIALS

Apparatus

The chromatographic analysis was carried out in a Gilson, high performance liquid Chromatograph (Gilson Medical Electronics, Villiers le Bel, France) equipped with a type 305 pump, a type 302 pump and a type 7125 Rheodyne Injector with a 20 μl loop. A Gilson 118, variable long wave ultra violet detector was also used.

The chromatographic separation was achieved with a Spherisorb S_{10} ODS_2 Chromatographic column, 10 μm. The integrator used was a Gilson 712 HPLC System Controller Software.

A Gilson 121 Spectrofluorometer was used for detection of the fluorescent adduct.

Reagents and Standards

Taurine and o-phthaldehyde were obtained from Sigma Chemical Co. L-Amino acids kit was also from Sigma (Kit No. LAA-21). Sulphosalicylic acid, boric acid, sodium hydroxide and mercaptoethanol, all p.a., were obtained from Merck. Methanol (LiCrosolv) was Merck "gradient grade".

Water used for chromatography possessed a resistance greater than 15 MΩ, and was filtered through a membrane of 0.45 μm porosity which was subsequently degassed.

Sample Preparation

After homogenisation, 5.0 g of infant formulae were dissolved in 40 mL of warm (40°C) water. 5.0 mL of the infant formulae solution or breast milk were added to 5.0 mL of sulphosalicylic acid solution 0.2 M, mixed thorougly, and allowed to stand for 10 min. All samples were filtered through W42 paper and thereafter, through 0.2 μm filter paper.

The use of sulphosalicylic acid as a protein precipitating agent has been shown to result in a higher % recovery of taurine.[11]

Derivatisation Procedure

Taurine was derivatised with o-phthalaldehyde (OPA, 40 mg, 0.8 mL absolute ethanol) and 2-mercaptoethanol (40μl) in 0.5 M borate buffer (10 mL, 3.1 g boric acid in 90 mL water, adjusted to pH 10.4 with 5M NaOH, and made up to 100 mL)[6] prior to injection onto a reversed phase column C_{18} ($S_{10}ODS_2$).

The derivatizing solution was prepared daily and filtered through a 0.20 μm filter before use.

A 100 μL volume of standard or sample solution (after preparation) was placed in an eppendorf and 100 μl of derivatizing reagent were added. The derivatization reaction was allowed to proceed for exactly 1.5 min, at which time an aliquot was injected into the HPLC.

Chromatography

The HPLC elution required a mixture of two solvents. Solvent A, 0.05 M phosphate buffer pH 5.3, and solvent B, methanol. The two solvents were filtered and degassed before use. Isocratic elution was carried out at a flow rate of 1.5 mL/min, using 60% solvent A combined with 40 % solvent B. The injection volume was 20 μl and the chromatographic analysis was conducted at ambient temperature.

Taurine concentration was determined at an absorbance of 350 nm, which is the maximum wavelength on the excitation spectrum of OPA-derivatized amino acids. Taurine peak was identified by coelution with a standard and by comparison of the retention time.

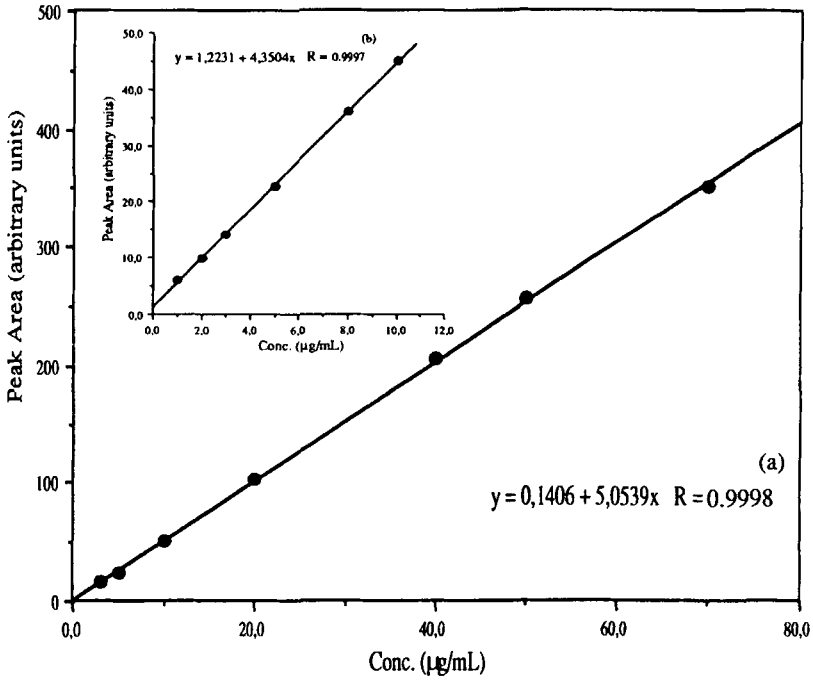

Figure 1. Standard curves for taurine. Taurine was derivatised and chromatographed by the method described in the text. Each point represents the average of triplicate determinations. (a) Sensitivity of 0.06 AUFS, detection limite 1 μg/mL. (b) Sensitivity of 0.03 AUFS, detection limite 0.3 μg/mL.

RESULTS

Analytical Curve and Detection Limit

Under the assay conditions described, a linear relationship between the concentration of taurine and the UV absorbance at 350 nm was obtained. This linearity was mantained over the concentration range 3-70 μg/mL with a sensitivity of 0.06 AUFS and 1-10 μg/mL with a sensitivity of 0.03 AUFS. The detection limit value was calculated as the concentration corresponding to three times the standard deviation of the background noise and was 1 μg/mL and 0.3 μg/mL, respectively. The calibration curves for taurine are ilustrated in Fig. 1.

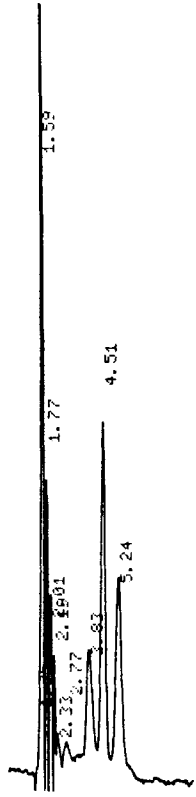

Figure 2. Typical chromatogram obtained for taurine extracted from infant formulae. Retention time of taurine 4.51.

Triplicate determinations were made on each of the calibration standards, the peak area values (arbitrary units) that were plotted are average values. The relative percent average deviations of triplicates were less than 3 % in all cases.

Validity of the Method

The repeatability of the derivatization procedure was examined. A standard taurine solution was derivatized and chromatographed six times. When reaction and chromatographic conditions were carefully optimized, the standard deviation was 0.02 (n=6 and concentration of taurine 20 μg/mL).

Table 1

Recovery of Taurine from Spiked Infant Formulae

	μg/mL Added	μg/mL Found[a]	Standard Deviation	CV%	Recovery %
Sample 1	2.00	2.03	±0.05	2.46	101.5
	4.00	3.92	±0.08	2.04	98.0
	6.00	5.82	±0.09	1.54	97.0
Sample 9	2.00	1.99	±0.03	1.51	99.5
	4.00	4.10	±0.07	1.71	102.5
	6.00	5.73	±0.06	1.05	95.5

[a]Mean value found for 3 assays for each studied concentrations.

The interference of other amino acids was examined next. For this purpose, different amino acids were injected after derivatization with OPA. They included L-Alanine, L-Valine, L-Leucine, L-Isoleucine, L-Proline, L-Phenylalanine, L- Tryptophan, L- Methionine, Glycine, L-Serine, L-Threonine, L-Tyrosine, L-Asparagine, L-Glutamine, L-Aspartic acid, L-Glutamic acid, L-Lysine, L-Arginine, L-Histidine. Different retention times were obtained for each amino acid.

Fig. 2 shows a typical chromatograph of taurine extracted from infant formulae. Peaks corresponding to several amino acids were detected in the chromatograms obtained for infant formulae determinations, but as shown, these peaks were well separated from those of taurine under the conditions used. A similar behaviour was observed with regards to breast milk.

The precision of the analytical method was evaluated by measuring the peak chromatographic area 10 times on the same sample. The relative standard deviation (RSD) ranged between 0.7 and 1.4 % (concentration of taurine in infant formula 42.6 and 28.6 mg of taurine/100 g of infant formula, respectively).

Recovery studies were carried out on two different samples of infant formulae, sample 1 with high concentration of taurine, and sample 9 with very low concentration of taurine. The samples were analysed in triplicate before and after the addition of known amounts of taurine and they were spiked with

Table 2

Comparison Between Taurine Determined in Infant Formulae and Breast Milk by HPLC-UV and a Comparison Method of Known Accuracy[a]

Sample No.	Taurine Determined (mg/100 g of Infant Formula) HPLC-UV(y)	Comparison method (x)
1	39.4 ± 0.7[b]	40.6 ± 0.5
2	34.4 ± 1.3[b]	32.7 ± 0.2
3	28.6 ± 0.3[b]	27.7 ± 0.7
4	39.0 ± 0.9[b]	40.3 ± 0.5
5	38.0 ± 0.2[b]	38.8 ± 0.6
6	44.7 ± 0.7[b]	43.1 ± 0.3
7	32.7 ± 0.9[b]	32.9 ± 0.4
8	30.2 ± 1.1[b]	31.0 ± 0.9
9	1.01 ± 0.08[c]	0.80 ± 0.04
10	3.99 ± 0.21[c]	4.32 ± 0.11
	Taurine Determined (mg/100 mL of Breast Milk)	
11	4.13 ± 0.06[b]	4.23 ± 0.10
12	4.25 ± 0.08[b]	4.32 ± 0.05
13	2.28 ± 0.11[b]	2.43 ± 0.08
14	2.18 ± 0.09[b]	2.08 ± 0.12

[a]Values are expressed as mean ± standard deviation of three determinations.
[b]Sensitivity of 0.06 AUFS.
[c]Sensitivity of 0.03 AUFS.
Samples 1 to 10 are from infant formulae, samples 11 to 14 are from breast milk.

three different concentrations of taurine. The results are listed in Table 1. The addition of taurine to the infant formulae was made before the protein removal. Thus, this procedure proved the effectiveness of the extraction step and the accuracy of the proposed method. Recovery values were between 95.5 and 102.5 %, which confirm no interference effects due to matrix composition.

In order to validate the accuracy of the analytical method and because there was no certified reference material available, not only the standard addition method was made, but also a comparison with a method of known accuracy.[11] The results of these determinations of taurine in infant formulae and breast milk are presented in Table 2. In both cases quantification was based on the external standard method.

No significant differences between the results obtained with the present method (y) and the comparison method (x) were obtained when determined by ANOVA methodology, followed by Fisher's PLSD test. (Differences were considered significant for $p < 0.01$). Regression lines between the two methods were $y = 0.996\ x + 0.108$, ($r = 0.997$) and $y = 1.01\ x - 0.042$, ($r = 0.993$) for infant formulae and breast milk, respectively.

CONCLUSION

An accurate and precise method to quantify taurine in infant formulae and breast milk is presented.

Despite the complexity of the matrix, the sample pre-treatment is simple and this approach only requires a basic HPLC system without extra reagent pumps, mixing manifolds, reaction coils, etc. which is an obvious advantage of precolumn derivatisation relatively to post column derivatisation.[7] However, it requires rigorous control of the OPA-reagent and reaction time in order to obtain the high degree of reproducibitily shown here.

The chromatographic run time of 5 minutes is comparable with the lowest reported time for fluorimetric determinations[11] and less than the run time reported for UV determinations.[7]

ACKNOWLEDGEMENT

This work received financial support from Junta Nacional de Investigação Científica Projecto PBIC/C/TPR/ 2564/95.

REFERENCES

1. I. Zelikovic, R. W. Chesney, "Taurine", in **New protective Roles for Selected Nutrients**, Alan R. Liss, Inc., pag. 253-294, (1989).

2. G. E. Gaull, Pediatrics, **83**, 433-442, (1989).

3. R. W. Chesney, Ped. Res., **22**, 755-759 (1987).

4. First Report of the Scientific Committee for Food related with the caracteristics of the infant formulae based on cow's milk. Commission of the European Communities, Luxemburg, 14th serie, EUR 8752 (1983).

5. A. A. Stampfli, O. Ballèvre, L. B. Fay, J. Chromatogr., **617**, 197-203 (1985).

6. B. R. Larsen, D. S. Grosso, S. Y. Chang, J. Chromatogr.Scienc., **18**, 233-236 (1980).

7. T. Hirai, H. Ohyama, R. Kido, Anal. Biochem., **163**, 339-342 (1987).

8. T. Yokoyama, T. Kinoshita, J. Chromatogr., **106**, 212-218 (1991).

9. T. Sakai, T. Nagasawa, (1992) J. Chromatogr., **114**, 155-157 (1992).

10. N. Masuoka, K. Yao, M. Kinuta, J. Ohta, M. Wakimoto, T. Ubuka, J. Chromatogr. B, **660**, 31-35 (1994).

11. C. J. Waterfield, J. Chromatogr. B, **657**, 37-45 (1994) .

12. Z. L. Chen, G. Xu, K. Specht, R. J. Yang, S. W. She, Anal. Chim. Acta, **296**, 249-253 (1994) .

Received August 6, 1996
Accepted August 26, 1996
Manuscript 4247