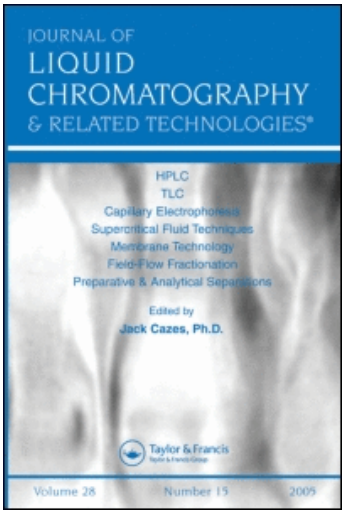
This article was downloaded by:
On: *25 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



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

HPLC Analysis of Taurine in Human Plasma Sample Using the Dabs-Ci Reagent with Sensitivity at Picomole Level

V. Stocchi[a]; F. Palma[a]; G. Piccoli[a]; B. Biagiarelli[a]; L. Cucchiarini[a]; M. Magnani[a]
[a] Istituto di Chimica Biologica "Giorgio Fornaini", Universitd degli Studi di Urbino, Urbino, Italy

To cite this Article Stocchi, V. , Palma, F. , Piccoli, G. , Biagiarelli, B. , Cucchiarini, L. and Magnani, M.(1994) 'HPLC Analysis of Taurine in Human Plasma Sample Using the Dabs-Ci Reagent with Sensitivity at Picomole Level', Journal of Liquid Chromatography & Related Technologies, 17: 2, 347 — 357
To link to this Article: DOI: 10.1080/10826079408013356
URL: http://dx.doi.org/10.1080/10826079408013356

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(2), 347-357 (1994)

HPLC ANALYSIS OF TAURINE IN HUMAN PLASMA SAMPLE USING THE DABS-Cl REAGENT WITH SENSITIVITY AT PICOMOLE LEVEL

VILBERTO STOCCHI*, FRANCESCO PALMA, GIOVANNI PICCOLI, BEATRICE BIAGIARELLI, LUIGI CUCCHIARINI, AND MAURO MAGNANI
Istituto di Chimica Biologica "Giorgio Fornaini"
Università degli Studi di Urbino
61029 Urbino, Italy

Downloaded At: 08:08 25 January 2011

ABSTRACT

A high-performance liquid chromatographic method for the evaluation of taurine in human plasma samples is presented. Two different extraction procedures have been employed: perchloric acid extraction and filtration using the CF-50 Amicon membrane, obtaining comparable results. After extraction the samples were derivatized with the 4-dimethylaminoazobenzene-4'-sulfonyl chloride (DABS-Cl) reagent and the DABS-derivatives, included DABS-taurine, analyzed by reversed-phase HPLC obtaining a sensitivity at picomole level. However, the plasma filtration using the CF-50 Amicon membranes is a quicker procedure (10 min) also allowing the simultaneous evaluation of acid-labile amino acids such as asparagine, glutamine and tryptophan and a higher recovery of taurine (>96%). The taurine levels in human plasma samples of ten normal adults (mean age, 20 years) using the perchloric extraction and the plasma filtration were 38±5 and 42±4 nmol/ml of plasma respectively.

*To whom correspondence and requests for reprints should be addressed.

Copyright © 1994 by Marcel Dekker, Inc.

INTRODUCTION

Taurine (2-aminoethanesulfonic acid) is an amino acid containing sulphur widley distributed in vertebrate tissues. It is present in relatively high concentrations in several vital organs where it could regulate some of their physilogical functions (1). The role of this amino acid in the enteroheptatic circulation of bile salts, in the renal transport and in the binding of calcium ions in the myocardial tissue is known (2). In the central nervous system taurine is important as a neurotrasmitter and a neuromodulator (3-6). Human, nonhuman primate species and cats present a poor ability to synthesize taurine so they are largely dependent on an exogenous source. The symptoms of a taurine depletion in these species are eye defects and growth retardation (7). Some authors have shown a possible relationship between a lower level of taurine in the plasma and a depressive illness (5) as well as differences between plasma levels of this metabolite in epileptic and non-epileptic children (8). Stephan et al. (9) using human hepatoblastoma cell line (Hep G2) have shown that taurine enhances the LDL receptor activity suggesting an interesting role of this metabolite on hepatic cholesterol catabolism. Taurine is present in tissues and physiological fluids in a very low concentration, ranging approximately from 1-50 μM. For this reason a method with high sensitivity is requirred for its evaluation. In the last

Downloaded At: 08:08 25 January 2011

few years high-performance liquid chromatography (HPLC) analysis has become the most successful method for taurine determination. Many authors have reported different HPLC methods for the evaluation of this amino acid. Reversed phase HPLC determination either a pre-column derivatization with o-phthalaldeyde (OPA) (10,11), o-phthalaldeyde + 2-mercaptoethanol (12,13), dansyl chloride (14,15), phenylisothiocyanate (PITC) (16) or a post-column derivatization (17) have been described; other authors have proposed ion-exchange HPLC chromatography (18-21).

Downloaded At: 08:08 25 January 2011

In this paper we report a procedure (extraction and RP-HPLC analysis) which allows to evaluate the concentration of this metabolite in human plasma samples with a sensitivity at picomole level. Furthermore this procedure allows the simultaneous analysis of the standard amino acids and some of their derivatives (22). The procedure proposed can also be applied to evaluate the distribution of this compound in other physiological fluids such as amniotic liquid or in the supernatant of cell cultures as well as in food samples.

MATERIALS AND METHODS

Chemical. DABS-Cl were purchased from Fluka (Buchs,Switzerland). Free L-taurine for the preparation of DABS-taurine

calibration mixture was obtained from Sigma (Sigma Chemical Co., St. Louis, MO). Acetonitrile, ethanol, isopropanol of HPLC grade and potassium dihydrogen phosphate and the other chemicals were purchased from Carlo Erba (Milan, Italy). CF50 Amicon membranes were obtained from Amicon (Lexington, MA). Triple-distilled water was prepared in the laboratory and used for the preparation of buffers. The buffers used for the HPLC analysis were filtered through a 0.22-μm Millipore filter (Millipore, Bedford, MA).

Downloaded At: 08:08 25 January 2011

HPLC analysis. A Gold liquid chromatographic system from Beckman (Beckman, Berkeley, CA) was used throughout this work. This system consisted of two Model 126 pumps, a PC-8300 solvent programmer, a Model 210 sample injection valve, and a Model 166 variable-wavelength uv-visible range detector, equipped with a 12-μl flow cell. Integration of peak areas was obtained by means of a Shimadzu C-R6A Chromatopac electronic integrator (Shimadzu Corp., Kyoto, Japan). The separation of DABS-amino acids was performed using a 3-μm Supelcosil LC-18 T column (15 cm x 4.6 mm i.d.) protected with a guard column 5-μm Supelcosil LC-18 T (2 cm x 4.6 mm i.d.) (Supelco, Bellefonte, PA). The mobile phases used were: 25 mM potassium dihydrogen phosphate, pH 6.8 (solvent A), and acetonitrile-isopropanol (80:20) (solvent B). The gradient program was:

1 min at 20% of solvent B, 4 min at up to 23% of solvent B, 7 min at 23% of solvent B, 11 min at up to 27% of solvent B, 7 min at 30% of solvent B, 9 min at up to 60% of solvent B, 1 min at up to 70% of solvent B, 5 min at 70% of solvent B. The gradient was then returned in a minute to 20% of solvent B and the initial condition restored in 6 minutes. The flow rate was 1.5 ml/min and the detection was performed at 436 nm. The separation of free amino acids present in human plasma, as DABS-derivatives, reported in Fig. 1, was obtained using the analytic conditions described. A calibration curve was performed injecting different amounts of DABS-taurine ranging from 1 to 50 pmoles. For more details concerning the HPLC separation of DABS-amino acids see *Stocchi et.al.* (22).

Downloaded At: 08:08 25 January 2011

Blood Collection. The human blood samples used for the experiments were obtained from 10 normal adult subjects with a mean age of 20 years. The blood was collected in heparin and immediately centrifuged at 3000 rpm x 10 minutes, at 4°C. The plasma was removed and used for the following extraction with perchloric acid or filtration through a CF-50 Amicon membrane.

Extraction with Perchloric Acid. To one milliliter of plasma 0.5 ml of 5% (v/v) perchloric acid solution was added. The sample was

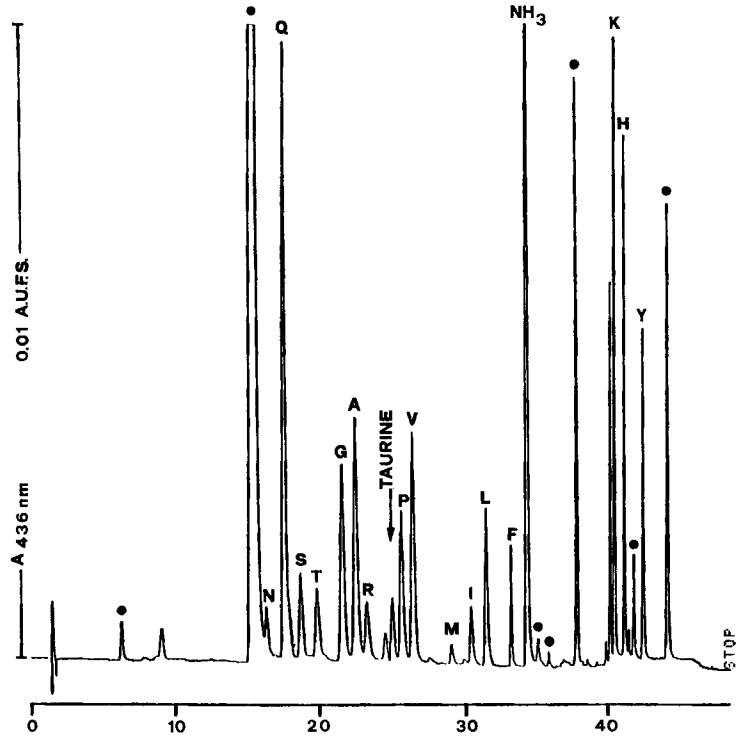


Fig. 1 Determination of DABS-taurine in human plasma by reversed-phase high-performance liquid chromatography using a Supelcosil LC-18 T (15 cm x 4.6 mm i.d.), 3-μm particles, column. The experimental conditions to obtain the separation are described in detail under "Materials and Methods". Solvent A, 25 mM potassium dihydrogen phosphate, pH 6.8; solvent B, acetonitrile-2-propanolo (80:20). Flow rate, 1.5 ml/min; room temperature. 5 μl of sample were injected for the HPLC analysis. ●, By-products originating from the excess of reagent. The chromatogram was obtained using a variable-wavelength detector, Model 166, from Beckman equipped with a 12 μl flow cell.

Downloaded At: 08:08 25 January 2011

centrifuged at 3000 rpm x 10 min, the supernatant removed and neutralized with K_2CO_3 3M. The solution was again centrifuged at 3000 rpm x 5 minutes and the supernatant was removed and brought to a final volume of 2 ml. 10 µl were used for the reaction with DABS-Cl as reported below.

Filtration on CF-50 Amicon membrane. 4 milliliters of plasma were placed on a CF-50 Amicon membrane and centrifuged at 2500 rpm x 10 minutes. 10 µl of the filtrate were used for the dabsylation procedure reported below.

Downloaded At: 08:08 25 January 2011

Derivatization with DABS-Cl. 10 microliters of plasma sample either perchloric acid extract or filtrate were derivatizated in an Eppendorf tube adding 10 µl of 0.1 M sodium hydrogen carbonate, pH 9.0 and 40 µl of DABS-Cl solution (4 nmol/ µl acetonitrile, freshly prepared). The reaction was performed at 70°C x 10 min and the volume was brought to 500 µl with 70% (v/v) ethanol solution. The sample was centrifuged at 14000 rpm x 3 min and 5 µl used directly for the HPLC analysis as previously described (22). Sample after perchloric extraction has half the concentration compared with the sample obtained after filtration on CF-50 Amicon membrane.

RESULTS AND DISCUSSION

The DABS-Cl reagent used for precolumn derivatization of primary and secondary amino acids can also be used for evaluation of L-taurine. For this metabolite a possible role has been proposed as neuromodulator and neurotransmitter also involved in many other biological processes (4-5). Furtermore, this metabolite is usually present in physiological fluids in very low concentrations (μMolar range) and therefore a method with high sensitivity for its evaluation is required. For this purpose the precolumn derivatization using the DABS-Cl reagent seems appropriate giving a sensitivity at picomole level.

In Fig.1 reports the complete RP-HPLC separation of amino acids present in human plasma (included L-taurine) as DABS-derivatives, using the method reported in this paper. The method shows a high reproducibility and a sensitivity at picomole level. Table I reports the plasma concentration of taurine of 10 normal adult human subjects. These data have been compared with those obtained by other authors (4-5) using different conditions of extraction and analysis (4-5). In our case we used a perchloric acid extraction and filtration of plasma with CF 50 Amicon membranes, obtaining the same results. However, plasma filtration is a more rapid procedure which also allows the simultaneous evaluation of plasma acid-labile amino acids such as

Downloaded At: 08:08 25 January 2011

TABLE I

TAURINE LEVEL IN HUMAN PLASMA OF NORMAL ADULTS

Reference	Taurine (nmol/ml)
Tachiki (3)	45±4
Perry (2)	56±2
Present study (perchloric extraction)	38±5
(plasma filtration)	42±4

Data are expressed as nmoles per milliliter of plasma and are the mean value (± standard deviation) of 10 blood samples, from normal adult subjects (mean age, 20 years), centrifuged immediately after collection.

Downloaded At: 08:08 25 January 2011

asparagine, glutamine and tryptophan. The procedure proposed (extraction and HPLC analysis) could also be used for the evaluation of this metabolite in other physiological fluids.

ACKNOWLEDGEMENTS

This work was supported by P.F. Chimica Fine II and P.F. Ingegneria Genetica, CNR, Italy.

REFERENCES

1. R. Huxtable and A. Barbeau, Taurine, Raven Press, New York (1976) 398-405.

2. I. Zelikowic and R. W. Chesney, Absorption and Utilization of Amino Acids, CRC Press, Boca Raton, vol. 1 (1989) 199-207.

3. A. Barbeau, N. Inoue, Y. Tsukada and R. F. Butterworth, Life Sci., 17 (1975) 669-672.

4. T. L. Perry, Taurine, Raven Press, New York (1976) 365-375.

5. H. K. Tachiki, C. H. Hendrie, S. J. Kellema and H. M. Aprison, Clin.Chim.Acta. 75 (1977) 455-465.

6. K. C. Hayes, Nutr.Rev., 43 (1985) 65-70.

7. J. A. Sturman, Sulphur Amino Acids: Biochemical and Clinical Aspects Progress in Clinical and Biological Research, Alan R. Liss, Inc., New York, vol.125 (1982) 281-295.

8. M. Eslami, D. J. Stuart and W. R. Dean, J.Liq.Chromatogr., 10 (1987) 977-995.

9. F. Z. Stephan, S. Lindsey, C. K. Hayes, J.Biol.Chem., 262 (1987) 6069-6073.

10. M. Patrizio, V. Gallo, G. Levi, Neurochem.Res., 14 (1989) 627-633.

11. C. Kisrakoi, K. Horvath, Proc.Int.Conf.Biochem.Sep., 2nd (1988) 275-284.

12. T. Haller, R. Lackner, Fish Physiol.Biochem., 3 (1987) 145-149.

13. L. L. Hirschberger, J. De la Rosa, M. H. Stipanuk, J.Chromatogr. 343 (1985) 303-313.

14. C. F. Saller, M. J. Czupryna, J.Chromatogr., 487 (1989) 167-172.

15. G. M. Anderson, T. A. Durkin, M. Chakraborty, D. J. Cohen, J.Cromatography, 431 (1988) 400-405.

16. S. E. Lippincott, A. L. Friedman, F. L. Siegel, M. R. Pityer, R.W. Chesney, J.Am.Coll.Nutr., 7 (1988) 491-497.

17. B. Saidi, J. J. Warthesen, J.Dairy Sci. 73 (1990) 1700-1706.

Downloaded At: 08:08 25 January 2011

18. C. A. Palmerini, C. Fini, M. G. Cantelmi, A. Floridi, J.Chromatogr. 423 (1987) 292-296.

19. L. Ahtee, D. J. Boullin and M. K. Paasoner, Br.J.Pharmacol., 52 (1974) 245-252.

20. T. T. Aoki, W. A. Muller, M. F. Brennan and G. F. Cahill, Diabets, 22 (1973) 768-775.

21. K. Fukuda and T. Usui, Hiroshima J.Med.Sci., 32 (1983) 163-170.

22. V. Stocchi, G. Piccoli, M. Magnani, F. Palma, B. Biagiarelli and L. Cucchiarini., Anal.Biochem., 178 (1989) 107-117.

Received: April 2, 1993
Accepted: July 8, 1993

Downloaded At: 08:08 25 January 2011