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Journal of Chromatography B, 730 (1999) 161–166

JOURNAL OF CHROMATOGRAPHY B

Ion chromatographic determination of taurine in medicine, nutrient capsule and human urine with electrochemical detection

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Received 3 November 1998; received in revised form 3 March 1999; accepted 26 March 1999

Abstract

A very simple and sensitive method for the determination of taurine by ion chromatography with electrochemical integrated pulsed amperometry is firstly described. Taurine was determined using 160 mmol/l NaOH as eluent and a Dionex CarboPac™ PA1 separation column (250×4 mm I.D.) without the interference with ten kinds of common amino acids. The peak area response of taurine was linear in the range 0.1–20 μg/ml, the detection limit was 0.034 μg/ml. The method has been applied successfully in the determination of taurine in medicinal granule, nutrient capsule and human urine. The content determined in medicinal granule is consistent with that marked by the manufacturer.

Keywords: Taurine

1. Introduction

Taurine (2-aminoethanesulphonic acid) is a free, non-protein, β-amino acid occurring in biological fluids and tissues, such as milk, urine and muscle tissue. The function of taurine in a variety of tissues is the subject of much research. It has been shown that taurine has wide physiological functions, which is able to act as a neurotransmitter [1], antioxidant [2], modulator of intracellualar calcium levels [3], and osmolyte [4]. Also it has a close relationship with many diseases [5,6]. For example, it has possible role in the development of cardiomyopathy [7,8]. It is also considered to be a major end-product of sulphur amino acid metabolism [9] which may be excreted in the urine. Some research involves the determination of taurine in human and rats urine to help decide the metabolism of diseases. In recent years, it has also been reported that taurine has much nutritive value such as in adjusting the metabolism of trace elements, improving the synthesis of DNA, RNA, and proteins in the brain and acting as a regulator in human cell proliferation [10,11]. So, researchers have suggested that taurine should be considered as a conditionally essential nutrient of the human body [12,13]. In the food industry, more attention is being paid to the use of taurine as a nutrient additive. Since taurine plays an important role in many biological process, it is necessary to develop a rapid and sensitive method for determining taurine in many kinds of real samples.

Many methods for measuring taurine have been published including amino acids analysis [14,15], gas chromatography [16], electrophoresis [17,18] and

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0378-4347/99/$ – see front matter
PII: S0378-4347(99)00148-6

high-performance liquid chromatography [19–26]. The common liquid chromatographic methods involve pre-column or post-column derivatization due to the lack of an ultraviolet chromophore, and the procedure is complicated and time-consuming. A large amount of amino acids exist in biological fluids and tissues and they often interfere with the assay of taurine [25]. Though taurine can be eluted out at less than 4 min, as in Catherine J. Waterfield's article, her method must utilize an initial clean-up procedure for samples using dual-bed Dowex resins to remove all interfering amino acids [25].

In this paper, an ion chromatographic method with electrochemical integrated amperometry detection for the determination of taurine is reported firstly. Compared with HPLC methods reported, derivatization is not necessary. The procedure is very simple, convenient and rapid, and also shows good sensitivity and selectivity. Under the experimental conditions, ten kinds of common amino acids do not interfere with taurine. The method has been applied successfully in the determination of taurine in medicinal granule, nutrient capsule in milk powder and human urine. The content determined in the medicinal granule is consistent with that marked by the manufacturer.

2. Experimental

2.1. Reagents

Taurine, HPLC grade, was purchased from Huzhou Biochemical Factory (China). All amino acids as biochemical standard were obtained from Kong Da Amino Acid Factory (Shanghai, China). NaOH was of analytical reagent grade. All standard solutions were prepared using distilled deionized water and were kept in refrigerator at 4°C.

2.2. Apparatus

A Dionex Model DX-500 ion chromatography (Sunnyvale, CA, USA) equipped with a 25 μl sample loop, a Dionex CarboPac™ PA guard column (25×4 mm I.D.) and a Dionex CarboPac™ PA1 separation column (250×4 mm I.D.) were used throughout. The Dionex ED40 electrochemical detector in electrochemical integrated amperometry detection mode was used. 0.785 mm^2 gold electrode was used as the working electrode and Ag/AgCl as the reference electrode. All the instrument control and data collection were performed by a Dionex PeakNet chromatography workstation.

3. Results and discussion

3.1. Electrochemical detection

Integrated amperometric detection at the Au electrode is capable of producing sensitive and reproducible detection responses for amines which, with at least one pair of non-bonded electrons on nitrogen, can be oxidized on the surface of the Au electrode. The detection mechanisms are believed to involve prior adsorption of amines at the oxide-free electrode surface. Detection occurs simultaneously with the formation of gold electrode surface oxide and the anodic mechanisms are believed to be catalyzed by the formation of surface oxide product (AuO) [27,28]. The electrode current is integrated while the potential is swept across the metal oxide formation wave and the oxide reduction wave throughout a rapid cyclic scan. The potential scan proceeds into (positive scan) and back out of (negative scan) the region of the oxide-catalyzed reaction for detection. Without the presence of an electrochemically active analyte, the net charge is approximately zero. The advantage of integrated amperometry relates to the maximization of the analyte signal and the minimization of baseline magnitude and drift for oxide-catalyzed detection [27]. Positive and negative cleaning pulse potentials E_{oxd} and E_{red} are added to keep the native activity of the ''cleaned'' noble metal following the integration period.

Taurine has a free NH_2^- group. By anion-exchange separation and integrated amperometry detection on a gold electrode, a sensitive oxidized current of taurine was obtained. Fig. 1 shows the optimizing potential-time waveform. In this waveform, the electrode current is integrated during the entirety of a rapid cyclic scan of E_{det} (−0.1V, 0.35V, −0.1V) followed by pulse positive (E_{oxd} 0.9V) and negative (E_{red} −0.9V) cleaning potentials. Nano-coulomb (nC shown on *Y* axis in chromatograms) represents the

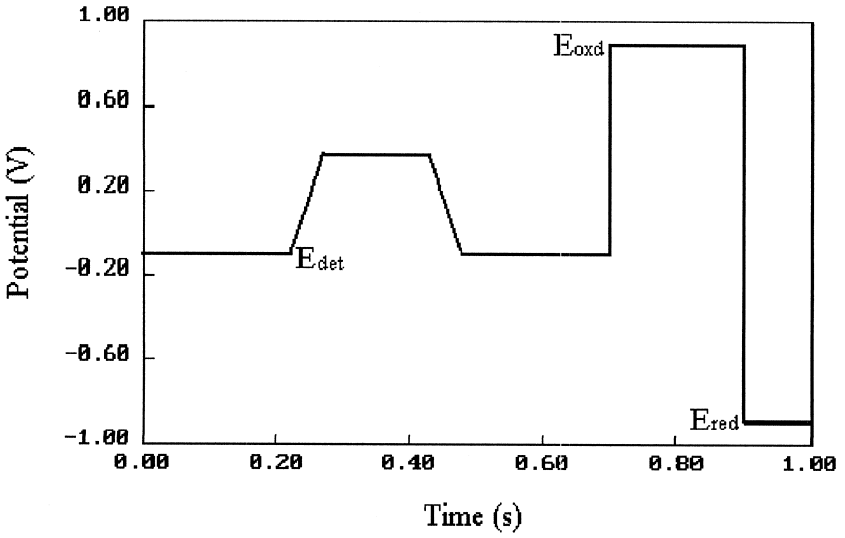

Fig. 1. Integrated amperometry potential-time waveform.

oxidization charge amount after potential-time integrating.

3.2. Choice of separation conditions

Taurine ($NH_2CH_2CH_2SO_3H$) is an organic weak acid, its dissociation constant pK_a is 4.96. Usually, ion-exclusion chromatographic separation is used for such kind of weakly ionized short chain organic acids but the sensitivity of suppressed conductivity detection of taurine is not high. Moreover, the use of the acidic eluent by ion-exclusion chromatography makes the integrated pulsed amperometry detection with gold electrode impossible. In the separation by ion-exchange chromatography, poor ionization of taurine results in its weak retention in common anion-exchange columns such as IonPac-AS4A and IonPac-AS11 column(Dionex), and it was eluted in a shorter retention time of about 2 min. To get a longer retention time, the eluent has to be very dilute, which can not meet the demand of high pH by integrated pulsed amperometry detection. Moreover, the use of very dilute eluents often results in the retention of large amount of the components in real samples, which makes the column overload easily. To maintain the eluent with high pH and obtain the satisfactory sensitivity and retention time of taurine, NaOH is chosen as eluent and the CarboPac column is considered as the separation column.

The CarboPac column is designed to separate reducing monosaccharides, disaccharides, oligo and polysaccharides, etc. The latex in the resin packed in the column carries the ion-exchange functional group and has significant hydrophobic character. It is the combination of anion-exchange and hydrophobic interaction that gives the incredible power for retaining the carbohydrate compounds, which have very high pK_a's and are poorly retained on other anion-exchange columns. The CarboPac column provides strong retention of taurine as it combines both anion-exchange and hydrophobic interaction mechanisms. Using CarboPac PA1 as the separation column, good separation results were obtained.

NaOH was used as eluent as the high pH can both keep taurine in anion form and meet the demand of integrated pulsed amperometry detection [28]. Fig. 2 shows the effect of NaOH concentration on taurine peak area and retention time. With the increase of NaOH concentration, the peak area does not change a lot and the retention time decreased obviously. A systematic dip appeared at 14 min in the chromatogram when a small Y axis scale was adopted. To avoid the influence of the dip, 160 mmol/l NaOH was preferred, which can keep taurine eluted before the systematic dip at less than 10 min. To get

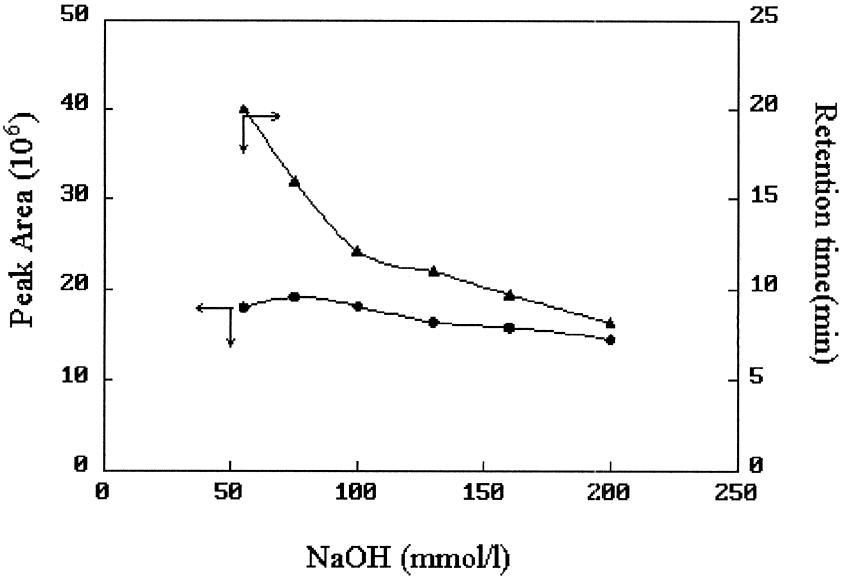

Fig. 2. The effect of NaOH concentration on the peak area and retention time of taurine.

satisfactory results in real sample analysis, the NaOH concentration can be chosen according to Fig. 2.

3.3. Interference

Due to the wide existence of amino acids in food and biological samples, their interference with taurine has to be considered. The mixed standard solution of ten kinds of amino acids (cystine, glutamine, lysine·HCl, glycine, leucine, isoleucine, proline, histidine·HCl, tryptophan, aspartic acid), and taurine with the same concentration (5 μg/ml) was separated (Fig. 3). Aspartic acid was eluted together with the water negative peak. Peak 1 in Fig. 3 represents cystine, glutamine and lysine·HCl eluted together, peak 2 represents glycine, leucine, isoleucine and proline co-eluted. Histidine·HCl and tryptophan were retained more strongly than taurine and were eluted after taurine (Fig. 3, peak 4).

3.4. Linearity, precision and detection limits

Under the experimental conditions, taurine shows good linearity in the range 0.1–20 μg/ml and correlation coefficient was 0.9996. The relative standard deviation (RSD) reaches 0.94% and its detection limit is 0.034 μg/ml (signal-to-noise ratio 3:1).

3.5. Analysis of samples

The granule acidi aminoethanesulfonici (sample A) was purchased from a local drugstore. The milk powder was purchased from a local market and it, along with a nutrient capsule in it, is sample B. 50 mg of samples A and B were dissolved directly into 50 ml deionized water respectively, and a diluted solution of 1:50 of samples A and B were prepared

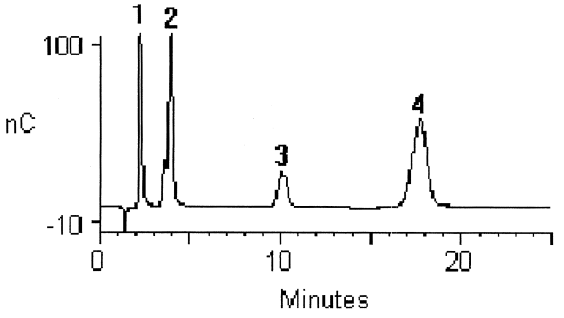

Fig. 3. Chromatogram of mixed standard solution of amino acids and taurine (10 μg/ml). Eluent: 160 mmol/l, flow-rate 1 ml/min. Peak (1): cystine+glutamine+lysine·HCl; (2): glycine+leucine+isoleucine+proline; (3): taurine; (4): histidine-HCl+tryptophan.

Table 1
Results of real samples and spiked recovery

Samples	Total content (mg/g or mg/ml)	Content (μg/ml)	Spiked (μg/ml)	Found (μg/ml)	Recovery (%)	RSD (%)
A	190.6	3.81	5	8.49	96.4	0.68
B	7.55	0.151	5	5.07	98.5	1.21
C	0.033	3.3	5	7.40	89.1	1.79
D	0.0094	0.94	5	5.69	95.8	1.61
E	0.125	12.5	5	15.18	89.6	2.13

before injection. Samples C, D and E are urine obtained from volunteers in our group. They were diluted 10 times by water before injection. All the samples were injected through a 0.45 μm filter membrane. The total taurine content of samples A, B, C, D and E were shown in Table 1. The results of sample A (190.6 mg/g) was consistent with the content marked by the manufacturer (200.0 mg/g). Spike studies were carried out using diluted samples added 5 μg/ml taurine. The recovery and relative standard deviation values for three replicated spike analyses were calculated, and the results shown in Table 1 were satisfactory. Figs. 4–6 are the chromatograms of diluted samples A, B and E respectively.

3.6. Discussion

Under the experimental conditions, using such high concentrations of NaOH as eluent, it is impossible to use the conductivity detection mode for the limited suppressor capacity of the Anion Self-Regenerating Suppressor-I (ASRS-I). It is also impossible to detect taurine directly with UV detection due to its lack of an ultraviolet chromophore. However, by integrated pulsed amperometry detection, the problems above were resolved satisfactorily.

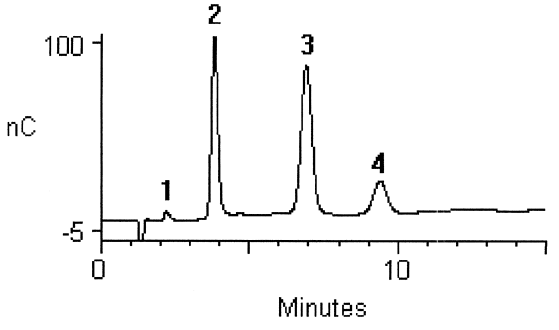

Fig. 4. Chromatogram of sample A diluted solution (1:50). Peak 1, 2, 3: unknown; 4: taurine. Chromatographic condition is the same as in Fig. 3.

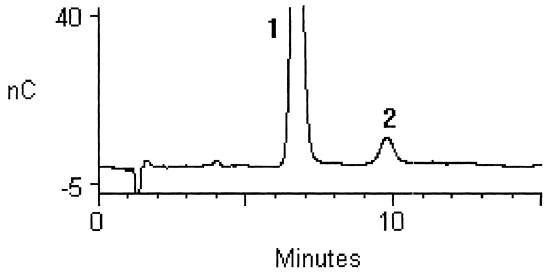

Fig. 5. Chromatogram of sample B diluted solution (1:50). Peak 1: unknown; 2: taurine. Chromatographic condition is the same as in Fig. 3.

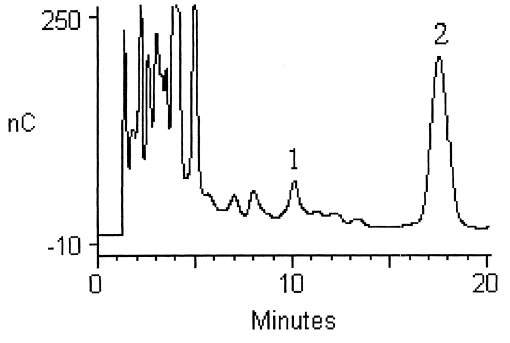

Fig. 6. Chromatogram of sample E. Peak 1: taurine; 2: unknown. Conditions see Fig. 3.

4. Conclusion

The combination of ion chromatography separation by CarboPac column and detection with integrated pulsed amperometry is an advantageous

method to determine taurine. Integrated pulsed amperometry detection makes the determination of compounds containing a NH_2^- group possible, where the dissociation constant pK_a is too large to be detected sensitively with conductivity detection or may not be detected by UV detection due to the lack of an ultraviolet chromophore.

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