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REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUE FOR TAURINE QUANTITATION*

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SUMMARY

Taurine (2-aminoethanesulfonic acid) was quantitated by reversed-phase chromatography on a C_{18} Resolve column using a linear gradient of 9–11% methanol in water. Glutamine was used as the internal standard. Pre-column derivatization of the amino acid with *o*-phthalaldehyde allowed the detection of as little as 0.1 pmol taurine. Dual ion-exchange column chromatography was employed to remove other amino acids and metabolic precursors of taurine from the samples. Cysteic acid and cysteine sulfinic acid did not interfere with taurine analysis by the high-performance liquid chromatographic method.

For sample deproteinization, boiling and picric acid precipitation were used. Recovery of taurine averaged 93.5 ± 5.0% ($\bar{x}$ ± standard error of the mean) from standard solutions and was not affected by the method of deproteinization.

Using this procedure, plasma taurine concentrations for the rat and chick were determined to be 100.7 ± 13.1 μM and 108.0 ± 0.3 μM, respectively. Recovery of taurine from plasma samples averaged 97.2 ± 4.7%.

INTRODUCTION

The presence and quantity of taurine (2-aminoethanesulfonic acid) has been reported in a wide variety of animal species and tissues[1]. The function of this non-protein amino acid in animal cells and fluids, other than bile acid conjugation in

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liver, is not known, although it has been suggested that taurine functions as a neuromodulator[2], membrane stabilizer[3], and antioxidant[4].

Early techniques used to quantitate taurine in biological samples included thin-layer chromatography[5], paper chromatography[6], and colorimetric determinations[7,8]. Applications of these techniques are limited because they require large sample size and lack sensitivity.

The application of high-performance liquid chromatography (HPLC) to the quantitation of taurine has overcome the limitations of the earlier techniques. Existing HPLC methods for taurine determinations lack resolution[9] or require complicated buffer systems for the analyses[10].

We have developed a new, simple HPLC method for the analysis of taurine in biological samples which has high sensitivity and resolution. The technique is based on adsorption chromatography of the *o*-phthaldehyde (OPA)-taurine derivative, using a linear gradient of 9–11% methanol in water. Separation of taurine from the internal standard glutamine is rapidly achieved.

MATERIALS AND METHODS

Equipment

The HPLC system consisted of a Perkin-Elmer Series 4 liquid chromatograph equipped with a microprocessor controlled solvent delivery system, a Rheodyne Model 7125 injector with a 20-μl injection loop, a Perkin-Elmer PC-75 absorbance detector and a Varian Model 4270 integrator. A Waters (Milford, MA, U.S.A.) C_{18} Resolve (5 μm) reversed-phase column was used. Scintillation counting was performed on a Beckman LS-1800 liquid scintillation counter (Fullerton, CA, U.S.A.).

Reagents

Taurine (external standard), L-glutamine (internal standard), *o*-phthalaldehyde (OPA) and β-mercaptoethanol were obtained from Sigma (St. Louis, MO, U.S.A.). Potassium borate buffer (fluoraldehyde reagent dilutent, pH 10.4) was obtained from Pierce (Rockford, IL, U.S.A.). Picric acid (2,4,6-trinitrophenol), HPLC-grade methanol and water were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.). HPLC-grade methanol was filtered with a Millipore (Bedford, MA, U.S.A.), HAWP, 0.45-μm filter. HPLC-grade water was filtered with a Millipore HVHP filter. Analytical ion-exchange resins AG 1-X8 (100–200 mesh, Cl^-) and AG 50W-X8 (100–400 mesh, H^+) were obtained from Bio-Rad (Richmond, CA, U.S.A.). Cysteic acid and cysteine sulfinic acid were obtained from ICN Nutritional Biochemicals (Cleveland, OH, U.S.A.). [1,2-^{14}C]taurine was obtained from New England Nuclear (Willmington, DE, U.S.A.). Scinti-Verse II scintillation fluid was obtained from Fisher Scientific.

Animals

Male Sprague-Dawley rats (200–250 g) were obtained from Charles River (Boston, MA, U.S.A.). Peterson male × Hubbard female chickens (five-weeks of age) were obtained from Rocco Hatchery (Harrisonburg, VA, U.S.A.).

Preparation of standards and derivatizing reagent

A 1.0 m*M* stock solution of taurine was diluted with HPLC-grade water to prepare the 5–30 μM standards. To 97 μl of the taurine standard, 3 μl of 0.4 m*M* glutamine was added.

The derivatizing reagent was formulated by adding 0.4 ml of β-mercaptoethanol and 50 mg OPA to 10 ml of HPLC-grade methanol. After gently shaking to dissolve the OPA, the volume of the solution was then brought to 100 ml with 0.4 *M* potassium borate buffer. This reagent was prepared fresh daily and stored at room temperature in a dark bottle during use.

Plasma collection

Rats were anesthetized with pentabarbital sodium (110 mg/kg body weight). Blood samples (6 ml) were drawn from the inferior vena cava using heparinized syringes. Chickens were anesthetized with chloroform. Blood samples (10 ml) were collected by cardiac punture using heparinized syringes.

Plasma from rats and chickens was obtained by centrifugation (1400 *g*, 10 min) of the whole blood. Samples from four rats and three chickens were analyzed in triplicate to determine plasma taurine levels.

Sample preparation

Plasma samples (1 ml) were deproteinized either by boiling for 15 min or addition of saturated picric acid (1 ml of 2 g/100 ml water). After centrifugation at 15 000 *g* for 10 min, the supernatants (200–500 μl) were removed and placed on dual-bed ion-exchange columns (0.5 cm I.D.) which were prepared by layering 2.5 cm AG 1-X8 over 2.5 cm of AG 50W-X8[9]. After loading the sample onto the column with 0.6 ml distilled water, the taurine fraction was collected using 2.0 ml distilled water. The eluants were placed in a drying oven at 70°C, and the dried samples were stored at room temperature until reconsitution with HPLC-grade water prior to analysis.

Standard taurine solutions used for estimation of recovery were treated identically to the tissue samples. To determine whether metabolic precursors of taurine interfere with the estimation of taurine, a solution of 1 mg/ml cysteic acid and 1 mg/ml cysteine sulfinic acid was subjected to analysis by the same method. To determine the volume of water needed to elute the samples from the ion-exchange columns, 0.002 μCi of [1,2-^{14}C]taurine was chromatographed and 0.5-ml fractions were collected and counted.

HPLC analysis

Standards or samples to which internal standard had been added just prior to the HPLC analysis were reacted for 1 min with an equal volume of the OPA reagent, then injected onto the HPLC[10]. A linear gradient of 9–11% methanol in water was run at a flow-rate of 1.0 ml/min for 10 min to achieve separation of taurine from glutamine. The absorbance of the OPA adducts of these amino acids were monitored at 340 nm, at a sensitivity of 0.01 absorbance units full-scale (a.u.f.s.). The taurine concentration of the sample was calculated by the peak area: weight ratio method from known concentrations of internal and external standards in the standard curve.

RESULTS

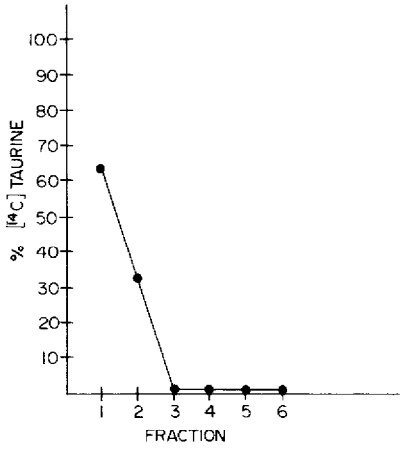
Elution of isotope from the preparative ion-exchange column with 2.0 ml of water resulted in recovery of 96.9% of the sample applied (Fig. 1).

A representative chromatogram, showing retention times for taurine (5.65 min) and the internal standard glutamine (2.69 min), is shown in Fig. 2. The standard curve is shown in Fig. 3. Taurine concentrations in 20 μl of the sample (injection volume) were calculated from the equation:

$$[\text{Tau}] = \frac{RT}{S} \cdot [\text{Gln}]$$

where RT = (peak area for taurine)/(peak area for glutamine), and S = slope of standard curve.

Recovery of taurine from standard solutions (10–30 μM) treated by either boiling or addition of picric acid, followed by ion-exchange chromatography and HPLC, was 93.5 $\pm$ 8.7% and 93.5 $\pm$ 5.0% [$\bar{x} \pm$ standard error of the mean (S.E.M.) for four added concentrations, each determined in triplicate], respectively. Recovery from plasma samples to which taurine (10–40 μM) was added did not significantly



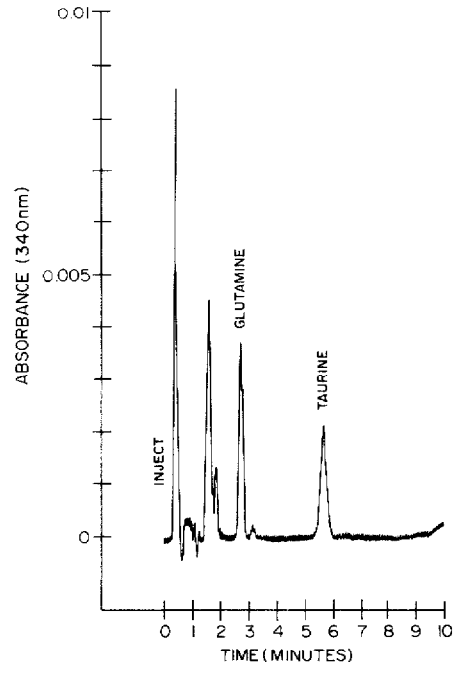
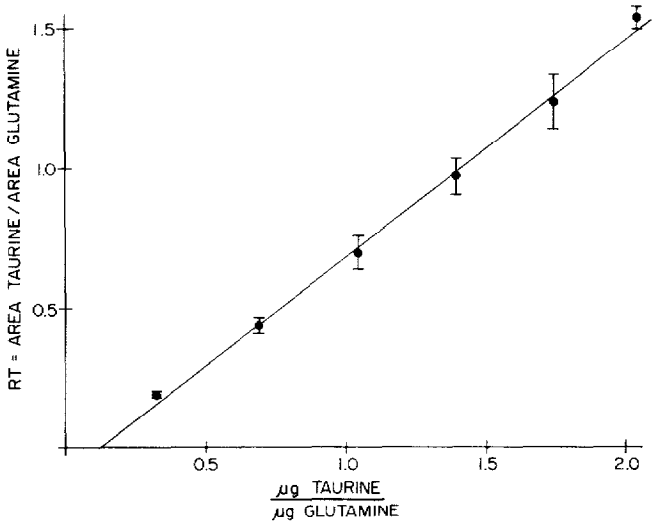


Fig. 1. Percentage of [1,2-^{14}C]taurine eluted by water from dual ion-exchange column in successive 0.5-ml fractions.

Fig. 2. Representative chromatogram showing elution of taurine and glutamine peaks. The absorbance detector was set at a wavelength of 340 nm, and a sensitivity of 0.01 a.u.f.s.



Fib. 3. Taurine standard curve. Values represent the mean ± S.E.M. of five analysis at each concentration. Slope = 0.780, intercept = −0.100.

differ by the method of sample deproteinization, and averaged 97.2 ± 4.7% ($\bar{x}$ ± S.E.M. for two added concentrations, each determined in triplicate).

Cysteice acid and cysteine sulfinic acid eluted in the solvent front, before the internal standard. However, when standards were subjected to preparative ion-exchange chromatography, peaks attributable to either of these metabolic precursors of taurine were not detected in the chromatogram.

Plasma taurine concentrations for the rat and the chick were determined to be 100.7 ± 13.1 μM and 108.0 ± 0.3 μM, respectively.

DISCUSSION

The HPLC procedure presented allows accurate quantitation of as little as 0.1 pmol of taurine. Lower quantities of taurine are detectable using our method, but baseline noise prevents accurate quantitation at these levels. A previous method which used isocratic elution of the taurine-OPA adduct reported a 5-pmol lower limit of detection[10].

Although recovery of taurine from biological samples which are deproteinized by boiling or picric acid did not significantly differ, the former method does offer some advantages. Picric acid explodes when rapidly heated or upon percussion[11], thus care must be exercised when using this compound. Also, for samples with high protein content (for example, rat plasma), multiple picric acid precipitation steps are required for complete deproteinization. This is necessary because use of inadequately deproteinized samples will accumulate on and decrease the life of the HPLC column.

As demonstrated previously[10], the sample preparation method which we used removes contaminating metabolic precursors of taurine such as cysteic acid and cysteine sulfinic acid. Use of the methanol–water gradient and the reversed-phase column provided excellent separation of the taurine and glutamine peaks.

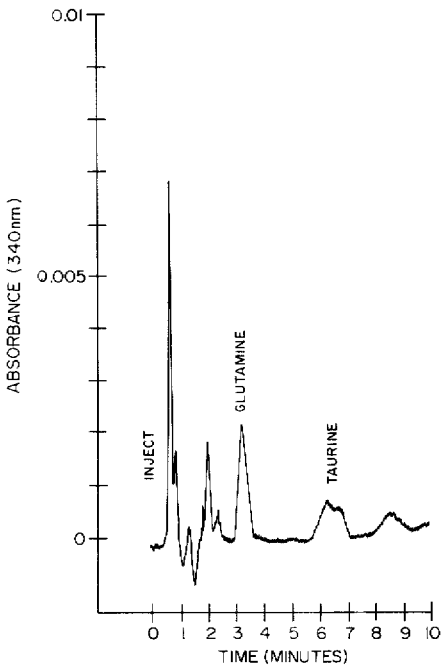


Fig. 4. Representative chromatogram showing shouldering of taurine peak caused by use of old OPA reagent.

To avoid shouldering of the taurine peak (Fig. 4), the OPA derivatizing reagent should be made fresh. Also, vigorous stirring of the OPA reagent should be avoided, since this caused shouldering of the taurine peak even if fresh derivatizing reagent was used.

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