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Short Communication

Simple, rapid and sensitive determination of plasma taurine by high-performance liquid chromatography using pre-column derivative formation with fluorescamine

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ABSTRACT

A simple, rapid and sensitive method for the determination of plasma taurine by high-performance liquid chromatography in the isocratic mode has been developed. The deproteinized plasma was treated with fluorescamine. These derivatives were separated on a LiChrospher 100 RP-8 column within 15 min. The detection limit for taurine was 0.2 μM. The plasma taurine contents of yellowtail fish, *Seriola quinqueradiata*, beef cattle, dairy cows and chickens were determined to be 125 ± 54, 5.6 ± 1.4, 2.2 ± 0.7 and 20.0 ± 9.6 μg/ml, respectively.

INTRODUCTION

Taurine (2-aminoethanesulphonic acid) is a major free amino acid in vertebrates. Various functions, such as bile acid conjugation, neurotransmission, and anti-oxidation, have been reported [1]. Taurine is usually analysed by amino acid analysis [2–4] or high-performance liquid chromatography (HPLC) [5–7]. However, these methods require the relatively time-consuming separation of taurine from other amines and amino acids. In some cases of post-column derivatization with ninhydrin, separation of taurine from other amines and amino acids is incomplete [8]. Sakaguchi *et al.* [9] have analysed taurine in some tissues of yellowtail fish, *Seriola quinqueradiata*, by cation-exchange chromatography followed by *o*-phthalaldehyde–urea reaction. This method can separate taurine from other amines and amino acid completely, but it requires a large amount of sample. Shihabi and White [10] have analysed taurine in cerebrospinal fluid by reversed-phase HPLC using pre-column derivative formation with fluorescamine [10], but their method cannot completely separate taurine from other amino acids and amines.

We have tried to develop an analytical method for the determination of taurine in the plasma of vertebrates by reversed-phase HPLC, using pre-column derivatization with fluorescamine. The method as developed can separate taurine from other amines and amino acids, especially hypotaurine and O-phosphorylethanolamine, and requires a total time of 15 min and 0.2 ml of plasma.

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EXPERIMENTAL

Equipment

A Model BIP I pump unit (Japan Spectroscopic, Tokyo, Japan) with a VL-614 injector (Japan Spectroscopic) was used. Chromatographic separations were performed using a LiChrospher 100 RP-8 column (250 mm × 4 mm I.D., Kanto Chemical, Tokyo, Japan). The column eluent was monitored at 400 nm (excitation) and 480 nm (emission) by a Model FP-210 fluorescence detector (Japan Spectroscopic).

Materials

Fluorescamine was obtained from Fluka (Buchs, Switzerland), and 25 mg were dissolved in 100 ml of acetone to make the derivatization reagent. Taurine, hypotaurine, D-glucosamine-6-phosphate and O-phosphoethanolamine were purchased from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade.

Samples

Plasma (heparinized) was collected from four yellowtail fish, *S. quinqueradiata* (average body weights 6.5 kg), six Japanese black beef cattle (average body weight 250 kg), six dairy cows (Holstein) (average body weight 370 kg) and four chicken (average body weight 2.2 kg). All samples collected were kept at –80°C until analysis.

Methods

Samples for analysis were prepared from 0.2 ml of plasma by deproteinization with 2.0 ml of 5% trichloroacetic acid (TCA). TCA was extracted with 2 ml of diethyl ether (three times) from the sample solution. The solution was evaporated to dryness *in vacuo*, and the residue was dissolved in 1.0 ml of water. A 20-μl volume of the solution was treated with 20 μl of phosphate buffer (200 μM, pH 7.8) and 100 μl of fluorescamine reagent for 15 min at room temperature, and centrifuged for 1 min at 5000 *g*. A 10-μl volume of the supernatant was injected into the column. The mobile phase consisted of 23% of acetonitrile in 15 mM phosphate buffer (pH 1.9). The flow-rate was 1.0 ml/min.

RESULTS AND DISCUSSION

As shown in Fig. 1A, when the pH of the mobile phase was 2.7, taurine was not separated from hypotaurine. Even when the pH of the mobile phase was decreased to 2.1, the resolution was still incomplete (Fig. 1B). However, as shown in Fig. 1C, when the pH of the mobile phase was decreased to 1.9, 100 μM each of D-glucosamine-6-phosphate and O-phosphoethanolamine, taurine and hypotaurine were clearly separated from each other. The separation was complete when 28% acetonitrile was present in the mobile phase and the pH of the mobile phase was 1.9. However, the separation was incomplete when a LiChrospher 100 RP-18 column was used (data not shown).

The calibration graph was linear for taurine concentrations in the range 1–100 μM with a correlation coefficient of 0.999 (n = 6). The coefficients of variation (within-day) were in the range 0.80–3.91%. From the fluorescence spectra of the fluorescamine derivative of taurine, excitation and emission wavelengths were determined to be 400 and 480 nm, respectively. The limit of detection was less than 0.2 μM at a signal-to-noise ratio of 2:1, which is lower than that achieved by Shihabi and White [10] (less than 1 μM).

Fig. 1D shows the chromatogram of the TCA-

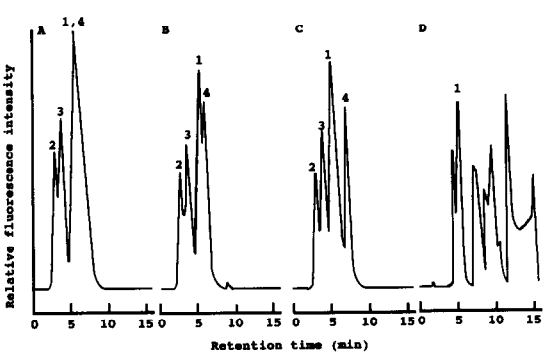

Fig. 1. Separation of taurine (1) from D-glucosamine-6-phosphate (2), O-phosphoethanolamine (3) and hypotaurine (4). (A) The pH of the mobile phase was 2.7; (B) the pH of the mobile phase was 2.1; (C) the pH of mobile phase was 1.9. (D) Chromatogram of a plasma sample of yellowtail fish (sample corresponding to 2 ml of plasma).

soluble fraction of yellowtail plasma. Taurine was clearly separated within 15 min from other peaks, under the isocratic conditions used. The recovery of taurine added to yellowtail plasma was 97.8 ± 1.7% (n = 5). The plasma taurine concentrations of yellowtail fish, beef cattle, dairy cows and chicken were 125 ± 54, 5.6 ± 1.4, 2.2 ± 0.7 and 20.0 ± 9.6 μg/ml, respectively. To our knowledge, this is the first report of the plasma taurine levels of these animals.

As mentioned above, a large amount of sample was previously required for the complete separation of taurine from other amino acids and amines [9]. However, the present method requires only 0.2 ml of plasma and only 15 min of analysis time. Therefore the present method may provide a useful tool for studying the physiological and nutritional significance of taurine.

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