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Note

Assessment of taurine in plasma and urine by anion-exchange high-performance liquid chromatography with pre-column derivatization

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Various methods are currently used for determining taurine in biological samples, including ion-exchange chromatography [1–3] and, more recently, reversed-phase high-performance liquid chromatography (HPLC) [4–8]. These techniques usually require long operating times or afford low sensitivity and are, therefore, of little interest for routine analysis.

In this paper an HPLC method that allows the rapid, precise and accurate determination of taurine in biological samples is presented. The method involves the extraction of taurine from plasma or urine samples with trichloroacetic acid, pre-column derivatization with 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NDB-Cl) and separation of the taurine derivative by anion-exchange HPLC with isocratic elution and fluorimetric detection.

EXPERIMENTAL

Materials

Taurine and NBD-Cl were analytical-reagent grade products from Sigma (St. Louis, MO, U.S.A.). Acetonitrile, methanol (HPLC grade) and all other chemicals (analytical-reagent grade) were purchased from Inalco (Milan, Italy). Sep-Pak C_{18} cartridges were obtained from Millipore (Milan, Italy). HPLC-grade water was purchased from Carlo Erba (Milan, Italy).

A stock 30 m*M* NBD-Cl reagent solution was prepared in methanol. A stock 10 m*M* taurine solution was prepared in 5% (w/v) trichloroacetic acid solution and a working solution (100 nmol/ml) was obtained by dilution with 5% (w/v) trichloroacetic acid solution. Stock solutions were stored at −20°C and used within one week.

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Blood and 24-h urine samples were obtained from healthy volunteers not submitted to a controlled diet.

Analytical procedures

Blood samples (5 ml) were collected in Vacutainer tubes containing 0.1 ml of 2% ethylenediaminetetraacetate solution and centrifuged at 2000 *g* for 10 min at 4°C. Plasma was carefully collected and 0.5 ml was mixed with an equal volume of 10% trichloroacetic acid solution. After centrifugation at 9000 *g* for 10 min at 4°C, the clear supernatant was withdrawn and used for analysis or stored at −20°C.

A 1-ml sample of 24-h urine was mixed with an equal volume of 10% trichloroacetic acid and centrifuged as above. A 1-ml aliquot of the supernatant was passed through a Sep-Pak C_{18} cartridge conditioned with 5 ml of methanol and 10 ml of water. The cartridge was then washed with 2 ml of 5% trichloroacetic acid to complete taurine elution. The eluate (3 ml) was collected and the volume was adjusted to 10 ml by addition of 5% trichloroacetic acid. The solution was immediately used for analysis or stored at −20°C.

An aliquot of 50 μl of acid-soluble extract of plasma, or urine purified on a Sep-Pak C_{18} cartridge, was mixed with 150 μl of 0.4 *M* sodium borate buffer (pH 9.0), then 50 μl of methanol and 100 μl of NBD-Cl solution were added. Derivatization was carried out at 60°C in the dark for 40 min. After dilution of the reaction mixture to 1 ml with cold mobile phase, 25–100 μl were injected into the chromatograph.

Ion-exchange HPLC

The chromatographic system consisted of a Violet Model Clar 002 chromatograph (Violet, Rome, Italy), a Model N 191 injection valve (Negretti and Zambra, Southampton, U.K.), a Partisil SAX column (25 cm×4.0 mm I.D., 10 μm particle size), a Waters Guard-PAK module with a CN Guard-PAK cartridge, which was used as a guard-column, and a Shimadzu (Kyoto, Japan) Model FC530 spectrofluorimetric detector, equipped with a 12-μl flow cell. The fluorimeter monochromators were set at 470 nm for excitation and 530 nm for emission. A Hewlett-Packard Model 7123A recorder (Hewlett-Packard, Frankfurt, F.R.G.) was employed for peak recording. Chromatographic separation was carried out isocratically at room temperature and a flow-rate of 1.3 ml/min by using as the mobile phase 0.025 *M* citric acid solution containing 10% (v/v) acetonitrile, the pH of which was adjusted at 2.9 with 1 *M* sodium hydroxide solution.

Quantification was performed by external calibration. The concentration of the analyte was determined by comparison with calibration graphs obtained by processing working standard solutions of concentrations up to 1 μmol/ml using the same procedure as for plasma and urine samples.

Conventional ion-exchange chromatography

This procedure, performed to check the accuracy of the HPLC methodology, was carried out on a Liquimat III amino acid analyser (Kontron Instruments, Milan, Italy), using a 250 mm×6 mm I.D. column packed with the resin MCI

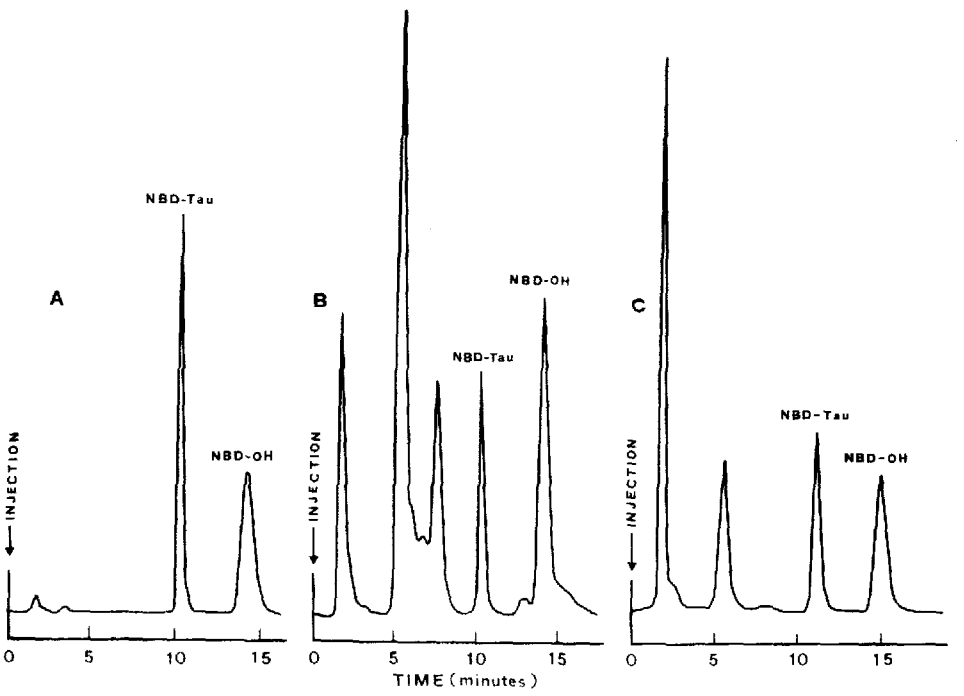

Fig. 1. Representative chromatograms for the analysis of (A) reference taurine (250 pmol), (B) plasma and (C) urine samples. The calculated amounts of taurine from chromatograms B and C are 155 and 110 pmol, respectively. Injection volume: (A) and (C), 50 μl; (B), 100 μl. Peaks: NBD-Tau = taurine derivative; NBD-OH = 7-nitro-4-chlorobenzofurazanol.

Gel 10 F, particle size 7 μm (Mitsubishi, Tokyo, Japan) and a method for physiological analysis with 0.2 *M* lithium citrate buffer (pH 2.8) [9].

RESULTS

The chromatographic profile, depicted in Fig. 1A, shows a typical separation of a standard taurine sample, derivatized with NBD-Cl; in addition to the peak of the derivative, a second component with a higher retention time is also present. As can be demonstrated by the chromatographic analysis of a reagent blank sample, this component is the reaction side-product 7-nitro-4-chlorobenzofurazanol [10], which does not interfere in the analytical process.

Fig. 1B shows the chromatographic profile of a plasma sample. The analyte derivative appears as a symmetrical peak and is well separated from all the other amino acid derivatives, which are less retained. Chromatographic profiles with almost the same characteristics are obtainable from analyses of derivatized urine samples (Fig. 1C).

The conditions adopted for the sample preparation, derivatization and chromatography allow the rapid determination of taurine in plasma and urine, with a sensitivity limit of 5 pmol on the column. The calibration graph is linear in the concentration range of 0–1 μmol/ml, with a correlation coefficient of 0.998. The

coefficients of variation, determined from eleven assays, are 3.0% for analyses of the same series and 3.6% between series.

Recovery experiments ($n=7$), performed by adding 40 nmol of taurine to 1 ml of plasma and 400 nmol of analyte to 1 ml of urine, gave recoveries of 96 ± 2 and $92 \pm 4\%$ (mean $\pm$S.D.), respectively.

Finally, the accuracy of the proposed HPLC method is satisfactory when the results are compared with those obtained by using a dedicated amino acid analyser: the results obtained with the two methods differed by less than 4% for plasma samples and 5% for urine samples.

Plasma and urine levels of taurine, obtained from eleven healthy adults under a non-controlled diet, were 52 ± 8 μmol/l and 579 ± 258 μmol/g of creatinine, respectively. The taurine concentrations obtained by the present method are in good agreement with previously reported values [8,11–13].

CONCLUSIONS

The described method allows the rapid, precise, accurate and sensitive determination of taurine in plasma and urine. In spite of the complexity of the biological matrix of the sample examined, the chromatographic analysis gives profiles in which none of the amino acid derivatives interfere with the analyte of interest. This was made possible by the optimization of the analytical conditions: first, the reaction with NBD-Cl produces highly fluorescent and stable amino acid derivatives; second, anion-exchange chromatography at acidic pH exploits the most important physicochemical feature of the taurine derivative, namely, the presence of a sulphonic group, which is still dissociated at the pH of the mobile phase. The last condition allows a good interaction of the taurine derivative with the functional group of the Partisil SAX anion exchanger; thus the taurine derivative has a longer retention time than the other amino acid derivatives, which do not have significant anionic characteristics at the pH value used in the chromatography. In addition, the selected chromatographic conditions allow an acceptable column lifetime: usually, over 100 analyses can be performed without a noticeable decrease in column efficiency if the CN cartridge is replaced after every 30 analyses.

The HPLC method described here can be used successfully for determining the levels of taurine in biological samples for clinical–biochemical or metabolic studies. The increasing interest in this amino acid, which is present in large amounts in tissues and in biological fluids [13], is due to its role in the biosynthesis of bile salts and in the regulation of various functions, especially in the excitable tissues [14–16].

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