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Note

High-performance liquid chromatographic determination of taurine in formulations as the dansyl derivative

PIER ANTONIO BIONDI*, ARMANDO NEGRI and ANTONELLA IOPPOLO
Istituto di Fisiologia Veterinaria e Biochimica, Via Celoria 10, 20133 Milan (Italy)
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Since retinal degeneration in the cat has been shown to be due to dietary deficiency of taurine (Tau) (2-aminoethanesulphonic acid)[1], there has been increasing evidence of its important role in the body of other mammalian species, including man. Hence the Tau requirement in primate nutrition has been reviewed[2] and its presence in formula diets has been studied[3].

Apart from a multi-step procedure involving gas chromatography[4], liquid chromatography was initially the technique mostly chosen for the determination of the ionic compound Tau. Procedures based on the amino acid analyser have been applied[5] but, in addition to problems of overlapping problems in the Tau zone of the chromatographic profile, the need for a complex apparatus appears excessive with respect to the simplicity of the analytical object. Pre-column derivatization and reversed-phase high-performance liquid chromatography (HPLC) have been the techniques mostly used for Tau determinations in recent years. In particularly, treatment with *o*-phthaldehyde (OPA) has been applied in different procedures and chromatographic systems[6–10]. In addition to the difficulty of resolving Tau from interfering compounds[6,9], the major disadvantage of the OPA derivative is its low stability, which necessitates rigorous control of the reaction and injection times in order to obtain high reproducibility[7].

The aim of this work was to test another conventional amino acid reagent, 5-dimethylaminonaphthalenesulphonyl chloride (dansyl chloride, DNS-Cl), for the determination of Tau in formulations by a rapid, simple and reliable procedure suitable for routine analysis.

EXPERIMENTAL

Materials

Tau and cysteic acid (CysA) were supplied by Sigma (St. Louis, MO, U.S.A.), DNS-Cl and HPLC-grade methanol by Merck (Darmstadt, F.R.G.) and all other reagents and solvents by Carlo Erba (Milan, Italy). DNS-Cl solution (1.5 mg/ml) in acetonitrile was maintained at −20°C and always kept wrapped with aluminium foil in reaction vials to exclude light. The tested formulations were powdered and capsule preparations containing 2% (w/w) and 250 mg per capsule of Tau, respectively.

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Chromatographic conditions

A Jasco (Tokyo, Japan) HPLC system equipped with a Twincle pump, Uvidec-100-III detector set at 254 nm and VL-611 injector was used. The separations were performed on a cartridge (25 cm × 4 mm I.D.) filled with a LiChrosorb RP-8 (7 μm) mounted on a Manu-Fix holder. Methanol–water (35:65) containing acetic acid (0.6%, v/v) and triethylamine (0.008%, v/v) was used as the eluent at a flow-rate of 0.5 ml/min.

Sample preparation

A 1-g amount of powdered formulation and 0.1 mmol of CysA, used as an internal standard, or the content of a capsule and 1 mmol of CysA were added to water (100 ml total volume), shaken for 5 min sonicated for 30 min, and filtered. An aliquot (5 ml) was withdrawn, washed with *n*-hexane (5 ml), treated with 10% trichloroacetic acid (1 ml) and centrifuged. The supernatant was neutralized with sodium hydroxide solution (5 mol/l) and brought to volume (50 ml) with 0.05 mol/l lithium carbonate buffer (pH 9.5). The capsule sample was again diluted 1:10 with carbonate buffer.

Derivatization

In PTFE-lined screw-capped vials an aliquot (1 ml) of the final buffered sample was treated with DNS-Cl solution in acetonitrile (0.5 ml). The mixture was maintained at 40°C for 10 min and, after cooling, the formed dansyl-Tau (DNS-Tau) and dansyl-CysA (DNS-CysA) were analysed by injecting 10-μl volumes of the sample into the chromatograph.

Quantitative analysis

Different volumes of 1 mmol/l aqueous Tau solution containing 10–200 nmol (*i.e.*, 1.2–25 μg) and always a constant volume (50 μl) of 2 mmol/l aqueous CysA solution, were mixed, brought to 1 ml with the above-mentioned carbonate buffer and treated according to the already described derivatization procedure. A calibration graph was obtained by plotting ratios between the peak heights of DNS-Tau and DNS-CysA (R_h) *versus* the amount of Tau. During formulations analysis the contents of Tau in the final samples were obtained from the R_h values.

RESULTS AND DISCUSSION

By exploiting the latest improvements in the dansylation of amino acid suitable for HPLC analysis[11], a derivatization technique alternative to that involving OPA was tested. In order to reduce the procedure time, the reaction temperature was brought to 40°C; under these conditions 10 min were sufficient to give the same maximum yields as obtained at room temperature in more than 30 min. At higher temperatures the reaction time became critical, owing to possible DNS-Cl hydrolysis and competing decomposition of DNS derivatives.

Fig. 1. shows a typical HPLC separation of DNS derivatives of Tau and CysA as internal standard. Under the described conditions the retention time of DNS-Tau relative to DNS-CysA was 1.12 and the overall instrumental analysis time was slightly more than 10 min. The stability of the DNS derivatives was tested by determining

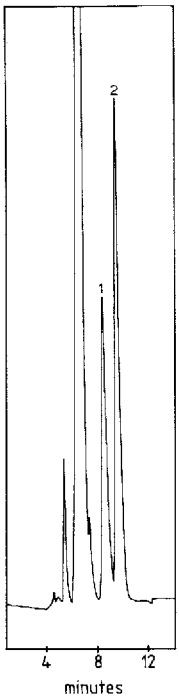

Fig. 1. Typical HPLC profile obtained from a sample analysis. Peaks: 1, DNS-CysA; 2, DNS-Tau.

the peak height of aliquots from the same sample solution kept at room temperature for different periods; no decrease was found for over 8 h. The only precaution necessary is to keep the reaction vials wrapped in aluminium foil to protect the samples from photodegradation, hence eliminating the need to analyse them immediately after derivatization step. The relationship between the DNS-Tau/DNS-CysA peak-height ratio and amount of Tau was linear. The intercept and correlation coefficient were −0.0120 and 0.9998, respectively, and the slope was 0.1672 and 0.0207 for amounts of Tau in micrograms and nanomoles, respectively. Assays performed in duplicate on at least three specimens of each formulation were gave recoveries of 98.5% and 97.6% (relative standard deviation 1.4% and 1.8%) for powdered and capsule formulations, respectively.

In conclusion, the procedure described here can be considered to be useful alternative to the previously reported methods and, with regard to its reliability and simplicity, it appears to be suitable for the routine determination of Tau in formulations. Further experiments are needed in order to assess the applicability of the proposed derivatization procedure to the determination of Tau in biological fluids.

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