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Assay procedures for Taurolin solutions using pre-column derivatisation and high-performance liquid chromatography with fluorescence detection

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Summary

A reverse-phase high-performance liquid chromatographic method using pre-column derivatisation and fluorescence detection has been developed for Taurolin solutions. The major component of commercial Taurolin, taurultam, was detected following pre-column derivatisation with 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD) chloride. Reaction conditions were 5 min at 95 °C, solutions being cooled rapidly and then chromatographed using methanol as eluent. Detection was at 465 nm (excitation) and 523 nm (emission). Taurinamide, a major metabolite of taurultam, was specifically detected following derivatisation with *o*-phthalaldehyde-ethanethiol (OPT) reagent (2 min at room temperature). Detection was at 359 nm (excitation) and 400 nm (emission) with methanol as eluent. Calibration graphs for both analyses were linear. The limits of detection was 7.35×10^{-7} M (taurultam) and 8.2×10^{-6} M (taurinamide). Spiked human plasma samples gave mean recoveries of 83.03% (taurultam) and 88.76% (taurinamide).

Introduction

Taurolidine (bis[1,1-dioxoperhydro-1,2,4-thiazinyl-4]-methane) is a broad spectrum, non-antibiotic chemotherapeutic agent widely used in Western Europe as a 2% m/V aqueous solution containing 5% m/V polyvinylpyrrolidone (Taurolin). This novel agent has been shown to possess significant anti-endotoxic (Pfirrmann and Leslie, 1979) and microbial anti-adherence properties (Gorman et al., 1987; Woolfson et al., 1987a), and is of particular value in the treatment of peritonitis (Browne, 1981).

Studies on taurolidine have to some extent been hindered by the lack of a sensitive and specific assay procedure capable of routine application both to clinical investigations and determination of the mechanism of antimicrobial action. However, PMR studies (Myers et al., 1980; Knight et al., 1983) have shown that taurolidine in aqueous solution exists largely (about 95%) as taurultam and hydroxymethyltaurultam (Fig. 1). A high-performance liquid chromatography (HPLC) method involving pre-column derivatisation with dansyl chloride and subsequent UV detection has been reported (Knight et al., 1981). This method was considered preferable to gas chromatography (Erb,

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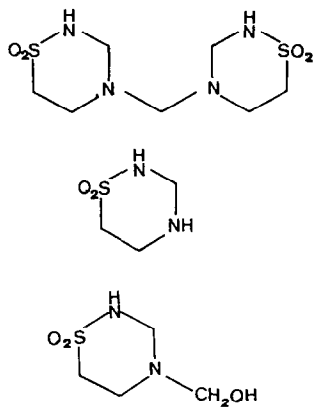

Fig. 1. Chemical structures of taurolidine (I), taurultam (II) and hydroxymethyltaurultam (III).

1983) since the higher temperature used in the latter resulted in ring-opening and consequent on-column degradation. A polarographic method for determination of taurolidine (as taurultam) has recently been reported (Woolfson et al., 1988) but lacks sufficient sensitivity for clinical applications. The present study therefore reports a sensitive and convenient HPLC assay of commercial Taurolin. Pre-column derivatisation with NBD chloride (7-chloro-4-nitrobenz-2-oxa-1,3-diazole) or, specifically for taurinamide ($H_2N \cdot CH_2 \cdot CH_2 \cdot SO_2 \cdot NH_2$, the major metabolite of taurolidine), OPT (*o*-phthalaldehyde-ethanethiol) were employed together with fluorescence detection.

Materials and Methods

Instrumentation

The HPLC system consisted of a Gilson Model 302 pump and manometric module (Gilson Model 803) used in conjunction with a Rheodyne Model 7125 injection valve equipped with a 20-μl loop. Detection was via a Perkin-Elmer Model LS-5 luminescence detector. Chromatograms were recorded on a Perkin-Elmer Model 56 chart recorder.

Chemicals

All solvents used were of HPLC grade. Water was reagent grade 1 (Milli-Q system, Millipore). Taurolin, taurolidine, taurultam and taurinamide were kindly supplied by Geistlich, Chester, U.K. NBD chloride and OPT reagent solution were obtained from Sigma U.K. All other chemicals used were of AnalaR or HPLC purity.

Chromatography, derivatisation and calibration

Separations were carried out on a stainless-steel chromatographic column (300 × 4.6 mm i.d.) packed with μ-Bondapak ODS, 10 μm (Waters). Methanol was used throughout as the mobile phase. For Taurolin, taurultam and taurolidine samples, derivatisation was by reaction with NBD. The sample was mixed (1 : 1) with NBD (20 mg · ml^{-1} in methanol), heated for 5 min at 95 °C, cooled in ice and injected onto the column. Fluorescence detection used wavelengths of 465 nm (excitation) and 523 nm (emission). For taurinamide, OPT reagent solution was mixed (1 : 1) with the sample solution and chromatographed after a reaction time of 2 min at room temperature. The mobile phase was methanol. Fluorescence detection was at 359 nm (excitation) and 400 nm (emission).

Linear calibrations were performed for taurultam between 2.94×10^{-3} M and 7.35×10^{-4} M, and for taurinamide between 1.13×10^{-3} M and 6.45×10^{-4} M.

Recovery of taurultam and taurinamide from plasma

Taurultam (7.35×10^{-4} mol) was dissolved in human plasma (10.0 ml). Plasma proteins were then precipitated by adding ice-cold methanol (20 ml) and vortexing for 30 s. Following centrifugation (3000 rpm, 15 min) the supernatant was diluted (1 : 9) with water, derivatised with NBD chloride and chromatographed. For taurinamide 8.06×10^{-3} mol was used and derivatisation was with OPT reagent. In each case the recovery was calculated from a linear calibration graph obtained using spiked plasma samples.

Determination of taurultam in Taurolin

Taurolin was diluted (1 : 499) with water. Aliquots (1.0 ml) of this solution were derivatised with NBD and chromatographed. The taurultam concentration was determined by reference to the previously prepared calibration graph.

Stability of aqueous taurolidine

Aqueous taurolidine (3.52×10^{-4} M) was freshly prepared and stored at ambient temperature. At 15 min intervals an aliquot (1.0 ml) was removed, derivatised with NBD and chromatographed. The taurultam concentration was calculated from the previously prepared calibration graph.

Results and Discussion

The analysis of Taurolin solutions is complicated by the presence of a number of related components, and by the lack of a suitable chromophore for spectrophotometric detection. Both taurolidine and taurultam are, however, secondary amines whereas the major metabolite of taurultam, taurinamide, has two primary amino functions. Derivatisation of amino acids and their consequent determination by HPLC with spectrophotometric detection is well established. NBD has been used to determine secondary amino acids by HPLC with fluorescence detection (Umagat et al., 1982). The amino function is generally present in a ring structure, the enhanced structural rigidity of the derivative thus being favourable to fluorescence. Therefore, the use of NBD as a fluorogen for the secondary amine components of Taurolin was investigated. Taurolidine and taurultam aqueous solutions gave well-defined chromatograms following NBD derivatisation (Fig. 2). Taurinamide, a primary amine, was not detected by this method. Although various mobile phase compositions were investigated it was found that there was a gradual loss of sensitivity, and a significant rise in back pressure, on injection of repeat samples when an aqueous component was introduced into the mobile phase. Use of methanol (100%) as the mobile phase produced, initially, a large signal followed by restoration of normal sensitivity. It appears that mobile phases with an aqueous component caused on-column precipitation of the

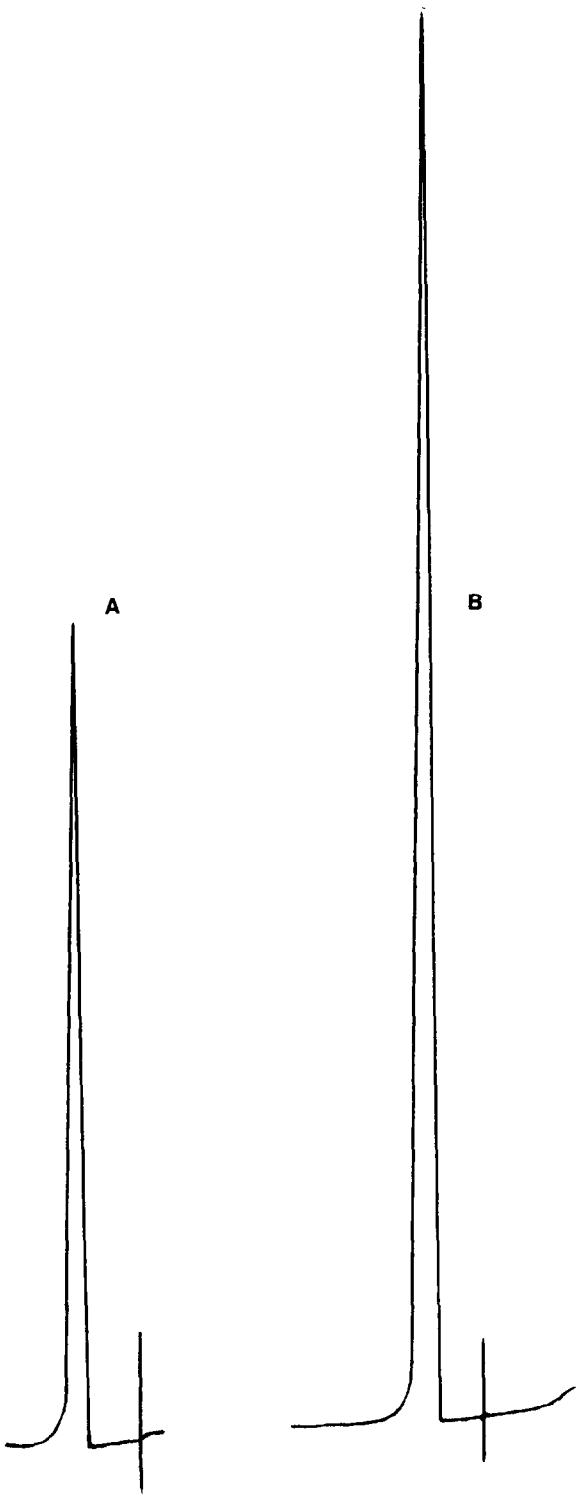

Fig. 2. Chromatograms of aqueous taurultam, 3.68×10^{-4} M (A) and aqueous taurolidine, 3.52×10^{-4} M (B). Eluent, methanol; flow rate, 1.75 ml·min^{-1}; sensitivity, 0.1 units f.s.d.

NBD-taurultam derivative with eventual blockage of the column, subsequently cleared by use of methanol as the mobile phase.

The pre-column derivatisation procedure is both rapid and simple, allowing a large throughput of samples. Both taurultam and taurolidine had identical retention times of 2.2 min at a flow rate (methanol) of 1.75 $ml \cdot min^{-1}$ (Fig. 2), providing further confirmation that the taurolidine dimer breaks down to the monomeric form in aqueous solution. Five taurultam standard solutions between 2.94×10^{-3} M and 7.35×10^{-4} M gave a linear calibration ($r > 0.99$) graph. The limit of detection for taurultam was found to be 7.35×10^{-7} M.

The method was applied to determination of the taurultam content of the commercial preparation, Taurolin, which is stated to contain 2% m/V taurolidine (0.141 M). Four replicate samples gave a mean taurultam concentration of 0.136 M with a relative standard deviation of 5.88%. This value for the breakdown of taurolidine to taurultam is in good agreement with a previous observation (Myers et al., 1980). Hydroxymethyltaurultam is estimated together with taurultam, to which it is largely broken down in aqueous solution (Knight et al., 1981).

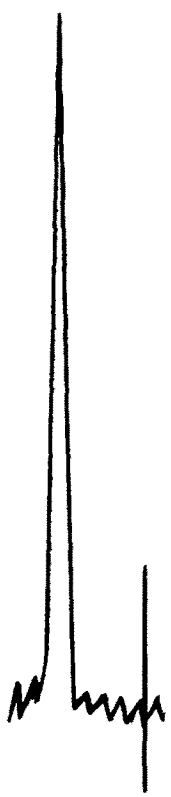

Fig. 3. Chromatogram of taurultam (7.35×10^{-2} M), plasma extract. Eluent, methanol; flow rate, 1.75 $ml \cdot min^{-1}$; sensitivity, 0.1 units f.s.d.

Commercial Taurolin solutions are prepared using autoclaving. Thus, the equilibria between taurolidine and its aqueous breakdown products are established at the point of manufacture. In order to follow the conversion of the dimer to monomeric taurultam a freshly prepared aqueous taurolidine solution was sampled at 15 min intervals over a 2 h period at ambient temperature and assayed for taurultam. A plot of taurultam concentration versus time was linear ($r > 0.99$). After 2 h the taurultam concentration corresponded to that found in commercial Taurolin solutions. The linear concentration–time plot indicated that the breakdown of the dimer can be modelled as zero-order kinetics with a rate constant of 3.20×10^{-6} $mol \cdot l^{-1} \cdot min^{-1}$. Although this model is not strictly correct considering the equilibrium nature of the reaction it is of practical value in that the breakdown reaction proceeds almost to completion. The reservoir of taurolidine thus present accounts for the zero-order kinetics of the breakdown reaction.

A preliminary investigation was made of the applicability of the method to determination of taurultam in plasma (Fig. 3). A linear calibration was obtained ($r = 0.985$). Four replicate spiked plasma samples gave a mean percentage recovery for taurultam of 83.03 (r.s.d. = 2.53%). This initial examination suggests that the method will be quite suitable for the routine determination of taurultam in patient plasma samples.

Taurinamide, the major metabolite of taurultam, is a primary amine. It was found not to react with NBD. Primary amino acids and amines can be derivatised readily by *o*-phthalaldehyde-ethanethiol, the product being both fluorescent (Umagat et al., 1982) and electro-active (Woolfson et al., 1987b). Taurinamide was thus readily derivatised by mixing equal volumes of the sample solution and OPT reagent (Fig. 4). The reaction is

complete within 2 min at room temperature and the fluorescent product as adjudged by chromatographic peak height (retention time 2.3 min) was stable for at least 1 h. The limit of detection for taurinamide in aqueous solution by this method was 8.2×10^{-6} M with a linear calibration ($r > 0.99$). The percentage recovery from spiked plasma samples (Fig. 5) was 88.76 ($n = 4$, r.s.d = 1.96). Since taurinamide is specifically detected by this method it offers good possibilities for future pharmacokinetic investigations of Taurolin, particularly when administered intravenously. The analytical methodology reported here may also be of value in studies on the mode of antimicrobial action of Taurolin.

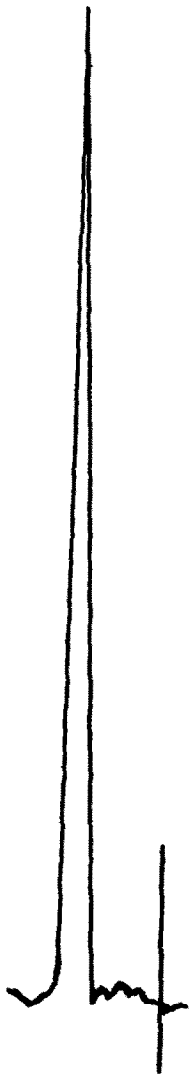

Fig. 4. Chromatogram of aqueous taurinamide (8.06×10^{-4} M). Eluent, methanol; flow rate, 1.75 ml·min^{-1}; sensitivity, 0.1 units f.s.d.

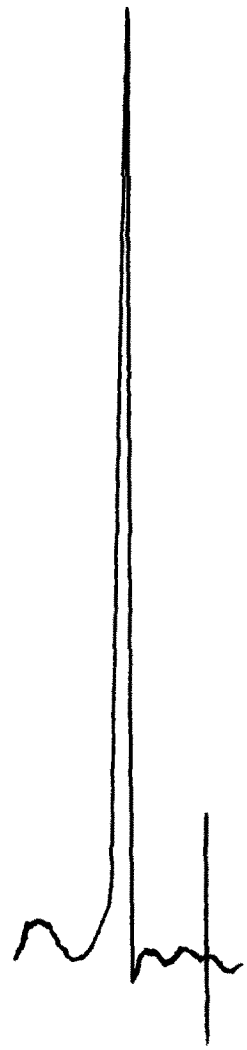

Fig. 5. Chromatogram of taurinamide (8.06×10^{-2} M), plasma extract. Eluent, methanol; flow rate, 1.75 ml·min^{-1}; sensitivity, 0.1 units f.s.d

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