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Note

Trace determination of trimetazidine in plasma by high-performance liquid chromatography using fluorescence detection

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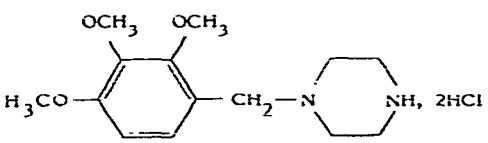
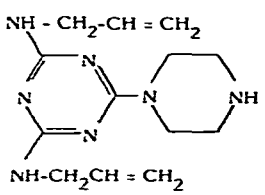
Derivatives of 5-dimethylamino-1-naphthalenesulphonyl chloride (dansyl chloride) have been widely used for thin-layer chromatographic determination of a large variety of amines [1—3]. The method is particularly useful for the analysis of trace components due to the high sensitivity of the products. The derivatives are usually separated by high-performance liquid chromatography (HPLC) using a fluorescence detector.

In the present paper, we describe preliminary results of the HPLC separation of dansylated trimetazidine and its fluorimetric determination in the nanogram range. Previous studies carried out by gas—liquid chromatography (GLC) with flame ionisation and nitrogen—phosphorus detectors enabled us to determine about 100—200 ng of trimetazidine per ml of plasma as the limit of detection. The level of determination was insufficiently sensitive to follow plasma pharmacokinetics in man.

Trimetazidine hydrochloride [1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride] regulates ionic and extracellular exchanges, correcting the abnormal flow of ions across the cell membrane caused by ischemia, and preventing cellular oedema caused by anoxia.



I



II

Fig. 1. Structures of trimetazidine hydrochloride (I) and internal standard (II).

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The structures of trimetazidine hydrochloride [I] and bis-2,4-(allyl-amino)-6-(1-piperazinyl)-triazine [II], used as the internal standard in the assay, are shown in Fig. 1.

MATERIALS AND METHODS

Reagents

Trimetazidine hydrochloride and its internal standard were supplied by Science Union & Compagnie, Suresnes, France. Acetone, cyclohexane, chloroform, isooctane, diisopropyl ether, methanol, 2 *N* sodium hydroxide and sodium hydrogen carbonate were supplied by E. Merck, Darmstadt, G.F.R. We used pro analysis quality. Dansyl chloride was supplied by Pierce, Rockford, IL, U.S.A.

High-performance liquid chromatography

The modular liquid chromatographic system used consisted of a Waters 6000A pump, a Rheodyne loop injector and a Schoeffel FS 970 LC fluorimeter (excitation wavelength, 252 nm; emission wavelength, >370 nm).

The chromatography column (15 cm × 2 mm I.D.) was slurry-packed with LiChrosorb Si-60 (5 μm). The mobile phase [4] was a mixture of solvents; namely, 950 ml of solvent A [isooctane—diisopropyl ether (50:50)], and 50 ml of solvent B [methanol—diisopropyl ether (50:50) + 2.6% of water]. The mobile phase was degassed with helium.

Fluorescence was measured using excitation at 252 nm and with an emission cut-off filter at 370 nm. The flow-rate (1 ml/min) was chosen to separate dansyl derivatives of reagents and endogenous plasma constituents from dansylated trimetazidine and dansylated internal standard.

Extraction procedure

Plasma samples (2 ml) were made alkaline with 300 μl of 2 *N* sodium hydroxide and extracted by stirring with three times 5 ml of cyclohexane—chloroform (3:1, v/v) for 15 min. The organic phases were separated and evaporated under a gentle stream of nitrogen. Before the last evaporation, 50 μl of internal standard solution (1 μg/ml in acetone) were added.

To dissolve the residue, 100 μl of aqueous sodium hydrogen carbonate solution (0.1 *M*) and 100 μl of dansyl chloride solution (0.4 mg/ml in acetone) were added. The tubes were capped and incubated in a water bath at 50°C for 30 min. After this time, the caps were removed and solutions were evaporated under a gentle stream of nitrogen. A 200-μl volume of mobile phase was then added and the mixture shaken for 2 min. A 20-μl aliquot of the organic phase was injected into the liquid chromatograph for the HPLC analysis.

The retention times for dansylated trimetazidine and dansylated internal standard were 10 min and 8 min, respectively (Fig. 2).

Calibration curve and reproducibility

A calibration curve was constructed by adding known amounts of trimetazidine hydrochloride and the internal standard to plasma, and these were then taken through the analytical procedure. The peak height ratio of dansylated tri-

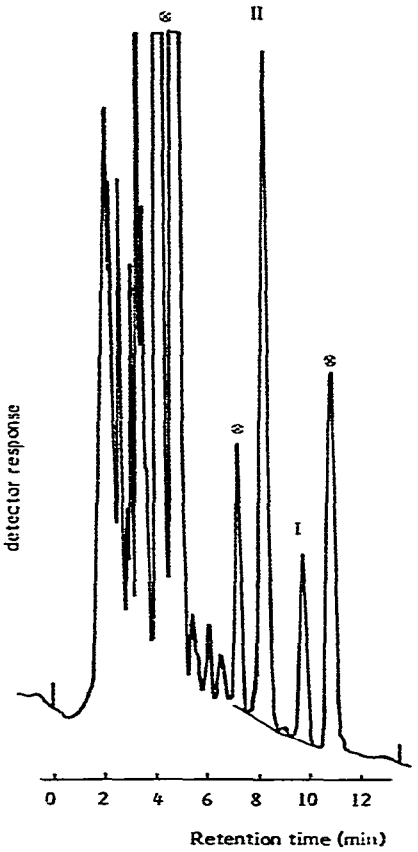

Fig. 2. Chromatogram obtained with plasma spiked with 25 ng/ml trimetazidine. I = Trimetazidine hydrochloride; II = internal standard. ⊗ = reagents and endogenous plasma constituents.

metazidine to the internal standard was plotted against the amount of trimetazidine added to construct the calibration curve. The linear regression equation $y = 0.0101x + 0.028$ describes the curve, where $r = 0.999$.

Replicates (six samples) of 20 ng of trimetazidine were carried through the procedure to determine the reproducibility of the method expressed by the standard deviation. Values of R [= (h trimetazidine)/(h internal standard)] were 0.284, 0.262, 0.271, 0.270, 0.295, 0.296, with $\bar{m} = 0.279 \pm 0.014$ equivalent to 20 ± 1 ng.

The reproducibility was further determined under the same conditions with plasma containing about 50 ng/ml of trimetazidine and with plasma containing about 2 ng/ml of trimetazidine. The results found were, respectively, 50 ± 2 ng/ml and 2 ± 0.5 ng/ml.

RESULTS

Limit of sensitivity

If the limit of sensitivity is defined as that signal which is three times higher

than the background signal, this method can be used to determine plasma containing about 1 ng of trimetazidine per ml.

Identification of dansylated trimetazidine

The structure of the compound that is analysed by HPLC has been confirmed as dansylated trimetazidine by GLC coupled with mass spectrometry. It shows a molecular peak at *m/z* 499 (Fig. 3). The molar mass of this derivative was also confirmed by chemical ionisation with the peak ($M^+ + 1$) = 500 *m/z*.

The structure of dansylated internal standard has been confirmed by GLC coupled with mass spectrometry. It shows a molecular peak at *m/z* 508. The molar mass of this derivative was also confirmed by chemical ionisation with the peak (M + 1) = 509 *m/z* (Fig. 4).

Conditions of derivatisation

The conditions for reaction of trimetazidine with dansyl chloride were optimised for pH, reagent concentration, temperature and time using published data for the reaction of the reagent with amines [2].

Pharmacokinetic profile in man

The method was applied to the determination of the pharmacokinetic profile of the drug during the clinical evaluation of trimetazidine in patients dosed orally. Fig. 5 shows results obtained for a patient who received a single 40-mg oral dose of trimetazidine hydrochloride.

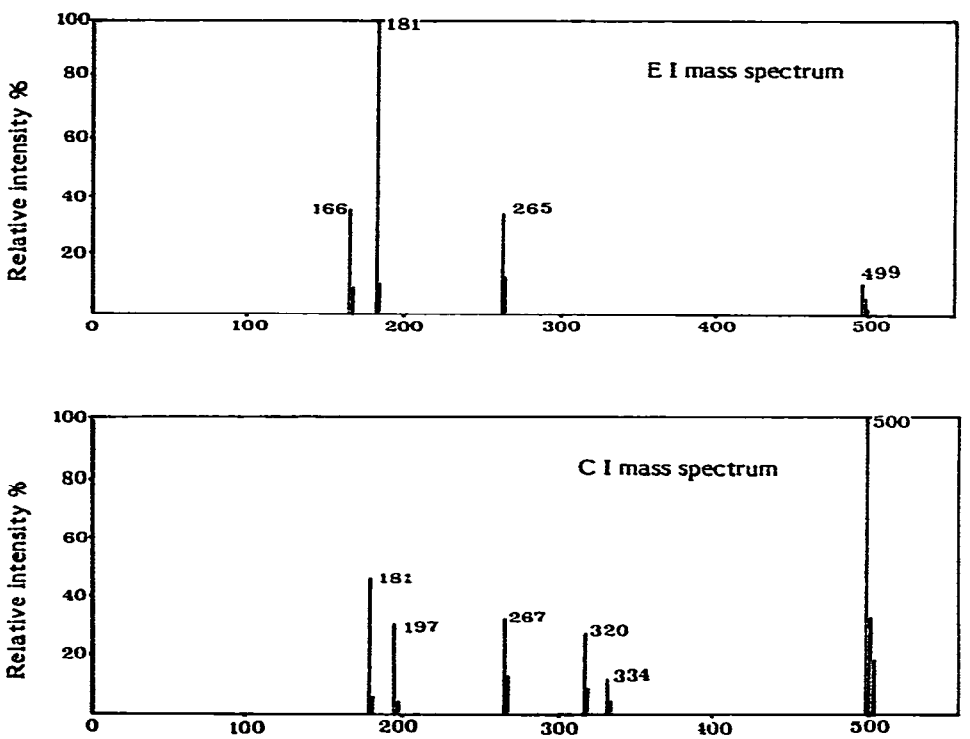

Fig. 3. Identification of dansylated trimetazidine by mass spectrometry.

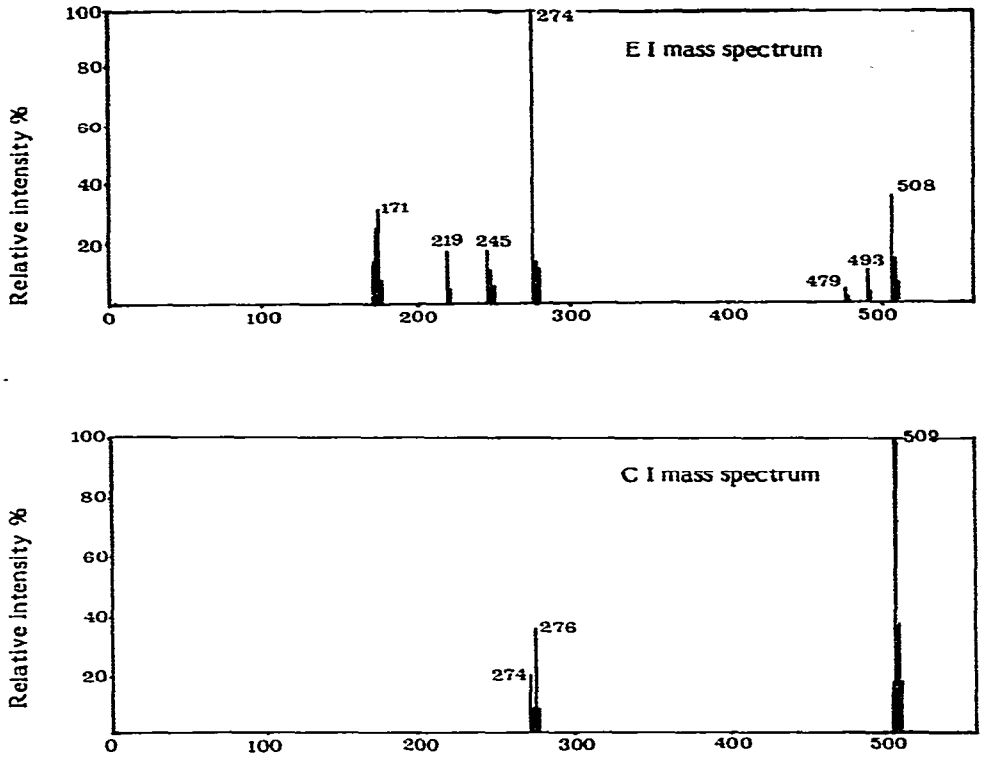

Fig. 4. Identification of dansylated internal standard by mass spectrometry.

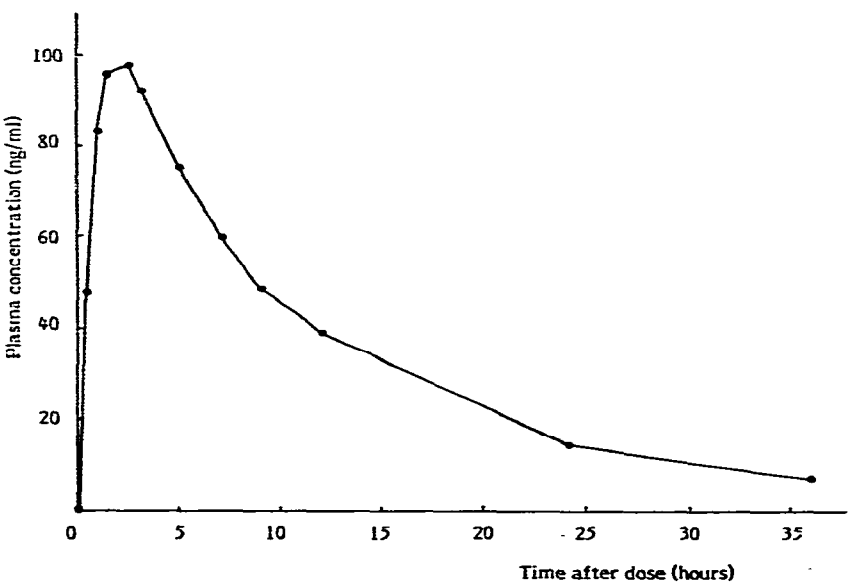

Fig. 5. Plasma trimetazidine profile after a single oral dose administered to a patient.

CONCLUSION

An HPLC procedure has been used successfully for the determination of trimetazidine in plasma at the ng/ml level. The sensitivity of the method described will allow plasma kinetics to be followed in hospitalized patients treated with trimetazidine, when plasma concentrations may range from 10 to 500 ng/ml.

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