Journal of Chromatography, 228 (1982) 205—213
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1136

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF PLASMA AND URINE METAPRAMINE AFTER DANSYLATION

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(First received August 6th, 1981; revised manuscript received September 24th, 1981)

SUMMARY

A high-performance liquid chromatographic method has been developed for the determination of metapramine in human plasma and urine. After selective extraction and derivatization with dansyl chloride, metapramine and the internal standard (maprotiline) are chromatographed on a reversed-phase LiChrosorb RP-18 column using a mixture of water—acetonitrile (35:65) as mobile phase. The eluted compounds are measured using a fluorescence detector. The detection limit of the assay for plasma and urine samples is about 1 ng/ml. The method has been successfully applied in a pharmacokinetic study following intravenous administration of 35 mg of metapramine.

INTRODUCTION

Metapramine is a psychoactive drug belonging to the class of tricyclic compounds. Chemically this substance differs from imipramine derivatives in that it contains only a methyl group on the intranuclear nitrogen atom and in addition a methylamino group at the C-10 position (Fig. 1). Owing to its

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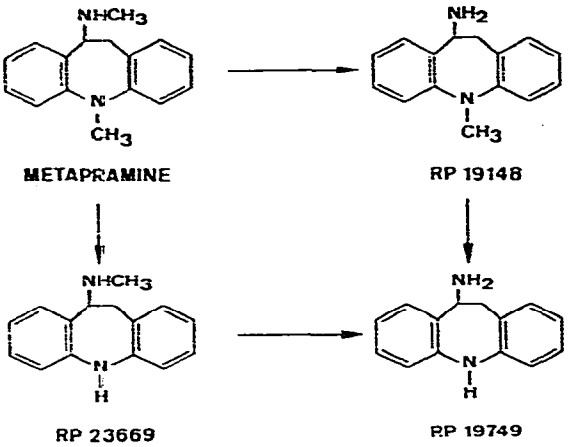

Fig. 1. Chemical structures of metapramine and its major postulated metabolites.

actions on cerebral amine metabolism, the pattern of the central activity of metapramine is different from that of other psychoactive agents [1].

Like other tricyclic compounds, metapramine has two characteristics which markedly affect pharmacokinetic studies. Its distribution in the human body is very rapid and it undergoes considerable metabolism in the liver [2], so that only low concentrations are found in blood or plasma. The chemical structures of metapramine and of its major postulated metabolites are presented in Fig. 1.

Several methods are available for the determination of metapramine in biological fluids, including gas chromatography, thin-layer chromatography and spectrofluorometry [3, 4]. So far, the gas chromatographic procedures are the most sensitive : 35 ng/ml could be measured using electron-capture detection (after derivatization with heptafluorobutyric anhydride) and 15 ng/ml with a nitrogen—phosphorus selective detector. These detection limits are insufficient for the pharmacokinetic investigation of metapramine following therapeutic doses.

Recent developments in the high-performance liquid chromatographic area, especially in the detection modes and derivatization techniques, have allowed the determination of some biologically and pharmacologically active compounds at concentrations below 5 ng/ml [5—15]. Fluorescence detection is now one of the most frequently used methods.

According to Viala et al. [4] metapramine, like imipramine and its derivative desipramine [5], possesses fluorescence characteristics, but it seems that its native fluorescence is not very intense, so that the minimal detectable concentrations were as high as 100 ng/ml. However, the presence of a secondary amino group in metapramine makes it accessible to derivatization with dansyl chloride, a known fluorophore which has been used for the preparation of fluorescent derivatives of a wide range of organic compounds [16—22].

This paper describes a high-performance liquid chromatographic method for metapramine determination involving dansylation and fluorescence detection. Maprotiline [N-methyl-9,10-ethano-anthracene-9(10H)-propanamine] has been used as internal standard.

EXPERIMENTAL

Reagents

All reagents were of analytical grade purity. Aqueous solutions were prepared with double-distilled water. Dipotassium hydrogen phosphate, pH 10 buffer solution, sodium carbonate and sodium hydroxide were obtained from Merck (Darmstadt, G.F.R.). The sulfuric acid used was concentrated sulfuric acid Ultrex (J.T. Baker, Phillipsburg, NJ, U.S.A.). Dansyl chloride was purchased from BDH (Poole, Great Britain), diethyl ether and hexane from the Solvant-Documentation-Synthèse company (Peypin, France). Methanol was methanol "RP Normapur" (Prolabo, Paris, France). Acetonitrile for the mobile phase was acetonitrile "RS" (Carlo Erba, Milan, Italy). Metapramine and maprotiline (as hydrochlorides) were provided by Rhône Poulenc (Antony, France).

Standard solutions

Standard solutions of metapramine and maprotiline were prepared in methanol. Working solutions (varying from 0.1 to 1 μg/ml) were freshly prepared, before analysis, by dilution in double-distilled water.

Extraction and dansylation (see flow diagram in Fig. 2)

To a 20-ml glass-stoppered centrifuge tube were added 10–1000 ng of

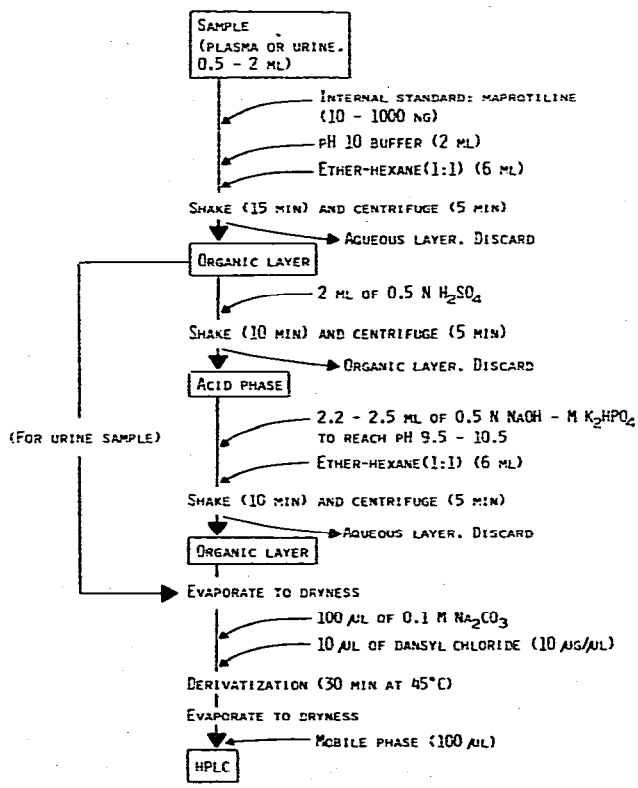

Fig. 2. Schematic flow diagram of metapramine analysis in human plasma and urine.

maprotiline (internal standard), 0.5—2 ml of plasma or urine, 2 ml of pH 10 buffer solution and 6 ml of diethyl ether—hexane (1:1). The mixture was shaken on a three-dimensional shaker (type EM 4; Desaga, Heidelberg, G.F.R) for 15 min and centrifuged for 5 min at 3750 *g*.

Procedure for plasma samples. The organic phase was transferred into another centrifuge tube containing 2 ml of 0.5 *N* sulfuric acid. The tube was shaken for 10 min and centrifuged for 5 min. The organic layer was discarded without removing any of the acid phase which was then adjusted to pH 9.5–10.5 by addition of 0.5 *N* sodium hydroxide solution containing 1 *M* dipotassium hydrogen phosphate. The mixture was extracted with 6 ml of diethyl ether—hexane (1:1) by shaking for 10 min and centrifuging for 10 min. The organic layer was transferred into a 10 mm × 100 mm reaction tube adjustable to a Teflon-lined screw-cap and then evaporated to dryness in a 45°C water bath under vacuum and a stream of nitrogen. To the residue 100 µl of 0.1 *M* sodium carbonate solution and 10 µl of dansyl chloride solution [1% (w/v) in acetone] were added. The tube was tightly capped, vigorously vortexed for 20—30 sec and placed on a 45°C water bath for 30 min. The solvent was evaporated and the residue redissolved in 100 µl of mobile phase (water—acetonitrile, 7:13). An aliquot of 10—50 µl was applied onto the column.

Procedure for urine samples. The organic phase was transferred directly into the reaction tube (without clean-up step), evaporated to dryness, dansylated as described above and injected.

Chromatographic conditions

The pumping system consisted of two Waters pumps (Models M45 and 6000A) (Waters Assoc., Milford, MA, U.S.A.) and of a Model 660 solvent programmer. Samples were loaded with a Waters Model U6K injector.

Chromatography was performed on a 12.5 cm × 4 mm LiChrosorb RP-18, 5 µm (Merck) reversed-phase column. The eluting solvent was an isocratic mixture of water—acetonitrile (35:65) at room temperature and a flow-rate of 2 ml/min.

The fluorescence of the eluent was monitored using a Schoeffel Model GM 970 fluorometer (Schoeffel, Westwood, NJ, U.S.A.) at an excitation wavelength of 248 nm and a emission wavelength of 470 nm.

Under these chromatographic conditions, the retention times of dansylated metapramine and maprotiline were about 16.2 min and 18.5 min, respectively.

Quantitative determination

Concentrations of metapramine in plasma or urine samples were directly calculated from the calibration curve. The standard calibration curve (ratio of peak areas of dansylated metapramine/dansylated maprotiline versus concentration of metapramine) was obtained by analysis of blank plasma or urine samples to which known quantities of metapramine were added together with a constant quantity of internal standard.

Concentrations of metapramine varied in this study from 2.5 to 350 ng/ml and from 350 to 900 ng/ml for plasma and urine samples, respectively. The quantities of metapramine and maprotiline used for the establishment of

the calibration curves were of the same magnitude. In all cases there was good linearity, with correlation coefficients > 0.998. Moreover, the regression curves practically passed through the origin.

RESULTS AND DISCUSSION

Optimization of the dansylation reaction

The conditions for the derivatization step have been studied as a function of the dansyl chloride concentration and of the reaction time. A set of mixtures containing 1000 ng of metapramine, 1000 ng of maprotiline and the extracts of 2 ml of plasma were derivatized using 10 μl of dansyl chloride solution of various concentrations (0.1, 0.25, 0.5, 1, 2.5, 5, 10 and 20 μg/μl) and 100 μl of 0.1 *M* sodium carbonate solution, and heating for 2 h at 45°C in a water bath. Another set was derivatized with 10 μl of dansyl chloride solution (10 μg/μl) in the presence of 100 μl of 0.1 *M* sodium carbonate for various periods of time (ranging from 2.5 min to 2 h). For each mixture, the same volume of aliquot was injected onto the column and the fluorescence was continuously recorded.

As can be seen in Fig. 3, the peak heights of dansylated metapramine (Met-Dns) and maprotiline (Map-Dns) and the peak height ratio of Met-Dns to Map-Dns are constant in the presence of dansyl chloride at concentrations of 5 μg/μl and above, and the dansylation reaction for metapramine and maprotiline was found to be complete within about 10 min at 45°C. Consequently, we used routinely 10 μl of a 1% dansyl chloride solution and a reaction time of 30 min for derivatizing 1000 ng or less of metapramine, 1000 ng or less of the internal standard, and an extract of 2 ml or less of

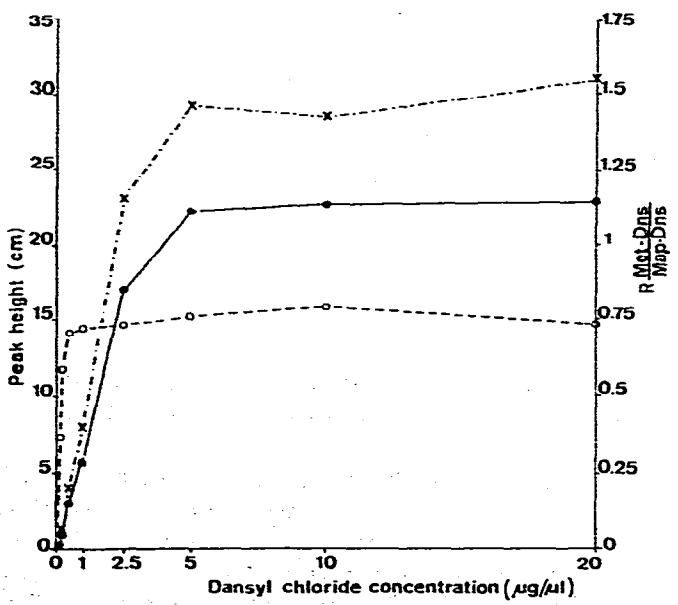

Fig. 3. Evolution of signal intensity observed for dansylated metapramine (•) and maprotiline (○) as a function of dansyl chloride concentration and peak height ratio (R) of dansylated metapramine (Met-Dns) to dansylated maprotiline (Map-Dns) (x).

plasma or urine. The amounts of dansyl chloride and the reaction time are at least twice those required to ensure completeness of derivatization.

Overall recovery and sensitivity

Using a diethyl ether—hexane (1:1) mixture as the organic solvent, metapramine is quantitatively extracted from an aqueous phase of pH 10 and the compound can be quantitatively back-extracted into 0.5 *N* sulfuric acid from this solvent. Overall recovery of metapramine from plasma samples after extraction by this method was higher than 95%.

This technique has allowed us to determine metapramine in concentrations as low as 2 ng/ml. The variation coefficient was 9.3% at this level of sensitivity. The detection limit for quantitative determinations of metapramine in plasma and urine using the method described is about 1 ng/ml. This limit is fifteen times lower than that obtained by a reported gas chromatographic method using a nitrogen—phosphorus selective detector [3].

Reproducibility of the assay

The reproducibility of the method has been determined at concentrations ranging from 2 to 1000 ng/ml. The results are summarized in Table I. The coefficients of variation (C.V.) ($s = 0.95$) within the range tested varied from 1.6 to 9.3%.

TABLE I

ACCURACY OF THE METHOD

Concentration of metapramine (ng/ml)	Sample	*n*	Standard deviation (%)	C.V. (%)
2	Plasma	6	8.5	9.3
10	Plasma	8	6.8	6.9
50	Plasma	7	4	3.9
200	Plasma	6	3.6	3.9
1000	Urine	6	1.5	1.6

Selectivity

Chromatograms of plasma and urine blanks show that no endogenous substances extracted and derivatized under the conditions described interfered with metapramine and maprotiline (Figs. 4 and 5).

No interference was observed from the major postulated metabolites of the drug (RP 19148, RP 23669 and RP 19749). Dansylated metapramine was completely separated from its metabolites (Fig. 6). The retention times of the three dansylated metabolites were approximately as follows: RP 19749, 4.3 min; RP 19148 and RP 23669, 7.5 min (metapramine, 16.2 min; and maprotiline, 18.5 min).

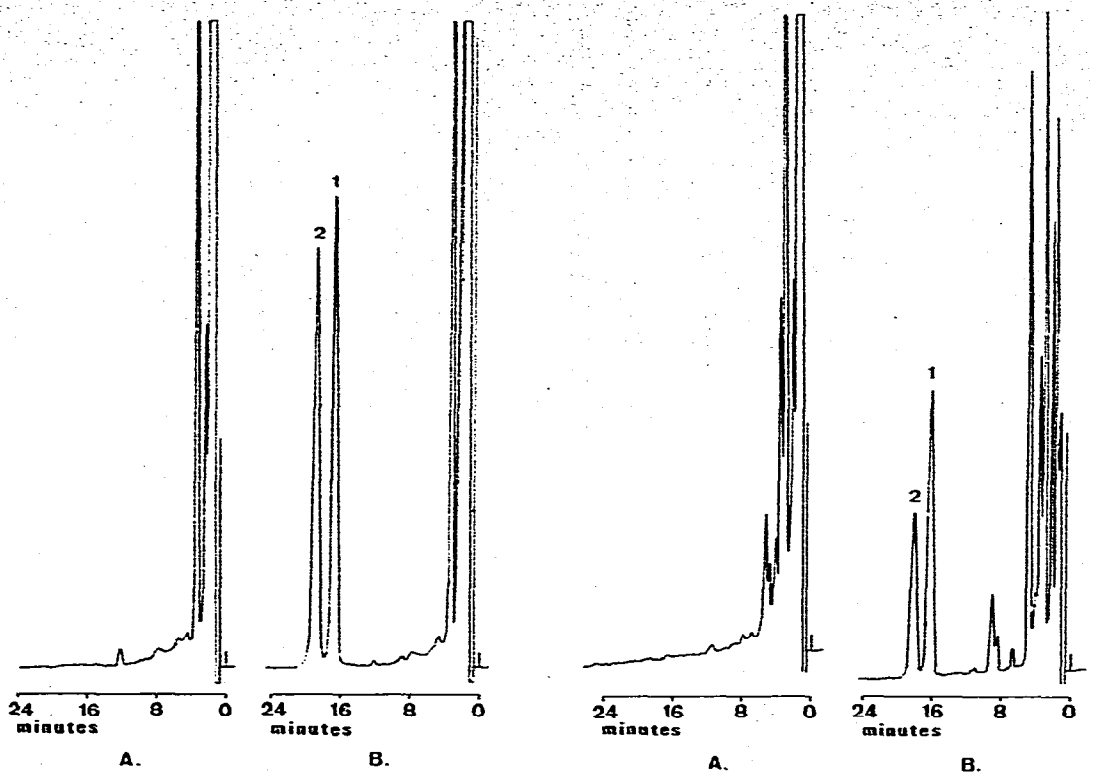

Fig. 4. Chromatograms obtained after extraction and derivatization with dansyl chloride. (A) Plasma of a subject 5 min prior to metapramine administration. (B) Plasma of the subject 5 min after intravenous injection of 35 mg of the drug with addition of 300 ng of internal standard maprotiline. 1 = Dansylated metapramine; 2 = dansylated maprotiline.

Fig. 5. Chromatograms obtained from urine samples. (A) Blank urine. (B) First 24-h urine of a subject receiving 35 mg of metapramine intravenously with addition of 300 ng of internal standard. 1 = Dansylated metapramine; 2 = dansylated maprotiline.

Mass spectrometric examination

Mass spectra of the dansyl derivatives of metapramine were examined using a Hewlett-Packard 5980A mass spectrometer equipped with a Hewlett-Packard 5934A data system by direct injection, and using the chemical-ionization mode. (Reagent gas methane, 70 eV ionization energy, emission current of 250 μA, source temperature of 200°C and source pressure of 1 Torr.) Identification was made using authentic dansylated metapramine and the extracted substance from the plasma of a subject receiving metapramine. The dansyl derivatives were isolated by liquid chromatography before mass spectrometric identification. Both mass spectra show $(M+1)^+$ peaks of the dansylated metapramine (M = 471). This confirms the presence of metapramine in the plasma of the subjects receiving this drug and shows the validity of our liquid chromatographic method.

Application to biological samples

This assay has been applied to the determination of metapramine plasma levels following intravenous administration of 35 mg of the drug to healthy

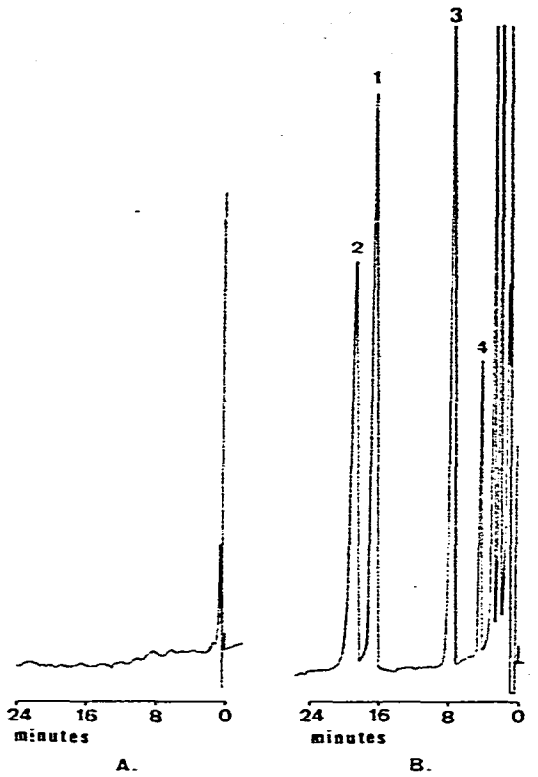

Fig. 6. High-performance liquid chromatographic separation of metapramine, its major metabolites and its internal standard maprotiline: (A) without dansylation; (B) after dansylation. 1 = MET-Dns; 2 = MAP-Dns; 3 = RP 19148-Dns and RP 23669-Dns; 4 = RP 19749-Dns.

human subjects. An example of these results is presented in Fig. 7. Plasma concentrations of metapramine decline at least tri-exponentially and decrease to 2.4 ng/ml at 24 h after dosing. In the first 20 min following administra-

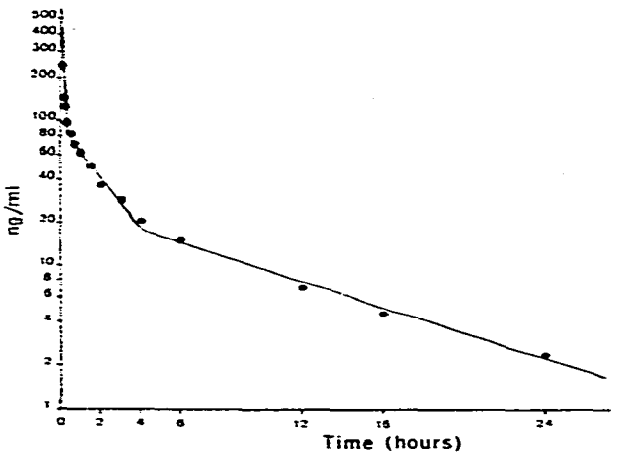

Fig. 7. Plasma concentration—time course of metapramine in one subject following a single intravenous dose of the drug. •, Experimental points; ——, simulated curve.

tion, metapramine plasma levels decrease very rapidly with a half-life of 4 min, showing a fast distribution of the compound into the tissues. In the second phase, metapramine is distributed more slowly with a half-life of 54 min. The apparent half-life of elimination and total plasma clearance determined in this subject were found to be 6.9 h and 90.3 l/h, respectively.

We have also determined the concentration of metapramine in the first 24-h urine of this subject. Only 1.2% of the drug administered was excreted in the urine as unchanged compound during the first 24 h. The appearance of metabolites could be observed.

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