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QUALITATIVE AND QUANTITATIVE DETERMINATION OF METAPRAMINE AND ITS METABOLITES IN BIOLOGICAL MATERIALS

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SUMMARY

The qualitative and quantitative determination of metapramine and its metabolites, after selective solvent extraction from biological materials, are achieved by gas–liquid chromatography with or without an internal standard, carried out directly or after derivatization, spectrofluorimetry and one- or two-dimensional thin-layer chromatography. The gas chromatographic procedures are the most sensitive for the quantitative determination of the unchanged compound: 15 and 35 ng/ml in plasma and 5 and 10 ng/ml in urine; the detection limit for thin-layer chromatography is about 100 ng. These techniques can be used for pharmacokinetic studies and therapeutic controls, and also in analytical toxicology.

INTRODUCTION

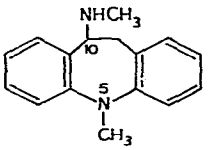
Metapramine (5-methyl-10-methylamino-10,11-dihydrodibenz[*b,f*]azepine) is presented as the fumarate or chlorhydrate. This psychoactive drug differs structurally from imipramine derivatives in that it has only one methyl group on the intranuclear nitrogen atom and one methylamine group at the C-10 position, (Fig. 1), and also on a pharmacological and biochemical basis because of the originality of its central activity[1]. The qualitative and quantitative determination of the unchanged compound and of its main metabolites (Fig. 1) in biological materials have been achieved by gas–liquid chromatography, spectrofluorimetry and thin-layer chromatography. We explain here the methodology we have developed and its various possibilities.

ANALYTICAL PROCEDURE

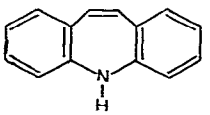
Reagents and standard solutions

All reagents and solvents must be extremely pure. Each aqueous solution is prepared with doubly distilled water. The glassware must be thoroughly washed and dried.

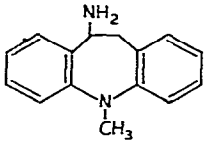
Reagents used were 1 and 3 *N* sodium hydroxide solution, concentrated



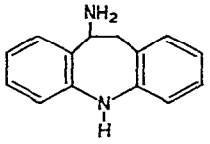
Metapramine



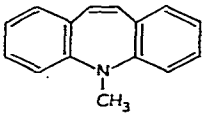
Compound G



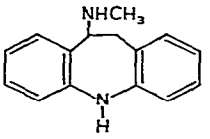
Compound A



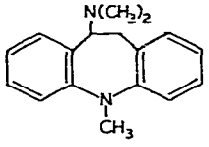
Compound H



Compound B



Compound I



Compound D

Desipramine

Fig. 1. Structure of metapramine, its derivatives and possible metabolites and desipramine (internal standard).

ammonia solution, diethylamine, triethylamine, 0.1 and 1 *N* hydrochloric acid, purified absolute ethanol, methanol, diethyl ether, cyclohexane, toluene, acetone, ethyl acetate and benzene.

Heptafluorobutyric anhydride, puriss p.a., was obtained from Fluka (Buchs, Switzerland) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) from Pierce (Rockford, Ill., U.S.A.)

Buffer of pH 9.8 was prepared by adding 34.9 ml of 0.1 *N* sodium hydroxide solution to 65.1 ml of a solution containing 12.37 g of boric acid and 100 ml of 1 *N* sodium hydroxide per litre. Buffer of pH 5.4 (McIlvaine buffer) contained 55.3 ml of 0.2 *M* disodium phosphate (35.60 g of $Na_2HPO_4 \cdot 2H_2O$ per litre) and 44.7 ml of 0.1 *M* citric acid (21.01 g of $C_6H_8O_7 \cdot H_2O$ per litre).

The purity of desipramine hydrochloride (Fig. 1) must be verified by thin-layer chromatography[2]. β-Glucuronidase/aryl sulphatase (Serva, Heidelberg, G.F.R.) from *Helix pomatia* was used.

Phosphoceric reagent[3]. Dissolve 3.126 g of ceric ammonium sulphate dihydrate in 50 ml of distilled water, then make up to 500 ml with orthophosphoric acid (sp.gr. 1.71) and shake until completely dissolved.

Standard aqueous solution of metapramine hydrochloride. Dilute a solution of 100 μg/ml of metapramine base in doubly distilled water to give solutions with concentrations between 0.05 and 1 μg/ml, which will be used to establish the calibration curve for the spectrofluorimetric determination.

Standard ethanolic solution of metapramine hydrochloride. Dilute a solution of 100 μg/ml of metapramine base in ethanol to give solutions with concentrations of 50 μg/ml (for thin-layer chromatography) or between 0.25 and 2 μg/ml (for gas–liquid chromatography).

Standard ethanolic solutions of desipramine hydrochloride. Containing 4 μg/ml of desipramine base.

Standard ethanolic solutions of compounds A,B,D,G,H and I (Fig. 1). These must be prepared in the same way as the standard ethanolic solution of metapramine hydrochloride.

Qualitative and quantitative determination of metapramine and its main metabolites in plasma and urine by gas–liquid chromatography

We describe below the method used previously for plasma[4]; it can also be used for urine.

In a centrifuge tube of adequate capacity, closed with a glass stopper, place a known amount (*ca.* 400 ng) of desipramine (internal standard) in ethanolic solution. Evaporate the solvent completely and add the sample of plasma (1–3 ml) or urine (1–10 ml). Shake for 15 sec in a vibrator. Add 2 ml of buffer (pH 9.8), then shake mechanically with 6–20 ml of diethyl ether (according to the volume of the sample used) twice for 10 min each time. Centrifuge, then combine the organic phases, concentrate them to *ca.* 1 ml and shake mechanically with 3 ml of 0.1 *N* hydrochloric acid. After centrifugation, neutralize the aqueous phase with 1 *N* sodium hydroxide solution, then add 1 ml of buffer (pH 9.8) and shake mechanically with 6 ml of diethyl ether twice (3 ml each time). Centrifuge and then combine the ethereal phases and evaporate to dryness under nitrogen and under vacuum. Dissolve the residue in 100 μl of ethanol.

Direct qualitative and quantitative determination. Inject 1–2 μl of the above solution into a chromatograph (such as a Hewlett-Packard Model 5710) equipped with a nitrogen-specific detector (Dual NP-FID) and a glass column filled with 3% of OV-1–OV-17 mixture (1:4/3:4) on Gas-Chrom Q (100–120 mesh) (column, 1.5 m × 2 mm I.D.; temperatures, column 230°, injector 200°, detector 300°; gas flow-rates, nitrogen 30, hydrogen 5.8 ml, air 50 ml/min). The retention times obtained under these conditions are between 4.40 and 8.67 min^{-1}, depending on the particular compound (metapramine, its derivatives or desipramine). Calculate the ratio of the peak areas (E/E_I, where E relates to the compound to be determined and E_I to the internal standard). Refer to a calibration graph obtained by similar treatment of standard plasmas to which had been added known amounts of metapramine or of the metabolite to be determined and a constant amount (*ca.* 400 ng) of internal standard, and calculate the amount of the compound of interest present in the sample.

Quantitative determination after derivatization with heptafluorobutyric anhydride. Following the above first operation, evaporate to dryness the ethanolic solution and dissolve the residue in 100 μl of cyclohexane plus 1 μl of heptafluorobutyric anhydride. Place the mixture in a vibrator, shake for 30 sec, then transfer it into an oven at 122° for 1 min in a closed tube. As soon as the mixture has been removed from the oven, evaporate it to dryness under nitrogen and under vacuum for 15 min. Dissolve the residue in 100 μl of cyclohexane and shake for 30 sec. Inject 1–3 μl of this solution into a chromatograph (*e.g.*, a Hewlett-Packard Model 5710) equipped with a nickel-63 electron-capture detector (ECD) and a glass column filled with the same mixture as for the direct determination (column, 1.2 m × 4 mm I.D.; temperatures, column 220°, injector 250°, detector 300°; carrier gas, 10% methane in argon; flow-rate, 25 ml/min). The retention times of the heptafluorobutyrated derivatives obtained under these conditions are between 1.50 and 9.80 min[4]; no derivatives are formed with compounds B and D. Calculate the ratio of the peak areas (E/E_I, where E relates to the heptafluorobutyrated derivative of the compound to be determined and E_I to the heptafluorobutyrated derivative of the internal standard). Refer to a calibration graph obtained by similar treatment of standard plasmas to which had been added known amounts of metapramine or of the metabolite to be determined and a constant amount (*ca.* 400 ng) of internal standard, and calculate the amount of the compound of interest present in the sample.

Silylation procedure. Evaporate to dryness under nitrogen and under vacuum an aliquot or the whole ethanolic solution remaining after the direct determination. Dissolve the residue in 50 μl of cyclohexane and 50 μl of BSTFA. Place the mixture in a vibrator, shake for 20 sec, then transfer it into an oven at 60° for 20 min in a closed tube. As soon as the liquid has been removed from the oven, evaporate it to dryness under the same conditions as above. Dissolve the residue in 100 μl of methanol and shake for 30 sec. Inject 1–2 μl of this solution into a chromatograph equipped with a nitrogen-specific detector. A quantitative determination can be made by comparing the peak areas corresponding with the silylated derivatives to the peak area of the internal standard and referring to the corresponding calibration graph drawn for the direct determination. The results are then expressed as metapramine.

Spectrofluorimetric determination of metapramine in plasma

In a centrifuge tube of adequate capacity, closed with a glass stopper, place the sample of plasma (1–3 ml) and 2 ml of buffer (pH 9.8), then shake the mixture mechanically for 10 min with 6 ml of diethyl ether twice (3 ml each time). Centrifuge, combine the organic phases, concentrate to about 1 ml and shake mechanically for 10 min with 3 ml of 0.1 *N* hydrochloric acid. Add 0.15 ml of 3 *N* sodium hydroxide solution to 2.5 ml of the acidic solution so as to obtain a pH of about 11.5. Measure the fluorescence at 398 nm with excitation at 280 nm. Refer to a calibration graph obtained with standard aqueous solutions of metapramine hydrochloride against a "blank" of the reagents on which the zero value is adjusted.

Identification of metapramine and its main metabolites in urine by thin-layer chromatography

In a centrifuge tube of adequate capacity, closed with a glass stopper, place the sample of urine (1–10 ml or more if necessary), add 2 ml of buffer (pH 9.8), then

shake the mixture mechanically with 10–25 ml of diethyl ether twice (10 min each time). Centrifuge, combine the organic phases and evaporate to dryness. Dissolve the residue in 50 or 100 μl of methanol for analysis by thin-layer chromatography.

One-dimensional procedure. Place some of the methanolic solution or all of it on a thin layer of non-activated silica gel. For comparison, place the standard ethanolic solutions of metapramine and, if necessary, those of compounds A, B, D, G, H and I, which are derivatives or possible metabolites of metapramine, on the thin layer. Elute the plate with toluene–acetone–concentrated ammonia solution (50:50:2). After drying, as none of these compounds is fluorescent in ultraviolet light, reveal the spots directly by spraying with phosphoceric reagent. The R_F values of the above compounds and the colours obtained are given in Table I. We noticed that compounds B and G give both a yellow coloration and a yellow fluorescence on spraying with phosphoceric reagent. Benzene–acetone–concentrated ammonia solution (50:10:5)* can also be used for elution; it improves the separation of some metabolites.

TABLE I

CHARACTERISTICS OF METAPRAMINE AND ITS DERIVATIVES AND POSSIBLE METABOLITES ON SILICA GEL THIN-LAYER CHROMATOGRAPHY WITH TOLUENE–ACETONE–CONCENTRATED AMMONIA SOLUTION (50:50:2)

Compound	R_F	*Coloration after spraying phosphoceric reagent*
Metapramine	0.33	Violet
A	0.45	Blue
B	0.64	Fluorescent yellow
D	0.56	Blue
G	0.61	Fluorescent yellow
H	0.41	Blue
I	0.37	Blue

Two-dimensional procedure. With the whole of the methanolic solution of the urinary extract or part of it, carry out two-dimensional separations on a thin layer of silica gel according to the method described by Populaire *et al.*[5], using cyclohexane–acetone–diethylamine (85:10:5) for the first direction and ethyl acetate–benzene–triethylamine (80:20:2) for the perpendicular direction. Reveal the spots by spraying with phosphoceric reagent; locate the colours and the possible fluorescences appearing under ultraviolet light.

Combination of thin-layer and gas–liquid chromatography. One-dimensional thin-layer chromatography on silica gel is carried out with part or the whole of the methanolic solution of the urinary extract (applied in stripes) in benzene–acetone–concentrated ammonia solution (50:10:5). Mark where the main spots are located, after an incomplete revelation with phosphoceric reagent, and scrape off the part of the silica gel layer corresponding to each unrevealed portion of the spots. Extract the scrapings of silica gel twice with 1 ml of ethanol, evaporate the ethanolic extracts to dryness and dissolve each residue in 50 or 100 μl of methanol. Inject 1–2 μl of each

* Shake the various compounds in a separating funnel and, after decanting, remove the excess of aqueous phase.

of these solutions into a chromatograph equipped with a nitrogen-specific detector. Evaporate to dryness the remainder of each methanolic extract and subject the residues to silylation. After dissolution in 100 μl of methanol, analyse 1–2 μl of each solution by gas–liquid chromatography with the same apparatus.

Thin-layer chromatography used directly on urine. A rapid detection of metapramine and its metabolites can be effected by direct thin-layer chromatography, without any previous extraction, on 100 μl of urine with the one-dimensional technique on silica gel, as described above. We have already used similar techniques for the detection of various organic poisons[6].

Enzymatic hydrolysis

The aqueous phase already extracted with diethyl ether and neutralized with 1 *N* hydrochloric acid, or a new sample of biological material, is adjusted to pH 5.4 by adding an appropriate buffer; 0.1 ml of β-glucuronidase/aryl sulphatase from *Helix pomatia* is added. After keeping in a water-bath at 37° in the dark for 15 h, so that the hydrolysis of the conjugated compounds can take place, the previous operations of extraction and chromatography are carried out.

VALUE OF THE TECHNIQUES

Gas–liquid chromatography

In a previous paper[4] we discussed the development of the extraction procedure, the choice of the internal standard and the value of the techniques of gas–liquid chromatography for which either a nitrogen-specific or an electron-capture detector after derivatization with heptafluorobutyric anhydride is needed. The limits of sensitivity are 15 and 35 ng/ml for plasma and 5 and 10 ng/ml for urine, respectively. The reproducibility is good. The specificity is better following derivatization than in the direct determination, but both methods are complementary. Silylation was considered for the analysis of possible hydroxylated metabolites in urine; we did not study it thoroughly because we lacked standards of the hydroxylated compounds for comparison. However, we verified that the silylation did not affect metapramine, or desipramine or the compounds A, B, D, G, H and I.

Spectrofluorimetry

We obtained the excitation and fluorescence spectra of metapramine with a Perkin-Elmer MPF 3 apparatus. We observed that the optimal wavelengths were 280 nm for the excitation and 398 nm for the emission, that the fluorescence was maximal in alkaline medium with a pH of 11.5, that it did not change over a long period and that the results were reproducible. However, the method lacks sensitivity: the detection limit is *ca.* 100 ng/ml in plasma.

Thin-layer chromatography

We confirmed that, after extraction, the method allowed the simple detection of up to 100 ng of metapramine (10 ng/ml in urine). This limit is 1 μg when the urine is placed directly on the chromatographic layer without previous extraction.

APPLICATIONS

The techniques developed gave us the opportunity of studying the kinetics and metabolism of metapramine in humans and animals. It can be applied not only to plasma and urine but also to other biological media such as faeces, bile, tissues and organs. It can also be used to diagnose possible voluntary or accidental poisonings and to follow their development. The direct thin-layer chromatographic analysis of urine is simple and gives rapid results; it can be of great help in analytical toxicology and for monitoring the administration of the drug to patients.

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REFERENCES

1 Specia Laboratories, Paris, personal communication.
2 A. Viala, F. Gouezo and C. Gola, *J. Chromatogr.*, 45 (1969) 94.
3 Y. Lemontey, J. Meunier and P. Lafargue, *Clin. Chim. Acta*, 30 (1970) 713.
4 A. R. Viala, J. P. Cano, A. G. Durand, T. Erlenmeyer and R. M. Garreau, *Anal. Chem.*, 49 (1977) 2354.
5 P. Populaire, B. Terlain, S. Pascal, B. Decouvelaere, G. Lebreton, A. Renard and J. P. Thomas (Laboratoires de Recherche de la Société des Usines Chimiques Rhone-Poulenc), personal communication.
6 A. Viala, M. Estadieu, A. Durand, A. Angeletti and J. P. Cano, *95ème Congrès de l'Association Française pour l'Avancement des Sciences, Marseille, July 5–10, 1976*, in press.