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Note

Capillary gas chromatographic assay for the routine monitoring of the antidepressant mepirzepine in human plasma

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The tetracyclic compound mepirzepine* (1,2,3,4,10,14*b*-hexahydro-2-methyl-pyrazino[2,1-*a*]pyrido[2,3-*c*][2]benzazepine; Fig. 1) is a potential antidepressant drug in clinical development. For clinical pharmacokinetic studies an assay method is required to determine mepirzepine in human plasma. Gas chromatography (GC) with nitrogen-sensitive detection is a reliable and sensitive method for routine monitoring of mianserin, a structurally related tetracyclic drug [1]. Since then, the application of capillary GC for routine monitoring of drugs has been made possible by the availability of fused-silica columns with cross-linked stationary phases. This type of column is chemically inert, thermally stable and compatible with a variety of injection solvents [2]. This paper describes a capillary GC assay with nitrogen-sensitive detection for the quantitation of mepirzepine in human plasma, using an isomer of mepirzepine as the internal standard (I.S.) throughout the assay procedure.

EXPERIMENTAL

Chemicals and glassware

Both mepirzepine and its I.S. were synthesized by the Organic Chemistry R&D Labs., Organon International (Oss, The Netherlands). [^{3}H]Mepirzepine (radiochemical purity >99.0%), used for recovery experiments, was obtained from the Organic Synthesis Group, Drug Metabolism R&D Labs., Organon International. All other chemicals, *n*-hexane, methanol and aqueous ammonia (25%,

*Mepirzepine has Organon lab. code Org 3770.

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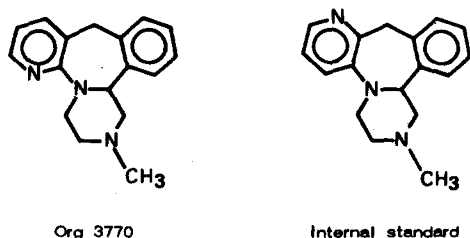

Fig. 1. Structures of mepirzepine (Org 3770) and the internal standard.

v/v), were obtained from Merck (Darmstadt, F.R.G.). The solvents *n*-hexane and methanol were of Uvasol quality, and aqueous ammonia was of Suprapur quality. Stock solutions of mepirzepine and its I.S. were prepared at a concentration of 1 mg ml^{-1} in methanol. Working solutions of mepirzepine and the I.S. were prepared freshly each week by dilution of the stock solutions with distilled water to final concentrations of 0.1 and 1.0 μg ml^{-1}. These aqueous solutions were used for plasma spiking. Solvent extraction of plasma was performed in 20-ml screw-capped disposable glass vials, and 10-ml conical glass tubes and 200-μl polypropylene conical vials were used for further processing and injection into the capillary gas chromatograph, respectively. For evaporation to dryness, a vacuum centrifuge (Speed Vac concentrator, Savant, New Brunswick Scientific, Hicksville, NY, U.S.A.) was used.

Capillary gas chromatography

A Hewlett-Packard Model 5880 gas chromatograph was used; it was equipped with a 7672A autosampler and a Model 18789 A N/P detector. A fused-silica capillary column, 5% phenylmethyl silicone cross-linked (purchased from Hewlett-Packard) was used (25 m$\times$0.3 mm I.D., film thickness 0.54 μm).

Analyses were performed using the splitless injection technique and temperature programming. The injection and detection temperatures were 300°C. The oven temperature programme was: initial temperature, 80°C (hold-up time, 1 min); programme rate, 30°C min^{-1} to 190°C (5 min); programme rate, 10°C min^{-1} to final temperature 270°C (10 min). Helium was used as carrier gas at a flow-rate of 6 ml min^{-1} and as detection make-up gas at a flow-rate of 10 ml min^{-1}.

Assay procedure

The design of the clinical study, the administered dose and the time of blood sampling were used to predict the mepirzepine plasma level. Variable volumes of the I.S. from an aqueous working solution were added to the clinical plasma samples in a concentration equal to that of the anticipated mepirzepine concentration. Usually 0.5–1.0 ml of plasma was processed. After thorough whirl-mixing (30 s) and 1 h of equilibration at ambient temperature the plasma samples were alkalinized with 100 μl of 25% aqueous ammonia per ml of plasma by thorough whirl-mixing (30 s). *n*-Hexane (10 ml) was added to the alkalinized plasma samples. The plasma samples were extracted by whirl-mixing (60 s) followed by

centrifugation at 1500 *g* for 10 min. The organic layer was transferred to 10-ml conical glass tubes and evaporated to dryness in a vacuum centrifuge. The wall of the glass tube was rinsed thoroughly with 200 μl methanol. This was transferred to 200-μl conical vials and again evaporated to dryness in a vacuum centrifuge. The residue was reconstituted in 20 μl of methanol. A 2-μl aliquot was auto-injected into the capillary gas chromatograph.

Assay validation and quality control

The assay was validated by assessing the accuracy (relative difference between mean measured and spiked mepirzepine plasma concentration) as a measure for the systematic error and the precision (percentage coefficient of variation) as a measure for the random statistical error. To that end, replicate analyses were performed on blank (free of drug) plasma samples spiked over a wide concentration range, viz. 0.5–100 ng of mepirzepine and I.S. per ml of plasma in a 1:1 ratio. For calibration purposes, a series of blank plasma samples spiked with mepirzepine and I.S. in different concentration ratios (4:1, 2:1, 1:1, 1:2, 1:4) was processed. In the routine analysis of clinical plasma samples, a calibration curve for each series of analyses was constructed from processed spiked plasma samples. Quality control on the results of routine analysis was performed by determining the accuracy and precision, using plasma samples spiked with a known amount of mepirzepine at selected plasma levels covering the anticipated concentration range of that series of analyses. When analyses turned out to be outside the range of the calibration curve, the analyses were repeated with adapted amounts of I.S.

Data processing

The peak heights of the analytical signals of interest (mepirzepine and I.S.) were temporarily, during one series of analyses, stored on-line on cartridge (HP 85 personal computer). For permanent storage, construction of the calibration curve by linear regression and calculation of the mepirzepine levels, the integrated data were transmitted to a DEC PDP 11/70 computer, using BASIC application programs.

Application of the assay

To demonstrate the applicability of the assay method, plasma samples from a Phase I clinical trial were analysed. This clinical trial was performed at Guy's Hospital (London, U.K.). Single night-time doses of 20 mg of mepirzepine were administered orally to male and female healthy volunteers. Serial blood samples were taken at regular intervals up to 96 h after dosing. The blood samples were centrifuged to prepare plasma, and the plasma samples were stored at −20°C until required for analysis.

RESULTS AND DISCUSSION

No interferences by endogenous plasma components were observed at the retention times expected for mepirzepine and its I.S., as shown in Fig. 2, which gives typical capillary gas chromatograms of individual, processed blank plasma,

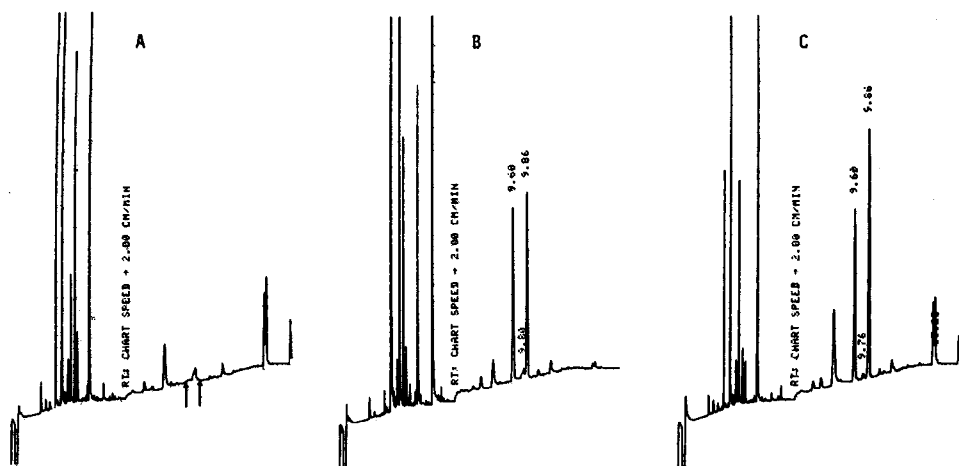

Fig. 2. Chromatograms of processed samples. (A) Drug-free plasma; the expected retention times of mepirzepine and internal standard are indicated with arrows. (B) Drug-free plasma, spiked with 20 ng of mepirzepine and 20 ng of internal standard per ml of plasma; retention time of mepirzepine = 9.60 min; retention time of internal standard = 9.86 min. (C) Clinical plasma, spiked with 20 ng of internal standard per ml of plasma; amount of drug administered = 20 mg; time after administration = 15 h. (Note: after 9 min the chart speed was changed from 0.5 cm min^{-1} to 2.0 cm min^{-1}.)

spiked plasma and a clinical plasma sample. It was assessed separately that the metabolites of mepirzepine did not interfere with the quantitative determination of mepirzepine.

Extraction experiments with [^{3}H]mepirzepine, when added to drug-free plasma, showed that 74.7% ($n = 7$; S.D. = 2.1%) of the spiked amount ended up in the methanolic solution, from which aliquots were subjected to capillary GC. The observed loss of 25.3% of mepirzepine during sample pretreatment includes extraction as well as manipulation (e.g. transfer of organic phase, reconstitution) losses.

The peak-height ratio of equal concentrations of mepirzepine and I.S. for unprocessed standards equals the slope of the calibration curve for processed plasma samples. Therefore, it can be concluded that the extraction recovery of

TABLE I

QUALITY CONTROL OF THE ASSAY

Mepirzepine level (ng ml^{-1})	Accuracy (%)	Precision (%)	n
0.5	−9.9	5.1	5
1	−13.3	7.4	5
2	9.1	2.9	5
5	4.6	2.6	10
10	4.9	3.0	10
50	9.8	1.7	10
100	7.1	2.7	10

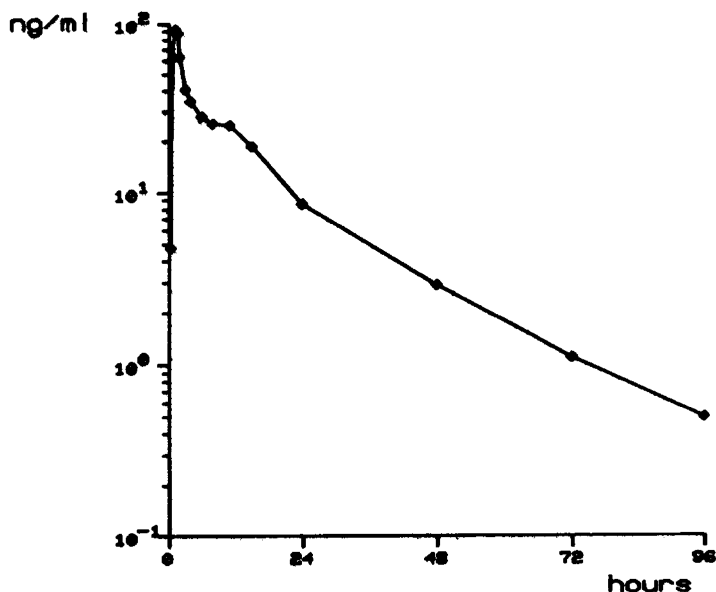

Fig. 3. Time course of mepirzepine plasma levels after oral administration of 20 mg of mepirzepine.

the I.S. is equal to that of mepirzepine. This demonstrates that the isomer of mepirzepine used as the I.S. is well suited.

The accuracy and precision of the assay were determined at 0.5, 1, 2, 5, 10, 50 and 100 ng of mepirzepine per ml of plasma by replicate analysis. These data are summarized in Table I. The accuracy and precision do not exceed 10%, except around the limit of detection.

The lower limit of detection is 0.5 ng of mepirzepine per ml of plasma with a signal-to-noise ratio of approximately 10.

An example of the time-course of mepirzepine plasma levels after oral administration of mepirzepine is given in Fig. 3. Mepirzepine plasma levels can be monitored at least up to 96 h after a single oral administration of 20 mg of mepirzepine.

In summary, a capillary GC assay with nitrogen-sensitive detection for the determination of mepirzepine in human plasma has been developed and validated. The assay can be applied for routine monitoring of mepirzepine in clinical plasma samples.

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