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Note

Determination of quinupramin in plasma and urine by capillary column gas chromatography–mass spectrometry

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Quinupramin, (3-quinuclidinyl)-5-dihydro-10,11(5H)-dibenzo(*b,f*)azepine (LM 208), is a therapeutic agent without pharmacologically active metabolites, used in the treatment of depression [1,2]. Owing to the intensity of its effects, this drug is administered orally in small doses, varying between 2.5 and 15 mg per day, depending on the treatment needs. At these doses, the circulating plasma levels are such that a very sensitive and specific assay is required. Previous trials to produce a bioassay of quinupramin by high-performance liquid chromatography (HPLC) with fluorescence detection [3] did not provide adequate sensitivity for the measurement of plasma drug levels observed in pharmacokinetic studies. The procedure described here provides an assay that combines gas chromatography and mass spectrometry (GC–MS), the sensitivity of which (0.5 ng of quinupramin per millilitre of biological fluid), enables plasma concentrations to be monitored over 48 h, after intravenous (2.5 mg) or oral (7.5 mg) administration of a single dose of the drug [4]. Urine samples are analysed in the same way.

EXPERIMENTAL

Standard and reagents

Quinupramin and the internal standard (IBF 28145) were purchased from Pharmuka Labs. (Gennevilliers, France). Sodium hydroxide (Prolabo, Paris, France), hydrochloric acid and doubly distilled water were used in the preparation of 1.0 *M* sodium hydroxide solution and 0.2 *M* hydrochloric acid, respectively. Ethyl acetate and isoamyl alcohol (Fluka, Interchim, France) of nanograde

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quality were used without further purification. Methanol was purchased from Carlo Erba (Milan, Italy).

Stock solutions

A 1.0 mg/ml quinupramin stock solution in methanol was prepared and dilutions of the stock solution in methanol were made to provide quinupramin solutions with concentrations of 500, 400, 300, 200, 100, 50, 20, 10 and 5 ng/ml. The internal standard solution was prepared by dissolving 10 mg in 100 ml of methanol to give a final concentration of 1 μg/ml. All solutions were stored at $-20°C$; no decomposition of the compounds was observed over 2 months.

Extraction procedure

In a 20-ml screw-capped tube, 1 ml of plasma (or urine) was supplemented with 150 μl of 1.0 *M* sodium hydroxide solution, 40 ng of internal standard (40 μl of 1 ng/μl in methanol solution) and 7 ml of *n*-hexane–isoamyl alcohol (99:1, v/v). The tube was shaken mechanically for 10 min and centrifuged for 10 min at 2500 *g*. In a 10-ml screw-capped tube, 6 ml of the organic phase were added to 1 ml of 0.2 *M* hydrochloric acid. After shaking for 10 min and centrifuging for 5 min at 2500 *g*, the organic phase was carefully discarded using a Pasteur pipette. The aqueous phase was made alkaline with 110 μl of 2.0 *M* sodium hydroxide solution and extracted with 7 ml of *n*-hexane. The mixture was shaken and centrifuged. The upper organic layer, transferred into another 10-ml screw-capped tube, was evaporated to dryness under a gentle stream of nitrogen at 30°C. The residue was dissolved in 30 μl of methanol and a 3-μl aliquot was injected into the chromatograph.

The retention times of the internal standard and quinupramin were 3.70 and 4.07 min, respectively.

Apparatus

Samples were analysed by mass fragmentography using a Hewlett-Packard HP 5985 hyperbolic quadrupole mass spectrometer with an HP 5840 gas chromatograph fitted with a solid injector (Ros model, Hewlett-Packard, Orsay, France). The fused-silica capillary column (12.5 m$\times$0.23 mm I.D.) was wall-coated with OV-1 methylsilicone (Hewlett-Packard) liquid stationary phase. The injection port temperature was set at 320°C and samples were injected at an initial oven temperature of 220°C. The temperature was programmed at the rate of 6°C/min up to 270°C. Helium was used as the carrier gas with an inlet pressure of 0.77 bar. The operating conditions of the mass spectrometer were as follows: separator temperature, 280°C; ion source temperature, 200°C; electron energy, 70 eV (electron-impact mode); and emission current, 300 μA.

Preparation of the calibration graph

A plasma calibration graph was constructed each day of the assay by spiking drug-free human plasma samples with known amounts of quinupramin ranging from 0 to 50 ng/ml.

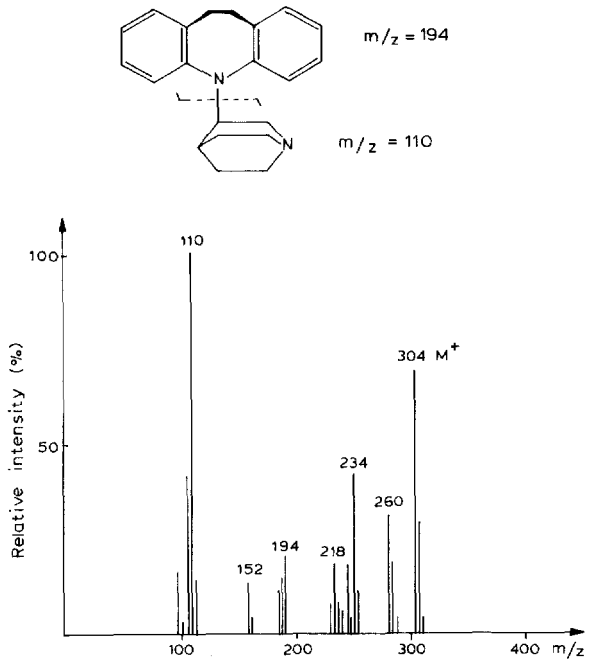

Fig. 1. Electron-impact mass spectrum and chemical structure of underivatized quinupramin.

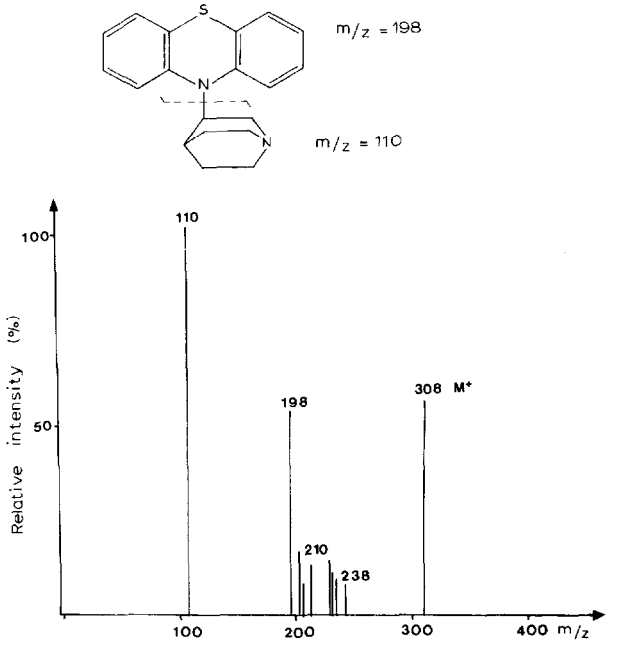

Fig. 2. Electron-impact mass spectrum and chemical structure of underivatized internal standard (IBF 28145).

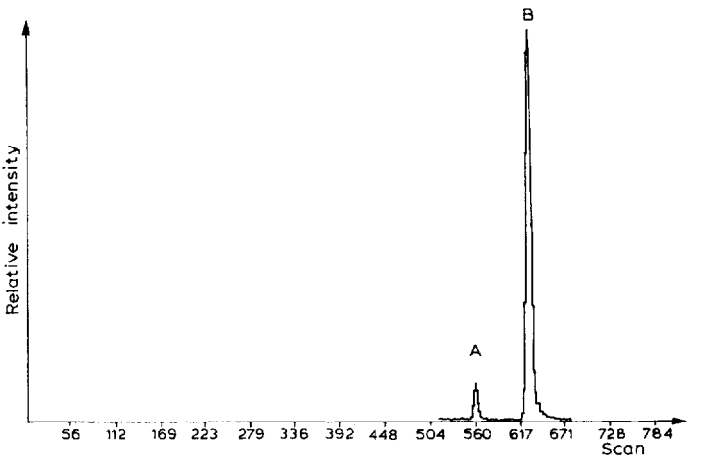

Fig. 3. Total ion current of a sample spiked with (A) quinupramin (1 ng/ml) and (B) internal standard.

Quinupramin in human plasma

The procedure was used first to study the pharmacokinetics of quinupramin following the administration of 2.5 and 3×2.5 mg single oral doses and a 2.5 mg intravenous dose, and second to evaluate the bioequivalence of the drug, in eight healthy male volunteers, following multiple-dose administrations of 7.5 mg of quinupramin in tablet form (every 24 h for 15 days) compared with 2.5 mg reference tablets applied every 6 h for 15 days. The two cross-over phases were separated by a 4-week wash-out period.

RESULTS AND DISCUSSION

Mass spectrometric analysis

Figs. 1 and 2 show the electron-impact mass spectra of underivatized quinupramin and the internal standard, respectively. A base peak appears at m/z 110 for the internal standard and quinupramin, which results from fragmentation by cleavage of the quinuclidinyl side-chain. Because of possible interference from endogenous material, this fragment was not suitable for ion monitoring so the molecular ions m/z 304 (quinupramin) and m/z 308 (internal standard), which

TABLE I

REPRODUCIBILITY ASSAYS PERFORMED WITH BLANK PLASMA SAMPLES ($n=5$) SPIKED WITH 0.5 AND 5 ng/ml OF QUINUPRAMIN

Theoretical concentration (ng/ml)	Mean measured concentration (ng/ml)	Standard deviation (ng/ml)	Coefficient of variation (%)	Mean error (%)
0.5	0.55	0.08	14.1	10.0
5.0	4.79	0.34	7.1	4.2

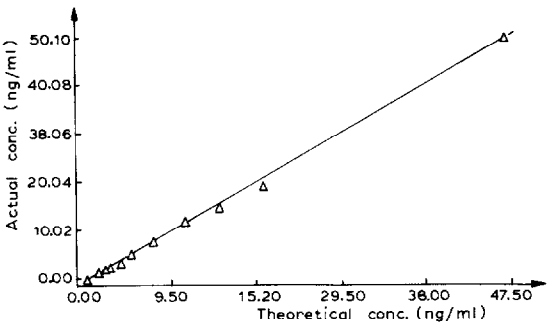

Fig. 4. Plot of theoretical concentrations of quinupramin in ten spiked plasma samples versus the measured concentrations. Linear regression analysis gives the equation $y=1.0484mc-0.1265$ (y = actual concentration; mc = theoretical concentration). The correlation coefficient was 0.996 for nine degrees of freedom.

are less intense but more specific, are preferred for quantification of the drug in biological specimens.

Fig. 3 shows a chromatogram obtained with plasma containing 1 ng/ml of quinupramin and 40 ng/ml of internal standard using total ion current detection.

Quinupramin concentrations in unknown samples collected during the various pharmacokinetic studies were calculated from the least-squares regression line of the calibration graph obtained by plotting the peak-area ratio (m/z 304 for quinupramin to m/z 308 for the internal standard) versus quinupramin plasma concentrations. The calibration graphs were straight lines over the range of concentrations studied. The equation of the calibration graph was

$$y=0.0315\,Cp-0.0054$$

where y = peak-height ratio and Cp = concentration of quinupramin added (ng/ml). The correlation coefficient was 0.9989 for six degrees of freedom.

Reproducibility assays were performed on two series of human plasma samples containing 0.5 and 5.0 ng/ml of quinupramin (Table I). The coefficients of variation were 14.1% at the limit of detection (0.5 ng ml) and 7.1% at the 5 ng/ml

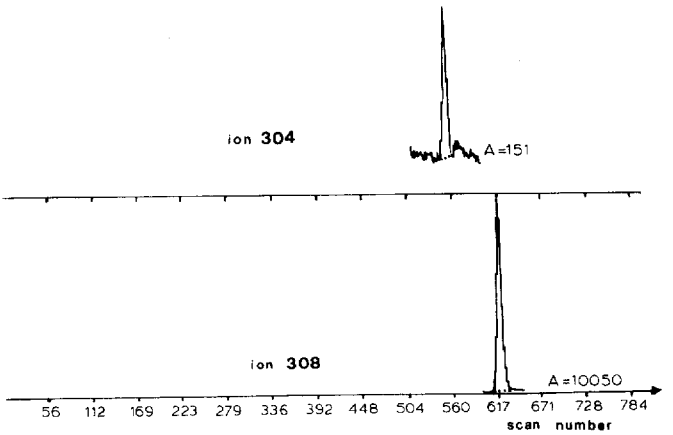

Fig. 5. Mass chromatogram obtained with a blank plasma sample spiked at the limit of detection (0.5 mg/ml of quinupramin).

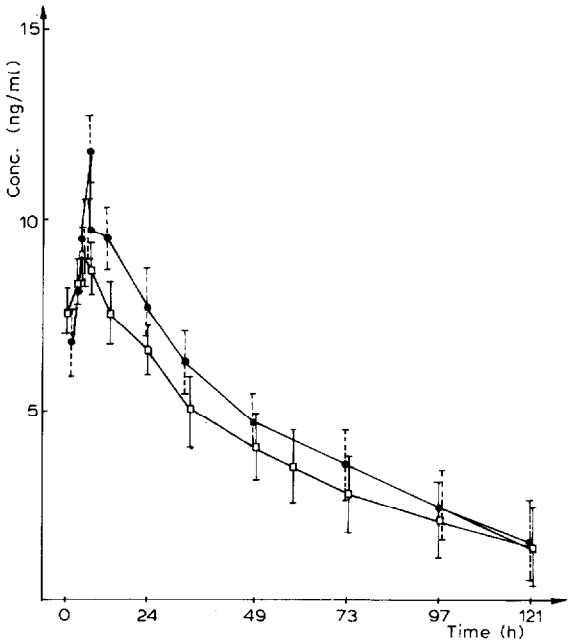

Fig. 6. Mean plasma levels (±S.E.M.) of quinupramin, following the last administration of (A) a 2.5-mg reference tablet every 6 h (□) and (B) a 7.5-mg tablet every 24 h (●) in eight healthy volunteers.

level. These values demonstrate the accuracy and precision of the method.

The reproducibility of the technique was calculated by statistical analysis of the slopes obtained from sixteen calibration graphs (0.5–50 ng/ml) prepared over a 2-month period. The result was 0.031 ± 0.001 (mean ± standard error of the mean, S.E.M.) and the coefficient of variation was 15.8%.

A plot of the measured concentration versus theoretical concentrations of ten quinupramin quality control samples analysed over the same period is shown in Fig. 4. Linear regression analysis gave a slope of 1.084 and an excellent correlation coefficient of 0.996 for nine degrees of freedom.

The limit of detection of the method is 0.5 ng/ml, with a signal-to-noise ratio of 4, when 1.0 ml of plasma was used (Fig. 5).

The procedure was applied to numerous plasma and urine samples in pharmacokinetic studies [4]. Fig. 6 shows the mean plasma concentration (±S.E.M.) of quinupramin over a 121-h period following the last administration of (A) a 2.5-mg treatment applied every 6 h and (B) a 7.5-mg treatment applied every 24 h to eight healthy volunteers for 15 days. The apparent elimination half-life was 42.2 ± 4 h (mean ± S.E.M.).

As can be seen, the technique allows the precise and reliable measurement of the drug at concentrations as low as 0.5 ng/ml in biological fluid.

In conclusion, the GC–MS method has been shown to be accurate and precise for the determination of quinupramin in the range of concentrations observed during pharmacokinetic studies [4,5].

ACKNOWLEDGEMENT

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