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Note

Determination of mequitazin in human plasma and urine by capillary column gas—liquid chromatography—mass spectrometry

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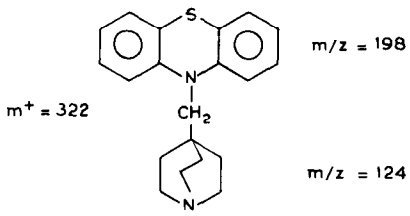
Mequitazin, 10-(3-quinuclidinylmethyl)phenothiazine (LM-209), is a new phenothiazine derivative which has been described as a potent H_1 antagonist with few or no sedative side-effects [1]. Related to the intensity of its pharmacological effects, mequitazin is administered orally at low dosages, varying between 5 and 10 mg per tablet. At these doses, the circulating plasma levels are such that a very sensitive and specific assay is required. Although the determination of radioactivity in rat and dog after oral or intravenous administration of ^{35}S-labelled mequitazin has been described [2], no chromatographic method has been published previously.



This work describes an assay using combined gas—liquid chromatography and mass spectrometry, the sensitivity of which (0.5 ng of mequitazin per ml of plasma) enables plasma concentrations to be monitored for over 72 h after the oral administration of a 10-mg single dose of mequitazin to healthy volunteers [3]. This assay was employed for a chronopharmacological study of mequitazin [4].

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EXPERIMENTAL

Reagents

LM-209 (mequitazin) and IBF-28145 (internal standard) were from Pharmaka Labs., Gennevilliers, France. Sodium hydroxide (Prolabo, Paris, France), hydrochloric acid and twice-distilled water were used in the preparation of 0.2 *M* hydrochloric acid and 2.0 *M* sodium hydroxide solutions. Diethyl ether (Merck, Interchim, Montluçon, France) and ethyl acetate (Fluka, Interchim, Montluçon, France) nanograde quality were used without further purification. Methanol and sodium chloride were from Prolabo.

Apparatus

Samples were analysed by mass fragmentography using a Hewlett Packard 5985 mass spectrometer equipped with a Hewlett Packard 5840 gas—liquid chromatograph, fitted with a solid injector (Ros model). The fused-silica capillary column (25 m × 0.23 mm I.D.) was wall-coated with the liquid phase CP Sil 5. The injection port temperature was set at 320°C and samples were injected at an initial oven temperature of 210°C. The temperature was programmed at a rate of 10°C/min up to 310°C. Helium was used as carrier gas at an inlet pressure of 0.9 kg/cm^2, which gave a constant flow-rate of 1.2 ml/min through the capillary column. The falling needle of the solid injector was cleaned every day to prevent drug adsorption. The operating conditions of the mass spectrometer were: separator temperature 280°C, source temperature 200°C, ionization energy 70 eV (electron-impact mode) and trap current 300 μA.

Extraction procedure

In a 20-ml screw-capped tube, 2 ml of plasma or 1 ml of urine were supplemented with 1 ml of 2.0 *M* sodium hydroxide saturated with sodium chloride, 5 ng of internal standard (50 μl of 100 ng/ml IBF 28145 in methanol solution) and 7 ml of diethyl ether. The mixture was shaken mechanically for 10 min and centrifuged for 10 min at 3000 *g* and 0°C. In a 10-ml screw-capped tube, 6 ml of the organic phase were added to 1 ml of 0.2 *M* hydrochloric acid saturated with sodium chloride. After shaking for 10 min and centrifuging for 5 min, the organic phase was carefully discarded using a Pasteur pipette. The aqueous phase was made alkaline with 0.8 ml of 2.0 *M* sodium hydroxide and extracted with 6 ml of diethyl ether. The mixture was shaken and centrifuged. The upper organic layer, transferred to a new 10-ml screw-capped tube, was evaporated to dryness under a gentle stream of nitrogen at 30°C. The residue was dissolved in 20 μl of methanol and an aliquot (2 μl) was injected into the chromatograph.

The retention times of internal standard (IBF 28145) and mequitazin were 5.0 and 5.75 min, respectively.

Mass spectrometric analysis

Figs. 1 and 2 show the electron-impact mass spectra of underivatized mequitazin and IBF 28145, respectively. A base peak appeared at *m/z* 124 and *m/z* 110, respectively, which resulted from fragmentation by cleavage of the side-

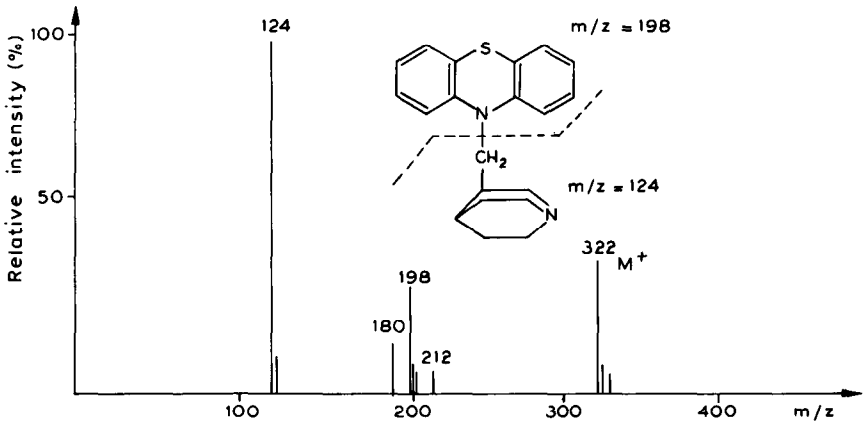

Fig. 1. Typical electron-impact spectrum and chemical structure of underivatized mequitazin.

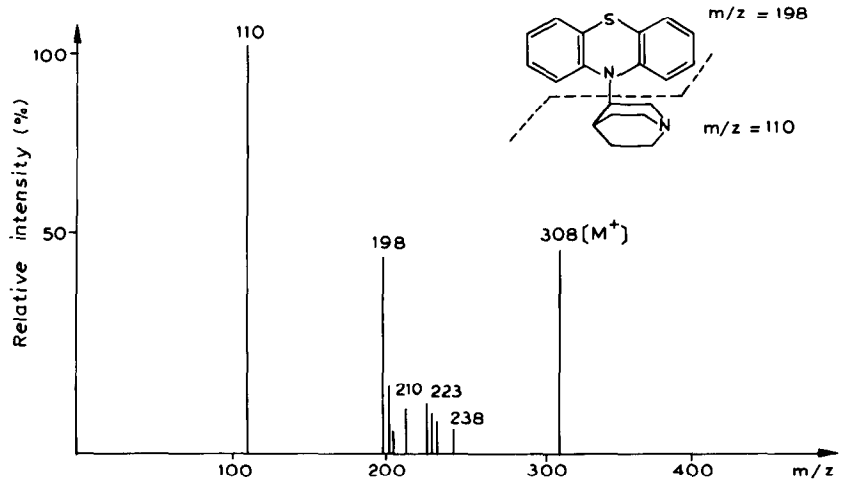

Fig. 2. Typical electron-impact spectrum and chemical structure of underivatized IBF 28145 (internal standard).

chain methylquinuclidinyl or quinuclidinyl. Because of possible interference from endogenous material, these ions were not chosen for quantification in biological samples; the molecular ions (*m/z* 322 and *m/z* 308) were used.

A standard curve (0, 0.5, 1, 2, 5, 10, 20, 40, 60, 80 and 100 ng of mequitazin per ml of plasma) was constructed from the simple linear relationship between the ion intensity ratios and the concentrations of mequitazin and internal standard.

The regression line of the data corresponding to the experimental points was drawn through the origin.

Mequitazin in human plasma

Eight healthy human subjects (six men and two women) were each given a single 5-mg oral dose of mequitazin (one tablet of 5.0 mg). Blood was collected in heparinized Vacutainer tubes at intervals during the 12 h following the dose. Plasma was separated by centrifugation and frozen at −20°C until assayed.

RESULTS AND DISCUSSION

Fig. 3 shows a chromatogram obtained with plasma containing 5 ng/ml internal standard and 15 ng/ml mequitazin using total ion current detection. Blank plasma or urine samples gave no interfering peaks on the chromatogram. A calibration curve prepared from a range of plasma levels (0, 5, 10, 20, 40, 60, 80 and 100 ng of mequitazin per ml of pooled plasma) indicated that intensity ratios of ions *m/z* 322 and 308 were linear when plotted against the concentrations of mequitazin. The lower limit of sensitivity was 0.5 ng of mequitazin per ml of plasma or urine.

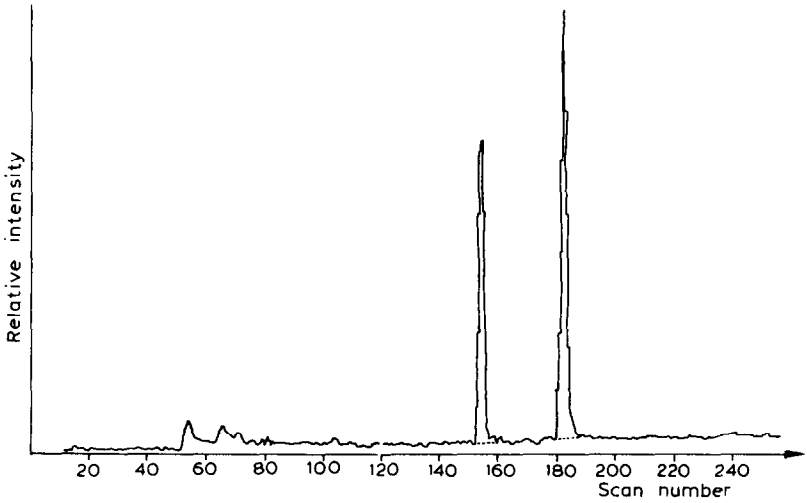

Fig. 3. Total ion current of a sample spiked with IBF 28145 (internal standard) and mequitazin.

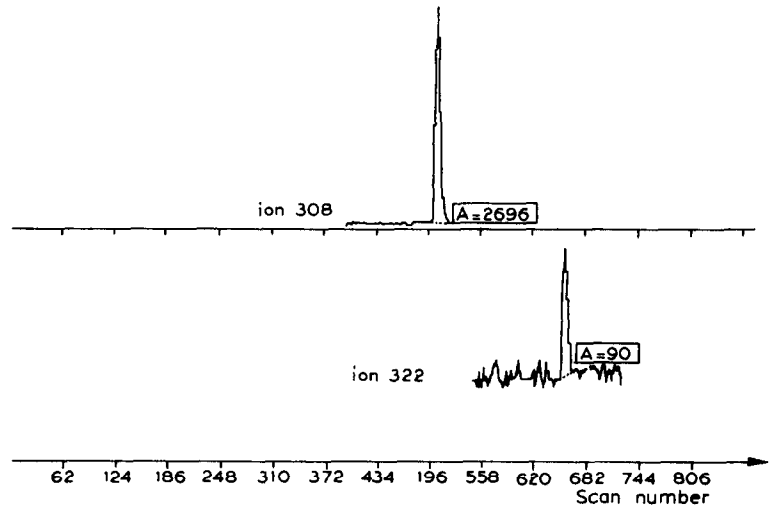

Fig. 4. Peaks obtained by mass fragmentography with plasma containing 5.0 ng/ml IBF 28145 (amplification 2800, *m/z* 308) and 0.5 ng/ml mequitazin (amplification 2800, *m/z* 322).

Fig. 4 shows a run obtained with a human plasma sample when 0.5 ng of mequitazin was added to 1.0 ml of plasma. This concentration gave for *m/z* 322 a peak area of 90 with an amplification value equal to 2800. The upper limit of linearity was at least 100 ng/ml.

The coefficient of correlation of the linear calibration was 0.9991 and the equation is of the type $Y = aX + b$, namely $Y = 0.1039X + 0.0124$.

Repeatability intra-day assays were performed on two pools of human plasma samples containing 1.0 and 10.0 ng/ml mequitazin (Tables I and II). The accuracy of the technique is good: the mean ratios of the peak areas (mequitazin/internal standard) were 0.034 ± 0.003 (± S.E.M.) and 0.693 ± 0.027 for the low- and high-concentration plasma samples, respectively. This mass fragmentographic method is presumed to be specific for the intact compound in urine and plasma.

The procedure was applied to numerous plasma and urine samples in pharmacokinetic studies. Fig. 5 shows the plasma concentration versus time run of mequitazin over a 252-h period, following multiple doses of 5 mg administered at 12-h intervals to healthy volunteers.

Fig. 6 represents a mass fragmentogram from a pharmacokinetic study corresponding to the plasma concentrations shown in Fig. 5.

TABLE I

REPEATABILITY ASSAY WITH MEQUITAZIN PLASMA LEVEL OF 1.0 ng/ml

Sample No.	Peak area ratio mequitazin/internal standard
1	0.038
2	0.032
3	0.027
4	0.033
5	0.046
6	0.039
7	0.024
8	0.030
Mean ± S.E.M.	0.034 ± 0.003

TABLE II

REPEATABILITY ASSAY WITH MEQUITAZIN PLASMA LEVEL OF 10.0 ng/ml

Sample No.	Peak area ratio mequitazin/internal standard
1	0.99
2	0.82
3	0.92
4	1.09
5	0.98
6	0.97
7	0.94
8	0.99
Mean ± S.E.M.	0.963 ± 0.027

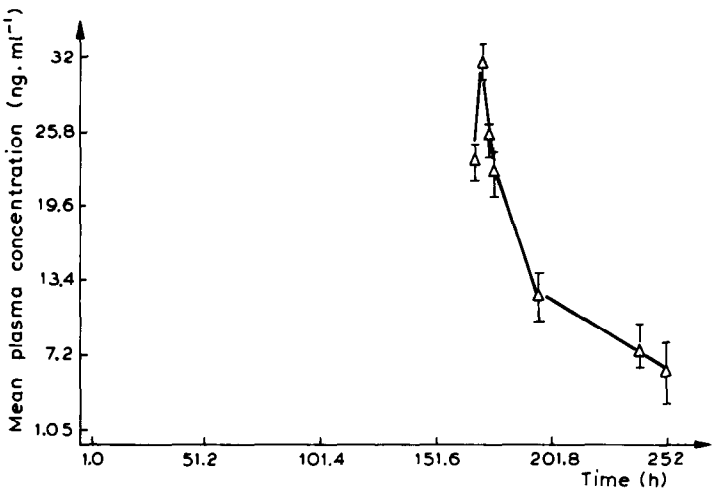

Fig. 5. Plasma concentrations of mequitazin, after multiple doses of 5 mg, every 12 h, over 252 h, to healthy volunteers. Each point represents the mean ± S.E.M. from six subjects.

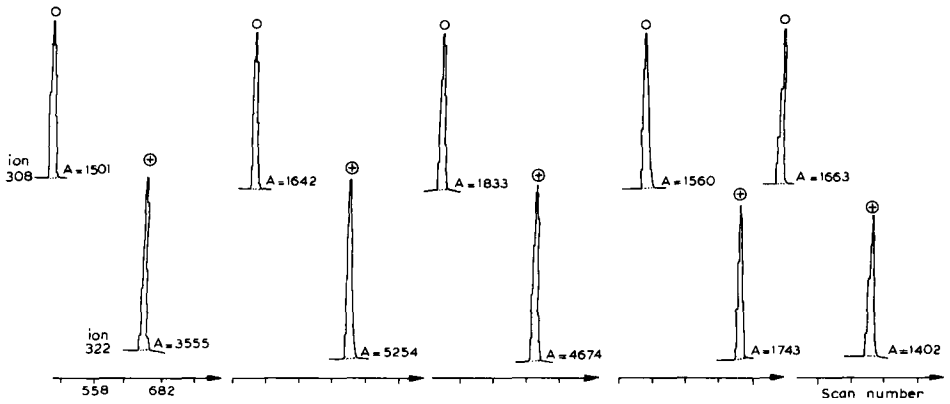

Fig. 6. Chromatograms obtained by mass fragmentography with plasma containing 5 ng/ml IBF 28145 (○) (amplification 2600, m/z = 308) and 23.5, 32, 26, 11.5 or 8.7 ng/ml mequitazin (⊕) (amplification 2600, m/z = 322).

Plasma mequitazin levels were determined in six healthy human volunteers over 32 h following a single 5-mg oral dose, and over 252 h after multiple oral doses of mequitazin. The terminal half-life was 48.4 ± 11.8 h (mean ± S.E.M.).

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