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Note

One-step extraction procedure for gas chromatographic determination of viloxazine as its acetyl derivative in human plasma

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Viloxazine hydrochloride (ICI-58834) is a morpholine derivative utilized in clinical practice as an antidepressant agent.

Whole blood [1—4] and plasma [5—7] levels of the drug in single-dose studies and in chronic administration have been investigated: concentrations of viloxazine fell after a single therapeutic dose to below 2 μg/ml, while repeated administrations gave plasma levels which largely exceeded this value.

Quantitative determination of the drug has been performed by gas chromatography (GC) with electron-capture detection of the heptafluorobutyryl derivative [8], or flame-ionization detection of its acetyl derivative [5]. ^{14}C-Labelling [3] or high-performance liquid chromatography with fluorescence detection [6] have also been employed for measuring blood viloxazine concentrations. The gas chromatographic methods, even if sensitive and selective, involve several steps before instrumental analysis (extraction, purification, derivatization), so they are time-consuming.

We have developed a simple and rapid GC procedure which allows determination of viloxazine in plasma after a single extraction step and on-column derivatization with acetic anhydride. The internal standard employed, *p*-tolylpiperazine, is derivatized simultaneously with the viloxazine in the chromatographic column.

EXPERIMENTAL

Reagents and standards

Viloxazine · HCl was supplied by ICI Pharma (Milan, Italy), and *p*-tolylpiperazine · 2HCl by the Department of Pharmaceutical Chemistry of Pavia University (Pavia, Italy); benzene, and methylene chloride, both

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pesticide grade, ethanol, acetic anhydride and borate buffer (pH 9.0) were purchased from Carlo Erba (Milan, Italy).

Apparatus and chromatographic conditions

A Carlo Erba Fractovap 2150 gas chromatograph equipped with a nitrogen–phosphorus detector was used. Viloxazine and *p*-tolylpiperazine were separated as their acetyl derivatives in a glass column (2 m × 2 mm I.D.) packed with 3% OV-17 on 100—120 mesh Gas-Chrom P silanized (Carlo Erba). Operating temperatures were: injection port 275°C, detector 275°C, column 242°C. The flow-rates were: nitrogen (carrier gas) 30 ml/min, hydrogen 35 ml/min, air 350 ml/min.

Extraction procedure

p-Tolylpiperazine (internal standard) was added to 1 ml of plasma (from citrated blood) in a centrifuge tube together with 2 ml of borate buffer (pH 9.0) and 6 ml of a mixture of benzene and methylene chloride (9:1). The tube was capped, shaken on a Vortex mixer for 3 min, then centrifuged at 1000 *g* and the supernatant transferred into a conical tube, in which it was concentrated at 50°C in a stream of dry nitrogen to a volume of about 20 μl for the determination of plasma levels of viloxazine in single-dose studies, or about 200 μl for the quantitative determination of the drug after chronic administration. Aliquots (1—3 μl) of these solutions were sampled using a 10-μl syringe previously loaded with 1 μl of acetic anhydride—ethanol (20:80) and injected on to the GC column.

Preparation of calibration curves

Two calibration curves were prepared by adding known amounts of viloxazine · HCl to 1 ml of blank plasma. Concentrations of the drug equivalent to 0.1, 0.5, 1, 1.5 and 2 μg/ml, and 1, 3, 5, 7 and 9 μg/ml as base, were measured, respectively.

An aliquot (100 μl) of internal standard aqueous solution was added to each spiked plasma. The internal standard concentrations were 0.75 μg/ml for the range 0.1—2 μg/ml viloxazine and 2 μg/ml for the range 1—9 μg/ml.

Clinical studies during chronic viloxazine administration

Five depressed patients were examined. Each subject received daily a 500-mg oral amount of viloxazine · HCl in divided doses (200 mg at 7 a.m., 200 mg at noon, 100 mg at 4 p.m.) for 26 days. Blood samples (10 ml), collected in polypropylene tubes containing about 300 mg of sodium citrate, were drawn at 3 p.m. on days 6, 11, 17, 21 and 26. The specimens were centrifuged and the plasma submitted to the GC procedure.

RESULTS AND DISCUSSION

The equations describing the standard curves, determined by linear least-squares regression analysis, were $y = 0.761x - 0.008$ and $y = 0.208x - 0.022$ for the ranges 0.1—2 and 1—9 μg/ml, respectively. The corresponding correlation coefficients (r) were 0.992 and 0.996. Table I shows the precision and accuracy of viloxazine determination in plasma.

TABLE I

ACCURACY AND PRECISON OF METHOD

First calibration curve*			Second calibration curve*		
Amount added (μg/ml)	Amount found (μg/ml, mean ± S.D.)	C.V. (%)	Amount added (μg/ml)	Amount found (μg/ml, mean ± S.D.)	C.V. (%)
0.1	0.11 ± 0.01	3.7	1	1.06 ± 0.07	6.6
0.5	0.51 ± 0.05	9.9	3	2.97 ± 0.10	3.4
1	0.98 ± 0.05	5.6	5	5.01 ± 0.25	5.0
1.5	1.51 ± 0.15	9.7	7	6.80 ± 0.32	4.7
2	2.00 ± 0.08	4.0	9	9.09 ± 0.30	3.3

*Five determinations for each point.

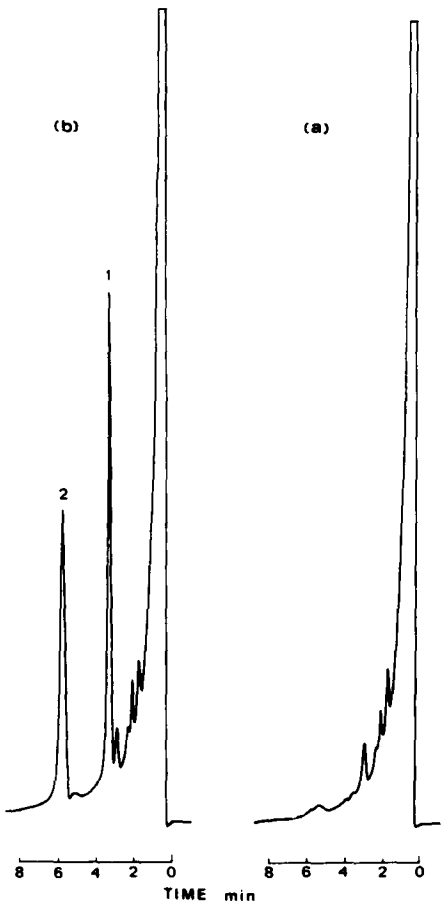

Fig. 1. Chromatograms obtained from a human plasma sample: (a) blank; (b) 2.5 h after oral administration of 50 mg of viloxazine · HCl. Peaks: 1 = internal standard (0.75 μg/ml), 2 = viloxazine (0.82 μg/ml).

The procedure employed gives a detection limit equivalent to 0.1 μg of viloxazine in 1 ml of plasma. Above this concentration, the plasma constituents do not interfere significantly with the internal standard and viloxazine, which were eluted as their acetyl derivatives after 3 min 27 sec and 5 min 47 sec, respectively. Typical chromatograms obtained from human plasma before and after a single oral administration of 50 mg of viloxazine are shown in Fig. 1. The mean (± S.D.) plasma levels found in the five depressed patients undergoing viloxazine therapy were 4.18 (± 1.41), 4.66 (± 2.72), 5.84 (± 2.43), 5.10 (± 1.33) and 5.00 (± 1.10) μg/ml after 6, 11, 17, 21 and 26 days of treatment, respectively.

Derivatization of viloxazine is required to shift the peak of the drug to a region of the chromatogram free from endogenous, interfering compounds. Thus, quantitative determination can be achieved after a single extraction step. Plasma concentration of viloxazine can also be evaluated without derivatization, although purification of the extract is required before GC analysis. In this case, another internal standard (chlorpheniramine) must be employed. The retention time of underivatized viloxazine in the chromatographic system described above (but with the oven temperature at 220° C) was 3 min 27 sec.

Derivatization before injection is not necessary. The acetylation of viloxazine and internal standard in the chromatographic column occurs readily and the speed of analysis is markedly increased. Injection of acetic anhydride causes a negligible influence on the response of the detector and a slight tailing of the solvent peak.

None of the other antidepressant drugs (amitriptyline, nortriptyline, imipramine, norimipramine, chlorimipramine, maprotiline, protriptyline and mianserin), checked using the same derivatization method, interfered with viloxazine and internal standard.

In conclusion, the proposed GC procedure for the quantitative determination of viloxazine in human plasma offers appreciable accuracy, precision and rapidity. The method is easily applicable to the monitoring of plasma concentrations of the drug during chronic treatment and is also sufficiently sensitive for its potential use in pharmacokinetic and bioavailability studies after a single administration.

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