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Note

Sensitive one-step extraction procedure for high-performance liquid chromatographic determination of viloxazine in human plasma

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Viloxazine hydrochloride (ICI-58834) is a morpholine derivative used in clinical practice as a non-sedative antidepressant agent with betamimetic properties [1]. Bioavailability studies of single doses of conventional or sustained-release viloxazine require high sensitivity and specificity. In addition, drug monitoring is useful for routine analysis in order to detect quickly the unresponsive patients, owing to the great intra- and inter-individual pharmacokinetic parameter differences.

Quantitative determination of the drug has been performed by gas chromatography (GC), with electron-capture detection of the heptafluorobutyryl derivative [2,3] or flame-ionization or nitrogen–phosphorus detection of its acetic anhydride derivative [4–6]. ^{14}C-Labelling [7] and high-performance liquid chromatography (HPLC) with fluorescence or UV detection [8,9] have also been employed for determination of blood viloxazine concentrations. The published GC methods, even if sensitive and selective, are time-consuming (extractions, purification, derivatization). Moreover, they often use benzene as the extraction solvent. The previous HPLC procedures either require a two-step extraction with fluorescence detection or offer only moderate accuracy and precision.

We have developped a quick, much less expensive semimicro HPLC procedure, without complex purification, which allows determination of viloxazine

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in plasma after a single extraction step, using disopyramide as an internal standard. The method is adequate for routine drug monitoring: an example from a recent pharmacokinetic study is described.

EXPERIMENTAL

Reagents and standard

Viloxazine·HCl was supplied by ICI Pharma (Cergy, France) and disopyramide·HCl by Roussel-Uclaf (Romainville, France). Triethylamine, *n*-hexane (both HPLC grade) and phosphate buffer (pH 2.5) were purchased from Merck (Darmstadt, F.R.G.). Acetonitrile (HPLC grade) was supplied by Baker (Deventer, The Netherlands). Analytical-reagent grade hydrochloric and orthophosphoric acids and sodium hydroxide were obtained from Prolabo (Paris, France).

Apparatus and chromatographic conditions

The chromatographic system consisted of a Waters Assoc. Model F 6000 A pump, connected to a Wisp 712 automatic injector (Waters Assoc.), a Varian 2050 UV spectrophotometric detector set at 220 nm (0.025 a.u.f.s.) and a Shimadzu C-R5A integrator (Touzard and Matignon, Vitry, France).

Separation was achieved at room temperature, using a reversed-phase C_{18} Micropak SP column (15 cm × 4.0 mm I.D.; particle size 5 μm) purchased from Varian (Les Ulis, France) preceded by a guard column (4 cm × 4.6 mm I.D.) packed with the same material.

The eluent was a degassed mixture of acetonitrile–0.1 *M* disodium hydrogenphosphate buffer adjusted to pH 2.5 with orthophosphoric acid–triethylamine (75:25:0.015, v/v). The flow-rate was set at 1.0 ml/min.

Extraction procedure

In a 10-ml centrifuge tube, 1 ml of plasma (from heparinized blood) alkalinized with 200 μl of 4 *M* sodium hydroxide was extracted by *n*-hexane (8 ml) after addition of disopyramide as an internal standard. The tube was capped, shaken horizontally for 15 min, then centrifuged at 3000 *g* for 10 min. A 300-μl volume of 0.01 *M* hydrochloric acid was added to the supernatant (6 ml) previously transferred in another tube, then vortexed for 1 min and centrifuged if necessary. The upper organic layer was discarded, and 100 μl of the acidic solution were injected into the HPLC system.

Preparation of the calibration curve

A standard curve was constructed for each assay by adding known amounts of viloxazine·HCl to 1 ml of drug-free human plasma. As the therapeutic range is wide, a single large calibration curve is employed for routine analysis. Con-

centrations of the drug equivalent to 100, 200, 400, 800, 1200, 1600 and 2000 ng/ml as base were measured.

An aliquot (150 μl) of internal standard aqueous solution (12.5 μg/ml) was added to each spiked plasma.

If the concentration found exceeds 2000 ng/ml, the plasma sample must be diluted before extraction with drug-free human plasma.

RESULTS AND DISCUSSION

Quantification, separation and plasma interference

The equation describing the standard curve, determined by linear least-squares regression analysis was y (peak-area ratio) $= 0.001215x$ (amount of viloxazine injected, ng) $+ 0.0132$. The corresponding correlation coefficient (r) was 0.9999.

Chromatograms obtained from blank plasma, from plasma spiked with viloxazine·HCl and its internal standard and from a sample obtained from a patient (following administration of viloxazine) are shown in Fig. 1. The retention times were ca. 4.0 and 7.2 min for viloxazine and disopyramide, re-

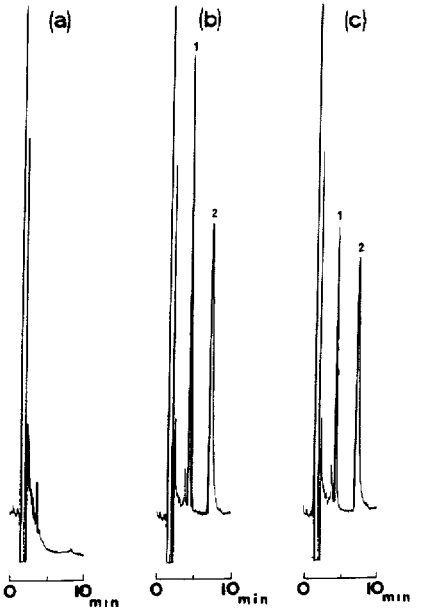

Fig. 1. Chromatograms obtained from (a) a blank human plasma sample, (b) a spiked human plasma and the internal standard and (c) at steady-state after chronic oral administration of 300 mg daily of viloxazine·HCl. Peaks: 1 = viloxazine (540 ng/ml); 2 = internal standard (12.5 ng/ml).

spectively. These two peaks were well resolved without any interference from endogenous compounds.

No interfering drug was detected after extraction and injection into the HPLC system of an ICL serum (Therapeutic Drug Control II) containing 34 drugs except, of course, for disopyramide.

Linearity, accuracy, recovery and sensitivity

Calibration curves obtained after extraction from plasma were linear up to 5000 ng/ml with a good correlation ($r=0.998$, $n=4$). Nevertheless, for a better precision in usual analysis, the calibration curve was limited to 2000 ng/ml.

Accuracy, precision and day-to-day reproducibility are shown in Table I. Accuracy, precision, intra-assay reproducibility and absolute mean recovery are shown in Table II.

The choice of detection wavelength (220 nm) was suggested by the remark-

TABLE I

ACCURACY, PRECISION AND DAY-TO-DAY REPRODUCIBILITY

Day-to-day assays were performed over a six-month period using spiked human plasma samples ($n=6$).

Viloxazine added (ng/ml)	Viloxazine found (ng/ml)	Coefficient of variation (%)
100	113	6.4
200	227	10.8
400	399	9.1
800	806	2.8
1200	1196	1.8
1600	1580	1.8
2000	1998	5.2

TABLE II

ACCURACY, PRECISION, INTRA-ASSAY REPRODUCIBILITY AND ABSOLUTE RECOVERY

Spiked human plasma samples were used ($n=10$).

Viloxazine added (ng/ml)	Viloxazine found (ng/ml)	Coefficient of variation (%)	Absolute recovery (mean ± S.D.) (%)
200	198	3.5	71.9 ± 5.0
1200	1186	2.7	68.0 ± 4.0
2000	1970	2.0	72.2 ± 1.8

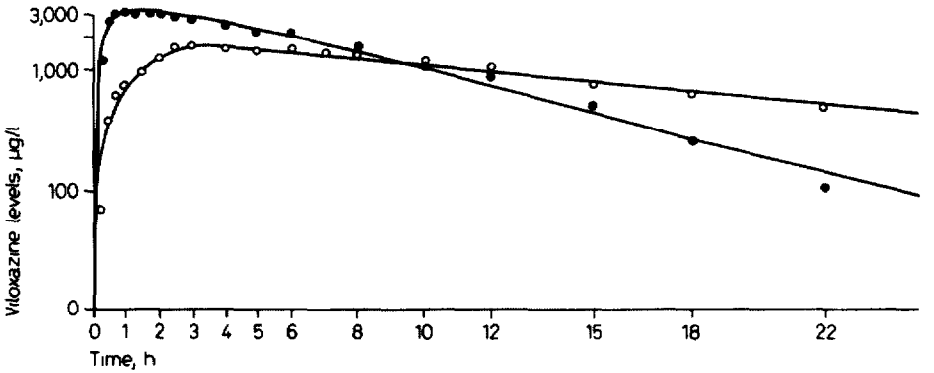

Fig. 2. Mean plasma viloxazine concentrations after administration of 300 mg of conventional (●) or sustained-release (○) viloxazine.

able increase of sensitivity compared with that obtained with other wavelengths as previously reported.

The lower limit of detection (at a signal-to-noise ratio of 3) was 20 ng/ml. The practical sensitivity with good accuracy was 40 ng/ml during routine analysis.

Stability

Viloxazine was stable in human plasma standard controls (500 ng/ml) for up to one month at −22°C. Spiked plasma samples extracted as usual and stored at +4°C remain stable for at least 24 h.

Applications

In our hands, fifty samples could be processed in a routine working day, and the method has been used to analyse several thousands of samples over the past three years. If the column is carefully cleaned with water and the frits are periodically changed, it may be used for more than one thousand plasma determinations.

As an example, the mean plasma concentrations of viloxazine observed in eleven healthy volunteers, after administration of 300 mg of conventional or sustained-release viloxazine, are reported in Fig. 2 [10].

CONCLUSION

The proposed HPLC procedure for the quantitative determination of viloxazine in human plasma offers rapidity, good accuracy and precision. The method is sensitive enough for clinical pharmacology studies and for drug monitoring. As this procedure is adapted from several HPLC determinations of tricyclic compounds in blood, the same system with a few modifications (mobile phase,

wavelength) allows drug monitoring of numerous tri- or tetracyclic antidepressants and their active metabolites. Finally, this method may be useful for disopyramide determination in plasma.

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