High-Pressure Liquid Chromatographic Determination of Viloxazine in Human Plasma and Urine

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Abstract □ A rapid, specific, high-pressure liquid chromatographic method is presented for the determination of viloxazine in plasma and urine. This method employs the high sensitivity of fluorescence detection with selective extraction and reversed-phase chromatography to measure concentrations as low as 25 ng/ml of plasma and 1.0 μg/ml of urine. Known metabolites of viloxazine do not interfere with the analysis, and experience with several hundred samples in a bioavailability study demonstrated the applicability and reliability of the method.

Keyphrases □ Viloxazine—high-pressure liquid chromatographic analysis, human plasma and urine □ High-pressure liquid chromatography—analysis, viloxazine, human plasma and urine □ Antidepressants—viloxazine, high-pressure liquid chromatographic analysis, human plasma and urine

Viloxazine hydrochloride [2-(2-ethoxyphenoxymethyl)tetrahydro-1,4-oxazine hydrochloride, ICI 58,834] is a psychotropic drug with antidepressant activity; its clinical pharmacology was summarized by Bayliss and Duncan (1). It was reported that viloxazine is absorbed completely and metabolized extensively following oral administration to human volunteers (2).

Previous procedures for the determination of viloxazine in plasma and urine employed GLC with electron-capture detection (3) following extraction and conversion to the heptafluorobutyrate or labeling with carbon 14 in combination with TLC (2). Although the GLC methods can be sufficiently specific and sensitive for pharmacokinetic and bioavailability studies, they are relatively complex and require a demanding prechromatographic derivatization.

The procedure reported here combines a selective two-step extraction with the separative capability of high-pressure liquid chromatography (HPLC) and the sensitivity of fluorescence detection. The method is rapid, is easily automated, employs an internal standard, separates the drug metabolites, and is of sufficient sensitivity (25-ng/ml plasma detection limit) for pharmacokinetic studies.

Table I—Retention Times for Viloxazine, Dextrorphan, and Viloxazine Metabolites

Compound	Code Number[a]	Retention Time, min	Retention Time Relative to Viloxazine, min
Viloxazine	58834	8.2	1.00
Dextrorphan	—	6.7	0.81
o-Hydroxyviloxazine	60169	3.0	0.36
2-(2-Ethoxyphenoxymethyl)-2,3,5,6-tetrahydro-5-oxo-1,4-oxazine	69322	7.4	0.90
n-Methylviloxazine	70098	16.0	1.95
m-Hydroxyviloxazine	71928	2.6	0.31
p-Hydroxyviloxazine	71976	3.0	0.36

[a] Taken from Ref. 2.

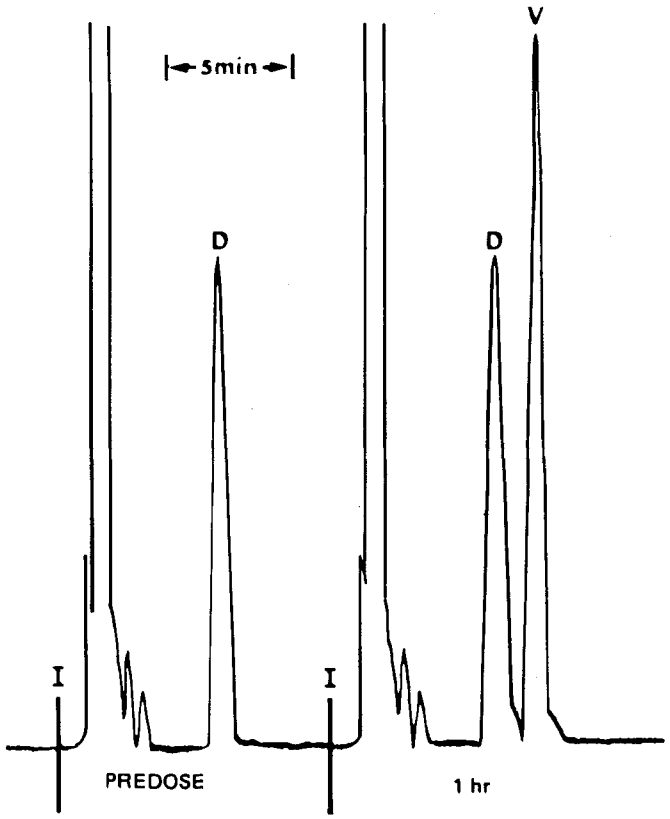

Figure 1—*High-pressure liquid chromatograms (excitation at 220 nm, no filter) of extracts before dosing and 1 hr after a 50-mg dose. Key: I, injection; D, dextrorphan; and V, viloxazine.*

EXPERIMENTAL

Materials—*n*-Butanol[1], *n*-butyl chloride[1], acetonitrile[1], triethylamine[2], dibasic sodium phosphate[3], phosphoric acid[4], and acetic acid[4] were used as received. Water was deionized and then distilled. Dextrorphan[5] (3-hydroxy-*N*-methylmorphinan), viloxazine[6], and the metabolites[6] of viloxazine were used as received.

[1] UV grade, Burdick & Jackson Laboratories, Muskegon, Mich.
[2] Reagent grade, Aldrich Chemical Co., Milwaukee, Wis.
[3] Reagent grade, Fisher Scientific Co., Fair Lawn, N.J.
[4] Reagent grade, Mallinckrodt, Paris, Ky.
[5] Hoffmann–La Roche Laboratories, Nutley, N.J.
[6] ICI Americas, Wilmington, Del.

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Table II—Accuracy and Precision

	Concentration, ng/ml									
	50.0	100.0	150.0	200.0	250.0	300.0	400.0	600.0	800.0	1000.0
n	9	27	10	24	11	20	30	27	23	16
Mean	54.7	100.9	153.3	193.2	247.9	297.2	391.8	587.1	804.5	1026.0
SD	5.1	5.5	8.8	8.2	16.0	15.3	17.9	27.4	35.4	73.4
CV, %	9.3	5.4	5.7	4.2	6.4	5.1	4.5	4.7	4.4	7.1

Apparatus—The HPLC system consisted of an automatic injector[7], a high-pressure pump[8], a bonded-phase column[9], and a fluorescence detector[10].

Chromatographic Parameters—Although several mobile phases were evaluated, the system containing 17.5% acetonitrile, 6 m*M* triethylamine, and 0.20 *M* phosphate buffer at pH 3.2 was optimal in this laboratory. The phosphate buffer was filtered through a 0.45-μm filter prior to mixing, as were all other reagent solutions. A flow rate of 1.3 ml/min at ambient temperature (usually 22°) was employed in most studies. Aliquots of 50–100 μl of the back-extraction were injected directly onto the column.

Analytical Procedure—*Plasma*—A 0.50-ml aliquot of plasma was pipetted into a 15-ml centrifuge tube and adjusted to pH 10.4 by adding 50 μl of saturated sodium carbonate solution. Eight milliliters of 10% *n*-butanol in butyl chloride containing 25 ng of dextrorphan/ml was added, and the capped tube was vortexed 1 min and then centrifuged. The upper organic phase was transferred to a second 15-ml centrifuge tube containing 300 μl of 1.0% acetic acid. The second tube was vortexed and centrifuged, and the upper organic layer was discarded. The acetic acid solution was injected into the HPLC system.

Urine—Twenty microliters of well-mixed urine was diluted with 0.5 ml of 0.139 *M* dibasic sodium phosphate buffer (pH 10.5) in a 15-ml centrifuge tube. This diluted aliquot was subjected to the same procedure as the plasma samples.

RESULTS AND DISCUSSION

Table I lists the retention times of viloxazine, dextrorphan, and the known unconjugated metabolites of viloxazine. All of the tested compounds were resolved from one another and the normal components of plasma and urine. The 8.3-min retention time for viloxazine allows a convenient processing of five or six samples per hour. Figure 1 shows chromatograms for two samples, one obtained prior to dosing and the other obtained 1 hr after a 50-mg oral dose. Although the metabolites do chromatograph and are detectable when injected directly onto the column, they do not extract efficiently and are not detected in plasma and urine.

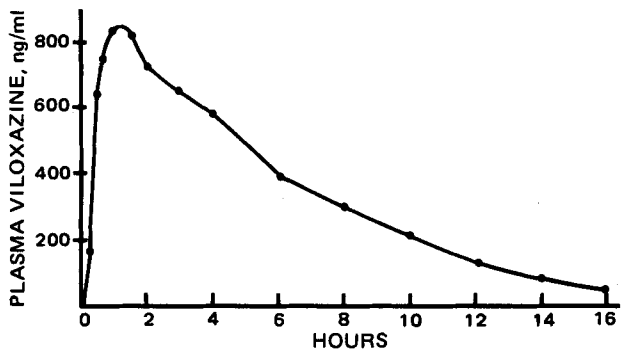

Figure 2—*Viloxazine concentration* versus *time curve in a human subject following a single 50-mg dose.*

The peak height ratio of viloxazine to dextrorphan varies linearly with the concentration of viloxazine added to pooled plasma. A typical standard curve obtained by assaying samples with 0, 50, 100, 150, 200, 250, 300, 400, 600, 800, and 1000 ng/ml had a linear regression coefficient of 0.999, a slope of 515.8 ng/ml, and an intercept of 11.6 ng/ml, where the peak height ratio was the abscissa and the viloxazine concentration was the ordinate. During the analysis of several hundred samples over 4 months, the slope and intercept values of frequent standard curves remained within ±20% of these stated values.

The precision and accuracy of the method are demonstrated in Table II. The analysis of 10 aliquots of a 50-ng/ml standard, assayed in batch, had a coefficient of variation of 9.7%, while a similarly processed 300-ng/ml standard gave a 6.9% coefficient of variation. When processing large numbers of samples, 25 ng/ml in plasma and 1 μg/ml in urine are practical lower limits of detection.

During the analysis of 576 plasma samples in duplicate from 12 healthy volunteers in a bioavailability study, no chromatographic peaks were observed to interfere with either viloxazine or dextrorphan. Figure 2 presents the plasma viloxazine concentration–time curve for one typical subject following a single 50-mg dose. The method clearly is sufficiently sensitive to describe the plasma curve in this dose range. Urine samples (252) from the same study also were assayed in duplicate, and no interfering chromatographic peaks were observed. The findings of the bioavailability study will be presented in a subsequent report.

As with most chromatographic procedures, the exact mobile phase composition required and the limits of detection will vary with the particular column and matrix of the sample. In this laboratory, the described method works well with several similar columns and for samples obtained from healthy volunteers. However, modifications may be required to achieve optimal separations in diseased patients, especially those receiving multidrug therapy. When the same column was used, the day-to-day change in the slope of the standard curve was <±1%; however, larger changes of up to 5% were observed when a different column was installed. Fortunately, properly maintained columns will allow several hundred determinations.

[7] WISP 710A, Waters Associates, Milford, Mass.
[8] Model 6000A, Waters Associates, Milford, Mass.
[9] Altex Scientific Inc., Berkeley, Calif.
[10] SPF 970, Schoeffel, Westwood, N.J.

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(3) D. E. Case, *J. Pharm. Pharmacol.*, **25**, 800 (1973).