High-Performance Liquid Chromatographic Determination of Ethmozin in Plasma

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Abstract □ A sensitive, specific high-performance liquid chromatographic procedure was developed for the determination of plasma ethmozin levels. Basic plasma samples were partitioned with methylene chloride. The organic extract was washed with water and then evaporated to dryness under reduced pressure. The residue was redissolved in 0.2 ml of the mobile phase, consisting of hexane–tetrahydrofuran–methanol–water (66:27:6.3:0.7 v/v), and then chromatographed on a microporous silica column. With a variable-wavelength UV detector set at 268 nm, 10 ng of ethmozin/ml of plasma was measured. The utility of the method for human pharmacokinetic studies was demonstrated.

Keyphrases □ Ethmozin—analysis, high-performance liquid chromatography, plasma □ High-performance liquid chromatography—analysis, plasma ethmozin levels □ Pharmacokinetics—plasma ethmozin levels, high-performance liquid chromatography, humans

Ethmozin, 10-(3-morpholinopropionyl)phenothiazine-2-carbamic acid ethyl ester hydrochloride, is an antiarrhythmic drug that suppresses atrial and ventricular premature depolarizations (1–4).

The reported assays for ethmozin are a nonspecific spectrophotometric method (5) and a TLC–spectrophotometry method (6). Both are unsatisfactory for pharmacokinetic studies because they lack either specificity or sensitivity.

This report describes a plasma assay for ethmozin that involves a single solvent extraction, a water wash, and an evaporation step. The assay is sufficiently sensitive to measure the compound at the 10-ng/ml level.

EXPERIMENTAL

Reagents—Ethmozin[1], borate buffer[2] (0.1 *M*, pH 9), tetrahydrofuran[3], methanol[3], hexane[3], and methylene chloride[3] were used without further purification.

Apparatus—The liquid chromatograph[4] was fitted with a valve-loop injector[5] with a 50-μl loop, a variable-wavelength UV detector[6] set at 268 nm, an electronic filter[7], and a microporous silica column[8]. The mobile phase flow rate was ~1 ml/min.

Mobile Phase—A solution containing hexane–tetrahydrofuran–methanol–water (66:27:6.3:0.7 v/v) was prepared as required.

Standard Curves—Standard curves were prepared from extracted, ethmozin-spiked plasma samples. Solutions of ethmozin in methanol (0.25, 0.50, and 1.0 μg/ml) were used to spike plasma samples for the preparation of the extracted plasma standards. Spiked plasma samples were prepared by pipetting 0.5 ml of each ethmozin solution into 50-ml, polytef-lined[9], screw-capped glass tubes[10]. The methanolic solutions were evaporated to dryness[11], and then 5.0 ml of control plasma was added to each tube. Control plasma from whole blood drawn in heparinized, evacuated tubes[12] was used because plasma from blood banks often contained an impurity that interfered with the assay.

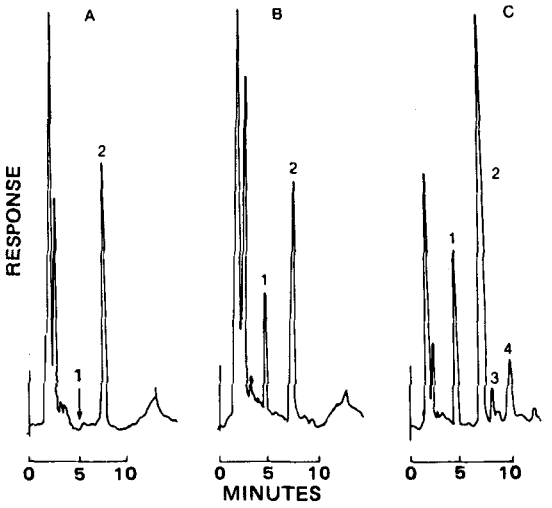

Figure 1—*Chromatograms of extracts from a plasma blank (A), spiked (0.10 μg/ml) plasma (B), and plasma from an individual taking ethmozin (C). Key: 1, ethmozin; 2, caffeine; and 3 and 4, ethmozin metabolites.*

The spiked plasma samples were adjusted to pH 9 using a few drops of 1 *N* NaOH and then were diluted with 5 ml of 0.1 *M* borate buffer (pH 9). Following the addition of methylene chloride (12 ml) to each tube, the tubes were capped and then shaken on a wrist-action shaker[13] for 30 min. The mixture was centrifuged for 10 min at 4000×*g*, and the aqueous phase was discarded. The organic phase was washed with 5 ml of distilled water in a clean 25-ml glass tube. The mixture was centrifuged for 5 min, and then 8.0 ml of the methylene chloride layer was evaporated to dryness in a 15-ml graduated centrifuge tube. All of the residue was dissolved in 0.2 ml of the mobile phase. Aliquots (50 μl) were injected into the column and chromatographed.

A standard curve was prepared by plotting the ethmozin peak heights from the extracted plasma samples *versus* the plasma ethmozin concentrations.

Recovery Study—Plasma ethmozin recoveries were determined by comparing the peak heights of the spiked plasma standards with those of the unextracted ethmozin standards (Table I) after correcting for the fact that only 8 ml of the original 12 ml of methylene chloride was analyzed.

Quantitation—A standard curve was constructed by plotting the ethmozin peak height of spiked plasma standards *versus* the plasma ethmozin concentration. Caffeine and several ethmozin metabolites elute near ethmozin, hindering use of an internal standard. To eliminate the need for several internal standards or an internal standard with a long retention time, external standards were used. Unextracted standards were chromatographed daily to monitor instrument performance and to calculate percent recoveries of the extracted standards.

Unknown samples were analyzed by comparing ethmozin peak heights from the plasma extracts to the extracted standard curve.

Plasma Level Study—Ethmozin (500 mg) was administered orally with 500 ml of water to three healthy human males, 59–75 kg, who had fasted overnight. Blood samples (15 ml) were withdrawn prior to dosing

[1] E. I. du Pont de Nemours & Co., Wilmington, Del.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] Burdick & Jackson Laboratories, Muskegon, Mich.
[4] Model 830, E. I. du Pont de Nemours & Co., Wilmington, Del.
[5] Model SVOV-6-3, Glenco Scientific, Houston, Tex.
[6] Model 1030B, Hewlett-Packard, King of Prussia, Pa.
[7] Model 1020, Spectrum Scientific Corp., Newark, Del.
[8] Microporasil, 3.9 × 300 mm, Waters Associates, Milford, Mass.
[9] Teflon, E. I. du Pont de Nemours & Co., Wilmington, Del.
[10] Corex, Corning Glass Works, Corning, N.Y.
[11] Evapo-mix, Buchler Instruments, Fort Lee, N.J.
[12] Vacutainers, Becton–Dickinson and Co., Missonga, Ontario, Canada.
[13] Model 75, Burrell Corp., Pittsburgh, Pa.

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Table I—Linearity of Unextracted Ethmozin Standards

Ethmozin[a], μg/ml	Peak Height, mm	Mean Peak Height, mm (*CV*)	Response Factor[b]
0.5	19	19 (3.1)	38.0
	20		
	19		
1.0	36	36 (0)	36.0
	36		
	36		
2.0	69	71 (2.1)	35.5
	71		
	72		
4.0	136	136 (1.1)	34.0
	134		
	137		

[a] As ethmozin hydrochloride. [b] Peak height divided by concentration.

Table II—Recovery of Ethmozin from Spiked Plasma

Ethmozin, μg/ml	*n*	Recovery, % (*CV*)
0.025	11	100.8 (12.5)
0.050	13	99.7 (7.5)
0.100	17	102.8 (9.0)
0.500	6	96.9 (7.0)
1.000	6	91.4 (6.3)
5.000	5	89.7 (2.6)

and at specified times up to 48 hr after dosing. The blood samples were centrifuged, and the plasma was stored frozen in clean plastic tubes[14]. The samples were assayed using the procedure described for ethmozin-spiked plasma. The elimination half-lives for these individuals were determined using linear regression analysis of the terminal plasma level–time data points.

RESULTS AND DISCUSSION

Under the described chromatographic conditions, ethmozin eluted from the column in ~5 min. However, ethmozin is similar to other phenothiazines in that it undergoes extensive metabolic transformation when given to humans[15]. There are large interindividual differences in the number and concentration of these metabolites. For this reason, a column cleanout time between samples was required and external standards were used. Representative chromatograms from blank and spiked human plasma extracts and a plasma extract from a subject given 500 mg of ethmozin are shown in Fig. 1.

A variable-wavelength detector at 268 nm was used to eliminate background contributions to the ethmozin peak at 254 nm in blank plasma extracts. The mobile phase was not used for more than 1 week because tetrahydrofuran oxidizes, reducing chromatogram quality.

The linearity of unextracted ethmozin standard response is shown in Table I. Peak heights were proportional to the standard concentrations from 0.5 to 4 μg/ml, as evidenced by the fairly constant response factors (34.0–38.0).

Recoveries of ethmozin from spiked plasma samples were nearly quantitative at plasma concentrations of 0.025–5.0 μg/ml, with average recoveries ranging from 89.7 to 102.8% and coefficients of variation ranging from 2.6 to 12.5% (Table II).

Figure 2 illustrates the plasma ethmozin concentration profile in three healthy volunteers given single oral 500-mg doses. Peak concentrations

[14] Model 2017, Falcon, Oxnard, Calif.
[15] C. C. Whitney and J. C. Gaylord, unpublished data.

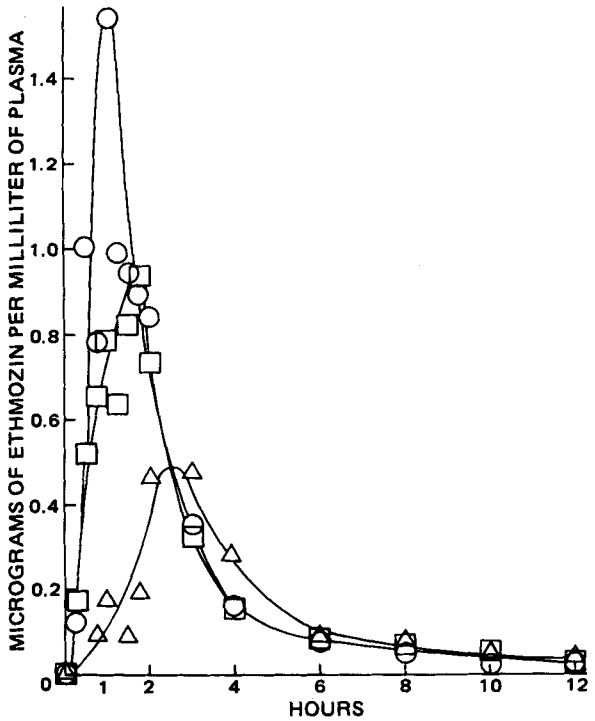

Figure 2—*Ethmozin profiles in three healthy adult human males following a single oral 500-mg dose.*

of 0.48–1.54 μg/ml occurred between 1 and 3 hr. The elimination half-lives were 2.8–4.3 hr.

Since ethmozin may be given concurrently with other drugs, several were examined to determine whether they would influence the ethmozin assay. No interference was observed with diazepam, hydralazine, prazosin, guanethidine, procainamide, quinidine, propranolol, chlorpromazine, fluphenazine, nicotine, caffeine, codeine, warfarin, phenytoin, cephalexin, aminopyrine, or perphenazine. Chlordiazepoxide, reserpine, trifluoperazine, and clonidine had approximately the same retention time as ethmozin.

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