Journal of Chromatography, 231(1982) 205—209
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1285

Note

Ion-exchange high-performance liquid chromatography in drug assay in biological fluids

I. Ethmozin

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(First received December 14th, 1981; revised manuscript received March 1st, 1982)

The reversed-phase sorbents that are most widely used in drug assay in biological fluids by high-performance liquid chromatography (HPLC) have some disadvantages because of their low selectivity. The retention on such sorbents depends mainly on the lipophilic properties of the substances to be analysed. On the other hand, biofluids, blood especially, contain considerable amounts of the various lipid substances, which are extracted, as a rule, with drugs and metabolites. When analysing such extracts on reversed-phase columns one often observes interference to the drug and/or metabolite peaks by endogenous substances. Ion-exchange sorbents are significantly more selective and practically do not retain non-ionogenic substances. This is why these sorbents seem to be highly specific for the assay of the drugs that form ions in solution. Many drugs exhibit such a property.

In this paper the assay method is presented for the new potent antiarrhythmic drug ethmozin (1, Fig. 1), which has been developed in the U.S.S.R. and is at present being studied in the U.S.S.R. and the U.S.A. [1—3]. The advantage of the cation-exchange column compared to the reversed-phase column is demonstrated. The method has a detection limit of about 0.01 μg/ml with a plasma sample volume of 1 ml, and is not time-consuming.

Recently two papers have been published [4, 5] which deal with the HPLC determination of some other phenothiazine drugs in plasma. Both methods use polar bonded-phase columns and require selective detectors: electrochemical detector [4] or fluorescence detector with a post-column photochemical reactor [5].

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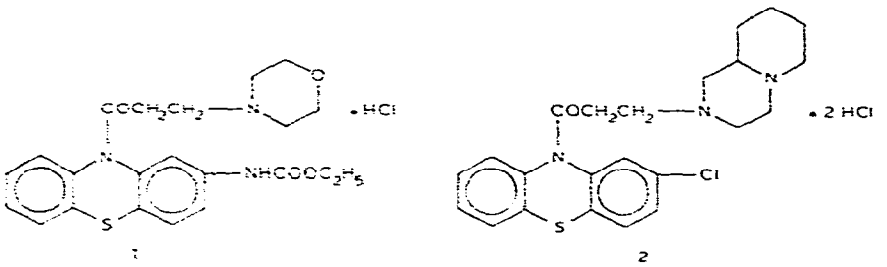

Fig. 1. Structural formulae of ethmozin (1) and nonachlazin (2).

EXPERIMENTAL

Apparatus and columns

The chromatograph used was Altex Model 322 with fixed-wavelength (254 nm) UV detector Altex Model 153 (Altex Scientific, Berkeley, CA, U.S.A.). The recorder was Linear Model 355 (Linear Instruments, Irvine, CA, U.S.A.). Chart speed was 12 cm/h, sensitivity was 10 mV/scale. The columns used were 250 × 3.2 mm packed with Partisil 10-SCX and LiChrosorb RP-18, both 10-μm particle size (Altex Scientific). The precolumns, 40 × 3.2 mm, were packed by us with the same sorbents. The columns were at ambient temperature.

Reagents and solvents

Acetonitrile, dichloromethane, isopropanol and *n*-heptane were analytical grade distilled in glass. Pure diethylamine was distilled twice over sodium hydroxide. The water was double-distilled in glass. Ethmozin and nonachlazin (internal standard) were obtained from the Institute of Pharmacology A.M.S. U.S.S.R. The stock solutions of drug and standard were prepared using double-distilled water and were stored at 4°C. They were stable for at least four months. Two concentrations were prepared: 0.02 and 0.2 μg/ml.

Extraction procedure

To 1 ml of plasma or 0.1 ml of urine in a 20-ml Pyrex tube was added 0.1 ml of standard solution (the concentration depends on the expected ethmozin level) followed by 1 ml of water and 0.1 ml of 0.1 *N* hydrochloric acid. The mixture was gently shaken for 20 min with 10 ml of dichloromethane—isopropanol (10:1). After centrifugation for 5—10 min at 500 *g* the lower layer was filtered into a conical tube and evaporated to dryness at 35°C under a gentle stream of air. To the residue 70 μl of acetonitrile were added followed, after brief mixing, by 70 μl of water. Then 0.5 ml of heptane was added to the tube which was shaken for several minutes to remove lipid particles. Then 100 μl of the lower layer were injected onto the column.

Quantitation

The internal standard method was used with nonachlazin (2, Fig. 1) as standard. For construction of the calibration curves the peak height ratios were plotted against the concentrations of ethmozin added to blank plasma and

urine. The recovery was estimated as the ratio of the ethmozin peak heights after analysis of the extracted drug and standard solution.

RESULTS AND DISCUSSION

A chromatogram of an extract of drug-free plasma on the reversed-phase column is presented in Fig. 2A. The conditions are given in the legend. Chromatogram B is the result of the analysis of the standard ethmozin solution (0.05 μg was injected) under the same conditions. It might be impossible to detect ethmozin in plasma at such a level as 0.1 μg/ml because of interference. Varying the conditions (mobile phase composition, column temperature) does not improve the separation. For the pharmacokinetic study of ethmozin the detection limit must be at least 0.02 μg/ml. Since interfering endogenous substances do not seem to be ion-generating in solution we tried to realize the separation on the cation exchanger Partisil 10-SCX. It has been found that ethmozin is retained on the column with such a sorbent. Drug-free plasma extract gives no peaks that could interfere with ethmozin (Fig. 2C); urine does not give such peaks either. This is why we chose Partisil 10-SCX for ethmozin determination. We tried several buffer systems as mobile phase but the ethmozin peak shape is quite satisfactory with diethylamine only. The organic modifier (acetonitrile) is necessary to shorten the retention time. The complete eluent composition is given in the legend to Fig. 2 (C).

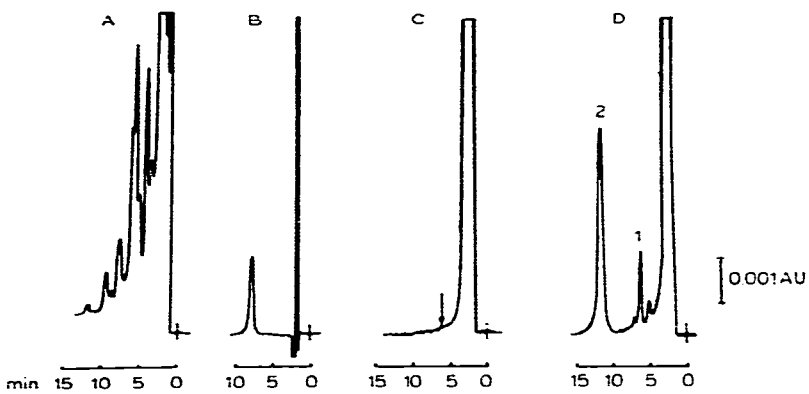

Fig. 2. (A) Chromatogram of a drug-free plasma extract on LiChrosorb RP-18. Eluent: acetonitrile—water—diethylamine (50:50:0.3). Flow-rate: 1 ml/min. Temperature: ambient. (B) Chromatogram of the ethmozin standard solution (sample size 0.05 μg) on LiChrosorb RP-18. Conditions are the same as in (A). (C) Chromatogram of a drug-free plasma extract on Partisil 10-SCX. Eluent: acetonitrile—water—diethylamine—acetic acid (27:73:0.18:0.18). Flow-rate: 1.5 ml/min. Temperature: ambient. The arrow marks the position of the ethmozin peak. (D) Chromatogram of an extract of plasma from a patient who received 300 mg of ethmozin in tablet form. Plasma sample was spiked with 0.5 μg of nonachlazin. Peaks: 1 = ethmozin; 2 = nonachlazin. Conditions are the same as in (C).

Nonachlazin (2, Fig. 1) was chosen as an internal standard since its absorbance spectrum is close to that of ethmozin and nonachlazin can therefore be detected at the same wavelength (254 nm). Its extraction

behaviour is also like ethmozin. The retention times of ethmozin and nonachlazin under the noted conditions are ca. 6 and 11 min, respectively. They depend on the mobile phase composition: the higher the acetonitrile and/or diethylamine—acetic acid content the shorter the rentention time of the drugs.

The calibration curves were plotted in the concentration range 0.02—5 μg/ml for plasma and 0.1—20 μg/ml for urine and were found to be linear (r = 0.97 and 0.98, respectively).

Table I gives data on the within-day and day-to-day accuracy of the ethmozin determination in plasma for four different concentrations. The accuracy of the ethmozin determination in urine was not greatly affected by concentration and was no more than 4% within-day and 5.5% day-to-day.

The ethmozin recovery from plasma was estimated at 0.05 and 2 μg/ml, and was equal to 73 ± 5% (n = 6) and 84 ± 7% (n = 7), respectively. The recovery from urine was not lower than 90%.

TABLE I

WITHIN-DAY AND DAY-TO-DAY ACCURACY OF ETHMOZIN DETERMINATION IN PLASMA

Concentration (μg/ml)	Accuracy (C.V. %, n = 5)	
	Within-day	Day-to-day
0.05	10.5	14.1
0.2	7.1	8.5
0.5	5.2	6.4
2.0	3.8	5.1

TABLE II

DRUGS WHICH DO NOT INTERFERE WITH ETHMOZIN DETERMINATION

Acetylsalicylic acid	Methoprolol
Amitriptyline	Methyldopa
Amobarbital	Mexiletine
Ampicillin	Nitrazepam
Caffeine	Nitroglycerine
Carbamazepine	Oxazepam
Chlorpropamide	Paracetamol
Diazepam	Phenylbutazone
Digoxin	Propranolol
Ephedrine	Quinidine
Furosemide	Sulfamethoxazole
Hydrochlorothiazide	Theophylline
Insulin	Verapamil
Isosorbide dinitrate	Warfarin
Lidocaine	

In Fig. 2D an example of the analysis of a human plasma extract after oral administration of 300 mg of ethmozin is presented. The concentration is found to be 0.087 μg/ml.

Thus the ion-exchange column had in this case obvious advantages over the reversed-phase column because the endogenous lipid substances from plasma, which were retained on the reversed-phase column, were practically not retained on Partisil 10-SCX.

The detection limit of the method is about 0.01 μg/ml and may be somewhat lower if the effluent is monitored at the ethmozin absorbance maximum (269 nm). Table II is a list of drugs which do not interfere in the determination of ethmozin by the method described. The extraction procedure is relatively simple. Up to 30 samples can be worked-up during a day. The residues after extract evaporation can be stored at 4°C for several days. The analysis time of one sample does not exceed 15 min. Provided the precolumn packing is changed regularly, the column can be used for at least two years without any noticeable change in its quality.

With a small variation in the ratio of the mobile phase components the method can be used for the determination of other phenothiazine drugs in biological fluids.

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