Journal of Chromatography, 490 (1989) 458–463
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4681

Note

Improved column liquid chromatographic method for the determination of moricizine in plasma or serum

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(First received October 31st, 1988; revised manuscript received January 16th, 1989)

Moricizine (Ethmozine®), a phenothiazine derivative (Fig. 1) with antiarrhythmic properties similar to those of lignocaine, was first developed in the U.S.S.R. and has been introduced into clinical trials in China as well as in the U.S.A. recently [1–6]. Few methods for its determination in biological fluids

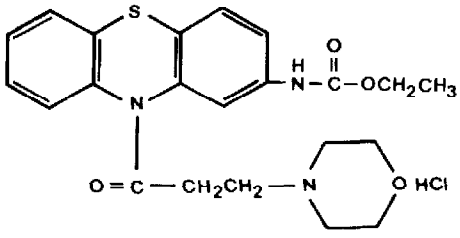

Fig. 1. Structure of moricizine hydrochloride.

0378-4347/89/$03.50

have yet been published. One of them described an ion-exchange high-performance liquid chromatographic (HPLC) assay [7] and other two dealt with a normal-phase [8] and an ion-pair partition [9] HPLC method, both using sodium hydroxide alkaline extraction. We found that moricizine was not stable under alkaline conditions. Based on our previous work [3], we now report an improved HPLC method involving a single ion-pair extraction of moricizine from plasma or serum. The assay requires a relatively short and simple sample preparation and a shorter analysis time.

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Waters 6000A pump, a U6K continuous-flow injector (Waters Assoc., Milford, MA, U.S.A.) and a variable-wavelength Hitachi 220-S UV detector with a chart recorder (Hitachi, Tokyo, Japan). Analyses were performed on a μBondapak reversed-phase C_{18} column (10 μm, 100 mm × 8 mm I.D.) housed in a Waters Z-module, linked to a C_{18} pre-column (30 μm, 75 mm × 4.6 mm I.D., Merck, Darmstadt, F.R.G.). Other apparatus included 10-ml centrifuge tubes with well fitting screw caps and 10-ml stoppered evaporation tubes with finely tapered bases (100 μl capacity). All glassware was cleaned by soaking it overnight in a 5% solution of Extran (Merck, Darmstadt, F.R.G.) in water, than rinsed thoroughly with methanol and hot tap water followed by distilled water. The tubes were subsequently silanized according to the previous procedure [10] in order to eliminate possible loss of drug owing to adsorption on the glass walls. A Hamilton syringe (25 μl) was used.

Materials and reagents

Moricizine hydrochloride was kindly supplied by Tianjing Pharmaceutical Industrial Institute (Tianjing, People's Republic of China) and clozapine, used as an internal standard, was obtained from Shanghai No. 19 Pharmaceutical Factory (Shanghai, People's Republic of China). The methanol was of HPLC grade from Mallinckrodt (Paris, KY, U.S.A.) and doubly distilled water was used. Pentanesulphonic acid (sodium salt) was purchased from Sigma (St. Louis, MO, U.S.A.). Diethyl ether and triethylamine from Merck were freshly distilled before use. Other reagents used were glacial acetic acid, ethanol and hydrochloric acid (AR grade).

Chromatographic conditions

The mobile phase was methanol–water–triethylamine (80:20:0.4, v/v) in which the final pH was adjusted to 6.5 with concentrated glacial acetic acid. The mobile phase was filtered before use using a Millipore vacuum filter sys-

tem equipped with a 0.22-μm filter (Waters Assoc.). Further degassing was found not necessary immediately after filtration.

Other operating conditions were: flow-rate, 1.8 ml/min; UV detector wavelength, 254 nm; temperature, ambient (25 ± 1 °C). The total run-time was ca. 7 min. The concentration of moricizine was calculated using the peak-height ratio of moricizine to the internal standard.

Standard solutions

Stock solutions of moricizine and clozapine were prepared by dissolving 10 mg of moricizine in 100 ml of distilled ethanol and 10 mg of clozapine in 100 ml of ethanol. When stored at 4 °C the stock solutions remained stable for more than two months. Daily working standard solutions of moricizine (20 μg/ml) and clozapine (40 μg/ml) were prepared by dilution of the stock solutions with ethanol.

Sample preparation

Into a 10-ml glass centrifuge tube, 20 μl of clozapine internal standard solution (800 ng) were added to the drug-containing plasma or serum (1 ml) for assay. Using a Pipetman® (Gilson, Villiers-le-Bel, France), 200 μl of 2.5 m*M* sodium pentanesulphonate were added and mixed briefly (3 s) using a Maxi-Mix® (Thermolyne, Dubuque, IA, U.S.A.). The sample was then extracted with diethyl ether (6 ml) by shaking vigorously for 2 min. After centrifugation for 5 min at 2500 *g* to break the emulsion, the organic extract was transferred to a 10-ml evaporation tube and evaporated to dryness at 45 °C in a water-bath. The residue was dissolved in 0.1 *M* hydrochloric acid or 0.1 *M* sulphuric acid (100 μl), and vortex-mixed for 30 s to facilitate dissolution of the sample. An aliquot (25 μl) was injected into the HPLC apparatus.

Quantitation

Calibration graphs were constructed by plotting the peak-height ratio of moricizine to the internal standard against the known concentrations of moricizine added to drug-free plasma to cover the range 0–5 μg/ml. The drug concentration was quantitated by relating the peak-height ratio to obtain the concentration from the calibration graph.

Recovery

To assess the assay recovery from plasma by the extraction procedure, the drugs were added to drug-free plasma (500 and 1000 ng/ml for moricizine and 800 ng for clozapine) and assayed as described. For comparison, the same concentrations of moricizine and clozapine were prepared in a diethyl ether solution, evaporated and assayed, but with the extraction step omitted; the assay recovery was computed.

RESULTS AND DISCUSSION

Performance of the HPLC system

The analytical peaks of moricizine and clozapine were well resolved with good symmetry (Fig. 2): the retention times were 3.6 and 5.6 min, respectively. No endogenous interference was observed.

Linearity, sensitivity and precision

Daily standardization curves for moricizine in plasma or serum resulted in a linear concentration–response relationship. Moricizine concentrations be-

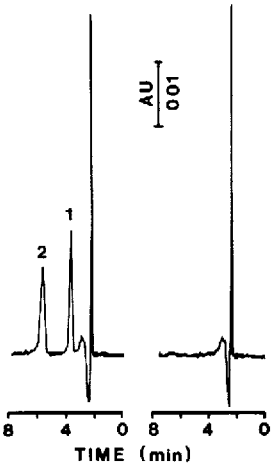

Fig. 2. Typical chromatograms of drug-free extract of plasma (right) and sample (left). Peaks: 1 = moricizine (1 μg/ml); 2 = clozapine (0.8 μg/ml).

TABLE I

CALIBRATION AND PRECISION OF THE HPLC ASSAY ($n = 6$)

Concentration (ng/ml)	Peak-height ratio (mean ± S.D.)	Coefficient of variation (%)
50	0.056 ± 0.005	8.9
100	0.104 ± 0.009	8.6
500	0.622 ± 0.033	5.3
1000	1.100 ± 0.018	1.6
1500	1.593 ± 0.053	3.3
2000	2.283 ± 0.140	6.1
3000	3.081 ± 0.056	1.8
5000	5.354 ± 0.073	1.4
Calibration graph	$y = 0.0011x - 0.0215$, $r = 0.9991$	

tween 50 and 5000 ng/ml were used for standardization and regression analysis. The mean correlation coefficient for these six standardization curves was 0.9991 (Table I).

The limit of determination of the assay has been evaluated as 5 ng (or 20 ng/ml), yielding a detector response approximately equal to three times the detector noise.

Precision data (Table I) were obtained by the repeated analysis of spiked plasma samples.

Recovery

The recovery of this extraction procedure with *n*-heptanesulphonic acid is high and constant for both moricizine (93.5% at 500 ng/ml and 96.9% at 1000 ng/ml) and clozapine (98.1% at 800 ng/ml).

Selectivity

To assess the selectivity of this method some commonly administered drugs were tested. The results are listed in Table II. Amidarone and caffeine were not resolved by the column under the conditions described for moricizine.

The improvements in this assay concern the extraction procedure and the mobile phase composition. We found in our previous work that the sample preparation procedure proposed by Pratt et al. [9] and Whitney et al. [8] was tedious, and moricizine was not stable under alkaline conditions. The pK_a of moricizine is 5.5 (our unpublished data). In view of the above factors we adopted the extraction with *n*-heptanesulphonic acid, which allows mild conditions (pH $\simeq$ 7) and is more suitable to avoid the breakdown of the drug. The presence of pentanesulphonic acid used in the extraction was essential because in its

TABLE II

RELATIVE RETENTION TIMES OF SOME DRUGS

Drug	Relative retention time
Ethmozine	0.612
Clozapine	1.00
Diazepam	0.622
Chlordiazepoxide	0.600
Methaqualone	0.48
Clonidine	0.34
Quinidine	0.52
Digoxin	0.35
Verapamil	0.52
Propranolol	0.43
Phenytoin	0.37
Aprindine	0.989

absence at pH 7, the recovery of both moricizine and the internal standard (clozapine) was low. The sample preparation is less labour-intensive than that used for the previously reported HPLC procedures [7–9], thus allowing for a sample through-put of up to 50 samples per day. We have been applying this method in our research work.

The mobile phase used in this study was also simpler than that previously reported [7–9]. With triethylamine in the mobile phase, the peak shapes of both moricizine and clozapine have been improved. The pH of the mobile phase has an apparent effect on the retention time of the drugs; with increasing pH the retention times of both drugs may be increased. The methanol/water ratio in the mobile phase may also influence the retention time, obeying the general principles of reversed-phase chromatography. The mobile phase can be recycled at least three times without causing any interference.

A determination limit of 20 ng/ml is sufficient to monitor plasma levels for three to five half-lives following an oral dose (300 mg) or intravenous bolus injection (100 mg). The sensitivity of the assay can be increased by using a larger sample size for injection (>25 μl) or by decreasing the volume of 0.1 *M* hydrochloric acid used for reconstitution (<100 μl).

In conclusion, an HPLC assay for moricizine determination in plasma or serum which is relatively simple, sensitive and selective and has a short run-time has been developed. Using the method outlined herein, the analysis of a standard curve and quality control, as well as many samples in the course of one day, is feasible, thus providing a useful research and clinical tool in the pharmacological studies of moricizine.

ACKNOWLEDGEMENTS

We are grateful to the Beijing–Hong Kong Academic Exchange Centre for supporting J.M. Yang's visit in Hong Kong. We thank Mrs. J. Ng for typing this manuscript.

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