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Short communication

Simultaneous determination of moricizine and its sulphoxidation metabolites in biological fluids by high-performance liquid chromatography

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Abstract

A simultaneous assay for moricizine, its two sulphoxidation metabolites, moricizine sulphoxide and moricizine sulphone, using high-performance liquid chromatography (HPLC) is described. The drug and metabolites and clozapine (internal standard) in biological fluids were extracted using pentanesulphonic acid into diethyl ether. The ethereal extract was evaporated to dryness and the residue was redissolved in the mobile phase (methanol–water–triethylamine, 65:35:0.5, v/v). The analyses were performed on a μBondapak reversed-phase C_{18} column housed in a Waters Z-module, linked to a C_{18} pre-column, with a run-time of 12 min. The retention times were 2.7, 3.5, 6.2 and 9.7 min for moricizine sulphone, moricizine sulphoxide, moricizine and clozapine, respectively. The recovery of the compounds from plasma ranged from 89.9% for the sulphoxide to 98.1% for clozapine. The limits of detection of the assay for moricizine, moricizine sulphoxide and moricizine sulphone were 20, 10 and 5 ng/ml, respectively.

1. Introduction

Moricizine hydrochloride (Ethmozine), a phenothiazine derivative with anti-arrhythmic activities similar to lignocaine, was originally developed in the USSR, tested in China [1–3] and recently approved by the US Food and Drug Administration for oral treatment of life threatening ventricular arrhythmias [4]. Several methods based on high-performance liquid chromatography (HPLC) have been published for the determination of moricizine in biological fluids. One described an ion-exchange assay [5], while the other two used a normal-phase [6] and an ion-pair partition [7] method, respectively. The latter two methods required preliminary extraction of the drug from the aqueous phase under alkaline extraction. We found that moricizine was unstable under alkaline conditions [3] and we subsequently modified the assay by incorporating an ion-pair extraction procedure using pentanesulphonic acid at the sample preparation step [8]. Recently we reported the characterisation of moricizine sulphoxide and moricizine sulphone as the sulphoxidation metabolites using

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a perfused rat liver preparation [9]. We now describe a HPLC procedure for the simultaneous determination of the parent drug and the two sulphoxidation metabolites in human and rat biological fluids.

2. Experimental

2.1. Materials and reagents

Moricizine hydrochloride was a gift from Tianjing Pharmaceutical Industrial Institute (Tianjing, China) and clozapine hydrochloride, used as the internal standard, was obtained from Shanghai No. 19 Pharmaceutical Factory (Shanghai, China). Sodium pentanesulphonate was purchased from Sigma (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from Mallinckrodt (Paris, KY, USA). Diethyl ether and triethylamine of Analar grade were purchased from Merck (Darmstadt, Germany). Double glass-distilled water was prepared in our laboratory and other reagents of Analar grade included ethanol and glacial acetic acid. The sulphoxide and sulphone of moricizine were prepared by Professor Yun-Feng Ren and Mr. Yi Zhang of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. These compounds were characterised in a separate study [9].

2.2. Apparatus

A Waters 6000A pump system linked to a U6K continuous-flow injector (Waters Assoc., Milford, MA, USA) and a variable-wavelength Hitachi 220-S UV detector with a chart recorder (Hitachi, Tokyo, Japan) were used. HPLC analyses were performed on a μBondapak reversed-phase C_{18} column (10 μm, 100×8 mm I.D.) housed in a Waters Z-module, linked to a C_{18} pre-column (30 μm, 25×4.6 mm I.D., Merck). Other apparatus used included: 10-ml centrifuge tubes with well fitting screw caps lined with PTFE liners, 10-ml stoppered evaporation tubes with finely tapered bases of 100-μl capacity, and a Hamilton glass syringe (25 μl). All glassware was cleaned by soaking in a 5% aqueous solution of Extran (Merck) overnight, then rinsed thoroughly with methanol and hot water followed by distilled water. The glassware was subsequently silanised according to a procedure previously described [10] using a 3% hexamethyldisilazane solution in chloroform in order to minimise possible loss of drug due to adsorption on the glassware.

2.3. Preparation of standard solutions

Stock solutions of moricizine, moricizine sulphoxide, moricizine sulphone and clozapine (internal standard) were prepared by dissolving accurately weighed quantities of the drugs separately in ethanol to make standard solutions of 1 mg/ml. When stored at 4°C the stock solutions remained stable for more than two months. Daily working standards were prepared by dilution of the stock solutions with ethanol.

2.4. Chromatographic conditions

The mobile phase was methanol–distilled water–triethylamine (65:35:0.5, v/v). The final pH was adjusted to range between 6 and 7 with concentrated glacial acetic acid. The mobile phase was filtered before use using a Millipore vacuum filter system equipped with a 0.22-μm filter (Waters). Further degassing was found not necessary immediately after filtration.

Other operating conditions were: flow-rate at 2.0 ml/min; UV detection at 254 nm; temperature, ambient (25 ± 1°C); the total run-time was about 12 min.

2.5. Sample preparation

Into a 10-ml glass centrifuge tube, 20 μl of clozapine solution (800 ng) were added to the drug containing bile, plasma or urine sample (1 ml) for assay. Using a Pipetman, 200 μl of a 2.5 mM solution of sodium pentanesulphonate (pH 6.0) were added and mixed briefly (3 s) using a whirly mixer. 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 ethereal extract was transferred to a 10-ml evaporation tube and evaporated to dryness at 40°C in a water bath under a gentle stream of nitrogen. The residue was dissolved in mobile phase (100 μl), and vortex-mixed for 30 s to facilitate dissolution of the sample. An aliquot (25 μl) was injected onto the HPLC apparatus.

2.6. Quantitation, recovery and precision

Calibration graphs were constructed by plotting the peak-height ratios of moricizine, its two sulphoxidation metabolites to the common internal standard (clozapine) against the known concentrations of the drugs added to drug-free plasma or urine samples to cover the concentration range 0–3000 ng/ml. The unknown concentrations were quantitated by relating the peak-height ratio to obtain the concentrations from the calibration graphs.

The recovery of the four drugs from plasma by the extraction procedure was assessed by adding them to drug-free plasma (500 and 3000 ng/ml for moricizine, the sulphoxide and sulphone metabolites and 800 ng/ml for clozapine) and assayed as described. For comparison, the same concentrations of the four compounds were prepared in a diethyl ether solution and assayed with the extraction step omitted. The peak heights of the peaks corresponding to the compounds were compared with and without extraction.

The in-between batch precision of the assay was assessed by including one standard sample of 500 ng/ml at the beginning and one at the end of processing a batch of 20 unknown samples. The between-batch standards data were used for calculating the between-batch coefficients of variation.

3. Results and discussion

3.1. Performance of the HPLC system

The analytical peaks of moricizine sulphone, moricizine sulphoxide, moricizine and clozapine

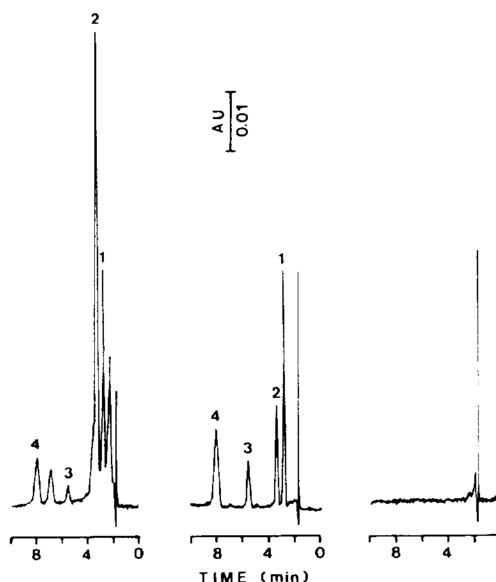

Fig. 1. Chromatograms from bile extract taken 2 h after intravenous administration of 0.72 mg moricizine-HCl in rat (left); peaks: 1 = moricizine sulphone (0.57 μg/ml) 2 = moricizine sulphoxide (3.68 μg/ml) and 3 = moricizine (0.34 μg/ml); and drug loaded bile extract after spiking 0.5 μg/ml each of 1, 2 and 3 and 0.8 μg/ml of 4 = clozapine (middle); and drug free bile extract (right).

were well resolved with good symmetry (Figs. 1 and 2). The respective retention times were 2.7 min, 3.5 min, 6.2 min and 9.7 min. No endogenous interfering peaks from bile, plasma or urine extracts were observed.

3.2. Linearity, sensitivity and precision of the assay

Standardisation graphs for moricizine and its two metabolites in plasma, bile or urine resulted in a linear concentration–peak-height-ratio relationship over the range 0–3000 ng. The linear equations and corresponding regression coefficients for the calibration of moricizine, moricizine sulphoxide and moricizine sulphone in plasma ($n = 6$) were $y = 0.000934x - 0.0072$ and $r = 0.997$, $y = 0.00228x + 0.0146$ and $r = 0.9999$, and $y = 0.00499x - 0.0539$ and $r = 0.9997$, respectively. Good in-between batch precision or accuracy was obtained. This was shown by the low difference between the mean

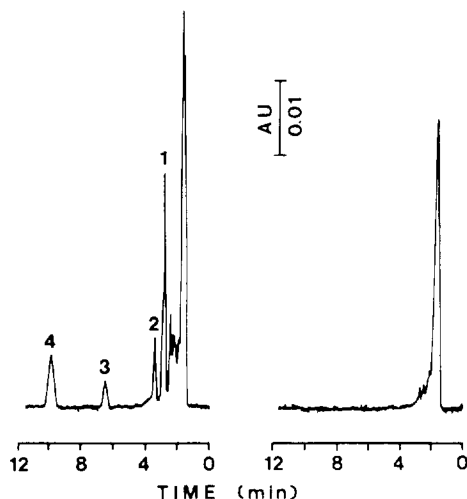

Fig. 2. Chromatograms from an extract of urine from a healthy subject 4 h after oral dosing of 300 mg moricizine tablet (left); peaks: 1 = moricizine sulphone (0.67 μg/ml), 2 = moricizine sulphoxide (0.45 μg/ml) and 3 = moricizine (0.42 μg/ml); and an extract of drug-free urine from the same subject (right).

peak-height ratios at 500 ng/ml of 8 batch measurements for moricizine (0.49 ± 0.03), moricizine sulphoxide (1.19 ± 0.07) and moricizine sulphone (2.53 ± 0.13). The corresponding coefficients of variation are 5.6, 6.1 and 5.0%, respectively.

The limit of detection of the assay was evaluated for moricizine, moricizine sulphoxide and moricizine sulphone as 20, 10 and 5 ng/ml respectively.

3.3. Recovery

The recovery of compounds from plasma samples after ethereal extraction with *n*-pentanesulphonic acid as the ion-pair is high and constant for all compounds (Table 1). No apparent difference was noted in the recovery of drugs from bile, plasma or urine samples.

3.4. Selectivity

As shown previously [8], 12 commonly prescribed drugs screened by the assay did not interfere with the analytical peaks although the present procedure utilised 65% instead of 80% methanol as the mobile phase with a flow-rate of 2 ml/min instead of 1.8 ml/min. The two sulphoxidation metabolites, being more polar than the parent compounds, were eluted more readily with shorter retention times (Figs. 1 and 2). We observed in our previous work [8] that the assay procedure for moricizine proposed by Pratt et al. [7] and Whitney et al. [6] was laborious and moricizine was unstable under alkaline conditions. The present procedure incorporated *n*-pentanesulphonic acid in the extraction, thus allowing more efficient uptake of the 4 compounds into the organic solvent. The presence of pentanesulphone was essential because in its absence at pH 7, the recovery of the compounds was very low. The mechanism of this extraction procedure was not investigated in the present study.

Table 1
Percentage recovery of moricizine, its sulphoxide and sulphone and clozapine from plasma

Drug	*n*	Concentration (ng/ml)	Recovery (%)	Coefficient of variation (%)
Moricizine	4	500	94.0	3.2
		3000	96.1	1.3
M-sulphoxide	4	500	89.9	1.4
		3000	93.2	2.1
M-sulphone	4	500	95.6	1.7
		3000	97.1	1.8
Clozapine	4	800	98.1	1.5

The inclusion of triethylamine (0.5%) in the mobile phase improved the peak shapes of the compounds in the chromatographic separation. The pH of the mobile phase also affected the retention times of the drugs; with increasing pH the retention times were prolonged. During the development of the assay, it was noted that the mobile phase could be recycled for at least 3 times, i.e. used to run 3 batches of 22 samples, without causing any interference.

Using this assay we have successfully determined the concentrations of moricizine and its sulphoxidation metabolites in plasma, bile and urine of rats in an isolated perfused rat-liver preparation [9].

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