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Determination of moclobemide in human plasma by high-performance liquid chromatography with spectrophotometric detection

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Abstract

A simple and sensitive high-performance liquid chromatographic (HPLC) method with spectrophotometric detection was developed for the determination of moclobemide in human plasma. Plasma samples were extracted under basic conditions with dichloromethane followed by back-extraction into diluted phosphoric acid. Isocratic separation was employed on an ODS column (250 mm × 4.6 mm, 5 μm) at room temperature. The mobile phase consisted of 5 mM NaH_2PO_4–acetonitrile–triethylamine (1000:350:10 (v/v/v), pH 3.4). Analyses were run at a flow-rate of 1.0 ml/min and ultraviolet (UV) detection was carried out at 240 nm. The method was specific and sensitive with a quantification limit of 15.6 ng/ml and a detection limit of 5 ng/ml at a signal-to-noise ratio of 3:1. The mean absolute recovery was about 98.2%, while the intra- and inter-day coefficient of variation and percent error values of the assay method were all at acceptable levels. Linearity was assessed in the range of 15.6–2000 ng/ml in plasma with a correlation coefficient of greater than 0.999. This method has been used to analyze several hundred human plasma samples for bioavailibility studies.

Keywords: Moclobemide

1. Introduction

Moclobemide (Fig. 1) is a selective and reversible inhibitor of monoamine oxidase type A (MAO-A), which is used in the treatment of depression [1]. Moclobemide is eliminated almost entirely by hepatic metabolism involving C- and N-oxidation of its morpholine ring to yield its two major metabolites in plasma, Ro 12-5637 and Ro 12-8095, which have little and no MAO-A inhibitory activity, respectively [1].

Some analytical techniques have been developed for the determination of moclobemide in biological fluids [2–7]. Gas chromatography [2–4] and high-performance liquid chromatography (HPLC) techniques with ultraviolet (UV) detection [5,6], or mass spectrometry (MS) [7] have been reported for monitoring plasma concentrations of moclobemide.

Previous HPLC methods [5–7] require solid-phase extraction of moclobemide from plasma. This paper describes a simple and rapid liquid–liquid extraction of moclobemide from plasma, which can be considered as an alternative to previous solid-phase extraction techniques.

2. Experimental

2.1. Materials and reagents

Moclobemide was prepared from Cipla Pharmaceutical Co. (Mumbai, India). Prazosin (as hydrochloride salt, Fig. 1B) was obtained from Omnichem Pharmaceutical Co. (Belgique). HPLC-grade methanol and analytical-grade triethylamine, sodium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium hydroxide and concentrated phosphoric acid were from Merck (Darmstadt, Germany). HPLC-grade acetonitrile and analytical grade dichloromethane were obtained from Carlo Erba Reagent (Italy).

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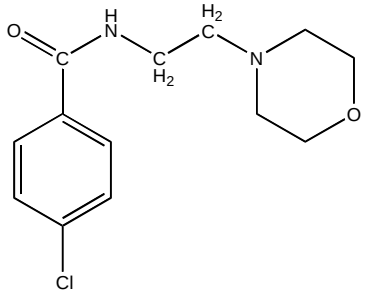
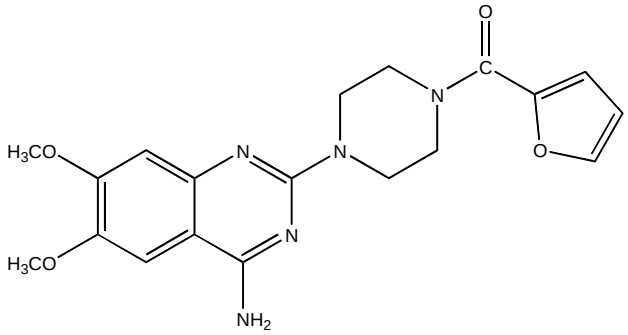


(A) Moclobemide



(B) Prazosin

Fig. 1. Structures of: (A) moclobemide and (B) prazosin (internal standard).

2.2. Chromatographic conditions

The HPLC system consisted of an LC-6A solvent delivery pump equipped with an SPD-6A UV-Vis detector (at 240 nm), a Shim-pack C_{18} guard column (10 mm × 4 mm, 5 μm), a Shim-pack VP C_{18} HPLC column (250 mm × 4.6 mm, 5 μm) and a C-R6A integrator (all from Shimadzu, Kyoto, Japan).

The samples were applied by a Rheodyne 7125 loop injector with an effective volume of 50 μl. Moclobemide and the internal standard (prazosin) were isocratically separated with 5 mM sodium dihydrogen phosphate–acetonitrile–triethylamine (1000:350:10 (v/v/v), pH 3.4), at a flow-rate of 1.0 ml/min. Analyses were run at room temperature and the samples were quantified using moclobemide to internal standard peak height ratios.

2.3. Preparation of standard solutions

Standard stock solutions of moclobemide and prazosin were prepared by dissolving 5 mg of them in 50 ml of methanol and stored at −20 °C. These stock solutions were subsequently used in the preparation of working standards on the day of analysis by further dilution with methanol.

2.4. Preparation of plasma standards

Plasma standards for calibration curves were prepared by spiking drug-free and fresh plasma with a known amount of moclobemide in the concentration range of 15.6–2000 ng/ml. Additional standards were prepared for the determination of accuracy and precision ($n = 3$) of the method.

The absolute recovery for moclobemide and the internal standard ($n = 3$) was estimated by comparing peak heights of directly injected drug solutions prepared in 1% phosphoric acid with peak heights of plasma standards.

Quality control (QC) samples were prepared by adding moclobemide to drug-free plasma to make two concentrations of 125 and 1000 ng/ml. QC samples were stored at −20 °C and extracted and analyzed during each analytical run.

2.5. Sample preparation

A 250 μl volume of plasma was transferred to a 1.5 ml polypropylene microcentrifuge tube. The internal standard (20 μl, equal to 100 ng prazosin) was added and vortex-mixed for 10 s. Extraction was performed by adding 100 μl of 0.1 M dipotassium hydrogen phosphate and 1 ml of dichloromethane and shaking for 2 min. After centrifugation at 11,300 × g for 3 min, the whole organic layer was separated and transferred into another tube. Then, 50 μl of 1% phosphoric acid solution was added. The mixture was vortex-mixed and centrifuged (2 and 3 min, respectively). Supernatant was transferred to another tube, and a 20 μl volume was injected into the chromatograph.

2.6. Application

The assay was used for a comparative bioavailability study of two tablets preparations containing 150 mg moclobemide (Aurorix, Roche, Germany and a generic moclobemide preparation from Arya Pharmaceutical Co., Tehran, Iran).

Fourteen healthy volunteers participated in the study. The study was conducted using a two-way crossover design, as a single dose, randomized trial. The two formulations were administrated on 2 treatment days, separated by a washout period of 7 days, to fasted subjects who received a single oral dose of one of the study medications. Food and drinks were not allowed until 3 h after ingestion of the tablet. Multiple blood samples (2.5 ml) were collected before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10 h post dosing. The plasma was immediately separated by centrifugation and frozen at −20 °C until analysis.

3. Results

Representative chromatograms of drug-free plasma, plasma spiked with moclobemide and a volunteer sample collected after oral dosing with moclobemide are shown in Fig. 2. The retention times for moclobemide and the internal standard were 6.7 and 9.8, respectively. The

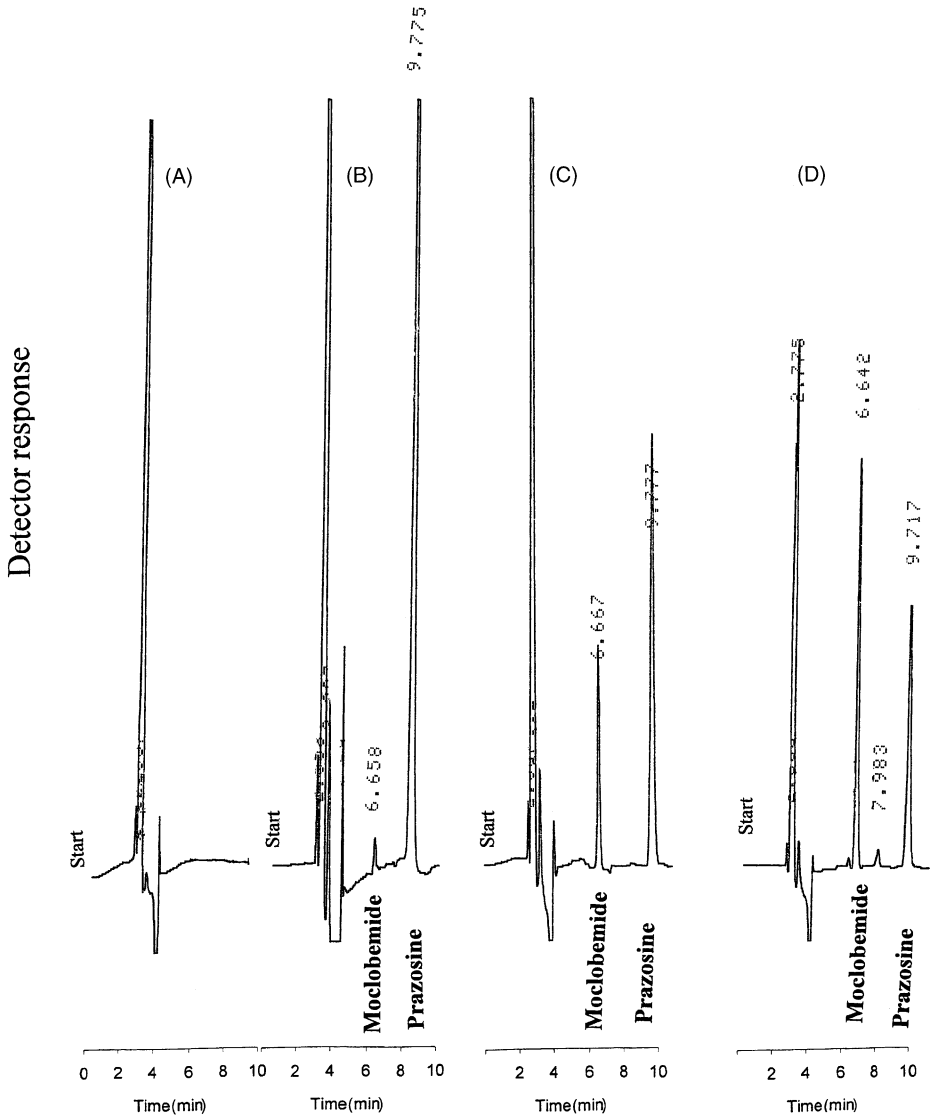


Fig. 2. Representative chromatograms of: (A) blank plasma without internal standard (AT = 1), (B) plasma spiked with 15.6 ng/ml moclobemide (AT = 0), (C) plasma spiked with 250 ng/ml moclobemide (AT = 2), (D) a volunteer plasma sample, 3 h after taking 150 mg tablet of moclobemide (877.5 ng/ml, AT = 3). The internal standard (prazosin, 100 ng) was added to all samples. AT: attenuation.

calibration curves were linear over the concentration range of 15.6–2000 ng/ml in human plasma, with a correlation coefficient greater than 0.999. The limit of quantification was 15.6 ng/ml, which is close to the LOQ of published methods (10–30 ng/ml) [2–7]. The limit of detection was about 5 ng/ml at a signal-to-noise ratio of 3:1. The mean absolute recovery of the moclobemide using the present extraction procedure was 98.2% (Table 1). The mean absolute

Table 1
The accuracy, within- and between-day precision and recovery data for the measurement of moclobemide in human plasma

Nominal concentration (ng/ml)	Recovery (%)	Within-day		Between-day	
		Precision (%)	Accuracy (%)	Precision (%)	Accuracy (%)
15.6	97.8	1.5	11.7	2.4	8.3
31.2	108.7	5.9	0.3	4.2	6.6
62.5	94.6	3.5	−4.9	1.9	0.6
125	94	3.8	−5.6	3.5	0.9
250	97.2	4.9	1.6	5.6	1.5
500	100.5	1.7	−1.3	2.6	−2.8
2000	94.3	5.5	0.65	2.1	−1.6

Table 2
The between-day precision of quality control (QC) samples measured in duplicate on 10 separate days

QC sample	Number of samples	Nominal concentration (ng/ml)	Mean measured concentration (ng/ml)	Coefficient of variation (%)
Moclobemide				
QC1	10	125	122.2	11.2
QC2	10	1000	955.9	5.1

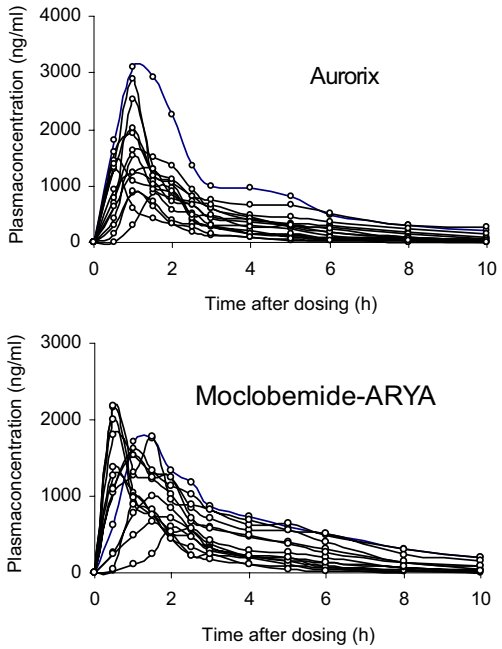


Fig. 3. Plasma concentration–time profiles of moclobemide in 14 healthy volunteers following oral administration of 150 mg tablet of Aurorix and a generic moclobemide in a crossover study.

recovery of the internal standard was 76%. It was observed that just before extraction, shaking plasma for 10 s after adding the internal standard improve the method reproducibility. The results of the method intra- and inter-day accuracy and precision are presented in Table 1. The results of QC samples are presented in Table 2. The method showed good stability and performance for a long time and was used successfully for a comparative bioavailibility study. The plasma moclobemide profiles for volunteers after taking the test or reference products are shown in Fig. 3. After dose adjustment, the obtained maximum plasma concentration and the elimination half-life of moclobemide in this study were close to previous results [5].

4. Discussion

Only solid-phase extraction has been used in previous HPLC methods [5–7] for the extraction of moclobemide from plasma. Geschke et al. [5] used expensive Extrelut 1 glass column to extract moclobemide, which is not economical. In another method [6] more cost-effective Bond Elut C_{18} cartridges were used, but the selectivity of the method was achieved by using MS rather than UV detection. Extraction of moclobemide by organic solvent has been reported in a GC method [2]. It involves double extraction with 10 ml of ethyl acetate, back-extraction into 1 ml of hydrochloric acid, alkalization and another extraction into ethyl acetate and finally, evaporation of the extract.

In our experience, we observed that dichloromethane is more suitable than ethyl acetate and eliminates the need for double extraction. Moclobemide showed poor recovery when dichloromethane extract was evaporated in polypropylene tube. However, back-extraction into diluted phosphoric acid gave high recovery (about 95%).

Use of single-use 1.5 ml polypropylene microtubes and low amounts of organic solvent (1 ml), makes the method simple and economical, whilst the method also offers high reproducibility, high recovery and clear chromatograms. It is also possible to use higher amounts of acidic phase (e.g. 100–200 μl) for back-extraction, to make it technically simpler.

There were no endogenous interferences in the chromatograms. However, 1–3 h after taking moclobemide tablet by volunteers, two to three unknown small peaks appeared in the chromatograms. The intensity of these peaks in comparison to moclobemide peak was very low, and they did not interfere with moclobemide or internal standard peak. It is possible that these unknown peaks were the metabolites of moclobemide.

It has been reported that the metabolites of moclobemide are highly HPLC column-dependent and most of commercially available HPLC columns are not suitable for chromatography of them [5]. It seems that the interaction of metabolites with free silanol groups of reversed-phase HPLC columns is more vigorous than that of moclobemide. However, it is possible that poor extraction of metabolites is also one of the reasons that unlike previous methods [5–7], the metabolites did not appear significantly in the chromatograms of the present method.

5. Conclusions

The present method provides a selective, accurate and precise means of assaying moclobemide in human plasma using simple liquid–liquid extraction technique. The method is time-saving and economical, and is suitable for pharmacokinetic studies of moclobemide.

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