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Short Communication

Automated high-performance liquid chromatographic method for the determination of mianserin in plasma using electrochemical detection

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ABSTRACT

An automated high-performance liquid chromatographic method for the determination of mianserin in plasma is described. Extraction and injection of the samples were automatically done by the Gilson ASPEC system using C_8, 100-mg Supelclean solid-phase extraction columns. The extracts were chromatographed on a reversed-phase C_{18} column (150 mm × 3.9 mm I.D.) with a phosphate buffer–acetonitrile–methanol mobile phase and the analytes detected electrochemically. Calibration curves were linear to at least 53.7 ng/ml at which the between-day relative standard deviation was 5% and the recovery 101%. The limit of quantification was 1.67 ng/ml at which the between-day relative standard deviation was 9% and the recovery 92% using a sample volume of 0.5 ml. The method was applied to the determination of mianserin in the plasma of normal human volunteers participating in a comparative bioavailability study.

INTRODUCTION

Mianserin is a tetracyclic antidepressant of the "second-generation group" with less anticholinergic activity and cardiotoxicity than the tricyclic antidepressants (Fig. 1). The determination of mianserin in plasma has been performed using gas chromatography [1–3]. High-performance liquid chromatographic (HPLC) methods using electrochemical detection [4] and UV detection [5,6] have been published. More recently a method has been described using fluorescence detection after on-line photochemical reaction [7]. None of these methods used automated solid-phase extraction.

Our objective was to develop an automated HPLC method for the determination of mianserin in plasma in order to perform a comparative

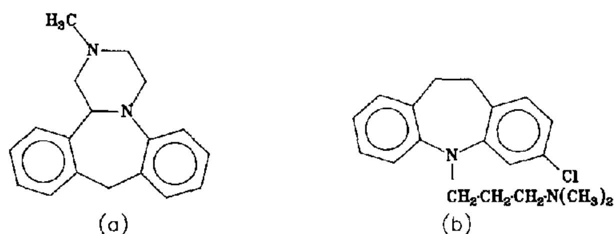

Fig. 1. Structures of mianserin (a) and clomipramine (b).

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bioavailability study of two mianserin hydrochloride tablet products in healthy subjects. This article describes a fully automated procedure using the ASPEC which provided us with a method sufficiently sensitive to determine mianserin for 48 h after a single 20-mg dose.

EXPERIMENTAL

Materials

Mianserin was obtained from Lennon (Port Elizabeth, South Africa) and clomipramine from Ciba-Geigy (Johannesburg, South Africa). Methanol and acetonitrile were HPLC grade (Waters Assoc., Milford, MA, USA). The potassium dihydrogenphosphate and disodium hydrogenphosphate were guaranteed reagent grade (Merck, Darmstadt, Germany). Water was purified and deionized using a Milli-Q reagent-grade water system (Millipore, Bedford, MA, USA).

Apparatus

A Series 1050 Hewlett-Packard pump (Hewlett-Packard, Avondale, PA, USA) and a Gilson ASPEC (Gilson, Villiers le Bel, France) were coupled to a 150 mm × 3.9 mm I.D. Waters Nova-Pak C_{18} stainless-steel column kept at 40°C in a Shimadzu CTO-6A oven (Shimadzu, Kyoto, Japan). The column effluent was monitored by using a Coulochem Model 5100A dual-electrode electrochemical detector (Environmental Sciences Assoc. Bedford, MA, USA) fitted with a Model 5011 analytical cell in the oxidative screen mode. The applied cell potential of the screen electrode was set at + 0.4 V and the sample electrode at + 0.7 V. The results were processed on a Hewlett-Packard Model HP 3396A integrator.

Chromatography

The mobile phase consisted of phosphate buffer–acetonitrile–methanol (436:380:185, v/v). The buffer was prepared by adding 325 ml of 0.07 *M* KH_2PO_4 to 148 ml of 0.07 *M* Na_2HPO_4. The final pH of the mobile phase was adjusted to 6.2 using phosphoric acid. A constant flow-rate of 1.0 ml/min was maintained through the HPLC column at 40°C.

Standards

The internal standard solution contained 200 ng of clomipramine in 0.5 ml of water. Blank plasma was spiked with a methanol stock solution of mianserin to produce working solution 1. This was then continuously diluted sequentially with blank plasma to produce calibration standards covering the range 1.5–100 ng/ml mianserin.

Extraction procedure

To 0.5 ml of plasma was added 0.5 ml of water containing clomipramine as internal standard. The ASPEC was programmed to condition each Supelclean LC-8 (100 mg, C_8 packing) extraction column (Supelco, Bellefonte PA, USA) with 1 ml of methanol followed by 1 ml of water just before use. The plasma mixture was loaded onto the column and washed with 1 ml of water followed by 1.5 ml of acetonitrile–water (1:1, v/v). The analytes were eluted with 300 μl of methanol, the eluate mixed with air, and 20 μl automatically injected onto the HPLC column.

RESULTS AND DISCUSSION

It has been shown that for basic drugs it is advantageous to use acetonitrile mixtures during the washing steps and methanol mixtures for elution [8].

Using this approach we were able to include a washing step using 1.5 ml of acetonitrile–water (1:1, v/v) without washing off any of the analytes and providing very clean extracts. Fig. 2 shows representative chromatograms obtained and demonstrates the lack of interfering compounds. Retention times of mianserin and clomipramine were 4.0 and 5.8 min, respectively. The recovery yields of mianserin carried through the whole extraction procedure with spiked plasma samples containing 50, 25, 12, and 2 ng/ml mianserin and using clomipramine as an external standard gave means ± S.D. of 82 ± 1.1, 88 ± 0.9, 74 ± 3.0 and 82 ± 2.9%, respectively. That of clomipramine at 240 ng/ml using mianserin as external standard was 70 ± 1.33%. In all cases, n = 3.

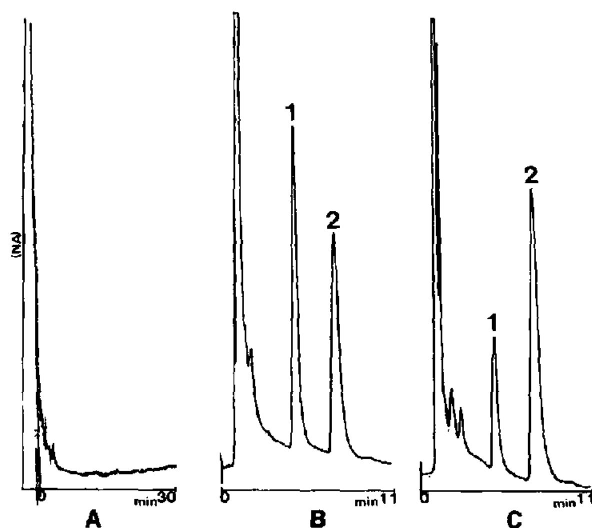

Fig. 2. Chromatograms of blank plasma (a), plasma standard of 53.7 ng/ml (b) and trial plasma sample equal to 19.1 ng/ml (c). Peaks: 1 = mianserin; 2 = clomipramine.

Accuracy, precision and linearity

The accuracy and precision of the assay procedure were continuously monitored during the period of assaying the biological samples by including quality control specimens containing known concentrations of the analyte. These *in vitro* quality control samples were stored under identical conditions (−20°C) to actual trial samples. Table I depicts the within-day and between-day accuracy and precision of the method as determined over a period of 25 days. The limit of quantification for this method was set at 1.67 ng/ml at which between-day precision has a coefficient of variation (C.V.) of 9%, a bias of 8% and a signal-to-noise ratio of 8:1. The calibration curves of mianserin/internal standard peak-height ratios *versus* concentrations were linear over the range 1.67–53.7 ng/ml using an unweighted linear least-squares fit (r^2 = 0.998, y = 0.398 + 0.452x). This equation represents the average of eight calibration curves, the slope giving a coefficient of variation of 12%. The highest standard when plugged into its own regression curve gave a coefficient of variation of 1.2% and a bias of 0.12% whereas the lowest standard gave a coefficient of variation of 5.4% and a bias of 0.22% (n = 8).

Stability

Additional blood was drawn from all subjects at predetermined times on the first day of the trial, and each sample was divided into aliquots. After analysing one aliquot of each sample, five samples that contained the analyte at concentrations spanning the expected range were selected and reanalysed at different times during the course of the analytical study. These *ex vivo* quality control samples were also stored under identical conditions (−20°C) to actual trial samples (Table II). No evidence of decomposition of mianserin in plasma stored at −20°C was observed over a period of 26 days.

TABLE I

WITHIN- AND BETWEEN-DAY PRECISION AND ACCURACY OF THE ASSAY

Nominal plasma concentration (ng/ml)	Within-day (n = 5)		Between-day (n = 4)	
	Nominal/found (mean) (%)	C.V. (%)	Nominal/found (mean) (%)	C.V. (%)
53.7	99	8	101	5
26.8	100	11	101	4
13.4	102	5	100	7
6.70	96	8	98	6
3.34	108	6	101	8
1.67	92	13	92	9

TABLE II

STABILITY OF MIANSERIN IN PLASMA STORED AT −20°C FOR 26 DAYS

A, B, C and D are *ex vivo* quality control samples

Day	Concentration found (ng/ml)			
	A	B	C	D
1	3.90	33.8	11.5	5.99
9	3.95	34.3	11.4	5.88
18	3.20	35.6	10.3	5.72
26	3.93	32.9	11.4	6.19
Mean	3.75	34.2	11.2	5.95
C.V. (%)	10	3	5	3

Application

Fig. 3 represents a concentration–time profile of the mean plasma mianserin values of eighteen healthy subjects after receiving an oral dose of 20 mg mianserin. The pharmacokinetic parameters obtained are summarised in Table III.

TABLE III

PHARMACOKINETIC PARAMETERS FOR ORAL ABSORPTION OF 20-mg MIANSERIN TABLETS

Values are means ± S.D. in eighteen normal trial subjects.

Parameter	Value
Peak plasma concentration (ng/ml)	25.8 ± 10.2
Time to peak plasma concentration (h)	1.81 ± 0.62
Area under the curve (ng h/ml)	218 ± 87.9
Plasma half-life (h)	16.3 ± 5.6

CONCLUSION

Compared to previous liquid–liquid extraction methods for the determination of mianserin in plasma [1–6] the solid-phase extraction procedure requires minimal sample preparation. The method using fluorescence detection after on-line photochemical reaction [7] had minimal sample preparation but the limit of quantification of 5 ng/ml was too high to do a bioavailability study

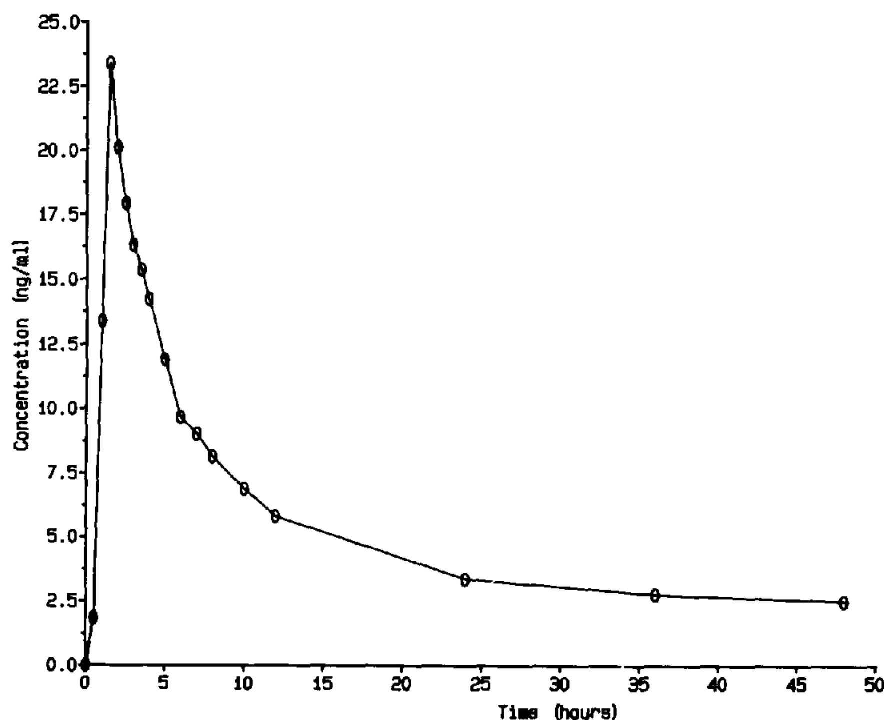

Fig. 3. Concentration–time profile of the mean mianserin plasma values of eighteen subjects after receiving a 20-mg mianserin hydrochloride dose.

after a single 20-mg mianserin dose. The automated solid-phase extraction compares favourably to liquid–liquid extractions regarding the precision, accuracy, limit of quantification and recovery.

The automated procedure using the ASPEC provided us with a method which is sufficiently sensitive to determine mianserin for 48 h after a single 20-mg dose. Because the extraction, injection and chromatography are completely automated, operators are freed from time-consuming, repetitive work enabling them to perform method development and validation tasks.

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