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Analytical method development and validation of mianserin hydrochloride and its metabolite in human plasma by LC–MS

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Abstract

Mianserin is a tetracyclic antidepressant drug and administered as racemate of *R* (−) and *S* (+) mianserin hydrochloride in a dose of 30–90 mg/day in divided doses. Liquid chromatography-mass spectroscopy (LC–MS) is a tool, which is widely used for determination of drug and their metabolites in biological fluids because of its high sensitivity and precision. Here we describe a liquid chromatography mass spectroscopy method for simultaneous determination of mianserin and its metabolite, *N*-desmethylmianserin, from human plasma using a liquid–liquid extraction with hexane:isoamylalcohol (98:2) and back extraction with 0.005 M formic acid solution. This method is specific and linear over the concentration range of 1.00–60.00 ng/ml for mianserin and 0.50–14.00 ng/ml for *N*-desmethylmianserin in human plasma. The lowest limits of quantification (LLQ) is 1.00 ng/ml for mianserin and 0.50 ng/ml for *N*-desmethylmianserin. Intraday and interday precision (%C.V.) is <10% for both mianserin and *N*-desmethylmianserin. The accuracy ranges from 94.44 to 112.33% for mianserin and 91.85–100.13% for *N*-desmethylmianserin. The stability studies showed that mianserin and *N*-desmethylmianserin in human plasma are stable during short-term period for sample preparation and analysis. The method was used to assay mianserin and its metabolite, *N*-desmethylmianserin, in human plasma samples obtained from subjects who had been given an oral tablet of 30 mg of mianserin.

Keywords: Mianserin; *N*-desmethylmianserin; Human plasma; Single ionization mode (SIM)

1. Introduction

Mianserin (1,2,3,4,10,14-b-hexahydro-2-methyl dibenzo [c, f] pyrazino [1,2-α] azepine) is a tetracyclic antidepressant drug. It is administered as a racemate of *R* (−) and *S* (+)-mianserin (Fig. 1) [1,2]. It is metabolized mainly by *N*-demethylation, aromatic hydroxylation, *N*-oxidation and *N*-glucuronidation [3,4]. *N*-desmethylmianserin is the major metabolite of mianserin in plasma and contributes substantially to the overall therapeutic effects of mianserin in patients [5,2]. Several methods are reported to detect mianserin and/or its metabolite by using HPLC, GC–MS, GC-NPD and capillary zone electrophoresis. Limits of detection as 2.64 ng/ml for mianserin and 2.50 ng/ml for *N*-desmethylmianserin with HPLC and limits of quantification as 1.00 ng/ml for mianserin with GC–MS, GC-SID were reported [2,6–10]. Liquid chromatography–mass spectrometry is a tool, which is widely used for determination of drug and their metabolites in biological fluids because of its high sensitivity and precision. In literature, no method is reported to determine mianserin and its active metabolite using liquid chromatography–mass spectrometry. We report a new rapid and highly specific method for quantification of mianserin and its active metabolite, *N*-desmethylmianserin, in human plasma by using liquid chromatography–mass spectrometry

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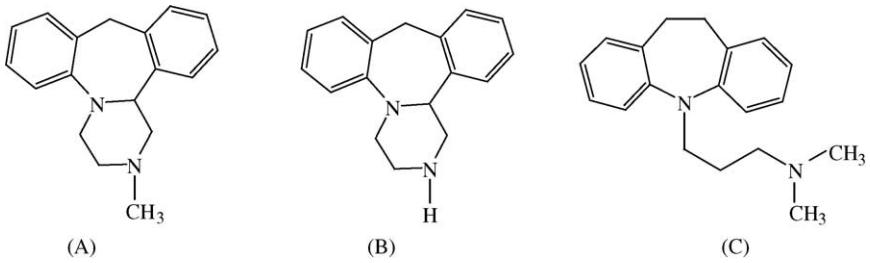

Fig. 1. Structure of mianserin (A), *N*-desmethylmianserin (B) and imipramine (C).

technique with lowest limit of quantification 1.00 ng/ml for mianserin and 0.50 ng/ml for *N*-desmethylmianserin.

2. Experimental

2.1. Materials and chemicals

Mianserin hydrochloride was a gift (Remedica, Limassol, Cyprus). *N*-desmethylmianserin was synthesized and purified in our center. Imipramine hydrochloride was used as internal standard (Torrent Pharmaceuticals Ltd., Ahmedabad, Gujarat, India). All solvents were of HPLC grade. Tris (hydroxymethyl) amino methane (Sisco Research Lab., Mumbai, Maharashtra, India) and ammonium acetate (S.D. Fine Chem. Limited, Mumbai, Maharashtra, India) of analytical grade were used. Human plasma was used as blank plasma (Prathma blood bank, Ahmedabad, Gujarat, India).

2.2. Synthesis of N-desmethylmianserin

1-Chloroethylchloroformate (1.03 ml, 0.0095 mol) in dichloroethane (5 ml) was added slowly to a rapidly stirring solution of mianserin (1.00 g, 0.0038 mol) in dichloroethane (20 ml) maintained at 0° temperature under nitrogen for an hour. The intermediate was unstable and rapidly converted to more stable carbamate product. The solvent was evaporated and methanol was added in to it. Reaction mixture was stirred for half an hour and refluxed for 30 h. The product was the mixture of *N*-desmethylmianserin hydrochloride and mianserin hydrochloride, which was separated by means of selective derivatization by forming acetyl derivative of *N*-desmethylmianserin and by using solubility property of acetyl derivative of *N*-desmethylmianserin in ether. The pure *N*-desmethylmianserin was recovered by simple hydrolysis with concentrated hydrochloric acid and 95% ethanol.

2.3. Apparatus and conditions

The HPLC–API-MS system was consisted of pump PU-980 (Jasco, Hachioji, Tokyo, Japan), autosampler AS-950-10 (Jasco, Hachioji, Tokyo, Japan) and mass detector API-165 (Perkin Elmer, Foster city, CA, USA). Chromatographic separation was achieved by using Kromasil RP-18, 50 mm × 4.6 mm; 5-μm column (Flexit Jour, Pune, Maharashtra, India). Mobile phase was (v/v) 75% acetonitrile, 5% methanol, 20% 10 mM ammonium acetate buffer and pH adjusted to 3.5 with formic acid. Flow rate of mobile phase was maintained at 0.4 ml/min. Stock solutions of drug, its metabolite and internal standard (100 μg/ml) were prepared in methanol and stored at 4 °C.

2.4. Extraction procedure

Fifty microlitres of internal standard (100 ng/ml of imipramine hydrochloride in methanol) was added to 1.00 ml of plasma and mixed with vortex for 10 s. To this 200 μl of 0.1 M Tris (hydroxymethyl) amino methane, pH 8.7 was added followed by 5 ml of mixture of hexane:isoamyl alcohol (98:2). The mixture was vortexed for 2 min and centrifuged for 10 min at 488 × *g*. Organic layer was transferred to conical stoppered tubes. To this 150 μl of 0.005 M formic acid solution was added and vortexed for 1 min. Again it was centrifuged for 10 min at 488 × *g*. Upper hexane layer was discarded and 50 μl of aqueous layer was injected to LC–MS.

3. Validation

3.1. Calibration curve

Calibration curves were prepared by adding known amount of mianserin hydrochloride (1.00, 2.00, 5.00, 10.00, 20.00, 40.00 and 60.00 ng/ml) and *N*-desmethylmianserin hydrochloride (0.50, 1.00, 2.00, 4.00, 8.00, 12.00 and 14.00 ng/ml) to 1 ml of blank plasma. An aliquot of 50 μl of the internal standard solution in methanol (100 ng/ml) was added to each sample. The samples were extracted as described above. The standard curves were constructed by plotting the peak area ratio of mianserin to internal standard and that of *N*-desmethylmianserin to internal standard versus their respective concentrations. The calibration curves were obtained by least square linear regression analysis.

3.2. Preparation of plasma quality controls (QC) to evaluate precision and accuracy of the assay method

The concentrations of mianserin and *N*-desmethylmianserin were 3.00, 15.00 and 50.00 ng/ml and 1.50, 6.00 and 13.00 ng/ml respectively, in human plasma to repre-

sent low, middle and high controls respectively. To prepare QC samples appropriate volumes from stock solutions of mianserin and *N*-desmethylmianserin were transferred to 50 ml flask and diluted to the mark with normal human plasma and then mixed. The QC solutions were stored at −20 °C. The QC samples were removed from storage at a specific time for analysis to determine intra-day and inter-day precision and accuracy.

3.3. Precision and accuracy

For the calculation of the intra-day precision and accuracy, six replicate samples of plasma with mianserin (3.00, 15.00 and 50.00 ng/ml) and *N*-desmethylmianserin (1.50, 6.00 and 13.00 ng/ml) from QC samples were extracted as described above and the concentrations were calculated from the standard curve. For the calculation of the inter-day precision and accuracy, six plasma samples of both mianserin (3.00, 15.00 and 50.00 ng/ml) and *N*-desmethylmianserin (1.50, 6.00 and 13.00 ng/ml) from QC samples were analyzed on three consecutive days, along with the standard calibration curve.

3.4. Recovery

In order to calculate recovery of the extraction procedure, six spiked plasma samples of mianserin (15.00 ng/ml) and *N*-desmethylmianserin (6.00 ng/ml) were analyzed, and the peak area ratios of mianserin to internal standard and *N*-desmethylmianserin to internal standard were compared with those of unextracted mianserin and *N*-desmethylmianserin reconstituted in 0.005 M formic acid.

3.5. Evaluation of bench top, autosampler and freeze thaw stability

QC samples of mianserin and *N*-desmethylmianserin were prepared in normal human plasma.

In bench top stability, three replicates of both mianserin (3.00 and 50.00 ng/ml) and *N*-desmethylmianserin (1.50 and 6.00 ng/ml) were analyzed at 0 and 24 h at room temperature. In autosampler stability, three replicates were analyzed at 0 and 24 h at 4 °C temperature for both mianserin (3.00 and 50.00 ng/ml) and *N*-desmethylmianserin (1.50 and 6.00 ng/ml). In freeze thaw stability, three replicates of the plasma samples containing the mianserin at concentration of 3.00 and 50.00 ng/ml and *N*-desmethylmianserin of 1.50 and 13.00 ng/ml were prepared and frozen at −20 °C and analyzed after 1, 2, 3 and 4 freeze-thaw cycles.

4. Results and discussion

Initially it was attempted to develop method to quantitate mianserin and *N*-desmethylmianserin by HPLC with UV–visible detector, but limits of quantification was found to be 10.00 ng/ml. For liquid chromatographic separation of n-desmethylmianserin, mianserin and internal standard, (v/v) 72% 10 mM ammonium acetate buffer solution pH adjusted to 4.5, 28% acetonitrile and 0.1% triethylamine was employed as mobile phase at a flow rate of 1.0 ml/min. The retention time of *N*-desmethylmianserin, mianserin and internal standard were 10.4, 14.3 and 21.9 min respectively with total run time of 30 min. The drug, metabolite and internal standard were attempted to extract by liquid–liquid extraction method using dichloromethane. The recovery of the extraction method was found to be 40 and 45% for drug and metabolite respectively. However, it was quite difficult to determine metabolite in human plasma by UV–visible detector because of low sensitivity of detector and availability of *N*-desmethylmianserin at low level in human plasma during pharmacokinetic studies. Then this method was optimized to LC–MS system. A simple and rapid sample preparation for the determination of mianserin and *N*-desmethylmianserin in biological fluid was developed. Reported time required for sample pretreatment was approximately 3.5–4.0 h per sample [6,7], while our sample pretreatment required approximately 0.5–1.0 h per sample.

Imipramine was chosen as the internal standard because it shows similar chromatographic behavior to mianserin and its metabolite with no interference by admixture in human plasma. Ammonium acetate was used as the buffer in mobile phase, because it does not corrode the LC–MS system. For the quick separation of mianserin and *N*-desmethylmianserin, (v/v) 75% acetonitrile, 5% methanol, 20% of 10 mM ammonium acetate buffer solution pH adjusted to 3.5 with formic acid was employed as mobile phase at a flow rate of 0.4 ml/min. The retention time of mianserin, *N*-desmethylmianserin and internal standard were 2.26, 2.19 and 2.40 min, respectively. Total run time of the analysis was 3 min per sample. In comparison to LC–UV, this optimized method has rapid sample pretreatment, better recovery, and high selectivity in SIM mode in mass detection with one tenth of total run time required per sample analysis.

4.1. Chromatograms

Fig. 2 shows typical selected-ion chromatogram of mianserin (A), *N*-desmethylmianserin (B) and internal standard (C) as $[M + H]^+$ at *m*/*z* 265.3, 251.0 and 281.4, respectively. Mianserin and its metabolite, *N*-desmethylmianserin, showed a good selectivity in the SIM mode. The procedure of back extraction in formic acid produced good specificity and sensitivity in mass detector in the SIM mode.

4.2. Linearity and lowest limits of quantification

The calibration curves were obtained by plotting the ratio of the peak area of mianserin to internal standard and that of *N*-desmethylmianserin to internal standard against the respective concentration. The calibration curves showed lin-

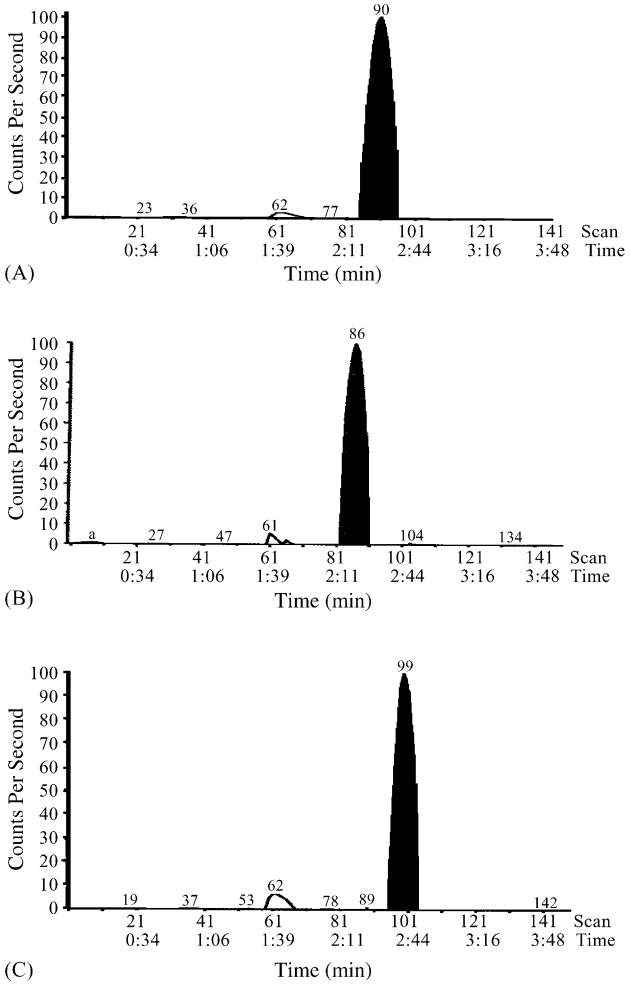

Fig. 2. Typical selected-ion chromatogram (A) 20.00 ng/ml of mianserin, (B) 8.00 mg/ml of *N*-desmethylmianserin and (C) 50.00 ng/ml of internal standard as $[M + H]^+$ at *m*/*z* 265.3, 251.0 and 281.4, respectively.

earity over the range 1.00–60.00 ng/ml for mianserin and 0.50–14.00 ng/ml for *N*-desmethylmianserin. The coefficient of correlation was found to be more than 0.99. The lowest limits of quantification (LLQ) is 1.00 ng/ml for mianserin and 0.50 ng/ml for *N*-desmethylmianserin.

4.3. Precision and accuracy

Intra-day and inter-day precision and accuracy were determined by QC samples at various concentrations as described in the experimental section. The intra-day precision (%C.V.) was found to be <10% ($n = 6$) for mianserin and *N*-desmethylmianserin and accuracy ranged from 98.33 to 112.33% for mianserin and 91.85–100.13% for *N*-desmethylmianserin when determined at concentrations of 3.00, 15.00 and 50.00 ng/ml for mianserin and 1.50, 6.00 and 13.00 ng/ml for *N*-desmethylmianserin, respectively (Table 1). The inter-day precision (%C.V.) was found to be <10% ($n = 18$) for mianserin and *N*-desmethylmianserin and accuracy ranged from 94.44 to 104.00% for mianserin and 92.72–98.22% for *N*-desmethylmianserin (Table 2).

4.4. Recovery from samples

The recovery of the extraction method for mianserin and *N*-desmethylmianserin determined using six replicates of 15.00 ng/ml of mianserin and 6 ng/ml of *N*-desmethylmianserin were found to be 40–50% and 50–60%, respectively.

4.5. Stability

The results of the short-term stability study suggested that mianserin and *N*-desmethylmianserin were stable on the

Table 1
Intra day precision and accuracy for mianserin and *N*-desmethylmianserin

Compound	Nominal concentration (ng/ml)	Estimated concentration (ng/ml)	Precision (%C.V.)	Accuracy (%)
Mianserin	3.00	2.95	6.73	98.33
	15.00	16.85	9.90	112.33
	50.00	50.44	8.86	101.14
N-desmethylmianserin	1.50	1.50	6.42	100.13
	6.00	5.57	6.72	92.83
	13.00	11.94	5.30	91.85

Data is expressed as mean ($n = 6$).

Table 2
Inter day precision and accuracy for mianserin and *N*-desmethylmianserin

Compound	Nominal concentration (ng/ml)	Estimated concentration (ng/ml)	Precision (%C.V.)	Accuracy (%)
Mianserin	3.00	2.83	4.06	94.44
	15.00	15.52	9.80	103.46
	50.00	52.00	4.14	104.00
N-desmethylmianserin	1.50	1.47	8.29	98.22
	6.00	5.56	5.39	92.72
	13.00	12.25	5.79	94.28

Data is expressed as mean ($n = 18$).

Table 3
Stability of mianserin and *N*-desmethylmianserin in human plasma

Compound	Nominal concentration (ng/ml)	0 h		24 h	
		Mean estimated concentration (ng/ml)	S.D.	Mean estimated concentration (ng/ml)	S.D.
Bench top stability					
Mianserin	3.00	3.29	0.55	2.57	0.01
	50.00	50.62	1.74	49.66	4.18
N-desmethylmianserin	1.50	1.62	0.11	1.41	0.14
	13.00	13.16	0.19	13.49	1.19
Autosampler stability					
Mianserin	3.00	2.53	0.01	2.82	0.14
	50.00	41.64	6.16	44.18	0.85
N-desmethylmianserin	1.50	1.34	0.08	1.40	0.16
	13.00	11.32	0.19	12.15	1.01

Data is expressed as mean and standard deviation ($n = 3$).

Table 4
Freeze thaw stability of mianserin and *N*-desmethylmianserin in human plasma[a]

Compound	Nominal concentration (ng/ml)	Mean estimated concentration (ng/ml)	S.D.
Freeze thaw stability			
Mianserin	3.00	2.87	0.18
	50.00	47.97	2.45
N-desmethylmianserin	1.50	1.47	0.08
	13.00	12.49	0.41

Data is expressed as mean and standard deviation of $n = 4$ cycles, each cycle of three replicates.
[a] Commercial plasma from Prathma Blood Bank (previously frozen).

benchtop at room temperature for 24 h (Table 3). Autosampler stability showed that the extracted samples were stable for 24 h at 4 °C temperature (Table 3). The results of freeze thaw stability for mianserin and *N*-desmethylmianserin showed that they are stable up to 4 cycles (Table 4).

4.6. Application

The method was used to assay mianserin and its metabolite, *N*-desmethylmianserin, in human plasma samples obtained from comparative bioavailability study after administration of an oral tablet of 30 mg of mianserin. During this bioavailability study, we observed nausea, vertigo, dizziness and headache at 1.50 h post dose. These symptoms became severe by 2.5 h post dose and all the volunteers started showing hypotension and hypoglycemia symptoms. So this study was terminated at 3.5 h post dose. The mean plasma concentrations of mianserin and its metabolite, *N*-desmethylmianserin, in six healthy volunteers are given in Fig. 3. The reported T_{max} of mianserin and *N*-desmethylmianserin is 1–3 h and 2–4 h, respectively [2]. Therefore, even from the truncated data, we were able to calculate T_{max} and C_{max} of mianserin and its metabolite, *N*-desmethylmianserin in human plasma. We got the T_{max} value of mianserin and *N*-desmethylmianserin as 1.0 and 2.5 h respectively and C_{max} of mianserin and *N*-desmethylmianserin as 42.08 and 9.08 ng/ml, respectively, which is quite comparable with the reported data [2].

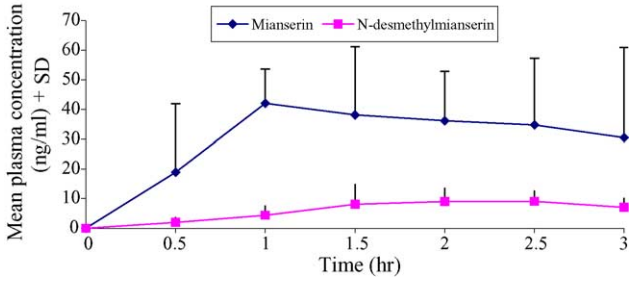

Fig. 3. Mean plasma concentrations + S.D. ($n = 6$) of mianserin and its metabolite, *N*-desmethylmianserin.

5. Conclusion

A rapid, highly specific and sensitive method has been developed and validated to determine mianserin and its metabolite, *N*-desmethylmianserin in human plasma. The stability studies showed that mianserin and *N*-desmethylmianserin in human plasma are stable during short-term periods for sample preparation and analysis. This method was applied for simultaneous determination of mianserin and *N*-desmethylmianserin in plasma samples of healthy volunteers after administration of oral tablet of 30 mg of mianserin. This method can be used for simultaneous quantitation of mianserin and its major metabolite, *N*-desmethylmianserin in human plasma during pharmacokinetics studies and clinical trials.

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