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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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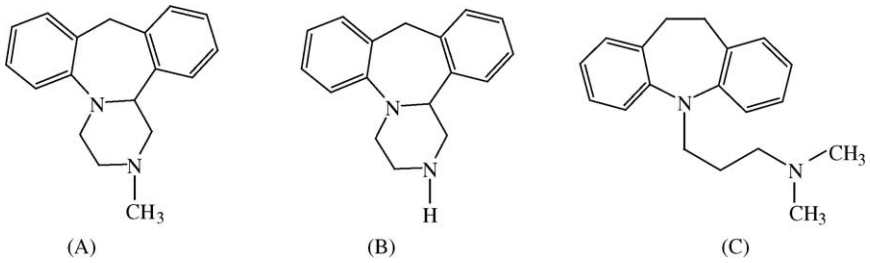

Fig. 1. Structure of mianserin (A), *N*-desmethylmianserin (B) and imipramine (C).

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-