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Determination of mesoridazine by liquid chromatography–tandem mass spectrometry and its application to pharmacokinetic study in rats

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ABSTRACT

The object of the present study was to develop and validate an assay method of mesoridazine in rat plasma using liquid chromatography-tandem mass spectrometry (LC–MS/MS). Plasma samples from rats were prepared by simple protein precipitation and injected onto the LC–MS/MS system for quantification. Mesoridazine and chlorpromazine as an internal standard (IS) were separated by a reversed phase C18 column. A mobile phase was composed of 10 mM ammonium formate in water and acetonitrile (ACN) (v/v) by a linear gradient system, increasing the percentage of ACN from 2% at 0.4 min to 98% at 2.5 min with 4 min total run time. The ion transitions monitored in positive-ion mode $[M+H]^+$ of multiple-reaction monitoring (MRM) were m/z 387 > 126 for mesoridazine and m/z 319 > 86 for IS. The detector response was specific and linear for mesoridazine at concentrations within the range 0.001–4 μg/ml and the correlation coefficient (R^2) was greater than 0.999 and the signal-to-noise ratios for the samples were ≥10. The intra- and inter-day precision and accuracy of the method were determined to be within the acceptance criteria for assay validation guidelines. The matrix effects were approximately 101 and 99.5% from rat plasma for mesoridazine and chlorpromazine, respectively. Mesoridazine was stable under various processing and/or handling conditions. Mesoridazine concentrations were readily measured in rat plasma samples after intravenous and oral administration. This assay method can be practically useful to the pharmacokinetic and/or toxicokinetic studies of mesoridazine.

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1. Introduction

Mesoridazine, (10-[2-(1-methyl-2-piperidyl)ethyl]-2-(methylsufinyl)phenothiazine), is an antipsychotic agent as phenothiazines class antipsychotics [1–5] and a metabolite of thioridazine by a side-chain sulfoxidation [6–10]. Although mesoridazine has also been used for the treatment of psychotic disease such as schizophrenia similar to thioridazine during a half century, this drug has its severe cardiac toxicity including irregular heartbeats and QT-prolongation. Because of its side effects, mesoridazine is under the situation of withdrawal in some market. One of the reasons for cardiac toxicity of mesoridazine was involved with QT interval prolongation by inhibition of potassium hERG (*human ether-a-go-go-related gene*) channels [11–14]. There have been several reports about the relationship between exposure level and toxicity of mesoridazine [15–18]. However, the pharmacokinetic and/or toxicokinetic analysis including the mechanisms of absorption, distribution, metabolism, and excretion (ADME) of mesoridazine has not been clear yet. The validated and advanced assay method in biological fluids can be helpful for these studies.

Mesoridazine was determined by the various assay methods including gas–liquid chromatography (GLC) [19–22], gas chromatography (GC) [23], and radioimmunoassay (RIA) [24], high-performance liquid chromatography (HPLC) [25–29]. Most of these methods are not good application to analyze mesoridazine levels in biological samples because of their low sensitivity and poor selectivity. Even though the determination of mesoridazine was also applied using liquid chromatography-tandem mass spectrometry (LC–MS/MS) methods with other Cytochrome P450 substrates or antipsychotics [30,31], these analytical methods were not enough for rapid and sensitive analytical method for further advanced pharmacokinetic studies using animal models for further

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pharmacological and toxicological studies because these methods were not focused on mesoridazine. An LC–MS/MS method has relatively short run times and higher sensitivity and selectivity in assays of various biological samples [32,33]. Multiple reaction monitoring (MRM) offers the selection of certain ions chosen by a specific precursor ion and collision-induced fragmental ion and a very powerful system for representative methods available for the determination of drug levels. There has been reported for LC–MS/MS method for mesoridazine including other antipsychotics. The method in the literature was not focused on mesoridazine, therefore, the analytical method showed time-consuming liquid–liquid extraction (LLE) method and less sensitive (~10 ng/ml) compared to this method.

The aims of this study, therefore, were to develop and validate a rapid, simple, selective and sensitive method to assay mesoridazine in rat plasma using one-step protein precipitation that is applicable to pharmacokinetic studies. Furthermore, this sensitive validated method can be applicable to further pharmacokinetic, toxicokinetic, and pharmacological studies for the safety usage of mesoridazine using LC–MS/MS equipped with an electro-spray ionization (ESI) mode.

2. Experimental

2.1. Chemicals

Mesoridazine, chlorpromazine, dimethyl sulfoxide (DMSO), polyethylene glycol (PEG) 400 and ammonium formate were purchased at high purity over HPLC, TLC or Bioreagent grade from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (ACN) of HPLC grade was from J.T. Baker (Phillipsburg, NJ, USA). Distilled water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA) in our laboratory.

2.2. Calibration standard and quality control samples

A stock solution of mesoridazine was prepared in DMSO at the concentration of 1 mg/ml. Working standard solutions were made by dilution of the standard stock solution in acetonitrile. Internal standard (IS) working solution (100 ng/ml chlorpromazine in ACN) was prepared from an IS stock solution (1 mg/ml). All solutions were stored at −20 °C before sample preparation. A standard calibration curve for mesoridazine was prepared by spiking working standard solution in rat plasma at the concentration ranges of 0.001, 0.003, 0.1, 1, 4 and 5 μg/ml. Quality control (QC) samples were prepared for mesoridazine concentrations of 0.003, 0.1 and 4 μg/ml in blank plasma.

2.3. Instrumentation and chromatographic conditions

The concentration of mesoridazine in rat plasma was quantified using Agilent 1200 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) with an API 4000 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with a turbo electrospray interface. The chromatographic separation of analyte was performed on a Hypersil GOLD C18 column (50 mm × 2.1 mm i.d., 3 μm; Thermo, Waltham, MA, USA) with a SecurityGuard C18 guard column (4 mm × 20 mm i.d., Phenomenex, USA) maintained at 35 °C. The separation was carried out a linear gradient of 10 mM ammonium formate in water and ACN (v/v), increasing the percentage of ACN from 2% at 0.4 min to 98% at 2.5 min. The flow rate was 0.4 ml/min and overall chromatographic run time was 4 min. The mass spectrometer was set in MRM mode using target ions at m/z 387 → 126 of mesoridazine and m/z 319 → 86 for chlorpromazine (IS). The optimized instrument conditions were as follow: ion spray voltage, 5500 V; source temperature, 550 °C; curtain gas (CUR), 10 psi; nebulizing gas (GS1), 50 psi; heating gas (GS2), 50 psi; collision energy (CE), 35 V for mesoridazine and 29 V for IS, respectively. Analytical data were performed with Analyst software Version 1.4.2. (AB Sciex).

2.4. Sample preparation

Before analysis, plasma samples were thawed at room temperature together with calibration standards and QC samples. A rat plasma sample (30 μl) was placed in a 1.5 ml microfuge tube and mixed with 270 μl of IS solution (100 ng/ml chlorpromazine in acetonitrile) to precipitate the plasma proteins. The mixture was vigorously mixed for 10 min using vortex mixer and followed by centrifugation at 10,000*g* for 10 min at 4 °C using temperature controlled microcentrifuge (Eppendorf, Hamburg, Germany). An aliquot of 5 μl supernatant was directly injected into the LC–MS/MS system for analysis after transferring to a fresh vial.

2.5. Validation

The analytical method for mesoridazine was determined with regard to selectivity, sensitivity, linearity, precision and accuracy. For selectivity validation, interference by endogenous compounds was assessed by comparison of the chromatograms for blank plasma samples. For sensitivity of mesoridazine, the limit of quantification (LOQ) was determined as the concentration with sufficient precision within 20% of the relative standard deviation (RSD) and acceptable accuracy between 80 and 120% of the theoretical value. In this study, the limit of detection (LOD) and LOQ values were 0.5 and 1 ng/ml with the signal-to-noise ratios over 3 and 10, respectively. To evaluate the linearity, using standard plasma solution containing mesoridazine in the concentration range of 0.001–4 μg/ml. Calibration curves were constructed by weighted ($1/x$) least squares linear regression of the peak area ratios (y) of mesoridazine to internal standard, versus the concentration (x) in μg/ml. Precision and accuracy of the method were estimated using replicate samples ($n = 5$). The intra- and inter-day precisions were estimated by analyzing the spiked samples at four different concentrations (0.001, 0.003, 0.1 and 4 μg/ml) in a single day and for five days, respectively. Precision was expressed as RSD at each concentration level. The percentage accuracy of deviation of the calculated concentration from theoretical concentration was expressed as the relative error (RE).

2.6. Matrix effect

The matrix effect for mesoridazine was assessed by analyzing 2 sets of standards at three concentrations (0.003, 0.1 and 4 μg/ml). The matrix effect was performed by comparing the peak areas of the analytes that was spiked into the post-precipitation matrix (set 1) with those of the reference standards prepared by spiking with the same concentration of mesoridazine in the mobile phase (set 2): matrix effect = mean peak area of an analyte added post-precipitation (set 1)/mean peak area of the same analyte standards (set 2) × 100. Each sample set was performed in triplicate.

2.7. Stability

The stability of mesoridazine was analyzed to evaluate the analyte stability in plasma samples under various storage or handling conditions. Assessment of mesoridazine stability in rat plasma included short- and long-term tests at the QC levels. To analyze short-term stability included: (a) freeze–thaw cycle stability; (b) exposure of samples to room temperature for 1 day; (c) exposure to 4 °C for 1 day; (d) exposure to −20 °C for 1 day; (e) exposure to

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