Abstract

A selective, sensitive and reliable method has been developed for quantification of Demethylphenidate in human plasma by using UPLC-MS/MS method. Validation was performed by USP general and specific requirements. The present method was developed for the determination of Demethylphenidate from human plasma subjected to solid phase extraction. Reversed phase Xerra RP 18 (250 x 4.6 mm, 5 µm) column with mobile phase acetonitrile-water (50:50) was used for separation. Detection was performed by mass spectrometer (AB Sciex API 4000). The analytes were separated using an electro spray ionization (ESI) in multiple reaction monitoring (MRM) mode with tandem mass spectrometry. Retention times were observed to be 1.60 min for Demethylphenidate and 0.81 min for internal standard. The calibration curves were linear over a concentration range of 0.210 ng/mL to 40.45 ng/mL for quantification of Demethylphenidate with the correlation coefficients demonstrating good linearity (0.9945-0.999). The lower limit of quantification was 0.210 ng/mL for Demethylphenidate. The instrument was found to be selective, precise and accurate. The stability parameters including specificity, sensitivity, precision, accuracy and stability were observed to be within the predefined limits. The method was validated in human plasma containing IS and was found to be suitable and validated for quantification of Demethylphenidate in human plasma for pharmacokinetic/biopharmaceutical studies.

Materials and Methods

Chemicals and reagents:

Demethylphenidate HCl was supplied by Pharcemex chemicals. Varilade HCl was supplied by Cipla Ltd. All solvents were used as HPLC grade. Acetonitrile and methanol of HPLC grade and obtained from J.T. Baker. Formic Acid and Ammonium Acetate were obtained from Merck. Distilled water was obtained from Saritas apparatus.

Standard solutions preparation

Stock solution preparation

To obtain a 1.0 mg/mL working stock solution of Demethylphenidate hydrochloride, 10 mg of Varilade HCl was dissolved in 10 mL of 0.1% HCl. The resulting solution was filtered and preserved for further use.

Preparation of standard solution

The Varilade standard solution (ST) was diluted by volumetric flask to obtain desired concentration.

Preparation of calibration curve (CC) standards and quality control (QC) samples

Appropriate dilutions of the stock solutions with water were made subsequently in order to prepare the working standard solution in the range of 0.010 mg/mL to 4.010 mg/mL. All the solutions were stored in a refrigerator between 2°C and 8°C. Calibration standards and quality control samples, in the range of 0.020 mg/mL to 0.040 mg/mL, were prepared for calibration. Accuracy, precision, and quality control stability were assessed by preparing 6 calibration standards, 5 controls and 2 samples for each batch of drug plasma with appropriate volume of working solution.

Sample preparation

Sample preparation was performed by acetonitrile-water (50:50) mixture taken in a centrifuge tube containing 700 µL of plasma. The mixture was vortexed and centrifuged at 3,500 rpm for 10 minutes. The supernatant was transferred into a volumetric flask.

Production of Solvent for Acetonitrile: (MeCN) and Water (H2O) 35:65 (v/v)

5.0 mL of acetonitrile and 35.0 mL of water were mixed well and transferred into a 50 mL glass vial. Then, 2.0 mL of acetic acid was added to get the mixture.

Sample preparation

Sample preparation was performed by solid phase extraction. To a 100 µL sample of plasma, 500 µL of acetonitrile-water (50:50) mixture was added, vortexed and centrifuged at 3,500 rpm for 10 minutes.

Sample extraction

After centrifugation for 10 min, the supernatant was transferred into a volumetric flask of 100 mL.

Results

Chromatographic and mass spectrometric conditions

UPLC separation was carried out on a Xerra RP 18 (4.6 x 150 mm, 5 µm) column using mobile phase acetonitrile-water (50:50) at a flow rate of 0.4 mL/min. The column temperature was maintained at 30°C. The injection volume was 10 µL. The acquisition time was 30 min. The data obtained from the analytical column was introduced directly to the MDSMS system using ESI source in the positive mode. Source specific and specific mass spectrometric parameters are given in Table 1.

Method Validation

Specificity and selectivity

Six plasma samples from six individual healthy donors receiving no medication were analyzed and assessed for the potential interference with endogenous substances. The apparent response at the retention time of drug and internal standard were compared with the response over the range of 50% at 150% spike level. The standard deviation and %CV between these peak areas for each analyte was calculated and the results were compared with the calibration. The results of the chromatograph and mass spectrometry for intact drug and its metabolite were found to be acceptable. The results of in vitro stability studies were found to be compatible with the in vivo stability studies and the results of the in vivo stability studies were found to be suitable and acceptable.

Precision and accuracy

For the high concentration samples, the precision and accuracy for three different levels were evaluated at 6 replicates. In the recovery study, 3 concentrations levels, in our study we got 95% and 98% recovery for Demethylphenidate and Varilade, which are within the acceptance criteria.

Reciprocity

The correlation coefficients observed between the peak areas of two different Demethylphenidate concentrations were evaluated by using SPE method. The results obtained were observed to be within the acceptance criteria. The results are depicted in Table 4.

Conclusion

The method was applied successfully to the analysis of plasma samples obtained for pharmacokinetic, bioavailability or biopharmaceutical study after therapeutic doses of Demethylphenidate and Varilade. The use of this method in the study of Demethylphenidate in human plasma. Because of the rapid short columns, the method is easy to follow and can be adapted for clinical drug monitoring.

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