Rapid Ultra flow liquid chromatography –tandem mass spectrometric method for quantification of Dexmethylphenidate in human plasma using Sold Phase Extraction

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Abstract

A selective, sensitive and reliable method have been developed for quantification of Dexmethylphenidate in human plasma by using UFLC–MS/MS method. Venlafaxine was used as an internal standard(IS). The extraction of the Dexmethylphenidate from human plasma was performed using solid phase extraction. Reverse phase- Xterra RP 18 (4.6x150mm,5µm) column was employed for chromatographic separation of Dexmethylphenidate and Venlafaxine(IS) for MS/MS detection at 1 ml/min flow with mobile phase combination using 90:10 (v/v) Methanol: 5mM Ammonium Acetate (pH 4.00). Detection was performed at transitions of m/z 234.200→84.000 for Dexmethylphenidate and m/z 278.100→ 58.100 for Venlafaxine by positive electro-spray ionization (ESI+) in multiple reaction monitoring (MRM) mode using tandem mass spectrometry. Retention times were observed with in 3.0 min for both Dexmethylphenidate and Venlafaxine. The calibration curves were linear over a concentration range of 0.201 ng/mL to 80.434 ng/mL for quantification of Dexmethylphenidate with the correlation coefficients demonstrating good linearity (0.996-0.999). The lower limits of quantification were 0.201 ng/mL for Dexmethylphenidate. The developed method was compared in the terms of validation parameters including specificity,linearity, sensitivity, precision, accuracy and stability. Matrix based samples were stable at room temperature for >8 hrs, processed samples were stable at least for >20 hrs and also stable at six freeze-thaw cycles. No significant effect was observed due to the presence of potentially interfering drugs like Paracetamol, Ibuprofen, Caffeine and Aspirin in the plasma sample and also presence of hemolysed or lipemic in plasma sample. The method was validated in human plasma containing K2EDTA as the anticoagulant. This validated method was successfully applied for quantification of Dexmethylphenidate in human plasma for pharmacokinetic bioequivalence study.

Materials and Methods

Chemicals and reagents :

Dexmethyl phenidate HCl was supplied by Pharchem chemicals. Venlafaxine HCl was commercially procured from Teva active pharmaceutical ingredients. All the solvents used were of HPLC grade. Acetonitrile and methanol were of HPLC grade and obtained from J.T.Bakers. Formic acid and Ammonium Acetate were obtained from Merck. Double distilled water is obtained from Sartorius apparatus.

Standard solutions preparation

Stock solution preparation

Approximately 10 mg of Dexmethyl phenidate / 10 mg of Venlafaxine (IS) working standard was weighed and transferred to 10.0 mL volumetric flask, to this 5.0 mL of Methanol was added and sonicated to aid dissolution and the final volume was made up with Methanol.

Preparation of internal standard dilution

The Venlafaxine internal standard (ISTD) dilution of about 500 ng/mL from the ISTD stock solution (IS stock) using (50:50methanol: water) as the diluent was prepared.

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

Appropriate dilutions of the stock solutions with diluent were made subsequently in order to prepare the working standard solution in the range of 10.0 ng /mL to 4.0 µg/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 0.201 ng/mL to 80.434 ng/mL were prepared for calibration. Accuracy and precision, quality control and stability assessment was done by spiking 0.5mL of drug free plasma with appropriate volume of working solution.

Solutions used:

50%v/v Methanol in Water: Prepared the diluent by mixing methanol and water in 1:1 ratio.

5 mM Ammonium Acetate(PH-4): Weighed accurately about 0.1925g of ammonium acetate into 500 mL volumetric flask and make up to the mark with HPLC Grade water, mixed well and transferred it into 500 mL reagent bottle. Adjusted the pH of buffer for 4.0 with formic acid.

Mobile phase Preparation: [MeOH : 5mM Ammonium Acetate(PH-4) (90:10)]:Taken 900 mL of methanol into 1000mL reagent bottle and added 100mL of 5 mM ammonium acetate(PH-4) mixed well and sonicated for about 10 minute and filter through 0.45µm nylon membrane filter.

Rinsing Solvent for Auto Injector **[MeOH : Milli – Q water (80:20)]**: Taken 800mL of Methanol in a 1000mL reagent bottle, added 200mL of water, mixed well, sonicated for 1 minute. Total preparation volume was adjusted according to the requirement, by keeping the ratios constant.

5% ammonia solution: Taken 5 ml of ammonia solution in to 100mL volumetric flask containing 30mL Milli-Q water, mixed well and make up to the mark with Milli-Q Water. Transfered it into a reagent bottle.

Sample preparation

Retrieved the frozen CC, QC and subject samples from the deep freezer and thawed in water bath maintained at room temperature, vortexed to mix. Removed the caps from the polypropylene tubes. An aliquot of 50 µL of CC, QC and subject samples in a ria vial tube was spiked with 20 µL of the IS solution (500 ng/mL) and vortex mixed for 30secs. Then 20 µL of 5 %v/v ammonia solution was added and vortex-mixed for 30 secs. SPE cartridges were conditioned with 1mL methanol, equilibrated with 1mL water and loaded the sample into SPE cartridges. The SPE cartridges were washed twice with 1mL of 0.1% formic acid and 1mL of Water (HPLC grade). The samples were eluted with 1mL methanol into pre labeled Ria vials. Then evaporated the samples to dryness under the nitrogen pressure with 50°C of temperature. The residual was reconstituted in 50 µL of a mobile phase and centrifuged at 4,000rpm for 5 min. Then, 10 µL aliquot was injected on to the LC-MS/MS system. For optimal stability, the auto-sampler temperature was set at 4 ˚C.

Data processing :

Chromatograms were acquired on a API 3200 of MDS Sciex tandem mass spectrometry equipped with Electrospray ionization (ESI) and connected to a PC runs with the standard software Analyst -1.5.1. Mass spectroscopic detection was performed on a Triple quadrapole instrument (API 3200 of MDS Sciex). The calibration curve is constructed by weighted 1/x² least-square linear regression analysis of the peak area ratio (drug/ISTD) vs. the concentration of drug.

Results

Chromatographic and mass spectrometric conditions

UFLC separation was carried out on a Xterra RP 18 (4.6x150mm, 5µm) with mobile phase A-methanol and B-5mM Ammonium Acetate(PH-4) (A:B=90:10) at a flow rate of 1 mL/min and the column temperature was maintained at 35˚C. The sample injection volume was 10 µL and the analytical run time was 3.0 min. The eluent from the analytical column was introduced directly to the MS/MS system using ESI source in the positive ion mode. Source specific and compound specific mass spectrometric parameters are given in Table-1.

Method Validation:

Specificity and selectivity

Six human plasma samples from six individual healthy donors receiving no medication were extracted and analyzed for the assessment of potential interferences with endogenous substances. The apparent response at the retention time of drug and internal standard were compared to the response at the lower limit of quantification (LLOQ) for drug to the response at the working concentration for internal standard. Observed retention times were about 1.925 min (Dexmethyl phenidate) and 0.688 min (Venlafaxine) respectively. No additional peak due to endogenous substances that could have interfered with the detection of the compounds of interest was observed. Representative chromatograms of extracted human blank plasma and extracted human blank plasma spiked with Drug and IS are shown in Fig.1.A and 1.B.

Linearity

The mean accuracy and precisions for back calculated concentrations of each standard calculated from calibration curves were tabulated as Table 2.

Recovery

The recoveries of Dexmethyl phenidate and Venlafaxine were evaluated with 6 replicates at 3 different concentration levels. In our method we got 94.8% and 91.6% recovery for Dexmethyl phenidate and Venlafaxine, which are within the acceptance criteria.

Precision and accuracy

The accuracy and precision for inter day and intra day was tabulated for drug in Table 3.

Stability

In our study quality control plasma samples were used subject to bench top (>8h), Auto injector (10–84 h), six freeze–thaw (-80 to +20 °C) cycles, wet extract (> 20 h) at room temperature, wet extract at 2-8°C (>20h) and long term (95 days) at deep freezer (at -80 °C) tests are performed. The values obtained for present stability studies are tabulated (Table 4), which are within the acceptance criteria.

Application of the method

The present method was applied for a randomized cross-over bioequivalence study of two different Dexmethyl phenidate preparations in 12 healthy male volunteers. After single oral administration of the drug blood samples were collected at a suitable time intervals. This method was successfully used to measure the plasma concentrations of Dexmethyl phenidate.

Parameter	Dexmethyl phenidate	Venlafaxine
Q1	234.200	278.100
Q3	84.00	58.00
Dwell Time(msec)	300 msec	300 msec
Declustering Potential (DP)	30	30
Entrance Potential (EP)	10	10
Collision Cell Entrance Potential (CEP)	16.44	17.89
Collision Energy (CE)	30	32
Collision Cell Exit Potential (CXP)	10.00	10.00
Scan Type	MRM	
Polarity	Positive	
Curtain Gas (CUR)	25.00	
Collision Associated Dissociation (CAD)	6.00	
Ion Spray Voltage (ISV)	5000	
Heater Temperature (TEM)	500 C	
Nebulizer Gas (GS1)	45.00	
Heater Gas (GS2)	40.00	
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Table-1:Source specific and compound specific mass spectrometric parameters

Nominal concentration (ng/mL)	Mean Accuracy (%)	Precision (% RSD)
80.434	93.4	2.3
70.000	94.6	7.1
55.006	96.8	1.8
40.154	90.8	4.2
20.077	101.5	4.3
5.019	95.3	5.6
0.402	98.6	3.8
0.201	92.8	1.3

Table 2: Back calculated concentrations from calibration curves

Nominal Conc. (ng/mL)	0.205	0.594	33.002	58.000
Intra-day accuracy(%)(day1)	92.3	98.5	99.1	95.6
Intra-day precision(%)(day1)	7.2	2.5	3.4	6.4
Intra-day accuracy(%)(day2)	93.1	95.8	99.8	104.8
Intra-day precision(%)(day2)	5.6	3.2	1.5	9.3
Intra-day accuracy(%)(day3)	97.6	93.5	104.5	92.4
Intra-day precision(%)(day3)	1.9	3.5	2.3	1.5
Overall accuracy (%)	94.3	98.7	94.3	97.1
Overall Precision (%)	3.2	4.3	5.1	6.6
Number of determinations	18	18	18	18

Table 3.Assessment of Accuracy and precision of the method

Nominal Conc. (ng/mL)	58.000		0.205	
	Precision	Accuracy	Precision	Accuracy
Freeze thaw stability	1.4	99.4	3.1	101.3
Bench top stability	3.2	101.4	4.6	102.3
Wet extract stability at room temperature	5.6	111.5	6.5	105.3
Wet extract stability at 2-8°C	9.1	99.5	4.7	98.7
Auto sampler stability	2.3	110.4	2.1	95.4
Long term stability	3.2	99.8	7.1	93.1
Interim storage stability at -25 °C	4.1	93.4	10.3	90.9

Table 4.Stability results

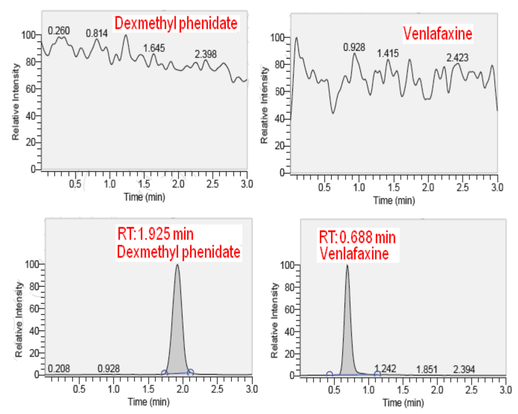

Fig.1.A and 1.B. Representative chromatograms of extracted human blank plasma and extracted human blank plasma spiked with Drug and IS

Conclusion

The method was applied successfully to the analysis of plasma samples obtained for pharmacokinetic, bioavailability or bioequivalence study after therapeutic doses of Dexmethyl phenidate. The established LC-MS/MS method is sensitive and suitable for the study of Dexmethyl phenidate in human plasma. Because of the relative short chromatographic runtime, the method is easy to follow and can be adopted for clinical drug monitoring.

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