Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 5, 2011

Research Article

A RUGGED AND ECONOMIC METHOD FOR THE ESTIMATION OF ARIPIPRAZOLE IN HUMAN SERUM BY LCMS/MS DETECTION FOR CLINICAL TRIALS

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Received: 12 Feb 2011, Revised and Accepted: 18 May 2011

ABSTRACT

A convenient method for the determination of Aripiprazole in human serum has been developed. Aripiprazole was extracted from an aliquot of human serum using liquid-liquid extraction method and then injected into a liquid chromatograph, equipped with mass spectrometry detector. Internal standard method was used for quantitation of Aripiprazole. The calibration curve for standard was linear over the range from 1 to 200 ng/ml. The limit of Quantitation (LOQ) was determined to 1 ng/ml. A common solid phase extraction procedure for the isolation of drug was developed from serum samples. The samples were analyzed on API 3200 Triple quadrapole mass spectrometer using Chromolith, RP-18e column in atmospheric pressure electro spray ionization. The mobile phase composition was an isocratic mixture of 5 m M Ammonium Acetate in water: Acetonitrile (25 : 75 %v/v). The method was validated over a linear range of 1 to 200 ng/ml and the limit of quantification was 1 ng/mL. Recoveries were observed above 65% for the analyte. The storage stability of Quality control samples was investigated under various conditions.

Keywords: Aripiprazole, LC-MS/MS, Pharmacokinetic studies

INTRODUCTION

Aripiprazole (Abilify™) is an atypical antipsychotic agent, recently approved by US Food and Drug Administration as the sixth second-generation antipsychotic for the treatment of schizophrenia, schizoaffective disorders, bipolar disorder and adjuvant therapy for major depression[1]. Dehydroaripiprazole, its main active metabolite, has an affinity for dopamine D_2 receptors and thus has some pharmacological activities similar to that of its parent compound[2,3].

Aripiprazole is considered a partial dopamine D_2 and D_3 receptor agonist, partial 5-HT_{1A} receptor agonist and $5HT_{2A}$ receptor antagonist[4,6]. The partial agonist activity at the D_2 receptor may explain its efficacy in the treatment of both positive and negative symptoms of schizophrenia and its low probability for extra-pyramidal symptoms[7]. Its side-effects includes weight gain, QTc prolongation and hyperprolactinemia. Neverthless, it is not devoid of side effects such as nausea, vomiting, lightheadedness, somnolence, constipation and postural dizziness[8]. Monitoring drug concentrations in serum may not only ensure effectiveness and safety, but also preclude side-effects, especially for psychiatric patients with poor communication skills and impaired selfcare. The commonly recommended therapeutic dosage prescribed for aripiprazole ranges from 10 mg/d to 30 mg/d, with the starting dose of 10 mg/d or 15mg/d[7]. However, the relationship between aripiprazole concentration in serum and drug effectiveness has not been well established[2].

Previous studies on detection and quantification of aripiprazole were mostly confined to high-performance liquid chromatography (HPLC) [9,10]. For example, HPLC with photodiode array detection[10], HPLC-tandem mass spectroscopy[11,12], LC-tandem mass spectroscopy[2,13,14] and ultra-performance LC-electrospray tandem mass spectroscopy[15].

The proposed LC-MS/MS method utilizes a simple and less time consuming liquid-liquid extraction procedure for sample extraction and allows the determination of aripiprazole at low concentration levels.

In this article, we have validated a simple and selective high performance liquid chromatography couples with mass spectrometry method for the detection of Aripiprazole from human serum as per USFDA guidelines [16]. Eplerenone was used as internal standard.

Aripiprazole was extracted from an aliquot of human serum using liquid-liquid extraction method and then injected into a liquid chromatograph, equipped with mass spectrometry detector. Internal standard method was used for quantitation of Aripiprazole. Linear regression with $1/X^2$ weighting was performed to determine the concentration of the drug from serum samples. All regressions and figures presented in this validation report were generated by analyst software version 1.4.1.

MATERIALS AND METHOD

Chemicals and Reagents

All reference standards were purchased from Synfine Research, Canada and Sigma Aldrich, USA. Ammonia, Acetonitrile, methanol ammonium acetate, Ethyl acetate and Dichloro methane were obtained from Qualigens (Worli, Mumbai) India. De-ionized water was prepared on Milliq Laboratory Plant (Millipore, Bedford. USA). Organic solvents and reagents used were of analytical grade.

Instrumentation

The chromatographic system consist of LC-2010HT (Shimadzu,Japan) equipped with SIL-HTc autosampler, DGU-14 A Vacuum Degasser, LC-10-VP Pump, CTO-20 A Column Oven. Mass Spectrometric analysis were conducted using API 3200 Q-trap Triple quadrapole instrument (Applied Biosystem, Sciex, Concord, Canada), equipped with a pneumatically assisted APCI(heated nebulizer) and ESI (electro spray) ionization source, which was operated in negative mode.. The whole system was controlled using Analyst software version 1.4.1(Applied-Biosystem-Sciex, Concord, Canada). HLB 30mg 1cc solid phase extraction cartridge (Oasis, Waters, USA) was used for sample clean up solid phase extraction procedure.

Liquid chromatography and mass spectrometry conditions

Chromatographic separation was achieved on Phenomenax C18 (50 * 4.6 mm) analytical column maintained at 25 °C temperature. Mobile phase composition was a mixture of 5 m M Ammonium Acetate in water and acetonitrile with ratio of 25:75 %v/v. Flow rate was maintained at 1.0 mL / min. The run time was about 2.25 minutes and the retention time of Aripiprazole as well as internal standard (Eplerenone) were about 1.61 and 0.78 minutes.

Mobile phase was introduced in to the mass spectrometer via the ESI source operating in the positive ion mode under multiple reaction monitoring conditions (MRM). Quantitation was performed using selective ion Monitoring (SIM) mode at m/z 448.06 for analyte. Nitrogen was used as the nebulizing and

curtain gas. Fragmentation was achieved with nitrogen. Dwell for each transition was 200 msec. The temperature was 650 °C and the resolution was set as unit and ion spray voltage was set at 5500 volts.

Stock solution, Calibration standard solutions and quality control standard solutions

Stock solution of Aripiprazole was prepared by accurately weighing and dissolving reference standards in methanol to give the final concentration of 100 µg/mL. Stock solution of internal standard i.e. Eplerenone was obtained in methanol at a concentration of 2000 ng/mL and was used directly for serum sample preparation. Stock solution of Aripiprazole was further diluted with methanol to give serial concentrations of 20, 40, 100, 200, 500, 1000, 2000, 4000 ng/mL to form working solution of calibration standards. Quality control standard solutions of Aripiprazole were prepared in methanol at concentration of 60, 300 and 3000 ng/mL. Working solution of analytes as well as internal standard was stored at 4 °C.

Sample Preparation

A common procedure for the isolation of Aripiprazole from serum samples prior to LC-MS/MS was developed. For analysis of analyte, 25 µL of Eplerenone, 2000 ng/mL and 475µL of 5% aqueous ammonia solution were added to 500 µL human serum. The mixture was vortexed for several seconds , 3 ml of extraction solvent (Ethyl Acetate : Dichloromethane (80:20 % v/v)) was added, then vortexed for a minute, centrifuged at for 2 minutes , transferred 2 ml of organic solvent in drying tubes to dry the evaporate the organic layer under nitrogen gas flow. The dried extract was further reconstituted in water : acetonitrile: methanol (20: 40: 40 % v/v).

Method Validation [16]

The method was validated for specificity, linearity, precision, accuracy, recovery and stability.

Specificity

Specificity was determined by analyzing six different lots to check interference at the retention time of Aripiprazole and Eplerenone.

Linearity

Linearity of calibration standards (n=9) for all three analytes were assessed by plotting peak area ratio (y) of analyte to internal standard against the concentration (x) of analytes. The calibration curves were constructed by weighted ($1/x^2$) least square linear regression.

Precision and Accuracy

To determine intraday precision, replicate analysis of quality control sample was performed on the same day. The run consisted of one set of calibration standards and five replicates each of low, middle and high concentration quality control sample. The inter-day precision was accessed by analysis of batches on different days. Precision was expressed as % CV.

Recovery

The extraction efficiency of Aripiprazole and Eplerenone were expressed in term of recovered concentration of analyte and internal standard added to a biological matrix prior to extraction (recovery QC) versus concentration obtained with biological sample where analyte and internal standard were added following extraction. All analysis was performed in triplicate at three analyte concentrations. Percentage drug recovery with corresponding % CV was determined for each serum sample fortified with analyte. To evaluate matrix effect, blank serum was subjected to sample pretreatment described above. The resulting solution was spiked with working standard solution to prepare solutions containing analytes at three different concentrations (lower, middle and high). Matrix enhancement/suppression of ionization was evaluated by comparing the peak areas of processed spiked samples with corresponding neat standard solutions prepared in mobile phase.

Stability

The stability of analyte was tested by short-term stability, long-term stability and freeze-thaw stability. To test stability of these analytes in serum, six replicates of each were stored under different conditions. The short term stability tests were performed at 25 °C temperatures for 24 hours. The autosampler stability tests were performed at 10 °C temperatures for 48 hours. Freeze-thaw stability testing was performed for three frozen and thawed cycles. "Freezing" was performed at -20 °C for 24 hours and "thawing" at room temperature. The results of freeze-thaw and short and long-term stability were compared with the average of intra-day calibration curves.

RESULTS

Limits of Quantitation

The lower limit of quantitation i.e., lowest standard level with a coefficient of variation less than 20 % was 1 ng/ml, for Aripiprazole with between-batch coefficient of variation 2.03 % and accuracy 100.01 %.

The Upper limit of Quantitation for Aripiprazole was 200 ng/ml and between-batch coefficient of variation of 5.91 % and accuracy were 95.68 %

Linearity and Sensitivity

Good linearity was achieved over the concentrations in the range of 1 to 200 ng/mL for analyte and coefficient of correlation were found to be better than 0.9975.

The data of linearity are listed in Table 1. The limit of quantification (LOQ) was 1 ng/mL using 500 µL of serum for Aripiprazole with accuracy, precision ≤ 20%. Back calculations were made from the calibration curves to determine analyte concentration of each calibration standard. The regression equations of these curves and their coefficients were calculated as follows:

Aripiprazole, $y = 0.0631(\pm 0.0196)x + 0.1765(\pm 0.3438)$, ($R = 0.9975$);

Where y is the peak area ratio of analytes to internal standard, X is the concentration of analytes.

Specificity

Presence of any interference from endogenous substances was estimated by analyzing human serum from six different lots of analyte (s) free human serum including hemolised and lipemic serum used for analysis .No significant interference was observed at the retention times of both analyte (s) and internal standard.

Precision, Accuracy and Recovery of Method

A good precision and accuracy was observed in this method. The intra and inter-day precision and accuracies are summarized in table 2 and 3. The intra-run CV (%) were less than 5.68, and inter-run CV (%) were less than 11.93. The intra-run accuracies (MRE) were found to be in the range between 89.98 % - 99.45 % for analyte the inter-run accuracies were found in the range of 94.81 % - 105.03 %.

Recovery

The recovery of the method was found above 65% for Aripiprazole as shown in table no 4. The data were found satisfactory for pharmacokinetic studies.

Samples Stability

Aripiprazole showed a good stability under the conditions used for storage and processing. The analytes were stable in human serum when stored at ambient temperature for at least 24 hours and showed good autosampler stability at 10 °C for 48 hours. Aripiprazole was stable under the influence of three freeze / thaw cycles. Stability data of analytes under various storage and freeze thaw conditions were mentioned in table 5.

Table 1: Table shows intermediate precision, accuracy and linear regression parameters of Aripiprazole determination in human serum by LC-MS/MS detection

Added Concentration (ng/mL)	Mean measured Concentration (n = 5) (ng/mL)	Precision SD	% CV	Accuracy (%)
1.00	1.00	0.02	2.03	100.01
2.00	1.96	0.24	12.33	98.11
5.00	5.59	0.67	12	111.75
10.00	10.07	0.95	9.45	100.74
20.00	21.78	2.41	10.15	108.90
50.00	52.59	2.21	9.67	105.18
100.00	106.5	8.66	8.13	106.50
200.00	191.37	11.3	5.91	95.68

Calibration curve
Slope 0.0631 ± 0.0196
Intercept 0.1765 ± 0.3438 Correlation Coefficient 0.9975

Table 2: Table shows intra- run precision and accuracy for Aripiprazole of QC (n = 5)

Added Concentration (ng/mL)	Mean measured Concentration (ng/mL)	Standard deviation	CV (%)	Accuracy (%)
Aripiprazole				
3.00	2.86	0.13	4.48	95.21
15.00	14.92	0.68	4.53	99.45
150.00	143.93	8.18	5.68	95.96

Table 3: Table shows inter- run precision and accuracy for Aripiprazole of QC (n = 36)

Added Concentration (ng/mL)	Mean measured Concentration (ng/mL)	Standard deviation	CV (%)	Accuracy (%)
3.00	2.99	0.18	5.99	99.83
15.00	15.75	0.99	6.26	105.03
150.00	149.71	12.95	8.65	99.80

Table 4: Table shows recovery of Aripiprazole from human serum

	LOW QC (3.00 ng/mL)		MED QC (15.00 ng/mL)		HIGH QC(150.00ng/mL)	
	Control Peak Response	Treated Peak Response	Control Peak Response	Treated Peak Response	Control Peak Response	Treated Peak Response
	25396	17401	116951	85755	1049033	753243
	25729	17533	119974	78563	1028633	715435
	25353	17465	119157	79879	1010426	747590
	26792	18303	122456	OL	1078843	727283
	25091	17693	118390	74654	1044066	727503
	25597	16586	123299	76890	1062804	682565
N	6.00	6.00	6.00	5.00	6.00	3.00
Mean	25659.67	17496.83	120037.83	79148.20	1045634.17	725603.17
SD	596.30	553.08	2429.26	4178.03	24249.93	25335.70
CV (%)	2.32	3.16	2.02	5.28	2.32	3.49
Recovery	68.19		65.94		69.39	

OL: Outliared, not included in analysis

Table 5: Table shows stability data of Aripiprazole.

Nominal Concentration (ng/mL)	Short term stability at about 25± 5 °C for 24 hours.		Autosampler stability at about -10 °C for 48 hours.		Three freeze / thaw cycles.	
	Lower Quality Control	Higher Quality control	Lower Quality Control	Higher Quality control	Lower Quality Control	Higher Quality control
	3.00 (ng/mL)	150.00 (ng/mL)	3.00 (ng/mL)	150.00 (ng/mL)	3.00 (ng/mL)	150.00 (ng/mL)
Mean found Concentration (ng/mL)	3.10	143.42	2.73	131.64	3.25	151.57
standard deviation	0.10	8.09	0.15	6.24	0.06	4.50
CV (%)	3.10	5.64	5.56	4.74	1.89	2.97
% change(bias)	5.64	-11.27	-6.41	-12.67	-10.04	10.11

DISCUSSIONS

A convenient method for the determination of Aripiprazole in human serum has been developed. The analytical method was validated as per the well defined standard operation procedure of Bioanalytical laboratory. The calibration curve for the standard was linear over the range from 1 to 200 ng/mL.

ACKNOWLEDGEMENT

I would like to thank the principal and my collegues of Smt. N.M. Padalia Pharmacy College for providing all support for carrying out this research study.

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