Journal of Chromatography B, 925 (2013) 20–25

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

SPE–UPLC–MS/MS method for sensitive and rapid determination of aripiprazole in human plasma to support a bioequivalence study

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ARTICLE INFO

Article history:
Received 11 November 2012
Accepted 20 February 2013
Available online 27 February 2013

Keywords:
Aripiprazole
Aripiprazole-d8
UPLC–MS/MS
Solid-phase extraction
Human plasma
Bioequivalence

ABSTRACT

An improved and rugged UPLC–MS/MS method has been developed and validated for sensitive and rapid determination of aripiprazole in human plasma using aripiprazole-d8 as the internal standard (IS). The analyte and IS were extracted from 100 μL of human plasma by solid-phase extraction using Phenomenex Strata-X (30 mg, 1 cc) cartridges. Chromatography was achieved on an Acquity UPLC BEH C18 (50 mm × 2.1 mm, 1.7 μm) analytical column using methanol: 10 mM ammonium formate (85:15, v/v) as the mobile phase with isocratic elution. Quantitation was done using multiple reaction monitoring in the positive ionization mode. The linearity of the method was established in the concentration range 0.05–80 ng/mL. The mean extraction recovery was greater than 96% across QC levels, while intra- and inter batch accuracy and precision (% CV) values ranged from 97.4 to 101.9% and from 1.20 to 3.72% respectively. The relative matrix effect in eight different lots of plasma samples, expressed as % CV for the calculated slopes of calibration curves was 1.08%. The stability of aripiprazole was studied under different storage conditions. The validated method was used to support a bioequivalence study of 10 mg aripiprazole formulation in 36 healthy Indian subjects.

1. Introduction

Aripiprazole (ARP) is a novel atypical antipsychotic drug with unique pharmacological properties and is used for the treatment of schizophrenia, acute manic and bipolar I disorder [1,2]. It acts as a partial dopaminergic agonist and acts both on postsynaptic dopamine D_2 receptors as well as presynaptic autoreceptors. Further, it displays partial agonism at serotonin ($5\text{-}HT_{1A}$/$5\text{-}HT_{2C}$) receptors and antagonism at $5\text{-}HT_{2A}$/$5\text{-}HT_7$ receptors [3]. Due to its unique pharmacological properties it is classified as a third generation antipsychotic with an ability to lower plasma prolactin levels coupled with less weight gain [4]. The oral bioavailability of ARP is about 87% and is highly protein bound (>99%), mainly to albumin. It is rapidly absorbed after oral administration and gets extensively metabolized in the liver by cytochrome P450 enzymes 3A4 and 2D6. The major active metabolite of ARP is dehydroaripiprazole, which represents 40% of the circulating dose of the parent drug [5]. ARP displays a linear pharmacokinetics for dose strength of 5–30 mg/day in healthy volunteers [6].

As the literature reveals, there are several methods to determine ARP in different biological matrices such as rat brain and plasma [7,8] human serum [9] and human plasma [10–19]. Majority of these methods are based on LC–MS/MS [8–11,15,16,18] and few others by HPLC-UV/DAD [7,13,14,17] or GC–MS [12]. A comparative summary of all chromatographic methods developed for ARP in different biological matrices is shown in Table 1.

The aim of the present work was to develop an improved, sensitive and rugged UPLC–MS/MS method compared to our previous LC–MS/MS [19] method by using a deuterated internal standard and solid phase extraction (SPE) for sample preparation. The method requires only 100 μL human plasma for processing and demonstrates excellent chromatographic efficiency (1.2 min per sample) and sensitivity. The proposed method has been successfully applied to support a bioequivalence study with healthy volunteers.

2. Experimental

2.1. Chemicals and materials

Aripiprazole (purity, 99.2%) and aripiprazole-d8 (IS, purity, 99.0%) (Fig. 1) were obtained from Clearsynth Labs (P) Ltd. (Mumbai, India). HPLC grade methanol, analytical grade reagent formic acid (90.0%) and ammonium formate were obtained from S.D. Fine Chemicals Ltd. (Mumbai, India). Deionized water was prepared using Milli-Q water purification system from Millipore (Bangalore, India).

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http://dx.doi.org/10.1016/j.jchromb.2013.02.022

Table 1
Salient features of chromatographic methods developed for aripiprazole in biological matrices.

S. no.	Detection technique	Extraction procedure; sample volume; internal standard	Mean extraction recovery	Column; mobile phase	Retention time; run time	Linearity (ng/mL)	Application	Ref.
1	HPLC-UV	LLE for rat plasma and PP for rat brain; 0.5 mL rat plasma and 1.0 mL brain homogenate; OPC-14558	89.2% (plasma) and 42.4% (brain)	Nova-pak phenyl (150 mm × 3.9 mm, 4.0 μm); ACN-methanol-20 mM sodium sulfate-acetic acid (27:25:48:1, v/v/v/v)	7.8 min; 15 min	10–2000 in plasma and 30–6000 ng/g in brain	Pharmacokinetic study in Sprague-Dawley rats at 10 and 30 mg/kg aripiprazole	[7]
2	UPLC–MS/MS	SPE; 0.1 mL rat plasma or brain homogenate; midazolam	77.8% (plasma) and 93.3% (brain)	Agilent eclipse plus C18 (50 mm × 2.0 mm, 1.8 μm); gradient of 0.1% FA in water-ACN	1.48 min (plasma) and 2.05 (brain); 2.0/3.0 min	0.5–100 in plasma and 1.5–300 ng/g in brain tissue	Pharmacokinetic study in albino Wistar rats at 30 mg/kg aripiprazole	[8]
3[a]	LC–MS/MS	PP; 0.03 mL human serum; dansyl-norvaline	95.2%	Luna C18 (50 mm × 2.0 mm, 5.0 μm); gradient of 0.1% FA in water-ACN	–; 10 min	2–1000	Clinical analysis of 22 Caucasian patients who received 9.75 mg aripiprazole	[9]
4[a]	LC–MS/MS	LLE; 0.4 mL human plasma; OPC-14714	92.5%	Chemcobond ODS-W (150 mm × 2.1 mm, 5 μm); 30 °C; 0.1% AA in water-ACN (65:35, v/v)	5.37 min; 7.5 min	0.1–100	–	[10]
5	LC-ESI–MS	LLE; 0.15 mL human plasma; estazolam	79.3%	Thermo hypersil Gold C18 (150 mm × 2.1 mm, 5 μm); ACN-water containing 0.1% FA and 30 mM AA (58:42, v/v)	2.8 min; 5.0 min	19.9–1119.6	Pharmacokinetic study with 10 mg aripiprazole twice a day in 11 schizophrenic patients	[11]
6[a]	GC–MS	SPE followed by derivatization; 0.5 mL human plasma; carteolol	75.4%	BPX5 5% phenyl polysilphenylene-siloxane capillary column (25 m × 0.22 mm i.d, film thickness 0.25 μm); carrier gas-helium	9.69 min; 17 min	15.6–500	Pharmacokinetic study with 10 mg aripiprazole per day in 7 psychiatric patients	[12]
7	HPLC-DAD	SPE; 0.6 mL human plasma; loxapine	95.7%	Varian Microsorb C8 (150 mm × 4.6 mm, 5 μm); 12.5 mM phosphate buffer, pH 3.5, containing 0.19% TEA-ACN (65:35, v/v)	9.6 min; 12 min;	70–700	Pharmacokinetic study with 15 mg aripiprazole once a day in schizophrenic patients	[13]
8[a]	HPLC-DAD	LLE; 1.0 mL human plasma; chlorohaloperidol	76.8%	X Bridge® C18 (100 mm × 4.6 mm, 3.5 μm); ACN-10 mM ammonium buffer, pH 8.35 (60:40, v/v)	6.8 min; 8.0 min	2.0–1000	Pharmacokinetic study with 10–30 mg aripiprazole daily dose in 34 psychiatric patients	[14]
9	LC–MS/MS	LLE; 0.2 mL human plasma; haloperidol-d4	61.6%	YMC ODS-AQ S (100 mm × 2.0 mm, 3 μm); gradient of 0.1% formic acid in water and acetonitrile	3.65 min; 5.0 min	2.0–400	Therapeutic monitoring of steady-state plasma aripiprazole in 8 human subjects	[15]
10[a]	LC–MS/MS	LLE; 0.5 mL human plasma; papavarine	104.5%	Phenomenex BDS Phenyl C18 (250 mm × 4.6 mm, 5 μm); methanol-AA buffer (70:30, v/v)	4.3 min; 6.0 min	0.1–600	Pharmacokinetic study with 5 mg aripiprazole in 12 healthy Chinese volunteers	[16]
11[a]	HPLC-UV Column switching	LLE; 1.0 mL human plasma; OPC-14558	73.6%	STR ODS C18 (150 × 4.6 mm, 5 μm); gradient of phosphate buffer, pH 4.5-acetonitrile- 60% perchloric acid	15 min; 20 min	1.0–500	Pharmacokinetic study with 6 mg aripiprazole in 4 healthy Japanese volunteers	[17]
12	LC–MS/MS	SPE; 0.5 mL human plasma; zolpidem tartrate	77.4%	Grace Smart C18 (100 mm × 4.6 mm, 3.0 μm); methanol -AA, pH 5.0 (95:5, v/v)	2.09 min; 3.5 min	0.2–60	Pharmacokinetic study with 10 mg aripiprazole in 15 healthy Indian volunteers	[18]
13	LC–MS/MS	LLE; 0.2 mL human plasma; propranolol	96.1%	Thermo aquasil C18 (100 mm × 2.1 mm, 5 μm); methanol-water containing 2 mM ATFA and 0.02% FA (65:35, v/v)	1.52 min; 2.4 min	0.1–100	Bioequivalence study with 10 mg aripiprazole in 27 healthy Indian subjects under fasting and fed conditions	[19]
14	UPLC–MS/MS	SPE; 0.1 ml human plasma; aripiprazole-d8	97.2%	Waters acquity BEH C18 (50 mm × 2.0 mm, 1.7 μm); methanol-AF, pH 4.0 with FA (85:15, v/v)	0.81 min; 1.2 min	0.05–80	Bioequivalence study with 10 mg aripiprazole in 36 healthy Indian subjects under fasting	PM

[a] Along with its active metabolite dehydroaripiprazole; LLE: liquid–liquid extraction; PP: protein precipitation; SPE: solid phase extraction; ACN: acetonitrile; FA: formic acid; AA: ammonium acetate; AF: ammonium formate; TEA: triethylamine; DAD: diode array detector; ATFA: ammonium trifluoroacetate; PM: present method.

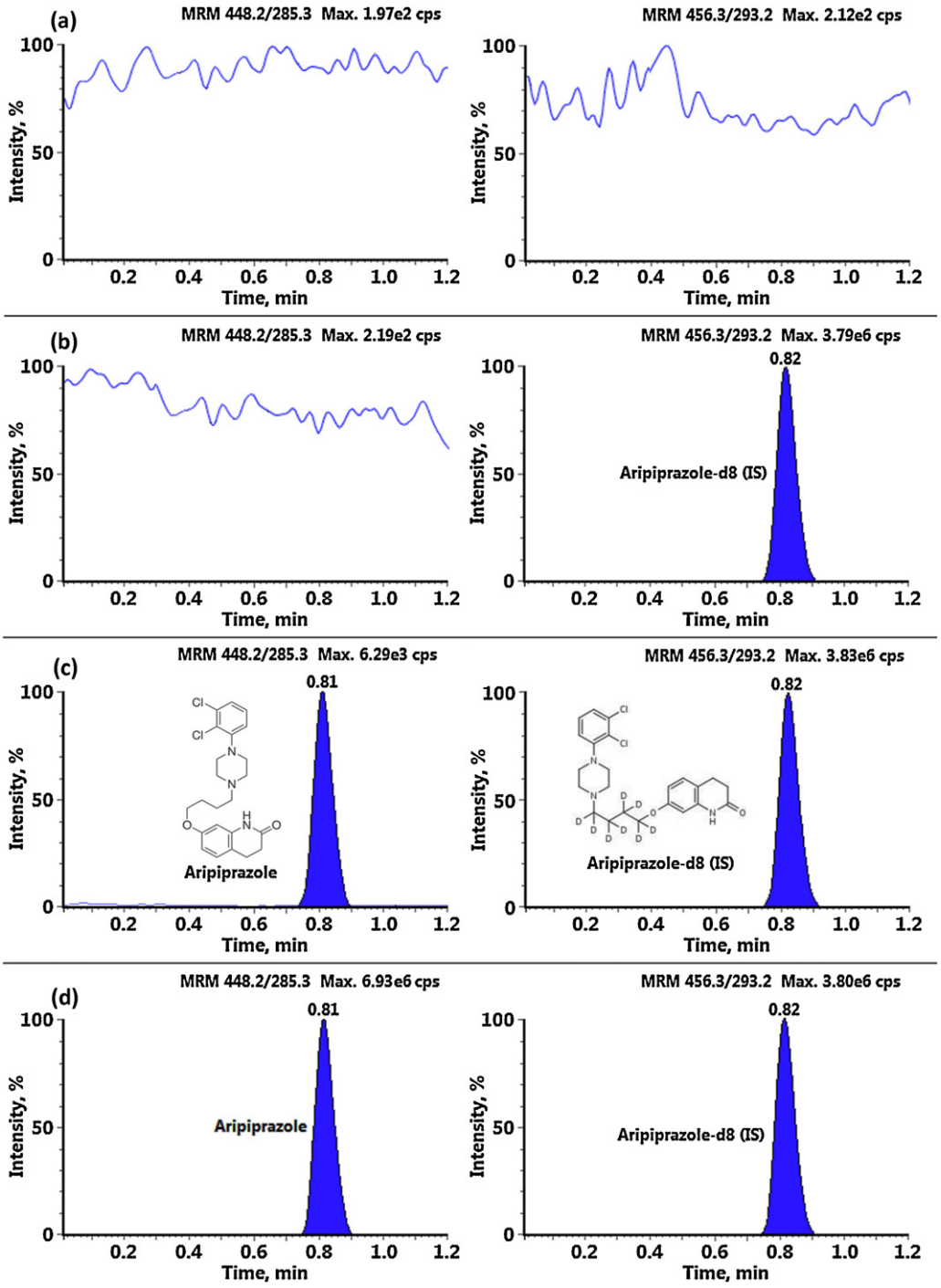

Fig. 1. Representative MRM chromatograms of (a) double blank plasma (without IS) (b) blank plasma with aripiprazole-d8 (IS), (c) aripiprazole at LLOQ and IS (d) real subject sample at C_{max} after administration of 10 mg dose of aripiprazole.

2.2. Liquid chromatographic and mass spectrometric conditions

Chromatographic analysis was carried out on Waters Acquity UPLC system (MA, USA) equipped with UPLC BEH C18 (50 mm × 2.1 mm, 1.7 μm) analytical column, maintained at 30 °C. Methanol and 10 mM ammonium formate, pH 4.00 adjusted with formic acid (85:15, v/v) was used as the mobile phase. It was delivered at a flow rate of 0.350 mL/min. Quantitation was done using multiple reaction monitoring for precursor → product ion transitions on Quattro Premier XE™ mass spectrometer from Waters–Micro Mass Technologies (MA, USA), in the positive ionization mode. The optimized mass parameters for ARP and IS are summarized in Supplementary Table S1. The dwell time was set at 50 ms and the data was processed by MassLynx software version 4.1.

2.3. Stock solution, calibrators and quality control samples

Standard stock solution of ARP (200 μg/mL) was prepared by dissolving accurately weighted reference standard in methanol. Calibration standards and quality control (QC) samples were prepared by spiking blank human plasma with stock and intermediate solutions (20.0 and 0.5 μg/mL in methanol–water (50:50, v/v). The IS stock solution of 10.0 μg/mL was prepared by dissolving requisite

amount of ARP-d8 in methanol. IS working solution (125 ng/mL) was prepared using the stock solution in deionized water. Calibration curve standards (CSs, 1–10) were made at 0.050, 0.100, 0.300, 1.00, 2.00, 4.00, 8.00, 16.0, 40.0, and 80.0 ng/mL concentrations respectively, while high, medium-1/2, low and lower limit of quantitation QC samples were prepared at 64.0, 32.0/2.40, 0.150 and 0.05 ng/mL concentrations respectively. The QC samples were prepared from separately weighted amount of analyte. The stock solutions were stored at 5 °C, while calibration standards and quality control samples were stored at −70 °C until use.

2.4. Sample preparation

To an aliquot of adequately thawed 100 μL of spiked plasma sample, 25 μL working solution of IS (125 ng/mL) was added and vortexed to mix. The samples were loaded on Phenomenex Strata-X (30 mg, 1cc) cartridges, after conditioning with 1 mL methanol followed by 1 mL of water. Washing of cartridges was done with 2 × 1 mL of 5% methanol in water and subsequently dried for 1 min by applying nitrogen (1.72×10^5 Pa) at 2.4 L/min flow rate. Elution of analyte and IS was done using 1 mL of methanol. The eluate was evaporated to dryness and reconstituted with 250 μL mobile phase and 10 μL was used for injection in the chromatographic system.

2.5. Validation methodology

The method was validated as reported in our previous work [19]. The method selectivity was studied in 10 different lots of blank human plasma including haemolyzed & lipemic plasma, collected with K_3EDTA as an anticoagulant. Carry over experiment was performed to verify any carryover of analyte, which may reflect in subsequent runs. The linearity of the method was determined by analysis of five linearity curves. The area ratio response for ARP/IS obtained from multiple reaction monitoring was used for regression analysis. Each calibration line was analyzed by least square weighted ($1/x^2$) linear regression.

Intra-batch accuracy and precision was determined by six replicates of high, medium and low QC samples along with calibration curve standards on the same day. The inter-batch accuracy and precision were assessed by analyzing six precision and accuracy batches on three consecutive days. Post-column analyte infusion experiment was done ion suppression/enhancement by infusing a standard solution of ARP (64 ng/mL) and IS into the mobile phase at 10 μL/min employing an infusion pump.

Recovery, matrix effect and process efficiency were evaluated as reported previously [20] for spiked samples at 64.0, 32.0/2.40 and 0.150 ng/mL concentrations in six replicates. Relative recovery was calculated by comparing the mean area response of samples spiked before extraction to that of samples spiked after extraction at each concentration. Absolute matrix effect was assessed by comparing the mean area response of samples spiked after extraction with the mean area response of neat standard solutions prepared in mobile phase. The overall 'process efficiency' was calculated as the product of relative recovery and absolute matrix effect/100. Relative matrix effect was determined by calculating the precision (% CV) of the slope of calibration lines from eight plasma batches (including haemolyzed and lipemic), which should not exceed 3–4% [21].

Stock solutions of ARP and IS were checked for short term stability at room temperature and long term stability at 4 °C. The solutions were considered stable if the deviation from nominal value was within ±10.0%. All stability results of spiked samples were evaluated by measuring the area response ratio (ARP/IS) of stability samples against freshly prepared comparison standards at identical concentrations. Bench top stability, processed sample stability at room temperature and at refrigerated temperature (4 °C), freeze thaw stability and long term stability at −20 °C and −70 °C were performed at 64.0 and 0.150 ng/mL concentration using six replicates. The stability samples were considered stable if the deviation from the mean calculated concentration of freshly prepared quality control samples was within ±15.0%.

Dilution reliability was evaluated by diluting a spiked standard containing 350 ng/mL concentration of ARP with the screened blank human plasma. Method ruggedness was tested on two precision and accuracy batches. The first batch was analyzed on two different columns, while the second batch was processed by two different analysts who were not part of method validation.

3. Results and discussion

3.1. Development of UPLC–MS/MS method

In continuation of our previous work on the analysis of ARP by LC–MS/MS in human plasma [19], we have now developed a more sensitive, rapid and a rugged method based on UPLC–MS/MS. Three aspects were considered to modify in our previously developed method, (a) instrumentation-UPLC (b) extraction procedure-SPE and (c) the internal standard (IS)-a deuterated analog. UPLC can serve as a superior alternative to HPLC, especially in reducing the analysis time when large numbers of samples are to be analyzed in a clinical setting. Moreover, it provides higher resolution and sensitivity, especially when coupled to mass spectrometer detector. The current UPLC–MS/MS method was more sensitive (0.05 ng/mL) compared to our previous work by two folds and ten times more sensitive compared to a similar UPLC–MS/MS method used for ARP determination in rat plasma and brain [8]. Additionally, reduction in the dwell time from 300 ms [19] to 50 ms showed to an improvement in signal to noise ratio for quantitative measurements. Correspondingly, the chromatographic run time was only 1.2 min (Fig. 1), which is much shorter, compared to all other methods reported for ARP in biological matrices. The reported UPLC–MS/MS method [8] required a chromatographic run time of 2.0 min and 3.0 min for ARP analysis in rat plasma and brain homogenate samples respectively.

The choice of mobile phase was extensively studied by varying the pH and organic modifiers in our earlier work on Thermo Aquasil C18 (100 mm × 2.1 mm, 5 μm) column (Supplementary Table S2). The best conditions were established using methanol:deionized water containing 2.0 mM ammonium trifluoroacetate and 0.02% formic acid (65:35, v/v) as the mobile phase, pH 3.61 to achieve superior peak shape, adequate retention and analyte response. Nonetheless, a mobile phase consisting of methanol:10 mM ammonium formate, pH 4.00 adjusted with formic acid (85:15, v/v) at a flow rate of 0.350 mL/min gave acceptable chromatography with retention time of 0.81 and 0.82 min for ARP and IS respectively on UPLC BEH C18 (50 mm × 2.1 mm, 1.7 μm) analytical column. Further, during our LC–MS/MS procedure propranolol was used as a general IS, which is easily available and is relatively inexpensive. The current method employed a deuterated analog, ARP-d8 as IS to maintain the overall efficiency of the instrumentation and thereby the accuracy of the data. Moreover, ARP and IS had almost similar recovery and retention time (unlike our previous work, 1.52 for ARP and 1.05 for propranolol), which helped to correct any experimental variability during sample preparation and analysis. Similarly, the method reported by Liang et al. [8] employed midazolam, a general internal standard which has been used for the analysis of other antipsychotics.

Several methods have employed liquid–liquid extraction (LLE) for sample preparation [7,10,11,14–17,19]. In our previous method

Table 2
Extraction recovery and matrix effect for aripiprazole at QC levels.

A (% CV)	B (% CV)	C (% CV)	Absolute matrix effect, [B/A × 100]	Relative recovery, [C/B × 100]	Process efficiency, [C/A × 100]
Low QC					
1743 (1.28)	1790 (1.53)	1723 (1.26)	102.7 (95.7)[a]	96.3 (96.5)[a]	98.9 (92.3)[a]
Medium QC-2					
28,445 (0.26)	28,634 (0.86)	28,125 (2.53)	100.7 (98.2)[a]	98.2 (95.3)[a]	98.9 (93.5)[a]
Medium QC-1					
380,158 (2.85)	381,243 (2.09)	370,255 (1.82)	100.3 (96.7)[a]	97.1 (97.6)[a]	97.4 (94.4)[a]
High QC					
760,265 (0.26)	771,548 (1.77)	749,658 (1.90)	101.5 (98.1)[a]	97.2 (96.5)[a]	98.6 (94.7)[a]

CV: coefficient of variation; A: mean area response of six replicate samples prepared in mobile phase (neat samples); B: mean area response of six replicate samples prepared by spiking in extracted blank plasma; C: mean area response of six replicate samples prepared by spiking before extraction.
[a] Values for internal standard, aripiprazole-d8.

LLE with methyl *tert*-butyl ether was successfully used with no serious matrix interference [19]. However, the recovery was inconsistent in several other solvents, especially at LOQ and low QC levels. Similar observation was also reported by Liang et al. [8], wherein significant ion suppression (more than 40%) was observed in positive ion ESI mode. Liang et al. [8] used a weak cation exchange mixed-mode SPE sorbent to selectively remove phospholipids and other interfering substances from rat plasma. However, the matrix effect varied from 0.90 to 1.14, giving an ion enhancement by 14% and about 10% ion suppression. Thus, SPE was tried on Phenomenex Starta-X (30 mg, 1cc) cartridges in the present work. The elution of ARP with 1.0 mL methanol was adequate unlike the previous work [8] which employed methanol–ammonium hydroxide (95:5, v/v) mixture (pH > 11) to obtain a mean recovery of 77.8% for ARP in rat plasma. The recovery obtained in the present work was consistent and quantitative (96–97%) at all QC levels with minimum matrix interference (2–3% ion suppression). The matrix effect was also calculated using the precision value of the slope of the calibration lines from eight plasma batches as the indicator for relative matrix effect as proposed by Matuszewski [21]. The % CV value calculated was 1.08%, which further suggests negligible matrix interference during analysis.

3.2. Validation results

The precision (% CV) for system suitability test for the retention time and the area response of ARP and IS and system performance (expressed as S/N ratio) is shown in Supplementary Table S3. There was practically insignificant carry-over (≤0.05% of LLOQ area) during auto-sampler carryover experiment. The chromatograms in Fig. 1 of double blank plasma, plasma spiked with IS, ARP at LLOQ and a subject sample at C_{max} demonstrates the selectivity of the method to differentiate and quantify the analytes from endogenous components in the plasma matrix.

The calibration curves showed good linearity ($r^2 \geq 0.9994$) through the studied concentration range of 0.05–80 ng/mL for ARP. The mean linear equation for calibration curve concentrations was $y = (0.03027 \pm 0.00013)x + (0.000027 \pm 0.000006)$. For CSs, the accuracy (%) and precision (% CV) values ranged from 97.1 to 101.7% and from 0.61 to 2.28% respectively. The lowest concentration in the standard curve (0.05 ng/mL) was measured at a signal-to-noise ratio (S/N) ≥ 30.

The intra-batch precision (% CV) ranged from 3.72 to 1.60 and the accuracy was within 97.4–101.9%. For the inter-batch experiments, the precision (% CV) varied from 1.20 to 2.64 and the accuracy was between 98.1 and 101.8% (Supplementary Table S4). The extraction recovery, absolute matrix effect and process efficiency data for ARP is presented in Table 2. The mean recovery across QC levels was 97.2 and 96.5% for ARP and IS respectively. Post-column infusion chromatograms (Supplementary Fig. S1) showed no assessable ion suppression or enhancement at the retention time of ARP and IS.

Samples for short-term and long term stock solution stability remained unchanged up to 12 h and for at least 24 days respectively with a % change ≤ 1.2. Acceptable results were obtained for other stability experiments in plasma as shown in Table 3.

The ruggedness of the method was evaluated by re-injection of analyzed samples on two UPLC BEH C18 (50 mm × 2.1 mm, 1.7 μm) columns, each from a different batch and also by two analysts. The precision (% CV) and accuracy values for different columns and analysts ranged from 0.7 to 2.1% and from 96.5 to 101.9% respectively for ARP across five QC levels. The precision values for dilution

Table 3
Stability of aripiprazole under different conditions ($n = 6$).

Stability	Storage condition	Nominal conc. (ng/mL)	Mean stability samples ± SD	% Change
Bench top	Room temperature (24 h)	64.0	65.8 ± 1.1597	1.95
		0.150	0.147 ± 0.0032	1.00
Processed sample	Auto sampler (4 °C, 82 h)	64.0	64.1 ± 1.1696	−0.09
		0.150	0.146 ± 0.0048	−2.21
	Room temperature (32 h)	64.0	63.4 ± 0.8764	−2.58
		0.150	0.153 ± 0.0029	3.66
Freeze and thaw	After 5th cycle at −20 °C	64.0	63.7 ± 1.9308	−0.38
		0.150	0.151 ± 0.0023	1.89
	After 5th cycle at −70 °C	64.0	63.3 ± 2.2289	2.79
		0.150	0.147 ± 0.0023	−3.16
Long term	198 days at −20 °C	64.0	65.5 ± 2.5607	−2.67
		0.150	0.153 ± 0.0049	−1.29
	198 days at −70 °C	64.0	63.3 ± 1.9235	0.73
		0.150	0.158 ± 0.0051	0.89

SD: standard deviation.
n: number of replicates at each level % change $= \frac{\text{mean stability samples} - \text{mean comparison samples}}{\text{mean comparison samples}} \times 100$

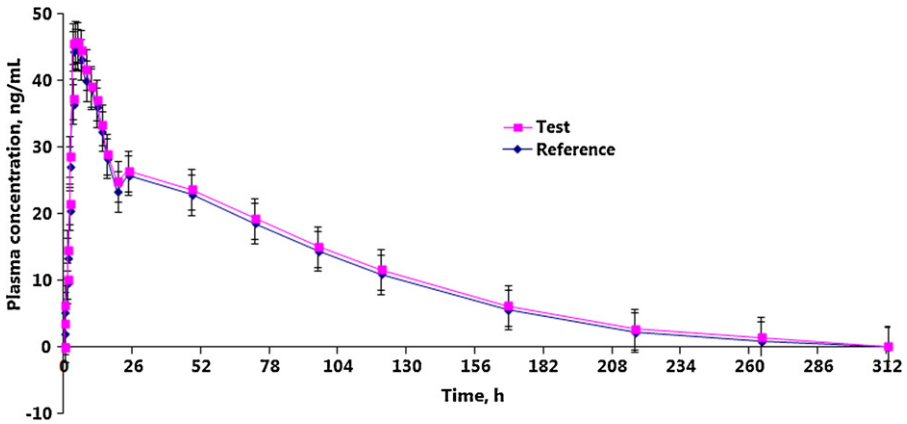

Fig. 2. Mean plasma concentration–time profile of aripiprazole after oral administration of 10 mg (test and reference) tablet formulation to 36 healthy Indian volunteers.

reliability for 1/5 and 1/10th dilution were 0.6 and 1.3%, while the accuracy results were 97.8 and 96.2% respectively.

3.3. Application of the method and incurred sample reanalysis

The validated method was applied to study the relative oral bioavailability of test (10 mg aripiprazole orally disintegrating tablets of a generic company) and a reference (ABILIFY® DISCMELT, 10 mg orally disintegrating aripiprazole tablets from Otsuka America Pharmaceutical, Inc., Rockville, MD, USA) formulation in 36 healthy Indian subjects under fasting. The procedures followed were based on International Conference on Harmonization, E6 Good Clinical Practice (ICH, E6 GCP) guidelines [22]. Blood samples were collected in vacutainers containing K_3EDTA anticoagulant at 0.0, 0.17, 0.33, 0.66, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10, 12, 14, 16, 20, 24, 48, 72, 96, 120, 168, 216, 264, 312 h of administration of drug. The mean pharmacokinetic profile for the test and reference formulation under fasting is shown in Fig. 2. The concentration of ARP corresponding to individual data points for both the formulations is summarized in Supplementary Table S5. The pharmacokinetic parameters of ARP namely C_{max}, AUC_{0-312}, AUC_{0-inf}, T_{max}, $t_{1/2}$ and K_{el} were estimated by non-compartmental model using WinNonlin software version 5.2.1 (Pharsight Corporation, Sunnyvale, CA, USA) (Supplementary Table S6). The values obtained were comparable with our previous work with 27 healthy subjects under fasting [19]. The ratios of mean log-transformed parameters and their 90% confidence intervals varied were within the defined bioequivalence range of 0.80–1.25. Further, the % change in 151 selected subject samples near the T_{max} and elimination phase for incurred sample reanalysis was within ±9%, which is within the acceptance limit of ±20% [23].

4. Conclusions

The aim of this work was to improve upon our previously developed LC–MS/MS method by changing the instrumentation, sample preparation technique and the internal standard. The newly developed UPLC–MS/MS method is highly sensitive, rapid and rugged compared to all other methods developed for ARP in biological fluids. The method can be useful in high throughput applications, especially in a clinical setting. It was successfully employed to support a bioequivalence study in healthy subjects with 10 mg ARP formulation. Further, the reproducibility in the measurement of subject samples is confirmed by incurred sample reanalysis.

Acknowledgement

The authors are thankful to the management of Veeda Clinical Research, Ahmedabad, India for providing infrastructure facility to carry out this work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2013.02.022.

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