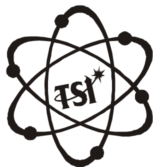

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A stability indicating RPLC method for aripiprazole

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ABSTRACT

A simple, sensitive isocratic RP-LC method has been developed for the quantitative determination of aripiprazole in both bulk drug and pharmaceutical dosage forms, used as a typical antipsychotic drug. The developed method also applicable for the related substances determination. Efficient chromatographic separation was achieved on a Kromasil C8, 150mm×4.6 mm, 5μm column with simple mobile phase combination delivered in an isocratic mode and quantification was carried out using ultraviolet detection at 215 nm with a flow rate of 1.0 mL min^{-1}. The mobile phase contains a mixture of buffer and acetonitrile in the ratio of 65:35 (v/v). Buffer consists of 30mM sodium dihydrogen phosphate monohydrate and 5mM hexane-1-sulfonic acid sodium salt, pH adjusted to 2.5 using phosphoric acid. In the developed HPLC method the resolution (Rs) between aripiprazole and for its potential five impurities in bulk drug was found to be greater than 2.0. Regression coefficient (r^2) value of greater than 0.99 for aripiprazole and it's all the five impurities shows good linearity of the developed method. This method was capable of detecting all five impurities of aripiprazole at a level of 0.009% with respect to test concentration of 0.5 mg mL^{-1} for a 5μL injection volume. The inter and intraday precision for all five impurities and aripiprazole were found to be within 2.0% RSD at its specification level. The method has shown good and consistent recoveries for aripiprazole (98.4-100.6%) and for it's all the five impurities (93.5-106.2%). The test solution was found to be stable in the diluent for 48h. The drug was subjected to stress conditions of hydrolysis, oxidation, photolysis and thermal degradation. Considerable degradation was found to occur in alkaline medium and in oxidative stress conditions. The stress samples were assayed against a qualified reference standard and the mass balance was found close to 99.4%. The developed RP-LC method was validated with respect to linearity, accuracy, precision and robustness.

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KEYWORDS

Column liquid chromatography;
Aripiprazole;
Forced degradation;
Stability indicating.

INTRODUCTION

Aripiprazole, 7-(4-[4-(2, 3-dichlorophenyl)-1-piperazinyl]butoxy)-3, 4-dihydro-2(1*H*)-quinolinone is an antipsychotic drug (Figure 1). It was used for the purpose of improving both negative and positive symptoms of schizophrenia without inducing extra pyramidal side effects. Aripiprazole was a novel atypical antipsy-

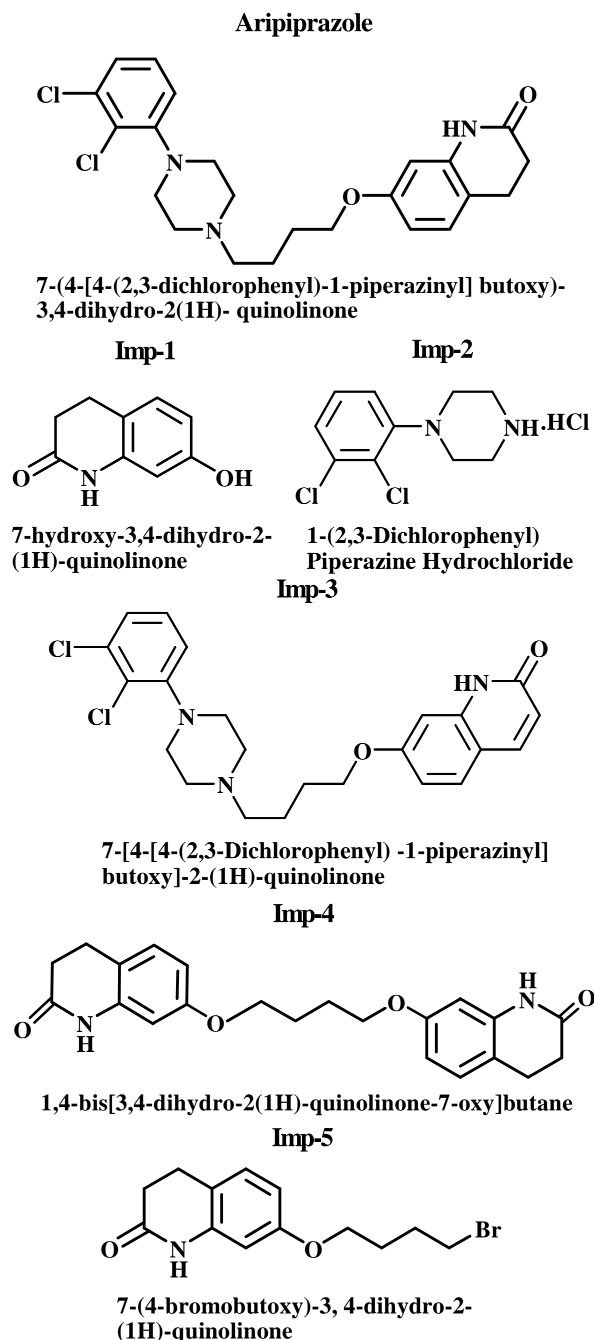

Figure 1 : Chemical structures and labels of aripiprazole and its impurities

chotic drug[1]. It was distinguished from all other antipsychotics by its unique pharmacologic profile i.e. partial agonist activity at dopamine D2 receptors[2], partial agonist activity at serotonin 5-HT1A receptors[3] and antagonist activity at serotonin 5-HT2A receptors[4]. In clinical studies, aripiprazole has shown to improve both positive and negative symptoms in patients with schizophrenia and schizoaffective disorder[5,6,7]. This novel agent has also demonstrated a favourable and excellent safety profile, tolerability with a low liability for extra pyramidal symptoms and sedation, no evidence for an increased risk of weight gain, prolactin elevation and QTc prolongation[8,9].

Extensive literature survey did not reveal any simple, sensitive and stability indicating method for the determination of aripiprazole in bulk drug and pharmaceutical dosage forms. Yoshihiko Shimokawa etal reported a liquid chromatography method for the determination of aripiprazole, in rat plasma and brain[10]. Further more Masanori Kubo et al reported a LC-MS-MS method for the quantitative determination of aripiprazole and its main metabolite, OPC-14857, in human plasma and in rat plasma[11]. As far as we are aware there is no stability-indicating LC method for determination of related substance and quantitative estimation of aripiprazole. In this paper we described validation of an assay and related substances method for accurate quantification of aripiprazole and its five impurities in bulk samples and in pharmaceutical dosage forms along with method validation as per ICH norms. Intensive stress studies were carried out on aripiprazole, accordingly a stability-indicating method was developed, which could separate various degradation products.

EXPERIMENTAL

Chemicals

Samples of aripiprazole and its related impurities were received from Gensen Laboratories Limited; Mumbai, India; commercially available 10 mg aripiprazole tablets (Abilify) were purchased. HPLC grade acetonitrile was purchased from Merck, Darmstadt, Germany. Analytical reagent grade sodium dihydrogen phosphate monohydrate and hexane-1-sulfonic acid sodium salt were purchased from Merck, Darmstadt, Germany. High purity water was prepared by using Millipore Milli-Q plus water purification system. All sample, standards and impurities used for this study were of more than 99.6% purity.

Equipment

The LC system used for method development, forced degradation studies and method validation was Agilent 1200 system (manufactured by Agilent Tech-

nologies, Germany) with diode array detector (DAD). The output signal was monitored and processed using chemstation software (Agilent) on Pentium computer (Digital Equipment Co).

Chromatographic conditions

The chromatographic column used was Kromasil C8 (150×4.6) mm with 5µm particles. The mobile phase contains a mixture of buffer and acetonitrile in the ratio of 65:35 (v/v). Buffer consists of 30mM sodium dihydrogen phosphate monohydrate and 5mM hexane-1-sulfonic acid sodium salt, pH adjusted to 2.5 using phosphoric acid.

The flow rate of the mobile phase was 1.0mLmin^{-1}. The column temperature was maintained at 27°C and the detection was monitored at a wavelength of 215 nm. The injection volume was 5µL. Acetonitrile and water (8:2 v/v) was used as diluent.

Preparation of solutions

1. Preparation of standard solutions

A stock solution of aripiprazole (5.0 mg mL^{-1}) was prepared by dissolving appropriate amount in the diluent. Working solutions of 500 and 100µg mL^{-1} were prepared from above stock solution for related substances determination and assay determination, respectively. A stock solution of impurities (mixture of imp-1, imp-2, imp-3, imp-4 and imp-5) at concentration of 0.5 mg mL^{-1} was also prepared in diluent.

2. Preparation of sample solution

Twenty tablets were weighed and the content transferred into a clean and dry mortar, grinded well (each tablet contains 10mg of aripiprazole and inactive ingradients includes Corn starch, hydroxylpropyl cellulose, lactose monohydrate, magnesium stearate and micro crystalline cellulose. Colorants include ferric oxide (red or yellow) and FD&C Blue No-2 Alumium Lake.). Then equivalent to 50 mg of drug was transferred to 100 mL volumetric flask, 70 mL of diluent added and kept on rotatory shaker for 10 min to disperse the material completely and sonicated for 10 min and diluted to 100 mL (500µg mL^{-1}). The resulting solution was centrifuged at 3,000 rpm for 5 min. 10 mL of Supernatant solution was taken and diluted to 100 mL with diluent (50 µg mL^{-1}). This was filtered using 0.45 µnylon 66-membrane filter.

Analytical method validation

1. Selectivity

Selectivity of the developed method was assessed by performing forced degradation studies. The terms selectivity and specificity are often used interchangeably. Selectivity is the ability of the method to measure the analyte response in the presence of its potential impurities. According to ICH[12] stress testing of the drug substance can help the intrinsic stability of the molecule and validate the stability indicating power of the analytical procedures used. The nature of the stress testing will depend on the individual drug substance and the type of drug product involved. Stress testing is likely to be carried out on a single batch of the drug substance. It should include the effect of temperatures (in 10°C increments (e.g., 50°C, 60^{0}C etc.) above that for accelerated testing), humidity (e.g., 75% RH or greater) where appropriate, oxidation and photolysis on the drug substance. The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension. Photo stability testing should be an integral part of stress testing. The standard conditions for photo stability testing are described in ICH Q1B.

The specificity of the developed LC method for aripiprazole was determined in the presence of its impurities namely imp-1, imp-2, imp-3, imp-4, imp-5 and degradation products. Forced degradation studies were performed on aripiprazole to provide an indication of the stability indicating property and specificity of the proposed method[13,14]. The stress conditions employed for degradation study includes light (carried out as per ICH Q1B), heat (60°C), acid hydrolysis (1N HCl), base hydrolysis (1N NaOH), water hydrolysis and oxidation (1 % H_2O_2). For heat and light studies, study period was 10 days whereas for acid, base, water hydrolysis and oxidation, it was 48 h. Peak purity of stressed samples of aripiprazole was checked by using diode array detector (PDA) of Agilent technologies. The purity angle was within the purity threshold limit obtained in all stressed samples demonstrates the analyte peak homogeneity. All stressed samples of aripiprazole [heat (60°C), acid hydrolysis (1N HCl), base hydrolysis(1N NaOH), water hydrolysis and oxidation

(1% H_2O_2)] were studied for extended run time of 100 min (with 90% acetonitrile and 10% buffer solution as mobile phase) to check the late eluting degradents.

Assay studies were carried out for stressed samples against qualified reference standard and the mass balance (% assay+% of impurities + % of degradation products) was calculated. Assay was also calculated for bulk samples and drug product by spiking all five impurities at the specification level (i.e. 0.15% of analyte concentration which is 500 μg mL^{-1}).

2. Method validation

Method validation was carried out as per general chapter <1225> Validation of compendial procedures[15].

2.1. Precision

Precision of the developed method was determined through repeatability (intra-day) and intermediate (inter-day) precision. The precision for related substances were checked by injecting six individual preparations of (500μg mL^{-1}) aripiprazole spiked with 0.15% each imp-1, imp-2, imp-3, imp-4 and imp-5. Precision of the method was expressed as percent relative standard deviation of results. The % RSD of area for each impurity was calculated.

The intermediate precision (ruggedness) of the method was evaluated by different analyst using different column and a different instrument in the same laboratory.

Assay method precision was evaluated by carrying out six independent assays of test sample of aripiprazole against qualified reference standard. The % RSD of six assay values obtained was calculated. The intermediate precision of the assay method was evaluated by different analyst using different column and by using different instrument from the same laboratory.

2.2. Sensitivity

Sensitivity was determined by establishing the limit of detection and limit of quantification for imp-1, imp-2, imp-3, imp-4 and imp-5 estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concentration. The precision study at LOQ level was also carried out by injecting six individual preparations of imp-1, imp-2, imp-3, imp-4 and imp-5 and calculated the % RSD for the area of each impurity.

2.3. Linearity

To establish linearity of the assay method, calibration solutions were prepared from stock solution at seven concentration levels from 25 to 200% of assay analyte concentration (25, 50, 75, 100, 125, 150 and 200μg mL^{-1}). The peak area versus concentration data was collected and performed regression analysis. by the method of least-squares

Linearity test solutions for chromatographic purity method were prepared by diluting the impurity stock solution to the required concentrations. The solutions were prepared at six concentration levels from LOQ to 200% of the permitted maximum level of the impurity (i.e. the LOQ, 0.015%, 0.0375%, 0.075%, 0.15%, 0.225% and 0.3% for an analyte concentration of 500μg mL^{-1}). Average peak area at each concentration level was subjected to linear regression analysis with the least square method. Calibration equation obtained from regression analysis was used to calculate the corresponding predicted responses. The residuals and sum of the residual squares were calculated from the corresponding predicted responses. The % y-intercept for both the assay and chromatographic purity method was calculated. Analytical range of the method was established from the analysis of sensitivity curves. Upper and lower levels of range were also established.

2.4. Accuracy

For determination of accuracy, recovery studies were carried out by spiking analysis. A known amount of the impurity stock solution was spiked to the previously analysed sample at i.e. 50,100 and 150μg mL^{-1} in bulk sample and drug product. The percentage of recoveries were calculated. Each concentration level was prepared for three times.

The bulk sample does not show the presence of imp-1, imp-2 and imp-5, it shows 0.02% of imp-3, 0.02% of imp-4. The study was carried out in triplicate at 0.075%, 0.15% and 0.225% of the analyte concentration (500μg mL^{-1}). The percentage of recoveries for imp-1, imp-2, imp-3, imp-4 and imp-5 were calculated.

The accuracy of the assay method was evaluated in triplicate at five concentration levels, i.e. 50, 75, 100,

125 and 150μg mL^{-1} in bulk sample and drug product. The percentage of recoveries were calculated.

3. Robustness

Robustness study was conducted by making small but deliberate changes to the optimized method parameters. Critical sources of variability in operating procedure such as percent organic strength, buffer strength, pH of the buffer, temperature of the column were identified. By making deliberate change in experimental conditions the resolution between aripiprazole, imp-1, imp-2, imp-3, imp-4 and imp-5 was evaluated. The flow rate of the mobile phase was 1.0 mL min^{-1}. To study the effect of flow rate on the resolution, 0.2 units changed (i.e from 1.0 mL min^{-1} to 0.8 and 1.2 mL min^{-1}). The effect of pH on resolution of impurities was studied by varying ± 0.1 pH units (at 2.4 and 2.6 buffer pH). The effect of column temperature on resolution was studied at 22^{0}C and 32^{0}C instead of 27^{0}C. In all the above varied conditions, the components of the mobile phase were held constant as that of initial. To study the effect of change in mobile phase composition by changing the organic phase ratio, organic content was changed by 5% (from 100% to 95% and 105%) keeping the buffer ratio as constant.

4. Solution stability and mobile phase stability

The solution stability of aripiprazole in the assay method was carried out by leaving the test solutions of sample in tightly capped volumetric flask at room temperature for 48 h. The same sample solution was assayed for 6 h interval up to the study period against freshly pepared reference standard solution. The mobile phase stability was also carried out by assaying the freshly prepared sample solutions against freshly prepared reference standard solutions for 6 h interval up to 48 h. Mobile phase prepared was kept constant during the study period. The % RSD of assay of aripiprazole was calculated for the study period during mobile phase and solution stability experiments.

The solution stability of aripiprazole and its impurities in the related substance method was carried out by leaving spiked sample solution in tightly capped volumetric flask at room temperature for 48 h. Impurity content was determined for every 6 h interval up to the study period. Mobile phase stability was also carried out for 48 h by injecting the freshly prepared sample solutions for every 6 h interval. Impurity content was checked in the test solutions. Mobile phase prepared was kept constant during the study period.

RESULTS AND DISCUSSION

Method development and optimization

All the impurities and aripiprazole solutions were prepared in diluent at a concentration of 100PPM and scanned in UV-Visible spectrometer; all the 5 impurities and aripiprazole were having UV maxima at around 215 nm. Hence detection at 215 nm was selected for method development purpose. Since aripipeazole was insoluble in water with its equilibrium solubility being about 0.00001% w/v, its pKa was established in 20% aqueous ethanol ; pKa = 7.6 (20% ethanol, at 25°C). As the pKa value of aripiprazole was 7.6, pH was selected below 7.0, for this activity buffer type and pH media such as sodium dihydrogen phosphate monohydrate buffer (pH 3-7) was selected.

Sodium dihydrogen phosphate monohydrate buffer with pH 2.5 and methanol (50:50, v/v) was chosen for initial trail with a 25cm length, 4.6mm ID and 5 micron particle size C-18 stationary phase. Flow rate was 1.0 mL min^{-1}.When aripiprazole sample spiked with all the impurites (system suitability) solution was injected the resolution between all the impurities and aripiprazole was good (>2.0) but the retention time of imp-5 was very high (~45 min) and the tailing of the aripiprazole peak was high (~1.6). Similar results were obtained with 25cm length, 4.6mm ID and 5 micron particle size C-8 column

To improve the retention time of imp-5 and tailing of aripiprazole peak, column length was decreased (Kromasil C-8, 150 mm x 4.6 mm i.d with 5 μm particles). When system suitability solution was injected in the above conditions the resolution between imp-1 and imp-2 was greater than 1.5, symmetry of the aripiprazole peak was also improved (tailing ~1.3) and retention time of imp-5 was found to be ~35 min. To further improve retention time and symmetry of aripiprazole peak, methanol was replaced with acetonitrile (buffer: acetonitrile 50:50 v/v), results were as follows: tailing of aripiprazole peak was ~1.0, the re-

tention of imp-5 was ~16 min, imp-1, imp-2 were co eluted, the resolution between imp-3 and aripiprazole was less than 2.0. To improve the resolution, acetonitrile content was decreased (buffer: acetonitrile: 65:35, v/v) and injected the system suitability solution, the resolution between imp-1 and imp-2 was observed greater than 1.2, the resolution between imp-3 and aripiprazole was greater than 2.0.In this conditions, stressed samples were injected. In peroxide degradation the resolution between aripirazole and one degradent (~1.1 RRT) was very less (less than 1.2). To further improve the resolution, 5mM hexane-1- sulfonic acid sodium salt was added to the above mobile phase and injected system suitability solution. Resolution between all the peaks was observed greater than 2.0, the symmetry of aripiprazole was observed ~1.1 and the retention of imp-5 was observed around 23 min.

Effect of buffer pH was also studied in the above conditions. When the pH of the buffer(sodium dihydrogen phosphate monohydrate) was adjusted to 7.2 with sodium hydroxide solution, the tailing factor of aripiprazole peak was 1.5 and the retention time of imp-5 was about 40 min. When pH increased towards acidic side (2.5) the symmetry of Aripiprazole peak was improved (tailing ~1.2) and retention of imp-5 too (23 min).

It indicates that the isocratic method with Kromasil C-8, 150 mm×4.6 mm i.d with 5µm particles (pH 2.5 sodium dihydrogen phosphate monohydrate and 5mM hexane-1- sulfonic acid sodium salt buffer: acetonitrile: 65:35, v/v) was successful in separation of drug and all chromophoric degradation products. In the above conditions system suitability solution was injected and results were given in TABLE 1.

The interference of excipients (Corn starch, hydroxyl propyl cellulose, Lactose monohydrate, Magnesium stearate and micro crystalline cellulose) was also checked by injecting sample solutions of excipients. There was no interference of excipients with impurities (imp-1, imp-2, imp-3, imp-4, imp-5) and aripiprazole peak.

In the optimized conditions aripiprazole, imp-1, imp-2, imp-3, imp-4 and imp-5 were well separated with a resolution of greater than 2 and the typical retention times of imp-1, imp-2, imp-3, aripiprazole, imp-4 and imp-5 were about 2.3, 3.3, 7.5, 9.8, 13.1and 23.5 min respectively. The system suitability results were given in TABLE 1.

TABLE 1: System suitability report

Comp.	USP Resolution (R_S)	USP Tailing factor	USP Theoretical plates (N)	Capacity factor (α)
Imp-1	--	1.3	8576	2.3
Imp-2	3.3	1.2	8197	3.8
Imp-3	10.2	1.1	8621	9.8
Aripiprazole	3.8	1.2	9415	13.1
Imp-4	8.7	1.2	7850	17.8
Imp-5	12.5	1.1	9560	32.5

Analysis was performed for different batches of bulk drug samples (n=3) and for pharmaceutical dosage forms (n=3). The assay results for bulk samples found to be 99.86% (APL0K701), 99.88 % (APL0K706) and 99.87% (APL0K708) where as for drug product, 99.73% (CKM0702), 100.06% (CKM0705), 99.41% (CKM0706). The content of imp-1 and imp-2 were not detected, imp-3, imp-4 were below 0.02% and imp-5 is below 0.01% in all the above drug substance and drug product batches, which shows all the results are well within the limit. Accelarated and long term stability study results as per ICH Q1A (R2)[16] for Aripiprazole were generated for 6 months and the results are well within the limits.

Analytical method validation

1. Precision

The %RSD of assay of aripiprazole during assay method precision study was within 0.2% and the %RSD of area of imp-1, imp-2, imp-3, imp-4 and imp-5 in related substance method precision study were within 1.5 % confirming the good precision of the method.

The %RSD of assay results obtained in intermediate precision study was within 0.3% and the %RSD of area of imp-1, imp-2, imp-3, imp-4 and imp-5 were within 2.0 %, Low RSDs indicated the repeatability and intermediate precision of the developed method.

2. Sensitivity

The limit of detection of imp-1, imp-2, imp-3, imp-4 and imp-5 were 0.001, 0.008, 0.009, 0.009 and 0.009% (of analyte concentration, i.e.500 µg mL^{-1}) respectively for 5 µL injection volume. The limit of quantification of imp-1, imp-2, imp-3, imp-4 and imp-5 were 0.003, 0.025, 0.028, 0.028 and 0.03% (of analyte concentration, i.e. 500µg mL^{-1}) respectively for 5µL

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injection volume. The precision at LOQ concentration for imp-1, imp-2, imp-3, imp-4 and imp-5 were below 2%.

3. Linearity

Calibration curve obtained by the least square regression analysis between average peak area and the concentration showed linear relation ship with a regression coefficient of 0.999 over the calibration ranges tested, i.e. 25- 200 μg mL^{-1} for assay calculation. The best-fit linear equation obtained was y = 36772x - 99837. At all concentration levels, standard deviation of peak area was significantly low and RSD was below 0.6%. Analysis of residuals indicated that residuals were scattered within ± 2% with respect to 100% concentration response. The % Y intercept is also within the limit (± 2% wrt 100% area response).The points in the sensitivity graph were scattered within ±2% with respect to 100% concentration response. (TABLE 2)

The results of linearity and range obtained for the five potential impurities were tabulated in the TABLE 2. Linear calibration plot for related substance method was obtained over the calibration ranges tested, i.e. LOQ to 0.3% for imp-1, imp-2, imp-3 imp-4 and imp-5. The correlation coefficient obtained was greater than 0.999 for all five impurities.

TABLE 2: Summary of forced degradation results

Stress condition	Time	% Assay of active substance	Mass balance (%Assay + %impurities + % Degradation products)
Acid hydrolysis (1N HCl reflux at 60 deg)	4 h	97.75%	99.4
Base hydrolysis (1N NaOH)	48 h	99.6	99.7
Oxidation (1% H_2O_2)	48 h	77.15%	99.4
Water hydrolysis (Reflux at 60 deg)	4 h	99.66%	99.7
Thermal (60°C)	10 days	99.53	99.6
Light (photolytic degradation)	10 days	99.82	99.9

4. Accuracy

The method showed consistent and high absolute recoveries at all the concentration levels by spiking method. The percentage recovery of aripiprazole in bulk drug samples ranged from 98.8 to 99.5 and in pharmaceutical dosage forms ranged from 98.4 to 100.6 %. The percentage recovery of imp-1, imp-2, imp-3, imp-4 and imp-5 in bulk drugs samples ranged from 93.5 to 106.2. HPLC chromatogram of spiked sample at 0.15% level of all five impurities in aripiprazole bulk drug sample are shown in figure 2(c).

5. Robustness

In all the deliberate varied chromatographic conditions carried out (flow rate, pH, change in mobile phase composition and column temperature) the resolution between closely eluting impurities, namely imp-1, imp-2, imp-3 and Aripiprazole was greater than 2.0, illustrating good robustness of the method.

6. Solution stability and mobile phase stability

The %RSD of assay of aripiprazole during solution stability and mobile phase stability experiments was within 1%. No significant changes were observed in the impurity content during solution stability and mobile phase experiments. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay and related substance determination were stable up to the study period of 48 h.

Selectivity

1. Results of forced degradation studies

Stress studies on aripiprazole under different stress

TABLE 3: Linearity results for related substances and assay of aripiprazole

	Impurity-1	Impurity-2	Impurity-3	Impurity-4	Impurity-5	Assay
Calibration equn	y =449.23x-480.82	y =92.575x-34.966	y =286.64x - 120.38	y =282.36x - 1321.9	y =279.96x - 922.59	y = 36772x - 99837
Linearity range	2.0-200%	16.7-200%	18.7-200%	18.7-200%	20-200%	25-200%
Regression coefficient	0.9999	0.999	0.9991	0.9996	0.9994	0.9999
Slope	449.2	92.575	286.64	282.36	279.96	36772
Intercept	-480.82	-34.966	-120.38	-1321.9	-922.59	-99837
%Intercept	-0.0109	-0.00389	-0.0042	-0.0487	-0.0328	-0.02787
Residual sum of squares	6208348.0	483121	3960452	2010129	2595907	2853361700

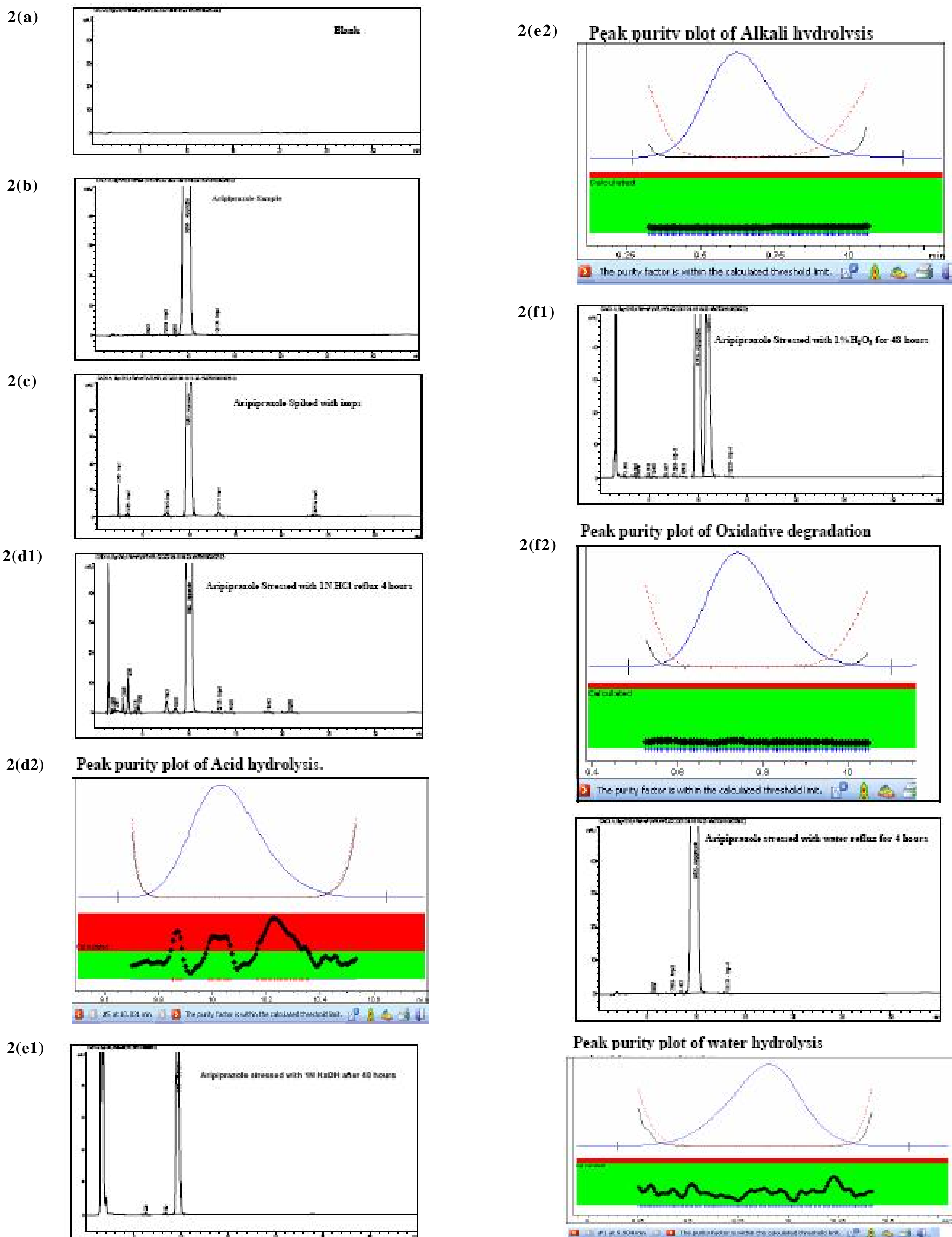

Figure 2: Typical chromatogram of blank, aripiprazole sample aripiprazole spiked with impurities at 0.15% level and stressed aripiprazole samples

conditions suggested the following degradation behavior.

2. Degradation in acidic solution

In 1N HCl at room temperature no major degradation was observed. Minor amount of degradation (~2.3%) was observed when stressed conditions were applied (1N HCl 4 hours reflux at 60°C) figure 2(d).

3. Degradation in basic solution

In 1N NaOH at room temperature after 48 h, no major degradation was observed. Aripiprazole is stable towards base hydrolysis.

4. Oxidative conditions

The drug was exposed to 1% hydrogen peroxide at room temperature for 48 h. The drug gradually undergone degradation with time in 1 % hydrogen peroxide and a prominent degradation was observed (~23%). Aripiprazole has shown significant sensitivity towards the treatment of hydrogen peroxide figure 2(e).

5. Degradation in neutral (water) solution

No major degradation products were observed after 48 h at room temperature. The drug was also stable in water on heating at 60°C for 4 h. The drug was stable to water hydrolysis.

6. Photolytic conditions

The drug was stable to the effect of photolysis. When the drug powder was exposed to light for an overall illumination of 1.2 million lux hours and an integrated near ultraviolet energy of 200-watt hours/square meter (w/mhr) (in photo stability chamber), no degradation of the drug was observed.

7. Thermal degradation

The drug was stable to the effect of temperature. When the drug powder exposed to dry heat at 60°C for 10 days, no decomposition of the drug was observed.

Peak purity test results derived from PDA detector, confirmed that the aripiprazole peak is homogeneous and pure in all the analyzed stress samples. Peak purity results for degrade peaks from PDA detector confirm that all-degradant peaks are homogeneous and pure in all analyzed stress samples.

Assay of all stressed samples were calculated using qualified standard of aripiprazole. Considering the purities from the respective chromatograms of stressed samples, mass balance (%assay + % degradants + % impurities) was calculated for each stressed sample. The mass balance of stressed samples was close to 99.4%. The assay of aripiprazole is unaffected in the presence of imp-1, imp-2, imp-3, imp-4 and imp-5 and its degradation products confirms the stability indicating power of the developed method. No degradants were observed after 30 min in the extended runtime of 100 min for all the aripiprazole stressed samples[heat (60°C), photolysis, acid hydrolysis (1N HCl), base hydrolysis (1N NaOH), water hydrolysis and oxidation (1% H_2O_2) with 90% acetonitrile as mobile phase (buffer: acetonitrile 10:90, v/v).

CONCLUSIONS

The isocratic RP-LC method developed for quantitative and related substance determination of Aripiprazole in both bulk drug and pharmaceutical dosage form was precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method was stability indicating and can be used for the routine analysis of production samples and also to check the stability of samples of aripiprazole.

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