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Development of stability indicating, validated HPLC method for quantitative determination of Aripiprazole and its impurities

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Abstract

The present paper describes the development of a simple, rapid and stability indicating reversed phase column liquid chromatographic method for 7-[4-{4-(2,3-dichlorophenyl) piperazine-1-yl}butoxy]-3,4-dihydro-1H-quinolin-2-one, a leading antipsychotic drug known by the generic name Aripiprazole in the presence of its impurities and degradation products generated from forced decomposition studies. Successful separation of the drug from the synthetic impurities and degradation products formed under stress conditions is achieved on Zorbax C18 (150 x 4.6x 5 micron) using a gradient elution of 0.2% trifluoroacetic acid in water and 0.2% trifluoroacetic acid in Methanol. The developed LC method is validated as per ICH guideline for validation parameters like specificity, precision, linearity, accuracy, limit of detection and limit of quantitation. Linearity of the impurities is established and correlation coefficient is found to be above 0.995 for every impurities. Recoveries of impurities are found between 100.87% and 103.68%. The proposed validated, stability indicating liquid chromatographic method for the related substances determination can be used to evaluate quality of Aripiprazole drug samples from regular production batches. It can also be utilized for testing the stability samples of Aripiprazole drug which will be of great help to quality control chemist and thus have robust industrial application.

Keywords: Antipsychotic, Stability indicating method, ICH guideline, Validation

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INTRODUCTION

Aripiprazole is a psychotropic agent belonging to the chemical class of benzisoxazole derivatives whose major therapeutic role is to treat the symptoms of schizophrenia. Aripiprazole is a selective monoaminergic antagonist with high affinity for the serotonin Type 2 (5HT2),

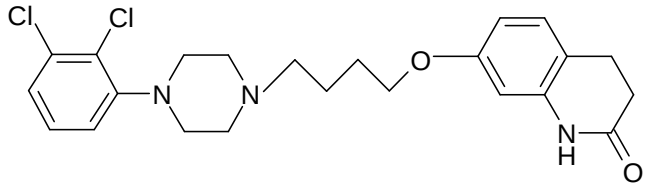
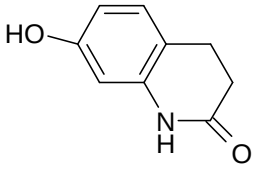
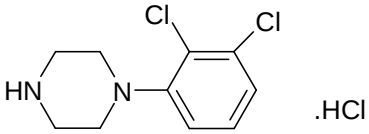
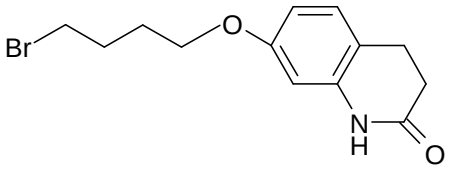
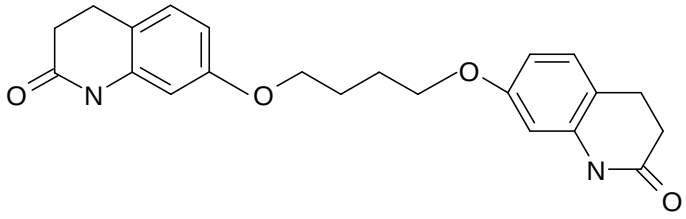
dopamine Type 2 (D2), 1 and 2 adrenergic and H1 histaminergic receptors. Aripiprazole acts as an antagonist at other receptors, but with lower potency. Antagonism at receptors other than dopamine and 5HT2 with similar receptor affinities may explain some of the other therapeutic and side effects of aripiprazole. Aripiprazole's antagonism of histamine H1 receptors may explain the somnolence observed with this drug. Aripiprazole's antagonism of adrenergic a1 receptors may explain the orthostatic hypotension observed with this drug.

The different analytical techniques reported so far for the determination of this drug and its metabolites in biological samples as well as in pharmaceutical formulations include LC–MS–MS previously published in [1–5]. The determination of aripiprazole in plasma by RP-LC [6] and that in bulk drug and solid dosage forms by RP-LC [7] were also reported. In one of the paper, the study on a LC method for the separation of substances related to aripiprazole [8] was described where in only two impurities namely Impurity-B (Imp-B) and Impurity-C (Imp-C) were considered. In the present study, other impurities namely Impurity-A (Imp-A) and Impurity-D (Imp-D) were considered for the validation. Organic impurities can arise during the manufacturing process and storage of the drug substances and the criteria for their acceptance up to certain limits are based on pharmaceutical studies or known safety data [9]. As per regulatory guidelines, the pharmaceutical studies using a sample of the isolated impurities can be considered for safety assessment. It is, therefore, essential to isolate and characterize unidentified impurities present in the drug sample.

Recently we have developed a process for the synthesis of aripiprazole in our laboratory. During the development of an analytical procedure, the RP-LC method was developed for the determination of in-house synthesized aripiprazole and the impurities arising during its synthesis. The potential Impurities in aripiprazole as shown in Table 1, are the process intermediates i.e. 7-hydroxy-3, 4-dihydro-2 (1*H*)-quinolinone (Imp-A), 1-(2, 3-dichlorophenyl) piperazine hydrochloride (Imp-B), 7-(4-Bromobutoxy)-3, 4-dihydro-2(1*H*)-quinolinone (Imp-C) and Dimer (Imp-D) formed as byproduct in the synthesis of 7-(4-Bromobutoxy)-3, 4-dihydro-2(1*H*)-quinolinone intermediate.

In this manuscript we describe determination of Relative Response Factors (RRFs) for the quantitative determination of impurities using a stability indicating isocratic RP-LC method. The proposed method was validated for Linearity, Precision, RRFs, Accuracy, Robustness, Specificity, LOD, LOQ and stability in the solution in accordance with ICH guidelines [10] and found to be suitable for quality testing and stability samples of aripiprazole drug samples.

Table 1: Aripiprazole drug substance and its impurities

Compound	Structure
Aripiprazole drug substance	
Impurity A (Imp- A)	
Impurity B (Imp- B)	
Impurity C (Imp- C)	
Impurity D (Imp- D)	

MATERIALS AND METHODS

Chemicals

Reference standard of aripiprazole and four impurities namely Imp-A, Imp-B, Imp-C and Imp- D were synthesized in the laboratory at Chemistry Department, Ruparel College, Mumbai 400016 and well characterized with the help of various spectroscopic and chromatographic techniques. This was used as reference standard for further work. All reagents used were of analytical–reagent grade unless stated otherwise. Milli Q water, HPLC grade Methanol and HPLC grade trifluoroacetic acid (TFA) were purchased from Merck (Darmstadt, Germany).

Equipment:

An Agilent HPLC System (1100 series) equipped with PAD and quaternary gradient pump was used for the study. All the data was acquired using Chem-station data acquisition and integration software.

A Shimadzu UVPC spectrophotometer was used for recording the UV spectrum.

Preparation of Solutions, Chromatographic Conditions and System Suitability

Parameters

Stock solutions of aripiprazole (1000 µg mL^{-1}) and all impurities (1000 µg mL^{-1}) were prepared in the mobile phase (MPh). The solutions were prepared having concentration 0.20, 0.40, 0.80, 1.0, 1.5, 2.0 and 2.5 µg mL^{-1} of all impurities in the mobile phase from stock solutions for the evaluation of the LOD, LOQ and linearity in accordance with ICH guidelines [8]. Aripiprazole sample solution from stock is spiked with 0.05, 0.10, and 0.20 % of all impurities to establish accuracy. The quantities of impurities were calculated from their peak areas.

Chromatographic Conditions

The mobile phase was prepared by 0.2 % TFA in water (MPh-A) and 0.2 % TFA in Methanol (MPh-B), both solutions filtered separately through a 0.45 µm PTFE filter and degassed by using an ultra-sonicator for 15 min prior to use. The system was equilibrated for 30 min with initial gradient composition of 60:40 % (MPh-A: MPh-B).

An analysis was carried out using a flow rate of 1.0 mL min^{-1} at a temperature of 25^0C. Chromatograms were recorded at 254 nm under gradient conditions by increasing percentage of MPh-B in gradient composition from 40 to 75 % over 0 to 20 min and hold it further for 20 min. Then the percentage Mph-B, in gradient composition is reduced from 75 % to 40 % over 5 min and continued further for 5 min.

System Solubility Parameters

Resolution between aripiprazole and each impurity from the chromatogram obtained with the system suitability solution is not less than 2.0.

Method Validation

The proposed method for estimation of aripiprazole and its Impurities is validated as per ICH guidelines, Q2 (R1) [10].

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities [9]. The specificity of the developed LC method for aripiprazole was carried out in the presence of its impurities namely Imp-A, Imp-B, Imp-C and Imp-D. Stress studies were performed for aripiprazole bulk drug to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition of UV light (254 nm, 24 h), heat (110 ^{0}C, 24 h), acid (1.0 N HCl, 60 ^{0}C, 48 h), base (1.0 N NaOH, 48 h) and oxidation (3.0% H_2O_2 60 ^{0}C, 2 h) to evaluate the ability of the proposed method to separate aripiprazole from its degradation product [9]. For heat and light studies, the study period was 24 h, for the acid and base 24 h and for oxidation 2 h. Peak purity test was carried out for the aripiprazole peak by using a PAD detector in stress samples. Assay studies were carried out for stress samples against qualified aripiprazole reference standard. Specificity was also demonstrated by spiking all four impurities in the aripiperazole sample.

Precision

The precision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. System precision, Method precision and Intermediate precision studies are conducted.

The precision of the method was evaluated by carrying out six independent determinations of aripiprazole test samples. The % RSD of the area for each impurity (Imp -A, -B, -C, -and D was calculated. The intermediate precision of the method was also evaluated using different analyst and different instrument in the same laboratory.

Limit of Detection and Limit of Quantitation

The detection limit is a characteristic of limit tests. It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated, under stated experimental conditions. LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The LOD and LOQ were determined by measuring the magnitude of analytical background. The LOD and LOQ were determined from signal to noise ratio. The LOD and LOQ for Imp-A, Imp-B, Imp-C and Imp-D were determined by injecting a series of dilute solutions with known concentrations [9].

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Linearity test solutions for the method were prepared by diluting stock solutions to the required concentrations. The solutions were prepared at six concentration levels starting from LOQ to 0.25%. The peak area versus concentration data was treated by least-squares linear regression analysis. Linearity test solutions for the related substance method were prepared. The % RSD value for the slope and Y-intercept of the calibration curve was calculated.

Accuracy

The accuracy study of impurities was carried out by spiking each impurity in triplicate at, 0.05, 0.10 and 0.20 % of the aripiprazole analyte concentration. The percentages of recoveries for the impurities were calculated.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

To determine the robustness of the developed method, experimental conditions were deliberately altered and the resolution between aripiprazole, Imp-A, -B, -C and -D was recorded. The flow rate of the mobile phase was 1.0 mL min^{-1}. To study the effect of flow rate on the resolution, flow was changed by 0.2 units from 0.8 to 1.2 mL min^{-1}. The effect of the percent organic strength on the resolution was studied by varying methanol by -5 to +5 %.

Solution stability

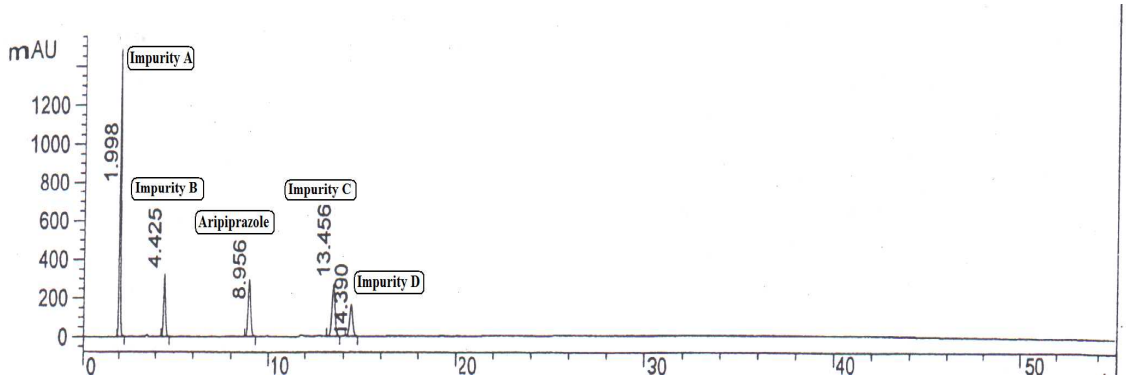
Study of Stability in analytical solution is carried out to know the stability of sample in analytical solution (diluent) over a period of time during routine analysis.

The solution stability of aripiprazole was carried out by keeping three precision sample solutions in tightly capped volumetric flasks at room temperature for 48 h. The content of Imp-A, -B, -C and -D were determined after 48 h with fresh standard preparations.

RESULTS

Optimization of chromatographic conditions, the main objective of the chromatographic method is to separate aripiprazole from Imp-A, Imp-B, Imp-C and Imp- D Impurities were coeluted using different stationary phases such as C18, C8 and cyano as well as different mobile phases. The effective chromatographic separation was achieved on Zorbax C18 150 x 4.6 mm, 5 µm column with binary gradient elution having mobile phase 0.2 % TFA in water in one port and 0.2 % TFA in methanol in second port. The flow rate of the mobile phase was 1.0 mL min^{-1}, at 25 ^{0}C column temperature, the peak shape of aripiprazole was found to be symmetrical with typical retention times were about 1.998, 4.425, 13.456 and 14.390 min, respectively (Fig 1).

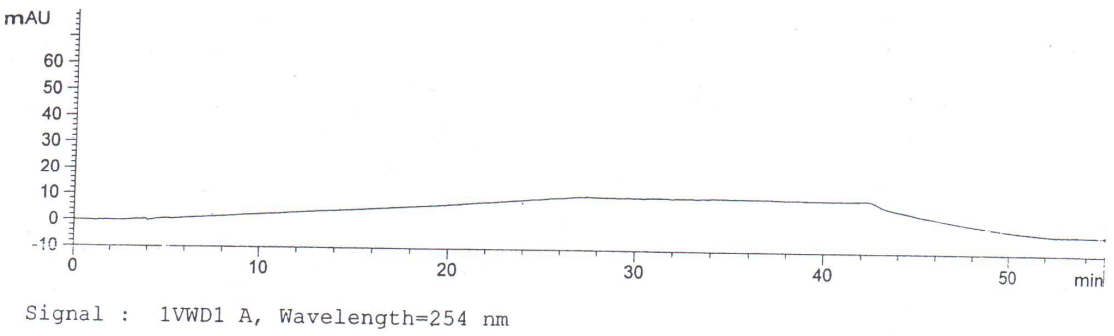
Fig. 1: Typical chromatogram of specificity



Specificity and Selectivity- Forced Degradation Studies results

To demonstrate the specificity and stability indicating characteristics of the method, samples of aripiprazole were subjected to various stress conditions such as 1 N acid, 1 N base, 30% v/v H_2O_2, Heat (105 ^{0}C), UV light (254 nm, 24 h). There was no degradation observed in aripiprazole samples when subjected to stress conditions like photolytic and aqueous hydrolysis. Aripiprazole was degraded to Imp-B under base and acid hydrolysis and unknown was observed in oxidation. No interference from blank at Retention time of Aripiprazole as seen in Figure 2. Selectivity of the method was performed by separately injecting individual impurities, and none of these impurities interfere with the aripiprazole peak with minimum resolution of 2.0 between any two peaks.

Figure 2: Typical chromatogram of blank



Linearity

Linear calibration plot for the method was obtained over the calibration ranges tested, i.e., LOQ (0.02 %) to 0.25 % for impurities Imp-A, Imp-B, Imp-C and Imp-D. The correlation coefficient obtained was greater than 0.995 as described in Table 2.

Table 2: Linearity data of aripiprazole and impurities

Level	Conc. µg mL^{-1}	Mean area of Aripiperazole	Imp-A	Imp-B	Imp-C	Imp-D
1	0.20	10284.02	9697.75	9516.25	9258.50	10067.39
2	0.40	18735.09	17092.75	18438.50	18715.50	20996.83
3	0.80	37356.73	33986.50	37753.75	38991.75	41818.54
4	1.0	75918.38	67139.00	75157.25	78706.50	83637.08
5	1.5	188339.1	166333.75	189356.75	197306.00	209092.70
6	2.5	384356.2	332172.25	382011.00	394191.75	418185.40
	Intercept	-749.99	915.31	-720.59	-574.86	-88.44
	Slope	38273.77	33116.27	38216.27	39499.69	41830.86
	Co-rell*	0.999	0.999	0.999	0.999	0.999

*Co-rell: Correlation coefficient

Precision

The % R.S.D for the area Imp-A, Imp-B, Imp-C and Imp-D in the related substance method precision study was within 1.49 % (Fig. 3). The % RSD of the results obtained in the intermediate precision study was within 1.21 %.

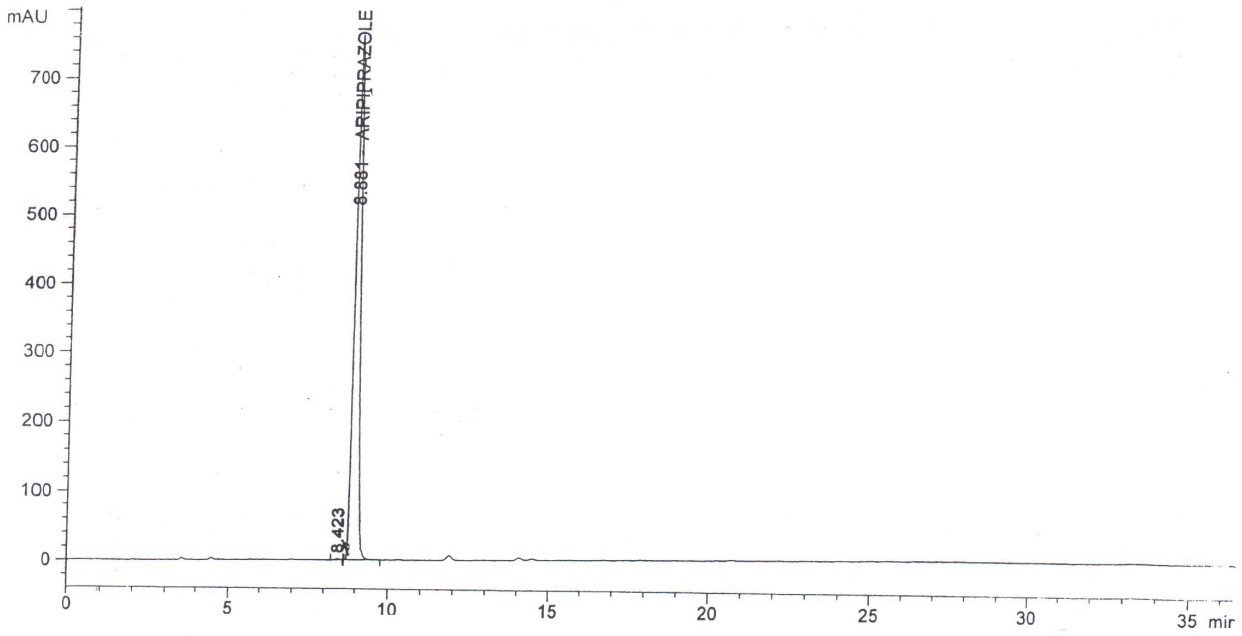
Limit of Detection and Limit of Quantification

The LOD of all the impurities namely Imp-A, -B, -C and -D was achieved at 0.01% for 20 µL injection volume. The LOQ for all the four impurities was achieved with in 0.02 % for a 20 µL injection volume. The precision at the LOQ concentrations for all the four impurities were below 1.75 %.

Accuracy

The percentage recovery of the impurities Imp-A, Imp-B, Imp-C and Imp-D in aripiprazole samples are Imp-A (101.39 %), Imp-B (100.87 %), Imp-C (103.68 %) and Imp-D (101.58 %) as described in Table 3.

Fig. 3: Typical chromatogram of method precision



Robustness

The robustness study was carried out by changing the Mobile phase composition, pH and flow rate. Samples of aripiprazole were analyzed for estimation of Impurities under these changed experimental conditions. It is observed that method is unaffected by small changes in experimental conditions with resolution greater than 2.0 confirming robustness of the method.

Solution Stability

Aripiprazole is stable at specified concentration in diluent solution for more than 48 h. No significant rise in impurity levels was observed, when sample at Impurity concentration were stored at ambient temperature under laboratory light conditions. Difference between 0 h and 48 h results is not more than 0.02 %.

Table 3: Accuracy data of aripiprazole and impurities (percentage recovery)

Sr. no.	Level	Imp-A	Imp-B	Imp-C	Imp-D
1.	Level-1 50%	101.28	103.06	106.06	102.34
2.		106.70	93.90	103.49	101.36
3.		100.22	98.29	104.78	101.09
4.	Level-2 100%	102.34	103.44	103.35	103.04
5.		100.06	100.39	103.31	101.25
6.		102.04	101.68	103.15	102.29
7.	Level-3 150%	100.69	103.94	103.81	102.81
8.		98.88	102.68	103.84	99.35
9.		100.28	100.42	101.34	100.68
	Mean	101.39	100.87	103.68	101.58
	SD*	2.25	3.17	1.27	1.17
	RSD** (%)	2.22	3.15	1.23	1.15

* SD: Standard deviation; **RSD: Relative standard deviation

DISCUSSION

In optimized gradient chromatographic conditions aripiprazole, Imp-A, -B, -C and -D were separated with a resolution greater than 2. The developed RP-LC method was found to be specific for Aripiprazole and its four impurities namely Imp-A, Imp-B, Imp-C and Imp-D. Peak purity test results obtained by using a PAD detector confirmed that the aripiprazole peak is homogenous and pure in all the analyzed stress samples. A minimum resolution of 2.0 was observed between any two peaks, reflecting the selectivity of the proposed method. The LOD and LOQ of impurities was established at 0.01% and 0.02 % for 20 µL injection volume respectively which is well within desired limits. Linearity of the impurities is well established with correlation coefficient found to be above 0.995 for every impurity. The above result show that an excellent correlation existed between the peak area and the concentration of all four impurities. The % R.S.D for the area in the related substance method precision study was within 1.49 % and 1.21 % for intermediate precision, conforming good precision of the method. Recoveries of impurities are found between 100.87% and 103.68%. The robustness of the method is evaluated by alterations in experimental conditions and confirmed with resolution greater than 2.0 achieved. Aripiprazole is stable at specified concentration in diluent solution for more than 48 h with no significant rise in impurity levels when stored at ambient temperature under laboratory light conditions. Analysis rresults indicate stability of the sample for at least 48 h.

CONCLUSION

Analytical method is found to be specific as proved by injecting known components into the chromatograph and by forced degradation study. Analytical method is found to be precise and accurate. LOD and LOQ for known impurities and unknown impurities have been established. Analytical method is found to be linear over a specified range. Analytical method is found to be rugged and robust. Samples prepared in analytical solution are found to stable for at least 48 h.

The RP-LC method developed for related substance determination of aripiprazole is precise, accurate, rapid and specific. The method facilitates the separation of four of known Impurities of the drug substance with a resolution of minimum 2.0. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method is stability indicating and can be conveniently used by analytical chemist in QC departments to determine the purity of drug substance, listed impurities and related applications like determination of stability samples of drugs.

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