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Research Article

A NEW STABILITY INDICATING VALIDATED METHOD FOR THE DETERMINATION OF ARIPIPRAZOLE IN BULK AND TABLET DOSAGE FORM USING RP- HPLC

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ABSTRACT

Objective: The present research work describe a rapid, simple and validated RP-HPLC method for analysis of Aripiprazole in bulk and tablet dosage form.

Method: The method employs a Purospher star C18 column (250× 4.6 mm, 5 µm) using 90:10 v/v mixture of methanol : water as mobile phase at a flow rate of 1mL.min^{-1}. And UV detector at 256 nm. The drug under study was subjected to different conditions like acid, base, alkali, temperature, light and oxidative stress conditions and also examined for solution stability for 48 hours.

Results: In the developed method Aripiprazole elutes at a typical retention time of 7.005 min. The detector response was linear in the concentration range of 5-25 µg/mL. The intraday and interday precision was found less than 2%, LOD and LOQ were 0.299 µg/ mL and 0.908 µg/ mL respectively. It was degrade significantly under all conditions except photolytic stressed condition, however the selectivity of the present method for Aripiprazole assay against their degradation product was confirmed and it was found to sufficiently stable in solution up to 48 hours.

Conclusion: The validated optimized method for analysis of Aripiprazole as per ICH Q2B guidelines was found to be simple, precise and reproducible. Undoubtedly present developed easiest, rapid and validated method can be applied routinely for the analysis of drug in bulk as well as tablet dosage form.

Keywords: Aripiprazole, RP- HPLC, Validation, ICH2QB, Forced degradation

INTRODUCTION

Aripiprazole chemically 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]-3,4-dihydro-2(1H)-quinolinone (chemical structure shown in Fig. 1) is a recent atypical antipsychotic drug that is effective for the treatment of patients with schizophrenia or schizoaffective disorder. It is belonging to the chemical class of benzisoxazole derivatives. It has potent partial agonist activity at dopamine (D2) receptors[1,2]. It is most commonly prescribed new drug world wide for the treatment of schizophrenic illness[3,4]. Aripiprazole in spectrophotometric[5,6] gas chromatography-mass spectrometry[7] , LC–MS/MS[8,9], capillary electrophoretic[10], methods are reported for the analysis in biological fluids. Few HPLC techniques are reported[11-14] for the determination of aripiprazole in pharmaceutical dosage form, and most of them used different buffers as a mobile phase[11-17] which is reducing the life span of a analytical column and preparation of buffer with the maintenance of proper P^{H} is cumbersome process .

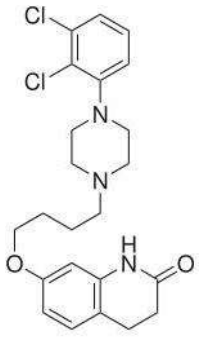

Fig. 1: Chemical structure of Aripiprazole

The above fact indicates there is need to develop a sensitive, stable and accurate method, the novelty of the present method involves the use a chief, simple solvent and well separated drug under study in presence of different degradate products. So the present RP-HPLC method for the determination of Aripiprazole in bulk and tablet dosage form can successfully use in the quality control laboratory for routine analysis.

MATERIALS AND METHOD

Instrumentation

The liquid chromatographic system was carried out using Purosper® Star (250 × 4.6 mm ID, 5 µm particle size) analytical column. A mobile phase of methanol and water (90:10; v/v) was pumped at a flow rate of 1 mL min^{-1}.

Chromatographic conditions

The mobile phase was prepared by mixing methanol and water (90:10; v/v), filtered through a 0.45 µm membrane filter and degassed by using an ultrasonicator for 15 min prior to use. The chromatographic separation was achieved on Purosper® Star (250 × 4.6 mm ID, 5 µm particle size) analytical column. The system equilibrated for 30 min and analysis was carried out under isocratic conditions using a flow rate of 1.0 mL min^{-1}. Chromatograms were recorded at 256 nm and the injection volume was 10 µL.

Chemicals

Aripiprazole standard was provided by Sun pharmaceutical industries, Mumbai. HPLC-grade methanol and acetonitrile were obtained from sigma-Aldrich. HPLC- grade water was obtained from Merck.

Preparation of standard solution

Stock solution of Aripiprazole was prepared in acetonitrile. Working solutions were obtained by diluting the stock solution with methanol and water (90:10 v/v).

Preparation of standard solution for Assay

A working standard solution with concentration 15µg/mL was prepared by pipeting 0.15mL from the stock solution into a 10 mL volumetric flask and diluted up to the mark with mobile phase. This solution was also used as standard for specificity, precision, LOD, LOQ and robustness.

Assay of Marketed formulation

"Arzu-10" a commercial formulation was taken for evaluating the drug content. Twenty tablets were weighed and triturated to a fine

powder and weighed accurately equivalent to 10 mg (i.e. 113.5 mg) from the powdered sample and dissolved in few mL of acetonitrile and diluted to 10 mL with acetonitrile. The solution was shaken well and allowed to stand for 15 minutes with intermittent sonication to ensure complete solubility of drug and filtered through a whatmann filter paper. From the filtrate, further dilution was made in a 10 mL volumetric flask by taking 0.15 mL of above solution and diluted to10 mL with mobile phase (methanol and water in the ratio of 90: 10) to get 15µg mL^{-1} Aripiprazole. Results of assay of marketed formulation given in figure 2, and table 1.

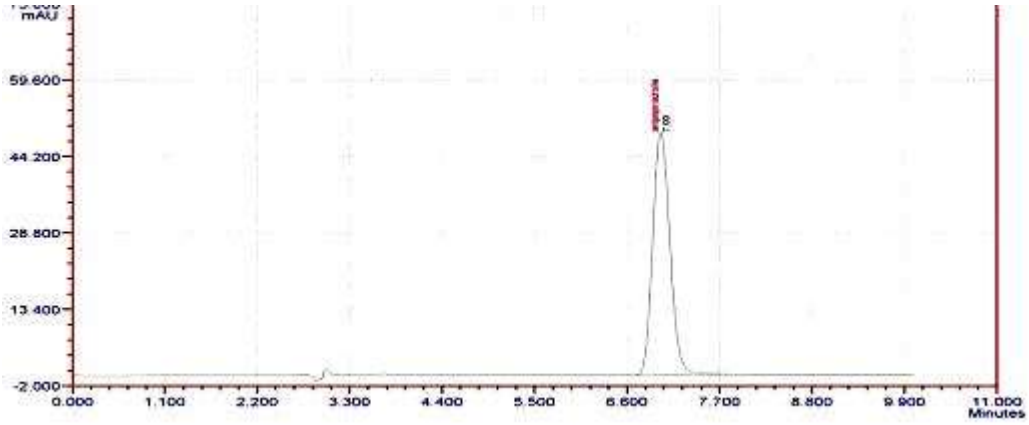

Fig. 2: Assay chromatogram of marketed formulation.

Table 1: Analysis of marketed Formulation

Formulation	Label claim (mg)	Amount found* (mg)	% Assay
Arzu-10	10	9.93	99.3

* Average of three readings.

Selection of wavelength

The working standard solution was scanned in UV region from 200nm- 400 nm using UV-visible spectrophotometer and from the obtained UV spectra a wavelength of 256 nm was selected as ideal wavelength used as detector wavelength in HPLC analysis.

Selection of mobile phase for method optimization and other experimental conditions

Several trial has been taken for the proper optimization of RP HPLC method by changing different mobile phase with different ratio. And finally the mobile phase for optimized condition was selected and given as follows.

Chromatographic conditions

Mobile phase	: Methanol: Water
Ratio	: (90 :10)
Column	: Purosper® Star RP-18,250×4.6mm ID, 5µm
Wavelength	: 256nm.
Injection volume	: 20µl
Temperature	: 30^0C (± 2^0C)
Run time	: 10 min
Flow rate	: 1.0ml/min.

Linearity

This study was performed by preparing the concentration of 5- 25 µg/mL by diluting the standard stock solution with different levels of Aripiprazole. Peak area and retention time was studied and constructed the calibration curve by plotting concentrations and peak area to check the correlation.

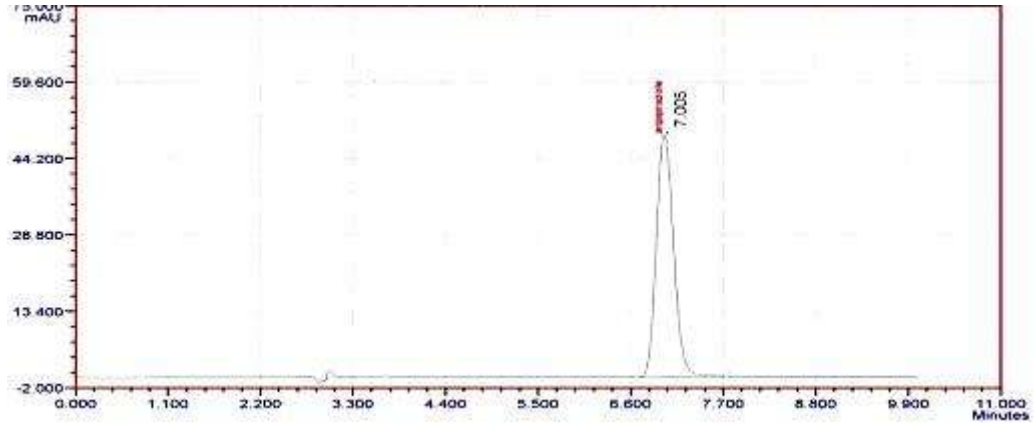

Fig. 3: Optimized chromatogram of Aripiprazole.

System suitability

This study was carried out to verify that the analytical system is working properly and can give accurate and precise results. It was carried out by injecting 10 µg/ml of Aripiprazole six times. A working standard solution with concentration 10 µg/mL of Aripiprazole was prepared by pipeting 0.1 mL from the stock solution into a 10 mL volumetric flask and diluted up to the mark

with mobile phase. The system suitability parameters like theoretical plates, peak area, retention time and asymmetric factor were evaluated.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre-analyzed sample), the reference standards of the drugs were added at the level of 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated.

Precision

The intra- and inter-day precision was determined by analyse Aripiprazole (5µg/mL, 10µg/mL and 15µg/mL) for six times on same day (intra-day study) and repeated on the second day (interday study). The chromatograms were recorded.

Specificity: The specificity study was done to check the interference of extraneous components for that a solution containing a mixture of tablet and standard was prepared using sample preparation procedure and injected into the system, to evaluate possible interfering peaks.

Table 2: Recovery study of Aripiprazole

Concentration of spiked level	Average peak Area	Amount Amount added(mg) found (mg)	% of Recovery	% Mean Recovery
80	16983	8 8.11	101.4	
100	18970.2	10 9.89	98.4	99.4
120	22771.9	12 11.79	98.3	

Robustness

Robustness of the method was demonstrated by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 ml/min to 0.8 ml and 1.2 ml/min. The wavelength was changed from 254nm to 256nm and 258nm. The mobile phase composition also changed from 90:10 to 87:13 and 93:7 and sample was injected to check their retention time and tailing factor.

Limit Of Detection (LOD) and Limit Of Quantitation(LOQ)

From the slope and standard deviation LOD and LOQ was calculated. Six replicates of the analyte was prepared in the range of 0.1- 10 µg/mL. The limit of detection was define as the concentration for which a signal-to-noise ratio of 3 was obtained and for Quantitation limit, a signal-to-noise ratio of 10 was considered.

Force degradation study of aripiprazole

Stress study was carried out using different ICH prescribed stress condition such as acidic, besic, oxidative, thermal and photolytic stresses. All study was carried out in 25 ml volumetric flask.

Acid degradation Study

This study was performed in environmental test chamber (Acamus Technologies, India) at 60^{0}C and 75% relative humidity using 1M HCL. 1 ml of standard stock solution was taken in 25 ml of volumetric flask, 1 ml of 1 M HCL was added to the flask, kept in environmental test chamber for 16 hour. After the stress period solution was neutralized using 1M NaOH and make up the volume with mobile phase.

Base degradation study

It was performed at 60^{0}C and 58% relative humidity using same environmental chamber. 1 ml of standard stock solution in 25 ml volumetric flask mixed with IM 1 ml of 1M NaOH for 16 hr. After the suitable stress period the solution was neutralized with I M HCL and make up the volume with mobile phase.

Oxidative degradation study

It was performed in versatile environmental chamber at 40 ^{0}C, 75% relative humidity using 6% H_2O_2. For this purpose 1 ml of standard stock solution was taken and 1 ml of 6% H_2O_2 was added in to flask and kept at 60^{0}C for 16 hr, finally make up the volume up t mark with mobile phase.

Thermal degradation study

This study also has been carried out in environmental chamber at 40^{0}C, 75% relative humidity in oven at 105^{0}C,1 ml of standard solution was taken in 25 ml volumetric flask and kept in chamber for 144 hr. and for dry heat thermolysis 1 mg of dry drug in solid form was placed in oven at 110^{0}C for 2 days.

Photo degradation study

This study was carried out in sunlight (60000- 70000 lux) during day time and in U.V light at 254 nm for the period of 48 hr. 1 ml of the standard solution in 25 ml volumetric flask, make up the volume up to the mark with mobile phase was used for the study.

Solution stability study

Stability of the sample solution was established by storage of sample solution at 25^{0}C for 24 hrs. Sample solution was reanalyzed after 12 and 24 hrs. time intervals & assay was determined for the Aripiprazole and compared against the fresh sample.

RESULTS AND DISCUSSION

Selection of wavelength and optimization of chromatographic condition

A wavelength of 256 nm was selected as ideal wavelength, which was used as detector wavelength in HPLC analysis. Various mobile phases with different ratios of Acetonitrile, buffer, methanol, water were tried before optimization. The samples were initially analysed using a mobile phase consisting of acetonitrile: water (50: 50 v/v) at a flow rate of 1 ml/ min and UV detection at 256 nm. Under this conditions, the peak shape was not optimal and retention time was too long. Acetonitrile: buffer(45: 55 v/v) showed broad peak at 5.19 minutes, methanol: water (80:20 v/v) showed longer retention time i.e. 27.462 min, with methanol: acetonitrile: water (60: 30: 10) and methanol: acetonitrile: water (70:20:10), peak tailing and peak fronting were observed. Methanol : water (90:10) showed less retention time with good peak symmetry was optimized as the mobile phase and optimized chromatogram showed in the figure 3. The optimized injection volume was 10 µL and detection wavelength 256 nm was selected. The analysis of Aripiprazole was achieved on a Purospher star C18 column (250 × 4.6 mm, 5 µm particle size) using 90: 10 v/v mixture of methanol and water as mobile phase with the retention time of 7.005 . The developed method was found to be specific and validated as per ICH guidelines.

Method Validation

In the study of specificity no peaks were detected in the retention time corresponding to analyte peak, which indicates no interference of excipients of the formulation. So the developed method is having the specificity. Proper results of system suitability study indicated that the analytical system worked properly and system suitability results was shown in table 3.

Table 3: Results for system suitability parameters of Aripiprazole

Parameters	Results
λmax(nm)	256
Beer's law limit	5-25
Correlation coefficient	µg/ml
Retention time(min)	0.996
Theoretical plates	7.0±0.5
Tailing factor	3869
Limit Of Detection(µg/ml)	1.16
Limit Of Quantitation(µg/ml)	0.299
	0.908

The curve proved to be linear over a concentration range of 5µg mL^{-1} to 25 µg mL^{-1} was shown in figure 4. The linear regression of concentration vs. peak area resulted in an average coefficient of determination (R^2) is 0.996 .

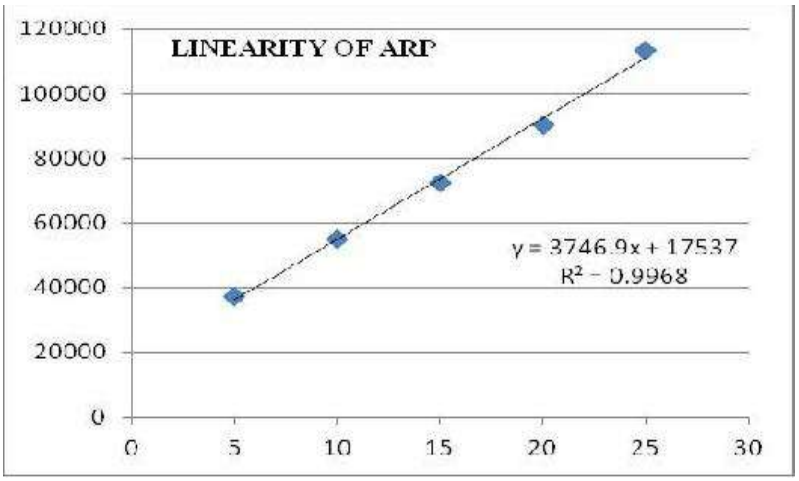

Fig. 4: Linearity graph of Aripiprazole

Accuracy of the method was determined by Recovery studies. To the formulation (pre-analyzed sample), the reference standards of the drugs were added at the level of 80%, 100% and 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug 99.4 was shown in table 2, indicates the accuracy of the developed method.

Intra-day precision was calculated from results obtained from six-fold replicate analysis of samples at three different concentrations (5, 10 and 15 µg mL^{-1}) of on the same day. Inter-day precision was calculated from results from the same samples analyzed on six consecutive days. The relative standard deviation of 6 determinations of Aripiprazole for intra and inter day precision found to be within the acceptance criteria of less than 2.0%. The results obtained are listed in table 5, with other validation parameters which indicates the developed method was precise and the LOD and LOQ of Aripiprazole were found to be 0.299 µg $mL^{-1}$and 0.908 µg mL^{-1} respectively which implies the sensitivity of the developed method

Table 4: Summary of Validation parameters

Validation Parameters	Results
Regression Equation	Y=37469X + 17537
Regression coefficient (R^2)	0.996
Precision (Intraday)	0.5-1.02
Precision (Inter day)	0.4-1.15
Recovery (%)	98.3-101.4
LOD	0.299
LOQ	0.908

The Robustness of the method was determined under different conditions including change in flow rate, wavelength and mobile phase composition. The results obtained by deliberately variation in method parameters and data are shown in table 6, which indicates little bid variation of the chromatographic condition does not affect the different parameters significantly.

Table 5: Results for Robustness of Aripiprazole

Robustness parameter with variation	t_{R^*}	SD	%RSD	T_{f^*}	SD	%RSD
Temp (± 5^0C)	6.86	0.04	0.63	1.27	0.02	1.54
M. P Composition(±3%v/v)	7.03	0.10	1.44	1.53	0.01	0.65
Flow rate (±0.2 ml)	7.00	0.04	0.59	1.53	0.02	1.36
Wavelength (+2 nm)	6.96	0.01	0.25	1.31	0.02	1.58

t_{R^*} = Average of three readings of Retention time. T_{f^*} = Average of three tailing factor.

SD= Standard deviation. %RSD=Percentage relative standard deviation

The chromatogram of the drug samples extracted from tablets did not show any change in the retention time and no extra peaks were observed indicates here was no interference from the excipients, which are commonly present in the tablets. The drug content was found to be 99.3% as shown in table 1. Force degradation study shows aripiprazole was degradate under acidic, basic, oxidation and dry heat stress condition with the rise of several degradation peaks, and it was stable under photolytic stressed condition, section A in figure 5 shows acid degradation pattern, B, C and D is for oxidative, thermal and base degradation pattern of aripiprazole respectively. The acidic stress condition degradated 9.98% of aripiprazole while 6.62% was in alkaline stress condition, in other stress condition like oxidative and thermal stress condition 3.39 and 2.18% of decomposition was found cited in table 6, but aripiprazole was well separated in all stressed conditions from the other degradation products, which shows the specificity of the method.

Table 6: Result of stressed degradation of Aripiprazole

Stressed Condition	% of Drug recovered*	% of drug decomposed*
Acidic hydrolysis	90.02	9.98
Alkaline hydrolysis	93.72	6.28
Oxidative hydrolysis	96.01	3.39
Thermal decomposition	97.82	2.18
Photolytic hydrolysis	99.98	0.02

*Mean of three replicates

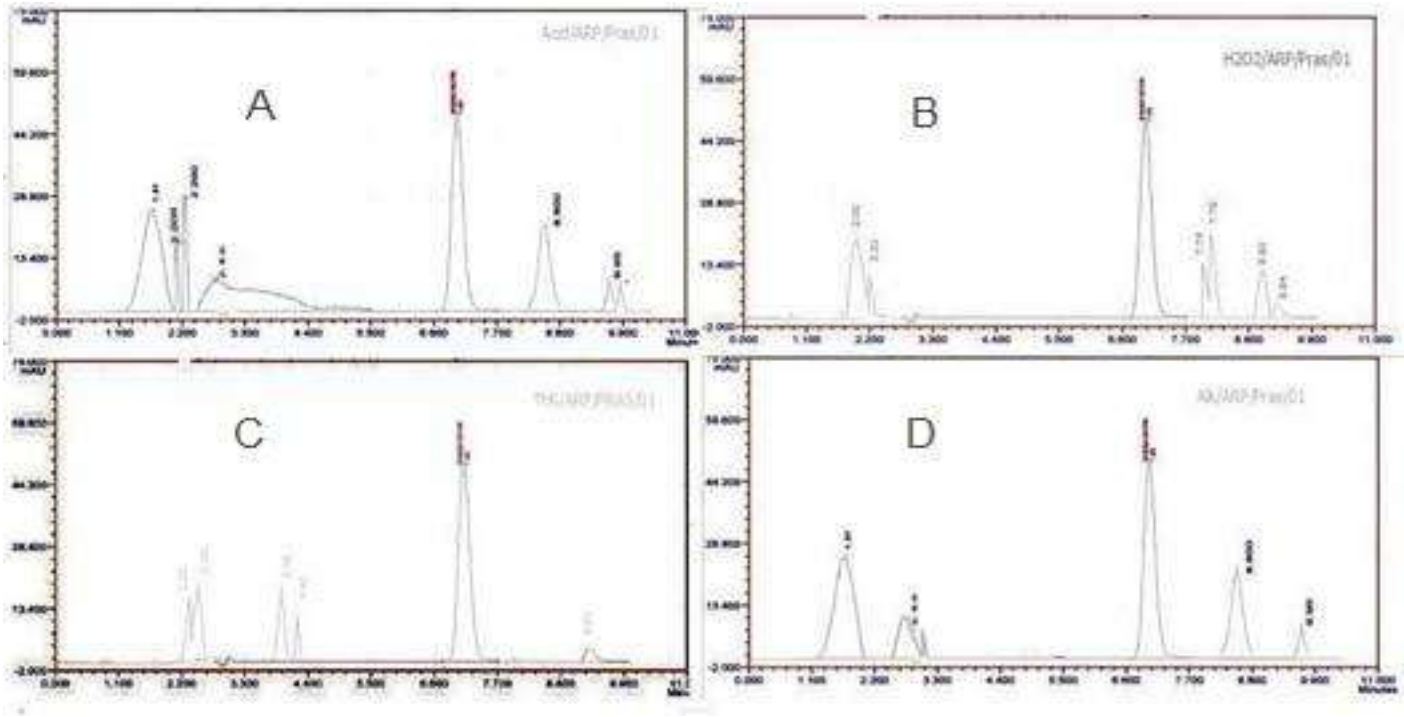

Fig. 5: Degradation behavior of Aripiprazole

Aripiprazole was found to be stable at specific concentration in dilute solution when stored more than 48 hour, at ambient temperature and laboratory condition indicates no significant rise of impurity. Assay results of Aripiprazole at different time interval on above stored condition indicates stability of the sample more than 48 hour. Results are cited in table no7.

Table 7: Solution stability study of Aripiprazole

Condition	Aripiprazole (% of assay)*
Initial	99.40
After 12 hours	99.38
After 24 hours	99.36
After 36 hours	99.31
After 48 hours	99.29

*average of three replicates

Therefore from the above experimental data it can be well concluded that the developed method is stable, accurate and economic and validated has ever developed and indicates the suitability of the method for the routine analysis of Aripiprazole in bulk and tablet dosage form.

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