Research Article

NEW STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE ESTIMATION OF ARIPIPRAZOLE IN BULK AND THEIR FORMULATIONS

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Access this article online

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ABSTRACT

A simple, specific, accurate, precise and stability-indicating reverse phase high performance liquid chromatography (RP-HPLC) method was developed for the estimation of Aripiprazole (ARI) in tablet dosage form. For the HPLC method, Symmetry® C-18 5μ Column consisting of 4.6 × 250 mm internal diameter in isocratic mode, with mobile phase containing Acetonitrile and methanol in the ratio of 35:65 v/v was used. The flow rate was 1mL min-1 and effluent was monitored at 254 nm. Aripiprazole was found to be 4.58±0.40 min, respectively. The method was validated in terms of Linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed that there was good linear relationship between response and concentration in the range of 10 - 100 μg/ml respectively. The LOD and LOQ values for HPLC method were found to be 0.4 and 10 μg/ml respectively. No chromatographic interference from tablet excipients was found. The proposed method was successfully used for estimation of ARI in tablet dosage form.

KEYWORDS: Aripiprazole, Development and Validation, RP-HPLC, Degradation study

INTRODUCTION

Aripiprazole (ARI) is chemically 7-{4-[4-(2,3-dichlorophenyl) piperazin-1-yl]butoxy}-3,4-dihydroquinolin-2(1H)-one. Fig.1 is an typical antipsychotic and antidepressant used in the treatment of schizophrenia, bipolar disorder, and clinical depression[1-2]. Literature survey revealed few analytic methods like HPLC for the estimation of ARI in formulation. Liquid chromatography/mass spectrometry (LC/MS) method has been reported for the determination of ARI in biological fluids[3- 4] The present work describes a novel stability indicating method for the determination of ARI in tablets using reverse phase HPLC. The proposed method was robust and hence suitable for routine determination of the drug in formulation.
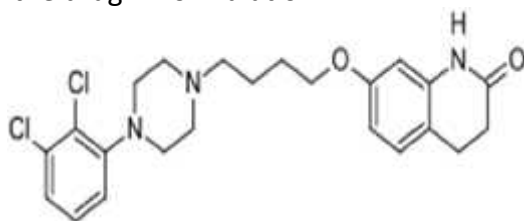

FIG.1: Chemical structure of aripiprazole

EXPERIMENTAL

Chemicals

Standard bulk drug sample of ARI was provided by Hetero Pharmaceutical Limited (Hyderabad, India). Pharmaceutical dosage form used in this study was **ARIP-MT** tablets labeled to contained 5 mg (Torrent Pharmaceutical Limited, India). Acetonitrile (HPLC grade), methanol (HPLC grade) and water (HPLC grade) were obtained from Qualigen Fine Chemicals, Mumbai, India. Sulphuric acid (LR grade), hydrochloride (LR grade) and glacial acetic acid (LR grade) were obtained from Ranbaxy Fine Chemicals Ltd, New Delhi, India. Ammonia solution (LR grade) was obtained from S.D. Fine Chemicals Ltd, Mumbai, India.

Apparatus and chromatographic conditions

HPLC method development and validation was done on a Shimadzu (Japan) Liquid chromatograph equipped with (LC-20 AD pump), LC-20A UV/Vis detector, (rheodyne) 7725i injection with 20µl loop and LC-Solutions software. Stationary phase used was Symmetry® C-18 5µ Column consisting of (4.6 × 250mm) i.d. and the mobile phase was acetonitrile and methanol in the ratio of 35:65 v/v was used. The mobile phase was filtered using 0.45µ membrane filter (Rankem Nylon membranes, New Delhi, India). The mobile phase flow rate was 1ml/min and injection volume was 20µl. All weighing's were done on Shimadzu electronic balance, BL-220 H (Shimadzu Corporation, Japan).

HPLC METHOD

Preparation of standard solution

Standard stock solution of ARI (100 µg ml^{-1}), and PAR (100 µg ml^{-1}) was prepared in Acetonitrile: Methanol (65: 35 % v/v).

Preparation of Calibration graph

Stock solution was diluted with mobile phase get a series of concentration ranging from 10-100 µg ml^{-1} each containing 10 µg ml^{-1} of Paracetamol as internal standard and analyzed in triplicate. The peaks obtained were integrated, peak areas were noted and Calibration graph was plotted using peak area ratios to internal standard peak areas vs. concentration of standard solutions.

Preparation of sample solution

Twenty tablets were weighed and average weight was calculated. Quantity equivalent to 10 mg ARI was weighed, transferred to 100ml standard flask, extracted with acetonitrile and made up with the same solvent. This solution was filtered through Whatman filter paper and suitable aliquots of formulation solutions were prepared to obtain concentration in the linearity range.

METHOD VALIDATION

The developed method was validated according to ICH guidelines for validation of analytical procedure. The linearity was evaluated by linear regression analysis. The calibration graph was plotted for the HPLC method (10-100 µg ml^{-1}). Precision studies were done in terms of repeatability (intra day precision) and intermediate (inter day precision) and was expressed as relative standard deviation (RSD) of a series of measurements. Intra day precision was calculated from six replicate readings at 3 concentration levels with-in the linearity range. Inter day precision was studied by comparing the results on three different days. To study the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre analyzed sample at 3 different levels i.e 50, 100 and 150%. The resultant solutions were then reanalyzed by the proposed method. LOD and LOQ was calculated using single-to-noise (S/N) ratio method. LOD was taken as a concentration of analyte where S/N is 3 and LOQ was taken as a concentration of analyte where S/N is 10. Robustness was evaluated by studying the influence of small deliberate changes of the analytical parameters on the retention times and peak shapes. The method should be robust enough with

respect to all critical parameters so as to allow routine laboratory use. Specificity of the method towards the drug was studied by determination of purity for drug peak in a mixture of stress samples using in a UV detector.

DEGRADATION STUDIES

PROCEDURE

The stress testing was conducted as per ICH[5] guidelines and USP[6] Forced degradation for the drug substance was carried out under acid / base / neutral hydrolysis, photolytic and oxidative stress conditions. Drug at a concentration 1 mg/ml was used in all degradation studies. In each study, blanks and control (zero-hour samples) were used to compare and calculate the %degradation. There were four samples prepared in each stress test,

1) Blank solution stored under normal condition
2) Blank solution subjected to stress like the drug
3) Zero-time sample containing the drug which is stored under normal condition (control) and
4) Drug solution subjected to stress.

HYDROLYTIC STUDIES

Acidic condition

The solution was prepared by dissolving the drug substance in water and the drug was subjected to accelerated degradation under acidic condition by refluxing with 0.05 N HCl at 100°C and the sampling was done at every five min till sufficient degradation was achieved. The resulting solution was neutralized, appropriately diluted and chromatograms were recorded.

Alkaline condition

The drug substance was dissolved in water and the drug was subjected to accelerated degradation under alkaline condition by refluxing with 0.05 N NaOH at 100°C and the sampling was done at every five min till sufficient degradation was achieved. The resulting solution was neutralized, appropriately diluted and chromatograms were recorded.

Neutral condition

For forced degradation study in neutral condition, drug dissolved in water was heated at 100°C samples were withdrawn at appropriate time intervals and subjected to HPLC analysis after suitable dilutions.

Oxidative studies

Initial oxidation studies were performed in 3% H_2O_2 at room temperature for 10 hrs, subsequently the drug was exposed to 6% H_2O_2 at room temperature and analyzed periodically. The resultant solution was appropriately diluted and chromatograms were recorded.

Photolytic studies

For photolytic stress studies, the samples of drug substance and drug product were exposed to sunlight during the "daytime" for 4 days. Samples were withdrawn at appropriate time intervals and subjected to HPLC analysis after suitable dilutions. Appropriate controls were also prepared and injected for each degradation studies.

RESULTS AND DISCUSSION

For the RP-HPLC, chromatographic conditions were optimized to get best resolution and peak shape. The selection of mobile phase was based on peak parameters, (symmetry, theoretical plates and capacity factor) ease of preparation and cost. Symmetrical peaks with good separation (retention time ARI 4.58±0.40 min and for PAR retention time 1.2±0.20 min) was obtained with C_{18} column and mobile phase consisting of acetonitrile and methanol in the ratio of 35:65 v/v was used at a flow rate

of 1ml/min. A typical chromatogram obtained from the analysis of drugs using the developed method is shown in the Fig. 2.
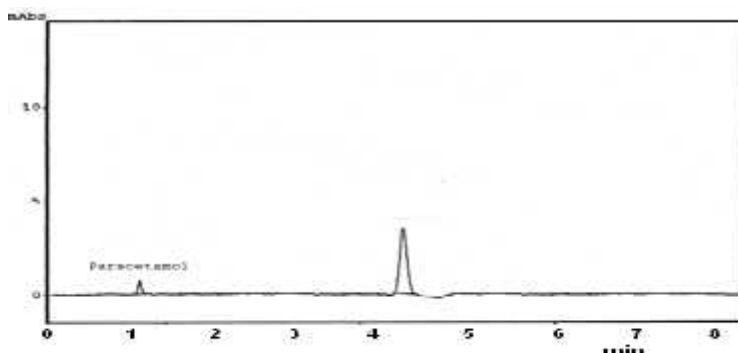

Fig. 2: Optimized Chromatogram of
Paracetamol (10 µg ml^{-1}), Aripiprazole (0.2 µg ml^{-1}),

The optimum wave length for detection and quantification was 254 nm, at which good detector response for both drugs were obtained. There was no interference from the diluents, excipients present in the pharmaceutical formulation. To check the Linearity, standard calibration curve for the drug was constructed by plotting using peak areas ratios of standard drug peak areas to internal standard peak areas vs. concentration of standard solutions and the curve showed good linearity over a concentration range of 10 – 100 µg ml^{-1}. The regression equations for the drugs were found by plotting peak area m Au(y) vs. concentration(x) µg/ml. Table 1 summarize the linearity range and the linear regression equation for the drug. The LOD and LOQ values of ARI were found to be 0.4 µg ml^{-1}and 10 µg ml^{-1} respectively. The Precision of the method was determined by repeatability (intraday) and intermediate precision (inter day). Precision was expressed as the RSD of the results. The values obtained for the precision studies presented (Table 1), indicates good repeatability and low inter day variability. The robustness of the proposed method was evaluated by slight modification in the organic composition of mobile phase and flow rate. During these studies, it was found that there was not much change retention time, area and symmetry of peak.

Table 1: Summary of validation parameters for proposed methods

Parameters	HPLC method
Linearity	10-100 µg ml^{-1}
Linear regression	
Intercept (c)	0.0921
Slope (m)	18.7660
Correlation coefficient (r)	0.9993
Limit of detection (LOD)	0.4 µg ml^{-1}
Limit of quantification (LOQ)	10 µg ml^{-1}
Precision (% RSD)	
Intraday (n=3)	0.2644
Interday (n=3)	0.9443
Repeatability of injection (n=10)	0.5497

Accuracy level (%)	% Recovery	% RSD
50	98.50	0.36
	98.52	
	99.12	
100	97.50	0.52
	98.52	
	97.95	
150	99.20	0.51
	98.52	
	98.22	

The recovery results studies ranges from 97-100 % show the accuracy of the method (Table 2).

Table 2: Recovery studies

The developed method was used for the assay of commercially available tablets and six replicate determinations were performed. Experimental values obtained for the determination of tablets are given in Table 3. The interference of excipients was studied by comparing the chromatography of standards and formulations. The same shape and retention times of peaks showed that there was no interference from the excipients.

Labeled amount, mg /tablet	Amount found, mg/ tablet	% RSD
10	9.899	0.12
	9.922	
	9.911	

Table 3: Analysis of formulation

The system suitability test of the chromatographic system was performed before each run. Five replicate injections of standards were made and peak asymmetry, theoretical plate number, RSD of peak areas determined. For all system suitability injections, asymmetry was < 1.5, theoretical plate number was > 5000 and RSD of peak areas is < 1%.

DEGRADATION STUDIES

Result and discussion

HPLC studies of samples obtained on stress testing of Aripiprazole under different conditions using Acetonitrile: Methanol (65:35% v/v) as mobile solvent system suggested the following degradation behavior.

Forced degradation studies

Acidic condition

The drug was subjected to 0.05 N HCl for 30 min at 100°C forming degradation products at retention time 1.01, 1.76, 2.71 and 4.5 min min Fig. 3b. The rate of hydrolysis in acid was faster as compared to that of alkali or water.

Alkaline condition

The drug was subjected to 0.05 N NaOH for 3 hrs at 100ºC Fig. 3c.

Neutral Condition
In neutral condition, significant degradation of the drug was achieved after heating the drug at 100°C for 8 hrs Fig. 3d.
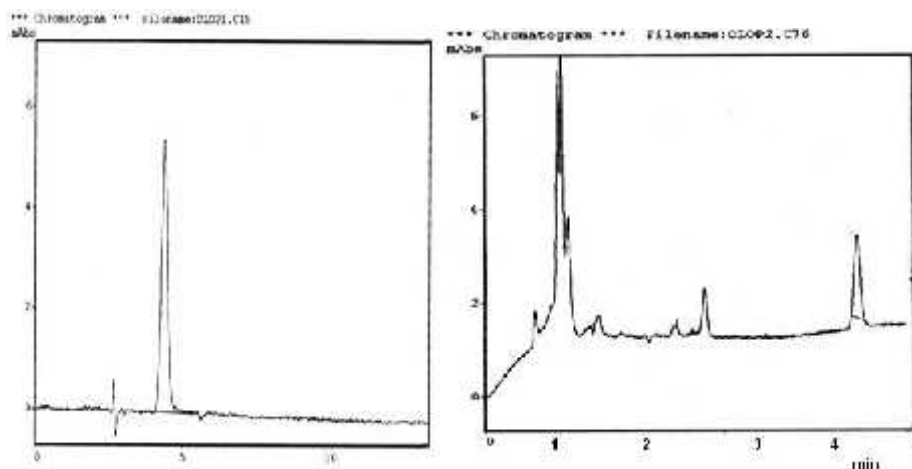**Oxidative studies**
In oxidative condition, significant degradation of the drug was obtained after exposure to 6% H_2O_2 for 24 hrs Fig. 3e.
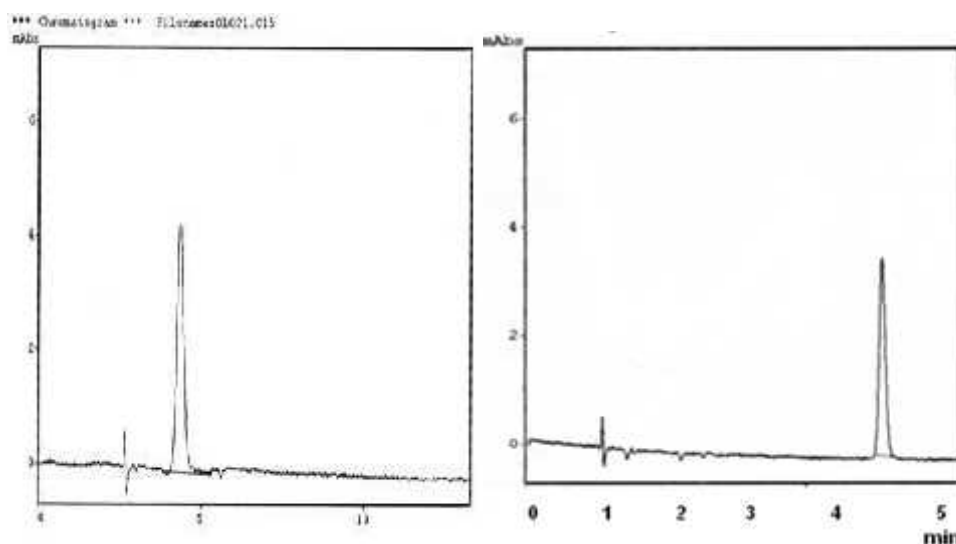**Photolytic studies**
Here sufficient degradation was achieved by exposing the drug product to sunlight during the "day time" for 3.5 days Fig. 3f. Forming degradation products at retention times 1.4, 1.7 and 4.5 min.
Fig. 3: HPLC Chromatogram of forced degradation samples of Aripiprazole
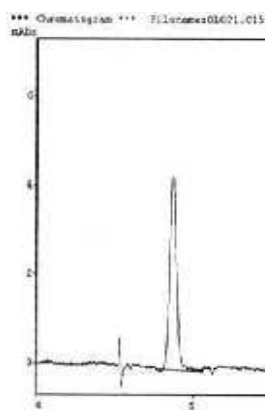

3a. Standard solution of
 Aripiprazole
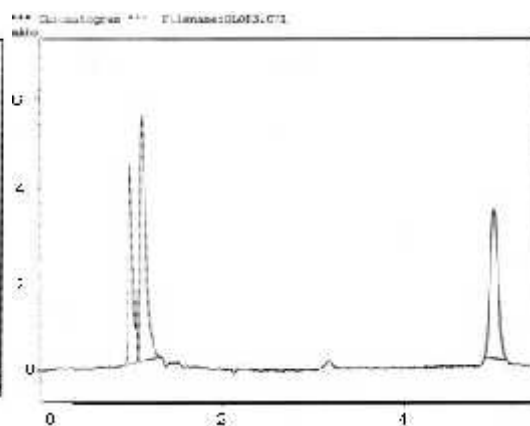
3b. Sample degraded in
 3N Hydrochloric acid

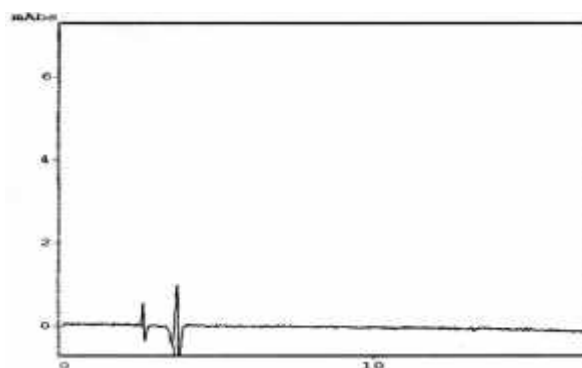3c. Sample degraded in
 3 N Sodium Hydroxide

3d. Sample heated in water

3e. Sample subjected to Oxidative degradation

3f. sample subjected to photolytic degradation

3g. Blank

In all degradation studies except, acid hydrolysis and photolytic studies there was no corresponding formation of degradation products when compared to the standard solution of the drug. This indicated that, may be the drug degraded to low molecular weight non-chromophoric compounds. Singh and Bakshi in their article, stress testing[7] suggested a target degradation of 20-80 % for establishing stability indicating nature of the assay method as even intermediate degradation product should not interfere with any stage of drug analysis. The drug showed extensive degradation in acid hydrolytic condition. UV/Vis detection was used as an evidence of the specificity of the method and to evaluate the homogeneity of the drug peak. Chromatographic peak purity data was obtained from the spectral analysis report and peak purity index value of 1.0000 indicates a homogeneous peak thus establishing the specificity of the assay method. The results of degradation study were summarized in the table 4. Hence the developed HPLC method was stability indicating and can be used for routine analysis of Aripiprazole.

Condition	Aripiprazole	
	% Assay	% Degradation
Acid	81.25	-18.75
Base	86.39	-13.61
Peroxide	90.18	-9.82
Photolytic	84.96	-15.04
Hydrolytic	85.15	-14.85

Table 4: Forced degradation and stability studies

CONCLUSION

Since the developed methods showed no interferences from the diluents like lactose, mannitol etc. and excipients like talc, magnesium stearate etc., and these methods can be used for the routine analysis of Aripiprazole from its dosage forms.

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