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## Development of Validated RP-HPLC Method for Quantitative Determination of Aripiprazole



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**K.PRASHANTHI, V. NAGA SRAVANI,  
G.SUGANDA, M.MOTHIRAM,  
B. SPANDANA, C.H.KRISHNA MOHAN**

*School of Pharmacy, Nalla Narasimha Reddy Education  
Society's Group of Institutions, Hyderabad, Telangana,  
India.*

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### ABSTRACT

A simple, selective, rapid, and economical reversed phase high performance liquid chromatography (RP-HPLC) method for the determination of Aripiprazole in the pharmaceutical dosage form has been developed and validated. The separation and quantification were achieved on waters 2695 5¼ PHENOMEX 150\*4.6mm column using a mobile phase of orthophosphoric acid: methanol (70:30) at a flow rate of 1 ml/min with detection of analyte at 245nm. The separation was achieved within 3.5min for aripiprazole sample. The method showed good linearity in the range of 1.25µg/mL- 3.75µg/ml. The recovery (mean  $\hat{A}\pm$ S.D.) of low, middle and high concentrations were 1.25µg/mL, 2.5µg/ ml, 3.75µg/ml respectively.



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## INTRODUCTION

Aripiprazole, (7-[4-[4-(2, 3-dichlorophenyl)-1-piperazinyl] butoxy]-3, 4-dihydrocarbostyryl (Figure 1), is a psychotropic agent belonging to the chemical class of benzisoxazole derivatives and is indicated for the treatment of schizophrenia<sup>1, 2</sup>. RP-HPLC method is the technique that most commonly used for the determination of aripiprazole. In this paper, we describe a simple, accurate, sensitive and validated RP-HPLC method for analysis of aripiprazole in tablet formulation. This method has been successfully used for quality-control analysis of drugs and for other analytical purposes.

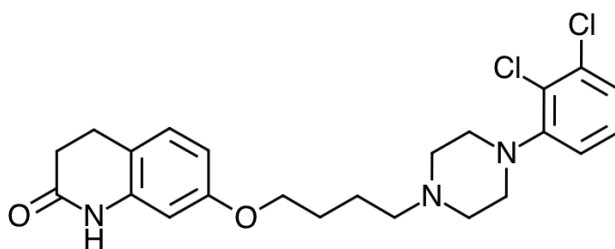


Figure 1. Structure of Aripiprazole

## MATERIALS AND METHODS

A reference standard of aripiprazole was obtained from apollo pharmacy (Hyderabad, India). Pharmaceutical product containing the same drug (10 mg per tablet), obtained from same laboratory and was used in the experiments. Acetonitrile (HPLC grade; CAN) and HPLC grade water were utilized for analysis. Mobile phase was filtered through a 0.45  $\mu\text{m}$  cellulose acetate papers were used for preparation of sample solutions.

### Chromatographic system and conditions

Analysis was performed with a waters 2695 5 $\frac{1}{4}$  PHENOMEX 150\*4.6mm column using a mobile phase of orthophosphoric acid: methanol (70:30) at a flow rate of 1.0ml/min with detection of analyte at 245nm under reverse phase conditions.

## **Calibration**

Calibration plots were constructed by analysis of appropriate working solutions of aripiprazole in the mobile phase and plotting concentration against peak area response for each injection. Unknown sample was quantified by reference to these calibration plots.

## **Sample preparation**

Twenty tablets were weighed and powdered; an amount of powder equivalent to 50 mg aripiprazole was accurately weighed and transferred to a 50ml. volumetric flask. Mobile phase (25ml) was added and the mixture was sonicated for 10 min for complete extraction of the drug and the solution was diluted to volume with mobile phase. The solution was centrifuged at 4000 rpm for 10 min and the clear supernatant was collected and filtered through a 0.2  $\mu\text{m}$  membrane filter. From this solution 2 ml was taken and diluted to 50 ml with mobile phase, to furnish a 40  $\mu\text{g ml}^{-1}$  solution, of which 10ml was injected for HPLC analysis.

## **RESULTS AND DISCUSSION**

### **Method development and optimization**

Column chemistry, solvent selectivity, solvent strength, additive strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so there was no interference with the aripiprazole peak from solvent or excipient peaks. Other criteria for example the time required for analysis, assay sensitivity, solvent noise and use of the same solvent system for extraction of the drug from formulation matrices during drug analysis were also considered. After each change of mobile phase the column was equilibrated by passage of at least twenty column volumes of the new mobile phase to investigate the appropriate wavelength for determination of aripiprazole, UV- visible spectra in the range of 200-400 nm were acquired from a solution of the drug in the mobile phase. From the UV spectra obtained the wavelength selected for monitoring the drug was 245 nm. Solutions of the drug in the mobile phase were injected directly for HPLC analysis and the responses were recorded at 245 nm. It was concluded that 245 nm was the most appropriate wavelength for analysis of the substance with suitable sensitivity.

## Chromatography

Symmetrical peaks were obtained for aripiprazole. Typical chromatograms obtained from a blank and from a solution of the drug are illustrated in figure 2. The retention time of aripiprazole was 3.5 min and the overall chromatographic run time was 6.0 min.

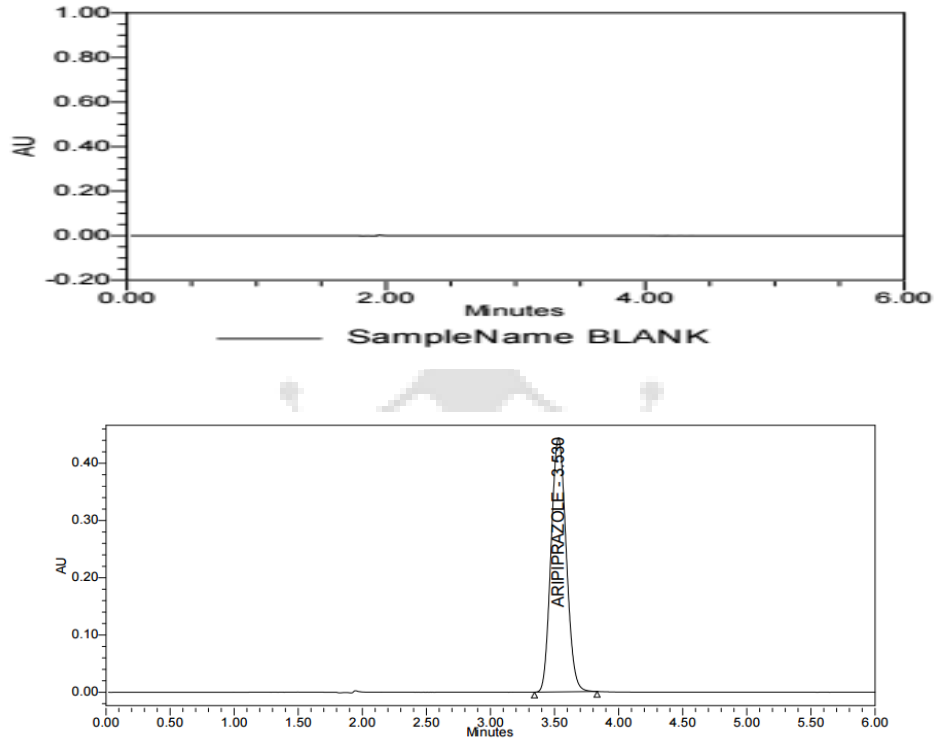


Figure 2. (A) Typical Chromatogram Obtained From Blank and (B) Aripiprazole Solution

## METHOD VALIDATION

### Linearity

The linearity of the method was tested the calibration solutions described above. Plot of concentrations against responses was leaner in the range of 50, 75,100,125,150  $\mu\text{g ml}$  (figure .3) Mean regression equation was  $y= 43363$  the correlation coefficient was  $R^2=0.999$ .

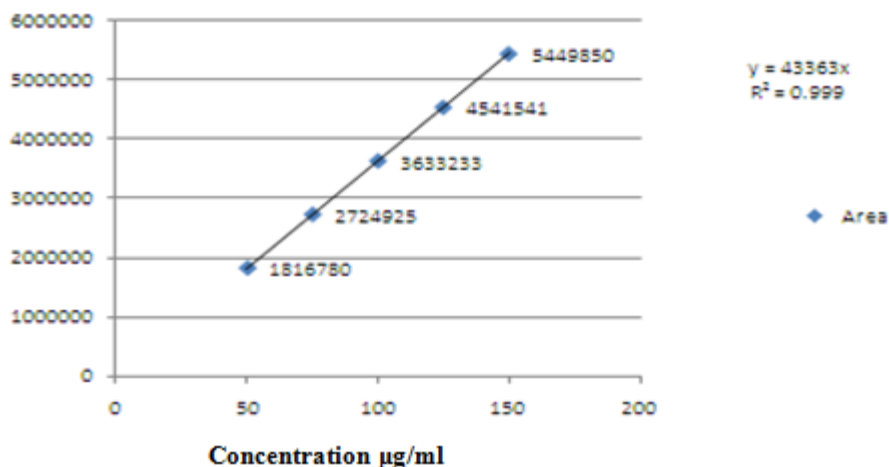


Figure 3. Calibration plot for aripiprazole

### Limit of detection and quantification

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be readily detected but not necessarily quantified. It is usually regarded as the amount for which the signal-to-noise ratio (SNR) is 3:1 the limit of quantification (LOQ) is defined as the lowest concentration of an analyst that can be quantified with acceptable precision and accuracy. It is usually regarded as the amount for which the SNR is 10:1. Two types of solution, blank solution and solutions containing known, progressively, decreasing concentrations of the analyte, were prepared and analyzed LOD and LOQ were 2.3 and 9.1  $\mu\text{g ml}^{-1}$  respectively.

### Accuracy

Recovery studies were performed in triplicate after spiking raw material in volumetric flasks with amounts of aripiprazole equivalent to 50, 100, 150% of the standard concentration of aripiprazole as in the analytical method. The results obtained indicate that recovery were excellent, not less than 99% and that relative standard deviations also less than 2%.

**Table 1. Results obtained from accuracy**

**Peak Name: ARIPIPRAZOLE**

	SampleName	Peak Name	RT	Area
1	ACCURACY 50%1	ARIPIPRAZOLE	3.516	1811149
2	ACCURACY 50%2	ARIPIPRAZOLE	3.520	1808810
3	ACCURACY 50%3	ARIPIPRAZOLE	3.518	1804688
4	ACCURACY 100%1	ARIPIPRAZOLE	3.524	3633901
5	ACCURACY 100%2	ARIPIPRAZOLE	3.526	3628450
6	ACCURACY 100%3	ARIPIPRAZOLE	3.528	3619700
7	ACCURACY 150%1	ARIPIPRAZOLE	3.531	5441920
8	ACCURACY 150%2	ARIPIPRAZOLE	3.529	5418445
9	ACCURACY 150%3	ARIPIPRAZOLE	3.531	5429201

### Precision

Intraday precision was calculated from results obtained from five replicate of samples at three different concentrations on the same day. Inter-Day precision was calculated from results from the same samples analyzed on five consecutive days. The results obtained are table 2.

**Table 2. Results obtained from precision**

s.no	Sample name	Peak names	RT	AREA
1	Precision 1	ARIPIPRAZOLE	3.535	3648855
2	Precision 2	ARIPIPRAZOLE	3.540	3656252
3	Precision 3	ARIPIPRAZOLE	3.529	3634460
4	Precision 4	ARIPIPRAZOLE	3.529	3660252
5	Precision 5	ARIPIPRAZOLE	3.527	3623027
6	Precision 6	ARIPIPRAZOLE	3.526	3632859

### Specificity

The specificity of the method was tested by chromatographing a mixture of commonly used tablet excipients for example starch, lactose and magnesium stearate (blank placebo) and comparing the chromatogram with that obtained from a mixture of drug and the same additives (placebo). The chromatogram obtained (figures 4 & 5) showed separation of the analyte from the

excipients was complete, i.e. there was no interference from the excipients under the chromatographic conditions used for the analysis.

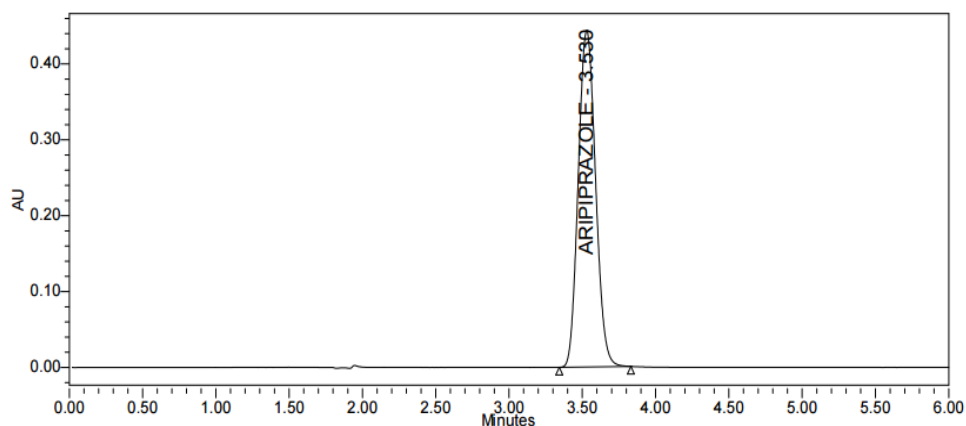


Figure 4. Chromatogram obtained from tablet sample

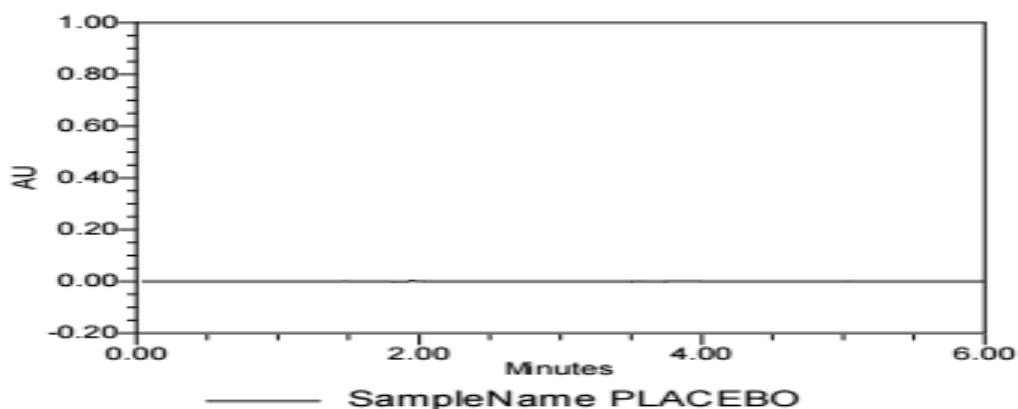


Figure 5. Chromatogram obtained from placebo

#### Application of the method to tablets

The method was used for determination of aripiprazole in a tablet formulation. The results obtained (table 3) showed the amount found was that accepted and RSD (%) values were low, which confirms the method is suitable for routine analysis of the compound in pharmaceutical preparations. A typical chromatogram obtained from analysis of a tablet formulation is shown in figure. 4.

**Table 3. Results from analysis of aripiprazole in tablets.**

	Weight of STD	UNITS	V1	V2	V3	Potency of STD	Mean Area
Standard Preparation-1 :	100.00	mg	100	3	25	99.8	3633233
Standard Preparation-2 :		mg					
Sample Preparation			100	3	25	L.C-1	10
						L.C-2	
						Average Wt	116.04

## CONCLUSION

This RP-HPLC method for analysis of aripiprazole in formulations is very simple, sensitive, and accurate. The run time is 6 min only; so many samples can also be processed and analyzed in a short period of time. The procedure described is suitable for the routine estimation of aripiprazole in pharmaceutical formulations

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