



Validated RP-HPLC Method for Analysis of Aripiprazole in a Formulation

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Abstract: A rapid, simple and validated reversed-phase high-performance liquid chromatographic method has been developed for analysis of aripiprazole in tablet dosage form. Aripiprazole was separated on an ODS analytical column with a 40:60 (v/v) mixture of acetonitrile and triethanolamine buffer (5 mM, pH 3.5 ± 0.05 adjusted by addition of 85% phosphoric acid) as mobile phase at a flow rate of 1.5 mL min⁻¹. The effluent was monitored by UV detection at 254 nm. Calibration plots were linear in the range of 20 to 60 µg mL⁻¹ and the LOD and LOQ were 0.411 and 1.248 µg mL⁻¹, respectively. The high recovery and low relative standard deviation confirm the suitability of the method for routine quality control determination of aripiprazole in tablets.

Keywords: Aripiprazole, RP-HPLC, Tablet analysis, Validation.

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^{1,2}. In the references, gas chromatography-mass spectrometry³, LC-MS/MS⁴, capillary electrophoresis⁶, methods are reported for the analysis of aripiprazole in biological fluids. HPLC is the technique that most commonly used for the determination of aripiprazole in plasma⁵⁻⁹, UPLC-MS in *in vitro* samples¹⁰ and RP-HPLC method in bulk drug and solid dosage forms by internal standard method¹¹ also reported. 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.

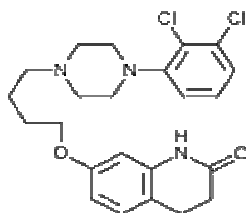


Figure 1. The structure of aripiprazole.

Experimental

A reference standard of aripiprazole was obtained from Orchid Healthcare (Chennai, India). A pharmaceutical product containing the same drug (30 mg per tablet), obtained from the same laboratory and was used in the experiments. Acetonitrile (HPLC grade; ACN) and water of HPLC grade were from Qualigens (Bombay, India) and orthophosphoric acid and triethanolamine (analytical reagent grade) were purchased from SD Fine Chemicals (Bombay, India). De-ionized water was used throughout the experiment. Before use, mobile phase was filtered through a 0.45 μm cellulose acetate filter from Millipore (USA). Whatman no. 41 filter papers (obtained commercially) were used for preparation of sample solutions.

Chromatographic system and conditions

Analysis was performed with a Shimadzu (Japan) chromatograph comprising an LC-10 AT VP solvent-delivery module, a SPD-10A UV-visible detector, 10 μL sample loop. Aripiprazole was chromatographed on a 250 mm \times 4.6 mm *i.d.*, 5 μm particle, Hypersil Gold ODS analytical column under reversed-phase partition conditions. The mobile phase was a 40: 60 (v/v) mixture of acetonitrile and triethanolamine buffer (5 mM, pH 3.5 ± 0.05 , adjusted by addition of 85% phosphoric acid). The flow rate was 1.5 mL min^{-1} and the analyte was monitored at 254 nm. The system was used in an air-conditioned HPLC laboratory (20 ± 2 °C). Before analysis the mobile phase was degassed by use of a Branson Ultrasonics (USA) sonicator and filtered through a 0.2 μm injection filter. The column was equilibrated before each injection.

Calibration

Calibration plots were constructed by analysis of appropriate working solutions (concentration 20, 30, 40, 50 and 60 $\mu\text{g mL}^{-1}$) of aripiprazole in the mobile phase and plotting concentration against peak-area response for each injection. Unknown samples were 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 50 mL volumetric flask. Mobile phase (25 mL) 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 μm 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 10 μL was injected for HPLC analysis.

Statistical calculations

Standard regression curve analysis was performed by use of Microsoft (USA) Office Excel 2003 software, without forcing through zero. Means and standard deviations were calculated by use of SPSS software version 9.5 (SPSS, Cary, NC, USA). Homoscedasticity for the calibration plots was tested by using GraphPad Prism software, demo version.

Results and Discussion

Method development and optimization

Column chemistry, solvent selectivity (solvent type), solvent strength (volume fraction of organic solvent(s) in the mobile phase), 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 200–400 nm were acquired from a solution of the drug in the mobile phase (Elico, India; model SL-164 spectrophotometer). From the UV spectra obtained the wavelength selected for monitoring the drug was 254 nm. Solutions of the drug in the mobile phase were injected directly for HPLC analysis and the responses (peak area) were recorded at 254 nm. It was observed there was no interference from the mobile phase or baseline disturbance at 254 nm. Therefore, it was concluded that 254 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(a&b). The retention time of aripiprazole was 3.8 min and the overall chromatographic run time was 8.0 min.

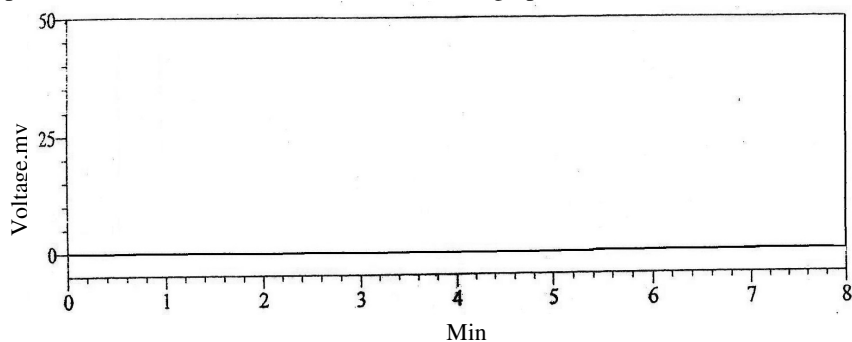


Figure 2(a)

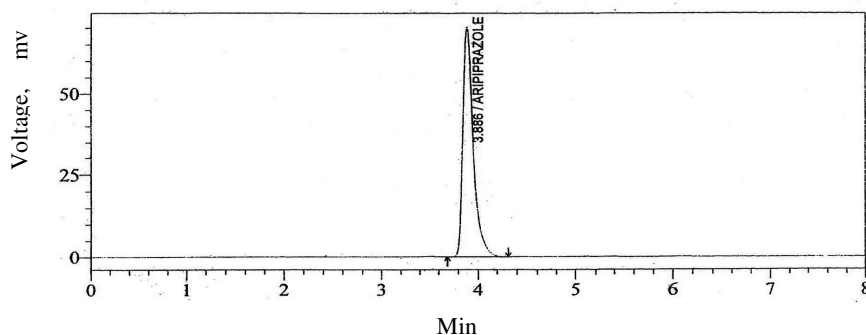


Figure 2(b)

Figure 2. (a) Typical chromatograms obtained from blank and (b) aripiprazole solution.

Method Validation^{12–15}*Linearity*

The linearity of the method was tested using the calibration solutions described above. Plot of concentrations against responses were linear in the range of 20–60 $\mu\text{g mL}^{-1}$ (Figure 3). The mean regression equation was $Y = 1.2958 \times 10^4 x + 1422.2$. The correlation coefficient was 0.9999.

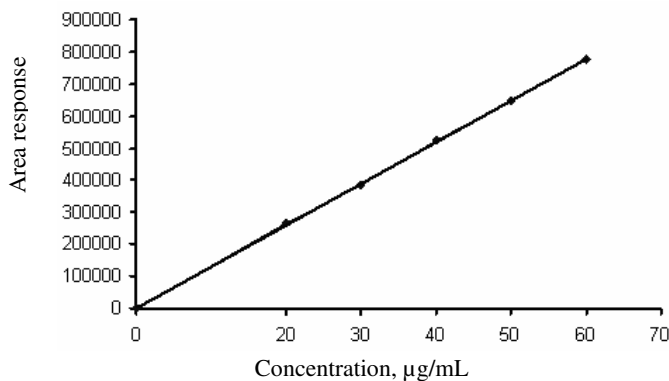


Figure 3. Calibration plot for aripiprazole.

Limits 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 quantitation (LOQ) is defined as the lowest concentration of an analyte 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 0.411 and 1.248 $\mu\text{g mL}^{-1}$, respectively.

Accuracy

Recovery studies were performed in triplicate after spiking raw material in volumetric flasks with amounts of aripiprazole equivalent to 80, 100 and 120% of the standard concentration of aripiprazole (40 $\mu\text{g mL}^{-1}$) as in the analytical method. The results obtained (Table 1) indicate that recovery were excellent, not less than 99% and that relative standard deviations also less than 2%.

Table 1. Accuracy of the method.

Drug	Spike level, %	Concentration added, $\mu\text{g mL}^{-1}$	Mean amount recovered, $\mu\text{g mL}^{-1}$, n=3	Recovery, %, n = 3	RSD, %, n = 3
Aripiprazole tablets	80	32	32.24	100.75	0.86
	100	40	39.93	99.82	0.95
	120	48	48.02	100.04	0.97

Precision

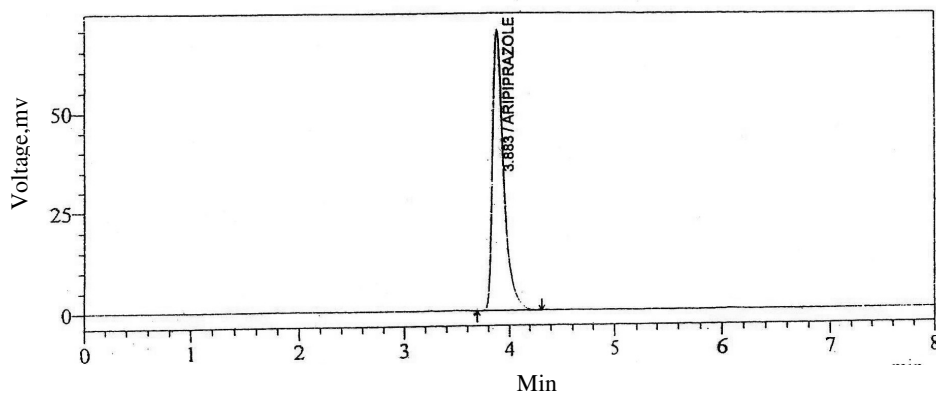
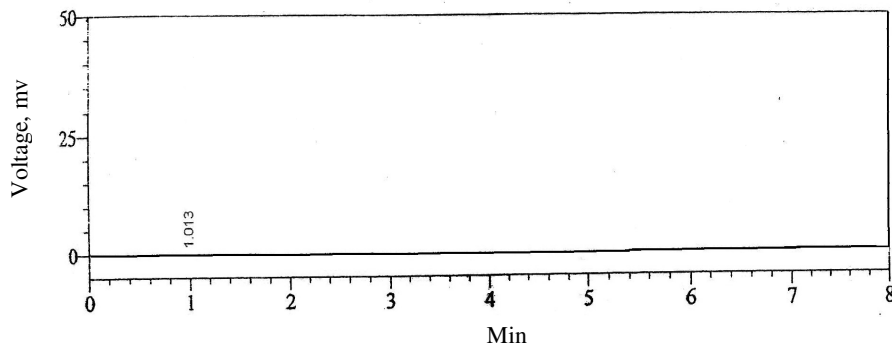
Intra-day precision was calculated from results obtained from five-fold replicate analysis of samples at three different concentrations on the same day. Inter-day precision was calculated from results from the same samples analyzed on five consecutive days. The results obtained are listed in Table 2.

Table 2. Intra-day and inter-day precision of the method.

Concentration Added, $\mu\text{g mL}^{-1}$	Intra-day precision		Inter-day precision	
	Mean amount found, $\mu\text{g mL}^{-1}$, n = 5	RSD, %, n = 5	Mean amount Found, $\mu\text{g mL}^{-1}$, n = 5	RSD, %, n = 5
32	32.04	0.98	32.61	0.99
40	40.12	0.95	39.99	1.07
48	47.98	1.01	48.12	0.96

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 chromatograms 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.

Stability

The stability of aripiprazole in solution was checked by determining the percentage deviation of the amounts present in solution after 72 h at room temperature in comparison with the amount at zero time. The results obtained after 72 h showed no significant variation; the percentage deviation was less than 2% of the initial amount. This is indicative of good stability of each component in the mixture over a period of 72 h.

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 expected 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.

Label claim, mg per tablet	30
Amount found, mg per tablet	29.98
Amount found, %, n = 6	99.93
RSD, %, n = 6	0.021

Conclusion

This RP-HPLC method for analysis of aripiprazole in formulations is very simple, sensitive, and accurate. The run time is 8 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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