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Research article

Development and validation of rapid HPLC method for determination of Aripiprazole in bulk drug and pharmaceutical formulation

Naved Ahmed, Obaid Shaikh*, Aateka Barrawaz, Sarfaraz Khan, Zahid Zaheer

Department of Quality assurance, Y.B. Chavan College of pharmacy, Aurangabad, Maharashtra, India-431001.

Key words: Aripiprazole, RP-HPLC, Chromatograph, Accuracy, Method validation.

***Corresponding Author: Obaid Shaikh,** Department of Quality assurance, Y.B. Chavan College of pharmacy, Aurangabad, Maharashtra, India-431001.

Abstract

A simple, sensitive, rapid and economical reversed phase high performance liquid chromatography (RP-HPLC) method was developed for the quantitative detection of aripiprazole in bulk and pharmaceutical formulation. The separation and quantification were achieved on waters spherisorb 5µ ODS 24.6mm x 250mm C18 column using a mobile phase of acetonitrile : methanol: buffer (20:40:40 v/v/v/) pH 3.5 at flow rate of 1.0 ml/min with detection wavelength at 254nm. The separation was achieved within 7.7±0.1 min for aripiprazole sample. The method shows good linearity between the ranges of 5-25µg/ml. The intra and inter day variation was found to be less the 2%. The mean recovery of drug from solution was 103.67%. This method can be applied directly for the estimation of drug content in pharmaceutical formulation.

Introduction

Aripiprazole (ARP) is a typical antipsychotic agent, which is used in a treatment of schizophrenia, bipolar I disorder and acute treatment of manic and mixed episodes [1-3]. It is also used in Tourette's disorder in pediatrics patients (16-18 years) in the dose range 5-20 mg/day patient weight less than 50 kg. It has chemical name 7-(-4-(4-2-3-dichlorophenyl)-1-piperazinyl)butoxy)-3,4-dihydrocarbostyril (Figure 1). It is effective in the treatment of both negative and positive symptoms of schizophrenia disorder. This agent belongs to the class of benzioxazole with dose 10-15 mg/day. It has partial agonist effect towards 5- HT1A receptor, dopamine D2 receptor and antagonistic effect on 5-HT2 receptor. Its sides effects including weight gain, QTc prolongation and hyperprolactinemia [4]. On the basis of literature survey few analytical methods reported for the detection of aripiprazole in pharmaceutical dosage forms and biological fluids include high performance liquid chromatography (HPLC), gas chromatography-mass spectroscopy (GC-MS), liquid chromatography-tandem mass spectroscopy (LC-MS/MS), capillary electrophoresis and spectrophotometric methods have been describe for the determination of aripiprazole in pharmaceutical preparations [5,7]. The purpose of the current study was to develop a validated HPLC method for determination of aripiprazole in bulk and pharmaceutical dosage form, to achieve more accuracy, specificity and precision. The method validation was perfomed according to ICH guidelines. The method designed for estimation of aripiprazole is more superior than previously reported methods and water is used as major part of solvents and less use of hazardous organic solvents.

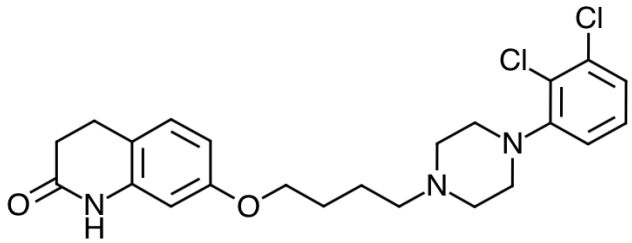

Figure 1. Structure of Aripiprazole

Experimental

Material

Aripiprazole (ARP) was obtained as gift sample from Medley pharmaceutical Ltd, Mumbai, India and acetonitrile and methanol of HPLC grade (Merck, Mumbai, India), Milli-Q-water (Rankem, India) were used. Aripiprazole tablets (ARIPRAZ-10, Vama pharma) were used for the study.

Instrumentation

A high performance liquid chromatography (SHIMADZU SCL-10 VP) with one an SCL-10 AT VP pump, with UV/VIS detector SPD-10 AVP, CTS-10 AS VP column (250 mm x 4.6 mm i.d particle size 5 µm) was used isocratic mode. HPLC system was equipped with the software class-VP 1c solutions (Shimadzu). The mobile phase was composed of acetonitrile: methanol: buffer (20:40:40 v/v/v).

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The prepared mobile phase was filtered through 0.45 µm Millipore HVLP filter paper and degassed by sonication process before use. A flow rate of 1.0 ml/min was set. Detection was carried out at 254 nm. The retention time was 7.7±0.1 minutes. The all validation parameters of the proposed method were also carried out according to ICH guidelines.

Method Development

To optimize the chromatographic conditions, the effect of chromatographic variables like pH, mobile phase, flow rate and solvent ratio were studied. Many solvent systems were tried for the development of a suitable HPLC method for estimation of aripiprazole in bulk drug and pharmaceutical formulations [8]. After an extensive literature survey it is prominent that acetonitrile and methanol were the constituents of almost every mobile phase here in this method we have developed a new mobile phase constituting buffer as a major part of mobile phase [9].

For the selection of mobile phase strength for specific separation problem can be determined by thin layer chromatography. TLC results are more readily transferable to HPLC system. The mobile phase tried for this purpose were acetonitrile: water (60:40 v/v), methanol: water (70:30 v/v), buffer: acetonitrile (40:60 v/v), methanol: water: acetonitrile (25:40:35 v/v/v) and acetonitrile: methanol: buffer (20:40:40 v/v/v). The effect of flow rate and pH was also seen on the symmetry and resolution was selected. The extraction of drug from the formulation was performed for quantification of a drug by extraction using different solvents.

Calibration Curve

Various concentrations (5-25 µg/ml) were made for the preparation of calibration curve from the stock solution. The mobile phase after filtration through 0.45 µm membrane filter was delivered at 1.0 ml/min for column standardization, the baseline was continuously monitored during the process. The UV visible spectroscopy scan of ARP was performed between 200-400nm and detection wavelength was selected at 254nm. The prepared dilutions were injected serially and areas under the peaks were calculated for each dilution. Check the stability of drug in solution during analysis was determined by repeated analysis of samples during the experimentation process on the same day and also after 48 hrs storage of drug solution at laboratory bench condition and in the refrigerator.

Method validation

Linearity

For quantitative analysis of ARP, the calibration curves were plotted for each concentration ranges. The linearity ranges for ARP found to be 5-25 µg/ml respectively.

Precision

The reproducibility of proposed method was determined by performing tablet assay at different time intervals (3 hour interval) on same day (Intra-day precision) and on three different days (Inter-day precision) ARP.

Accuracy as recovery

The accuracy was determined by standard addition method. Three different levels (80%, 100% and 120%) of standards were spiked to commercial tablet in triplicate. The mean of percentage recoveries and the % relative standard deviation (%RSD) was calculated.

Specificity

The specificity of the method was tested by some impurities and mixture of generally used tablet excipients such as lactose, starch, magnesium stearate (blank placebo). It is calculation of the percentage recovery of each component in the presence of other interfering substances.

Robustness

The Robustness study was carried out by determining effect of small variation in wavelength and in Ruggedness; sample was analyzed by two different analysts. The pH of buffer solution was deliberately changed slightly from PH 3.5 to 4.

Assay of commercial dosage form

The quantity equivalent to 10 mg tablet powder was weighed accurately and transfered into 10 ml volumetric flask and dissolved in 5ml acetonitrile and volume is make up to the mark. The solution is ultrasonicated for 30 minutes and then filtered through Whatman filter paper (No. 41). It gives 1000 µg/ml of ARP named as stock solution.

0.25 ml stock solution was taken and transfer into 10ml volumetric flask. Volume was made with mobile phase up to the mark. It gives 25 µg/ml of ARP the sample was injected corresponding to 25µg/ml of ARP and peak area was recorded.

Results and Discussion

Development and optimization of the HPLC method

The proposed HPLC process was optimized with a view to developed a suitable analytical method. The various mobile phase were tried for this purpose such as acetonitrile: water (60:40 v/v), methanol: water (70:30 v/v), buffer: acetonitrile (40:60 v/v), methanol: water: acetonitrile (25:40:35 v/v/v), acetonitrile: ammonium acetate (90:10 v/v). The chromatogram obtained with acetonitrile: methanol: buffer (20:40:40 v/v/v) solvent system was found to have very good symmetry with Rt (7.7±0.01) and sharp well defined peak. The mixture of acetonitrile: methanol: buffer (20:40:40 v/v/v) was chosen as mobile phase. The drug was stable in the mixture of mobile phase for a period of 48 hrs at laboratory temperature and under refrigerator condition.

Method validation

Linearity

A 20:40:40 v/v/v mixture of acetonitrile, methanol and buffer was used and dilution was made in the range of 5-25µg/ml for ARP. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 5-25µg/ml for ARP. The relevant data are summarized in Table 1 and Calibration curve was shown in Figure 2. The regression equations of this curves was computed.

Table 1. Linearity of Aripiprazole by HPLC

Sr. No.	Conc. (µg/ml)	Mean peak area
1	5	376.86
2	10	801.41
3	15	1206.48
4	20	1571.35
5	25	2006.56

LOD and LOQ

The limit of detection and limit of quantification for ARP was found to be 0.1009µg/ml and 0.3058µg/ml respectively, which indicate the sensitivity of the method.

Precision

Precision of the method was performed by intra-day and inter-day studies. The % RSD values obtained from peak area for ARP was observed and shown in the Table 2.

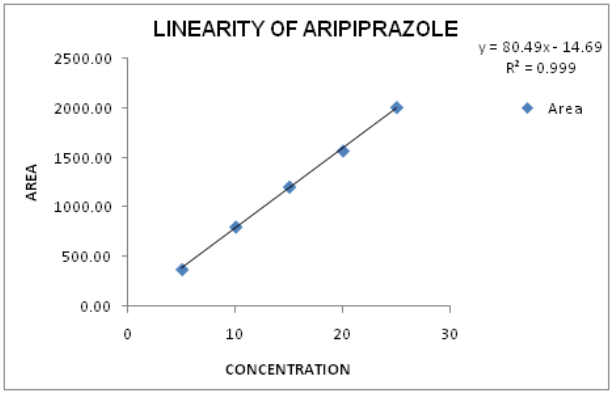

Figure 2. Calibration Curve of Aripiprazole by HPLC

Accuracy as recovery

The accuracy of the method was determined by recovery studies and the percentage recovery was calculated. The recoveries of ARP were found to be in the range of 103-104%. The proposed liquid chromatographic method was applied to the determination of ARP in their dosage forms (Aripraz-10 tablet). The results for ARP comparable with the corresponding labeled amounts. From the amount of drug percentage recovery was calculated. The relevant results are furnished in Table 3.

Table 2. (a) Precision study of Aripiprazole (Inter day)

Sr No.	Conc.	Mean Peak Area	Amount found	%Amount found	SD	%RSD
1	10	780.34	9.87	98.77	8.27	1.06
2	15	1235.57	15.53	103.55	3.79	0.31
3	20	1553.29	19.48	97.40	8.42	0.54

Table 2. (b) Precision study of Aripiprazole (Intraday)

Sr No.	Conc.	Mean Peak Area	Amount found	%Amount found	SD	%RSD
1	10	785.34	9.93	99.39	1.20	0.15
2	15	1230.57	15.47	103.13	3.28	0.27
3	20	1563.29	19.60	98.02	8.42	0.54

Table 3. Accuracy by the Recovery Study of Aripiprazole

Statistics	Level of Recovery		
	80 %	100 %	120 %
Amount Present (µg/ml)	10	10	10
Amount of Standard Added (µg/ml)	8	10	12
Mean Peak Area	1452.46	1593.29	1755.01
Total Amount Recover (µg/ml)	18.23	19.98	21.93
% Recovery	103.30	104.35	103.38
SD	0.04	0.04	0.08
% RSD	0.19	0.21	0.35
Mean % Recovery	103.67		
Mean SD	0.053		
Mean % RSD	0.25		

Specificity

The specificity of the method was tested by some impurities and mixture of generally used tablet excipients such as lactose, starch, magnesium stearate (blank placebo). It is calculation of the percentage recovery of each component in the presence of other interfering substances. The results are presented in the Table 4, which confined that separation of analytes from other interfering excipients was completed.

Table 4. Specificity study of Aripiprazole

Added, µg/ml	Recovered, µg/ml	Recovery %
7.5	7.60	101.42
7.5	7.58	107.19
7.5	7.50	100
7.5	7.48	99.76
Mean	7.54	
RSD %	0.78	

Table 5. Robustness Study for Aripiprazole

Flow Rate	**Flow-0.6 ml**		**Flow Rate**	**Flow-0.8 ml**	
Sr No.	Conc. (µg/ml)	Area	Sr No.	Conc. (µg/ml)	Area
1	15	1198.27	1	120	1186.69
2	15	1200.32	2	120	1192.87
Mean		1199.30	Mean		1189.78
SD		1.45	SD		4.37
%RSD		0.12	%RSD		0.37
Mobile phase Volume :	**20:35:45**		**Mobile phase Volume :**	**20:45:35**	
Sr No.	Conc. (µg/ml)	Area	Sr No.	Conc. (µg/ml)	Area
1	15	986.69	1	120	1128.25
2	15	980.54	2	120	1135.65
Mean		983.62	Mean		1131.95
SD		4.35	SD		5.23
%RSD		0.44	%RSD		0.46
Wavelength Change	**253**		**Wavelength Change**	**255**	
Sr.No.	Conc. (µg/ml)	Area	Sr.No.	Conc. (µg/ml)	Area
1	15	1180.25	1	120	1112.89
2	15	1185.69	2	120	1120.36
Mean		1182.97	Mean		1116.63
SD		3.85	SD		5.28
RSD		0.33	%RSD		0.47

Robustness

Robustness was studied by determining effects of small variation of mobile phase composition (±1%), Wavelength and Flow rate, no significant change in R_F value and relative standard deviation of peak area indicating the robustness of method. Results are shown in Table 5.

The method was used for determination of ARP in Aripraz-10 (Vama pharma, India). The results obtained (Table 3) shows that percentage recoveries were high and %RSD value were low, which confirm that method is suitable for routine estimation of aripiprazole in its pharmaceutical formulations. Figure 3.

A typical chromatogram obtained from analysis of aripraz-10 branded formulation. Figure 4 shows chromatogram of blank sample. Stability of ARP in solution was checked by determining the percentage deviation of the amounts present in solution after 48 hrs at room temperature in comparison with the amount of zero time. After the completation of 48 hrs the obtained results showed no significant variation, the percentage deviation was less than 2% of the initial amount. This is confirming that good stability of each component in the mixture over a period of 48 hrs.

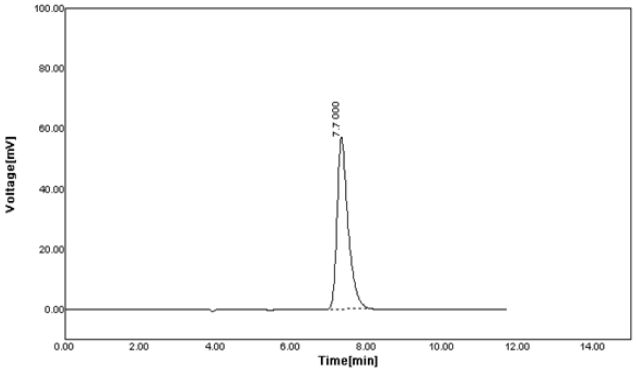

Figure 3. Typical HPLC Chromatogram of Aripiprazole

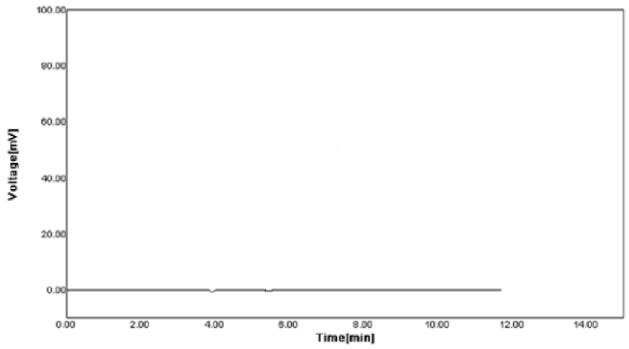

Figure 4. HPLC Chromatogram of Blank

Table 6. Summary of Validation Parameters by HPLC

Parameters	Data for Aripiprazole
Linearity Range	5-25 µg/ml
Line of regression	y = 80.49x -14.69
Correlations coefficient	$R^2 = 0.999$
Retention Time ($R_{f\ Value}$)	7.7
Tailing factor(T_f)	1.2469
Limit of Detection(µg/ml)	0.1009
Limit of Quantification(µg/ml)	0.3058
Accuracy	103.67%
Intra-Day precision	0.32
Inter-Day precision	0.64
Robustness	Robust
Ruggedness	%RSD is less than 1

Conclusion

The RP-HPLC method was developed, validated and applied to pharmaceutical analysis for estimation of ARP in bulk and tablet formulation. This HPLC method using common reagents and simple sample preparation procedure is particularly appropriate for analysis of ARP in pharmaceutical dosage form. This method has advantages of simplicity, precision, accuracy, sensitivity and quantification of ARP compared with other reported methods. The retention time is 7.7 min only so many samples also be analyzed in short period of time.

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