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Development, Estimation and Validation of Aripiprazole in Bulk and Its Pharmaceutical Formulation by HPLC Method

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Abstract: A new, simple, specific, sensitive, rapid, accurate and precise RP-HPLC method was developed for the estimation of Aripiprazole in bulk and pharmaceutical formulations. Aripiprazole was chromatographed on a INERTSIL C18 column (250x4.6mm I.D., particle size 5 µm) in a mobile phase consisting of 0.02 M Sodium Dihydrogen Orthophosphate: Methanol in the ratio 30:70 v/v. The mobile phase was pumped at a flow rate of 0.8 ml/min with detection at 283 nm. The detector response was linear in the concentration of 48-145µg/ml. The intra and inter day variation was found to be less than 2%. The mean recovery of the drug from the solution was 99.39%. The proposed method is simple, fast, accurate, precise and reproducible hence, it can be applied for routine quality control analysis of Aripiprazole in bulk and pharmaceutical formulations.
Keywords: RP-HPLC, Aripiprazole, precision, accuracy.

Introduction:

Aripiprazole is chemically 7-(4-(4-(2,3-Dichlorophenyl) - 1- piperainyl) butoxy) - 3, 4-dihydro-2(lH) quinolinone as in **fig 1.** It is freely soluble in chloroform. The molecular formula is $C_{23}H_{27}Cl_2N_30_2$ and molecular weight is 448.39. Aripiprazole is a benzisoxazole derivative used as Antipsychotic. Aripiprazole acts as a D2 partial agonist. Aripiprazole is also a partial agonist at the 5-HT1A receptor, and like the other atypical antipsychotics displays an antagonist profile at the 5-HT2A receptor[1,2]. Literature survey reveals that few spectroscopic and chromatographic methods have been reported for the quantitative estimation of Aripiprazole in bulk drug and pharmaceutical formulation[3-8]. Hence an attempt has been made to develop novel HPLC method for its estimation in bulk and pharmaceutical formulation with good precision, accuracy, linearity and reproducibility.

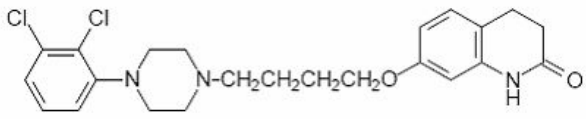

Fig. 1: Chemical structure of Aripiprazole

Experimental

Materials and methods:

A binary gradient high pressure liquid chromatography (SHIMADZU LC-2010 HT HPLC) with one an LC-10 AT VP pump, with UV/VIS detector SPD-10 AVP, CTS-10 AS VP column oven (shimadzu), and an inertsil C-18 Column (250 mm x 4.6 mm i.d. particle size 5 µm) was used. The HPLC system was equipped with the software class class-vp lc solutions (Shimadzu).

Chemicals and solvents

All the chemicals used were of HPLC grade and A.R.grade. Distilled water was used for making the dilutions and reagent solutions. The commercially available Aripiprazole tablets were procured from the local market.

Preparation of phosphate buffer (pH 3.0)

3.9421 gm of Sodium Dihydrogen Orthophosphate and 0.5gms of Sodium hexane sulphonic acid in 1000ml of milli Q water dissolved and adjusted the p^H to 3.0 with dilute O-phosphoric acid. Filtered through 0.45µm or a finer porosity membrane filter and degassed.

Preparation of mobile phase and diluents:

Mixed Buffer and Methanol in the ratio of 30:70 v/v respectively and degassed used as mobile phase and solvent as well.

Procedure

The content of the mobile phase was 0.02 M Sodium Dihydrogen Orthophosphate: Methanol in the ratio 30:70 v/v and same the diluents as well. The mobile phase was filtered through 0.45 µm membrane filter and sonicated for 15 min. The flow rate of the mobile phase was maintained at 0.8 ml/min. The column temperature was set 35°C and the detection was carried out by UV/VIS detector wavelength at 250 nm. The run time was set at 15 min and the volume of the injection loop was 20 µL, prior to injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system and the run time was set at 15 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 6.0 min. A typical chromatogram showing the separation of the drug is given in Fig. 3.

Calibration plot

About 25 mg of Aripiprazole was weighed accurately, transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 30:70 v/v mixture of 0.02 M Sodium Dihydrogen Orthophosphate: Methanol. The solution was sonicated for 15 min and the volume made up to the mark with a further quantity of the diluent to get a 100 µg/ml solution. From this, a working standard solution of the drug (48 µg/ml) was prepared by diluting 4.8 ml of the above solution to 10 ml in a volumetric flask. Further dilutions ranging from 48-145 µg/mL were prepared from the solution in 10 ml volumetric flasks using the above diluent. 20 µl of each dilution was injected six times into the column at a flow rate of 0.8 ml/min and the corresponding chromatograms were obtained. From these chromatograms, the average area under the peak of each dilution was computed. The calibration graph constructed by plotting concentration of the drug against peak area was found to be linear in the concentration range of 48-145 µg/mL of the drug. The relevant data is furnished in Table-1. A typical calibration plot showing the separation of the drug is given in Fig.2. The regression equation of this curve was computed. This regression equation was later used to estimate the amount of Aripiprazole in tablets dosage forms.

Table-1: Calibration data of the method

Concentration (µg/ml)	Mean peak area (n=5)
48	1077932
72.0	1618394
96.0	2157691
120.0	2690542
145.0	3194404

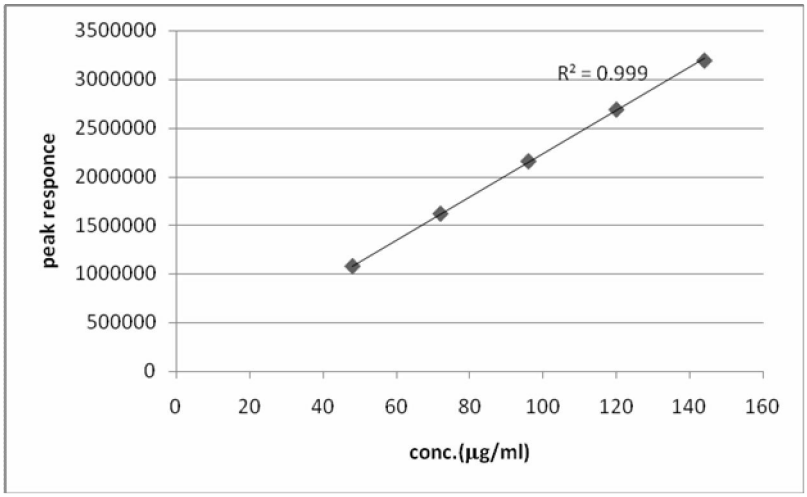

Fig 2: Line of best fit of Aripiprazole

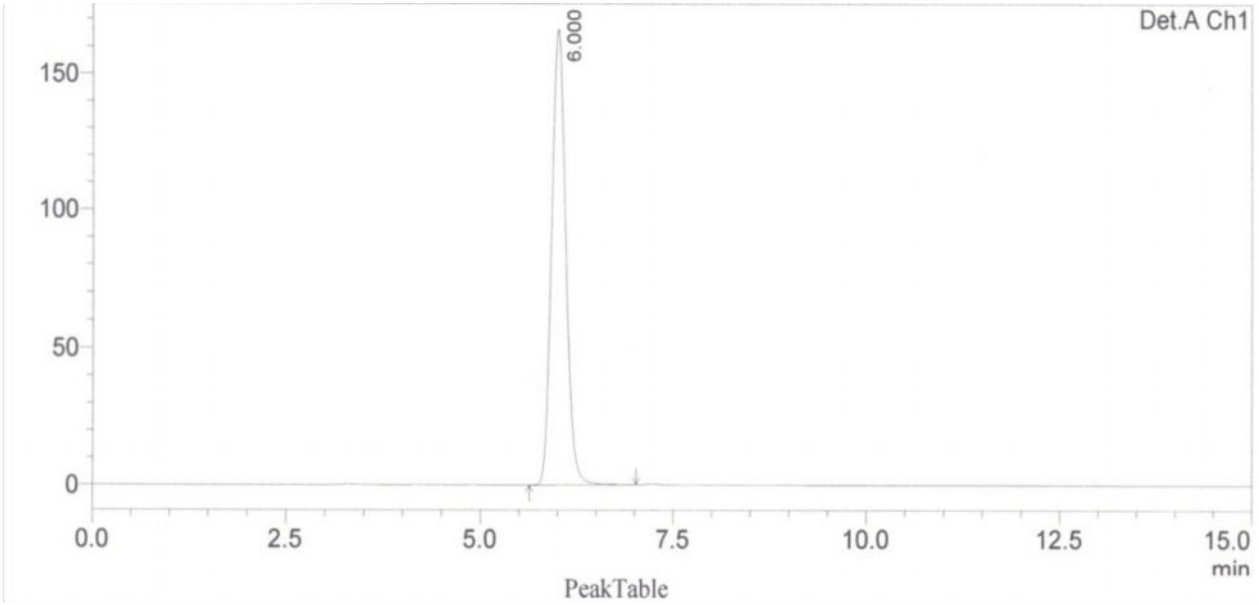

Fig. 3: Typical chromatogram of Aripiprazole

Table-2: Precision of the proposed HPLC method

Concentration of Aripiprazole(48µg/ml)	Peak area	
	Intra day	Inter day
Injection-1	1077932	1080630
Injection-2	1080467	1077932
Injection-3	1074762	1077832
Injection-4	1080627	1076732
Injection-5	1072656	1072656
Average	1077289	1077322
Standard Deviation	3518.0	2896.4
%RSD	0.32	0.26

Table-3: Accuracy studies:

Spike level	Avg.recovery (µg/ml)
50%	98.7
75%	99.3
100%	98.9
125%	99.1
150%	97.9

Validation of the proposed method

The specificity, linearity, precision, accuracy, limit of detection, limit of quantification, robustness and system suitability parameters were studied systematically to validate the proposed HPLC method for the determination of Aripiprazole. Solution containing 40 µg/ml of Aripiprazole was subjected to the proposed HPLC analysis to check intra-day and inter-day variation of the method and the results are furnished in Table-2. The accuracy of the HPLC method was assessed by analyzing solutions of Aripiprazole at 50, 100 and 150% concentrated levels by the proposed method. The results are furnished in Table-3. The system suitability parameters are given in Table-4.

Estimation of Aripiprazole in tablet dosage forms

Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate Aripiprazole in tablet formulations. Twenty tablets were weighed and powdered. An accurately weighed portion of this powder equivalent to 25 mg of Aripiprazole was transferred into a 100 ml volumetric flask and dissolved in 25 ml of a 30:70 v/v mixture of phosphate buffer and methanol. The contents of the flask were sonicated for 15 min and a further 25 ml of the diluent was added, the flask was shaken continuously for 15 min to ensure complete solubility of the drug. The volume was made up with the diluent and the solution was filtered through a 0.45 µ membrane filter. This solution containing 60 µg/ml of Aripiprazole was injected into the column six times. The average peak area of the drug was computed from the chromatograms and the amount of the drug present in the tablet dosage form was calculated by using the regression equation obtained for the pure drug. The relevant results are furnished in Table-5.

Table-4: System suitability parameters:

Parameter	Result
Linearity range (µg/ml)	48-145µg/ml
Slope	22084.65
Intercept	20112.44
Regression coefficient (r2)	0.99988
Limit of Detection (µg/ml)	0.22
Limit of Quantification (µg/ml)	0.66
Tailing factor	1.20
Theoretical plates	4813

Table-5: Assay and recovery studies

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Formulation 1	15	15.013	100.0
Formulation 2	20	19.992	99.97

Results and Discussion

In the proposed method, the retention time of Aripiprazole was found to be 6.0 min. Quantification was linear in the concentration range of 48-145µg/ml. The regression equation of the linearity plot of concentration of Aripiprazole over its peak area was found to be Y=20112.44+ 22084.65x (r^2=0.99988), where X is the concentration of Aripiprazole (µg/ml) and Y is the corresponding peak area. The number of theoretical plates calculated was 4813 which indicates efficient performance of the column. The limit of detection and limit of quantification were found to be 0.22µg/ml and 0.66µg/ml respectively, which indicate the sensitivity of the method. The use of phosphate buffer and methanol in the ratio of 35:65 v/v resulted in peak with good shape and resolution. The high percentage of recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram of the formulation within the run time indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

Conclusion

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Aripiprazole and can be reliably adopted for routine quality control analysis of Aripiprazole in its tablet dosage forms.

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