INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Analytical Method Development Report for Aripiprazole Tablets 2mg, 5mg, 10mg, 15mg, 20mg and 30mg

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ABSTRACT

A novel stability indicating liquid chromatographic assay method was developed for the quantitative estimation of Aripiprazole in tablets 2mg, 5mg, 10mg, 15mg, 20mg,30mg. An isocratic reverse phase LC-method was developed using The chromatographic column Zorbax 150mm×4.6mm, C18 column with 5µm particles. The LC method employs solutions a mixture of Ammonium acetate buffer with Acetonitrile and Methanol as mobile phase. A 50mM Ammonium acetate, filtered through 0.45µm filter mixed with Acetonitrile-HPLC grade and Methanol-HPLC grade in the ratio 50:40:10(v/v/v). The flow rate of the mobile phase was 1.5 ml/min with a post run time of 10 min. The column temperature was maintained at 25°C and the detection was monitored at a wavelength of 254 nm. The injection volume was 10µl. The Proposed method was found to be Linear, precise and accurate for the quantitative estimation of Aripiprazole in tablet formulations and can be used for commercial purposes.

Keywords: Aripiprazole, Liquid Chromatography, Method validation.

INTRODUCTION

Aripiprazole is an atypical antipsychotic agent belonging to the chemical class of benzisoxazole derivatives and is indicated for the treatment of schizophrenia. Aripiprazole is a selective monoaminergic antagonist with high affinity for the serotonin Type 2 (5HT2), dopamine Type 2 (D2), 1 and 2 adrenergic, and H1 histaminergic receptors. Aripiprazole appears to mediate its antipsychotic effects primarily by partial agonism at the D2 receptor. In addition to partial agonist activity at the D2 receptor, Aripiprazole is also a partial agonist at the 5- HT1A receptor, and like the other atypical antipsychotics, Aripiprazole displays an antagonist profile at the 5-HT2A receptor. Aripiprazole has moderate affinity for histamine and alpha adrenergic receptors, and no appreciable affinity for cholinergic muscarinic receptors1-2. Aripiprazole is available in market as conventional tablets , oral disintegrating tablets . Oral solution and intra muscular injection . In the present study attempts were made to develop a rapid, economical, precise and accurate stability indicating method for estimation of Aripiprazole in tablet formulations.

EXPERIMENTAL

Chromatographic System

Due to the above reasons there was a need for a new chromatographic system and the following conditions were optimized after conducting several experiments. The chromatographic column used was a Zorbax 150mm×4.6mm, C18 column with 5µm particles. The LC method employs solutions a mixture of Ammonium acetate buffer with Acetonitrile and Methanol as mobile phase. A 50mM Ammonium acetate, filtered through 0.45µm filter mixed with Acetonitrile-HPLC grade and Methanol-HPLC grade in the ratio 50:40:10(v/v/v). The flow rate of the mobile phase was 1.5 ml/min with a post run time of 10 min. The column temperature was maintained at 25°C and the detection was monitored at a wavelength of 254 nm. The injection volume was 10µl.

Diluent Selection
Aripiprazole itself is soluble in aqueous media having a concentration ranging from 0.05mg/mL to 0.29mg/mL in the pH range of 1.0 to 8.0 as per Annexure-1. And also it is having a good solubility in alcohols such as methanol and also in Acetonitrile. In order to obtain a clear solution of Test preparation in presence of placebo, different trials are taken with various aqueous and organic mixtures. Finally diluent was finalized having aqueous and organic in the ratio of 50:50 (v/v). The aqueous part was chosen as 1%AcOH and for organic part Acetonitrile was chosen. A summary of Diluent optimization along with recovery studies is presented in Annexure-2

Standard preparation
Weigh 40 mg of Aripiprazole Standard in a 100mL volumetric flask, dissolve in 10mL Acetonitrile and make upto the volume with diluent, mix well. Filter a portion through 0.45µm Nylon filter.

Test Preparation
Tablets weighed with optimum quantity as per USP from which average weight is calculated. Entire portion is crushed to a fine powder in a mortar and pestle to achieve maximum homogeneity and prepared a solution with a concentration of 0.4mg/mL by dissolving appropriate amount in diluent by sonication process and finally diluting to get a concentration of 0.04mg/mL with diluent and chromatographed.

Finalized Test Procedure
Mobile Phase preparation
Solution-A : 1.54g of Ammonium Acetate is dissolved in 500mL and made to 1000mL with HPLC grade water. Add 0.5mL of glacial acetic acid and mix well. Filter through 0.45µm Nylon 0,47mm membrane filter, **Solution-B:** HPLC grade Acetonitrile, **Solution-C:** HPLC grade Methanol.
Mobile Phase composition: Mix 50%Sol-A+40%Sol-B+10%Sol-C and degas under Sonication
Chromatographic conditions
Column: Zorbax XDB C18, 150 x 4.6mm, 5µm, **Flow rate**: 1.5mL/min, **Wave length**: 254 nm
Column Temperature: 25°C, **Run time**: 10min, **Injection volume**: 10µL
1% Acetic acid Solution: Dilute 10mL of Glacial Acetic acid to 1000mL with HPLC grade water

Diluent Preparation: 50% of 1%acetic acid solution + 50% ACN.

Standard Preparation: Weigh 40mg of Aripiprazole Standard in a 100mL volumetric flask, dissolve in 10mL Acetonitrile and make upto the volume with diluent, mix well. Filter a portion through 0.45µm Nylon filter.

Test Preparation : Weigh 40mg Equivalent of Aripiprazole in a 100mL Volumetric flask, add 10mL of Acetonitrile sonicate to disperse for 5minutes add 60mL of diluent and sonicate for 20minutes and make upto the volume with diluent, Filter a portion through 0.45µm Nylon filter.
Procedure: Inject filtered Diluent as blank followed by standard solution and test preparation into the chromatographic system and measure for major responses.
System suitability: Inject Standard solutions for five times,
%RSD from the five injections for Aripiprazole shall be not more than 2.0% and USP tailing for Aripiprazole peak shall be not more than 2.0

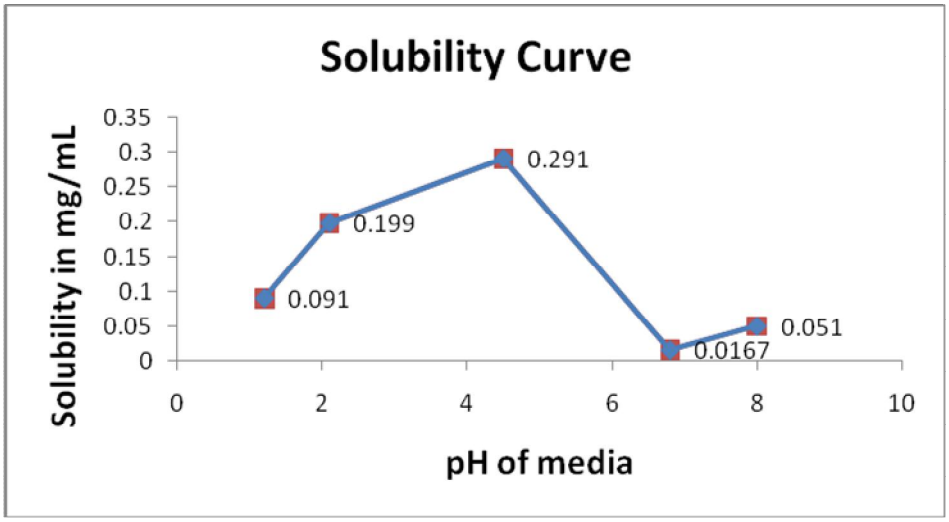
RESULTS AND DISCUSSION
Linearity
In order to prove that the response of the active with respect to concentration is linear within the concentration range employed for analysis. Linearity solutions are prepared from a stock solution having a concentration of 1mg/mL at five different concentration levels ranging from 25% to 200% of assay analyte concentration (25%, 50%, 100%, 150% and 200% i.e. from 100µg/mL to 800µg/mL). The peak area versus concentration data was performed by least-squares linear regression analysis. The correlation coefficient was found to be 1. A Plot of Linearity curve is presented in Annexure-3.

Specificity
Impurities interference was performed by spiking Test preparation with known amount of Impurities and chromatographed. There was no interference with main peak RT i.e. all the impurity peaks were well resolved from main peak. The RRTs obtained are presented in Annexure-4.

Calculations

$$\% \text{ Assay} = \frac{\text{Test area x std wt (mg) x Test dilution x Avg wt x Potency x 100}}{\text{Std area x std dilution x Test wt (mg) x Label claim x 100}}$$

Annexure-1

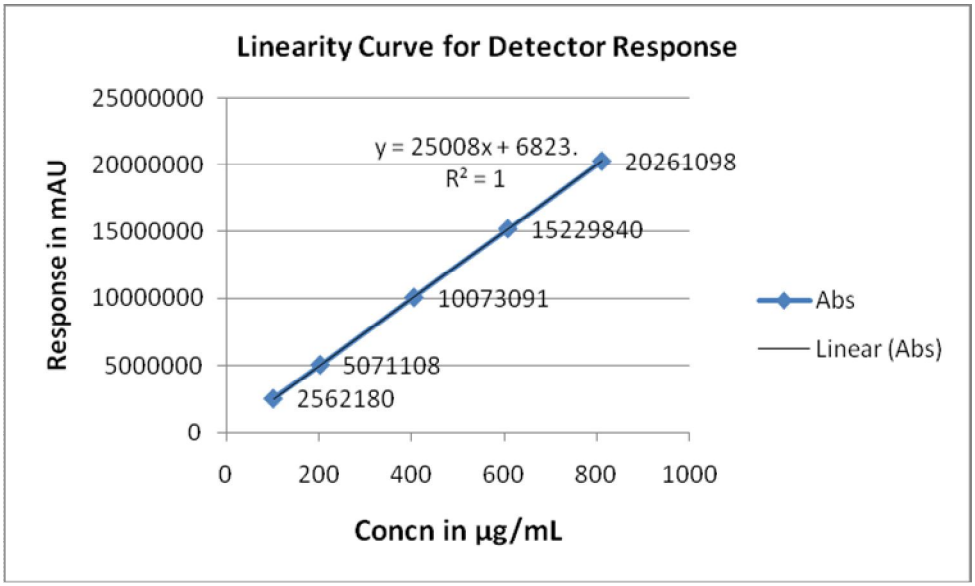


S. No.	Buffer used	Solubility in mg/mL
1	pH1.2 with HCl	0.091
2	pH2.1 with HCl	0.199
3	pH 4.5Acetate buffer	0.291
4	pH 6.8Phosphate buffer	0.017
5	pH 8.0Phosphate buffer	0.051

Annexure-2

S. No.	Diluent name	%Level	%Recovery
01	Aqueous/Acetonitrile (50/50-v/v) (With highest Dosage form of average weight 150mg contains 30mg)	50%	101.5
		100%	101.7
		150%	101.1
		200%	94.7
	Aqueous/Acetonitrile (50/50-v/v) (With lowest Dosage form of average weight 95mg contains 2mg)	50%	98.2
		100%	100
		150%	94.7
		200%	76.3
02	Aqueous (1%AcOH)/Acetonitrile (50/50-v/v) (With lowest Dosage form of average weight 95mg contains 2mg)	50%	100.6
		100%	100.6
		200%	101

Annexure-3



S. No.	Conc. in µg/mL	Response in mAU
1	101.2313	2562180
2	202.4626	5071108
3	404.9252	10073091
4	607.3878	15229840
5	809.8504	20261098

Annexure-4

Name	RT
Imp-A	1.053
Imp-B	1.213
Imp-C	1.380
Imp-D	3.440
Imp-E	4.493
Aripiprazole	5.427

Placebo Interference and Recovery studies were conducted with a new placebo and results are presented below.

S. No.	Name of the solution	%Assay
1	Recovery-50% solution	100.3
2	Recovery-100% solution	100.3
3	Recovery-200% solution	100.8
4	Placebo Solution	0.0

CONCLUSION

As Forced degradation was not performed yet, the method shall be considered as release method and after evaluating Forced degradation studies the method shall be considered as stability indicating method if the no interferences are found at known peaks RT in forced degradation studies, or else method shall be further optimized.