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Development and Validation of Stability Indicating Method for the Estimation of Aripiprazole in Oral Solution by RP-HPLC

Angati Krishnaveni[1], Dr. P.V. MADHAVI LATHA[2]
#1 Master of Pharmacy Scholar, Department of Pharmaceutical Analysis,
#2 Professor, Department of Pharmaceutical Analysis, Viswanadha Institute of Pharmaceutical Sciences, Visakhapatnam, AP, India.

Abstract: *The objective of the present research work was to Develop and Validate of stability indicating method for the estimation of Aripiprazole in Aripiprazole oral solution by stability indicating RP-HPLC method using Methyl paraben and Propyl paraben as preservatives. The mobile phase composition was phosphate buffer and Acetonitrile with gradient elution and was passed at 1.0 ml/min through a Zorbax eclipse XDB C18 column (250 x 4.6 mm, 5 µ) with UV detection at 256 nm. This method was validated for specificity, linearity, precision, accuracy, robustness, system suitability. Retention time of Aripiprazole peaks was observed at 8.42. The proposed method obeyed linearity in the range of 10-60 µg/ml with excellent correlation coefficient (0.9999).LOD and LOQ were calculated from the result obtained from calibration curve. The robustness of the method was checked by varying flow rate (±0.1 mL/min), buffer composition (±1 %), and temperature (±5 ˚C) and Detection wavelength (±0.2 nm) found that system suitability parameters were within the limit at all variable conditions, hence the method was robust. The system suitability parameter also revealed that the values within the specified limit for the proposed method. From accuracy studies it was found that recovery value of pure drug was between 98 % and 102 % for the formulation which indicates that the method was accurate and also revealed that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed method. The stability indicating method was checked by subjecting the drug to stress conditions like acidic, basic, oxidative, humidity and thermal and was found that the degradation peaks were well separated.*

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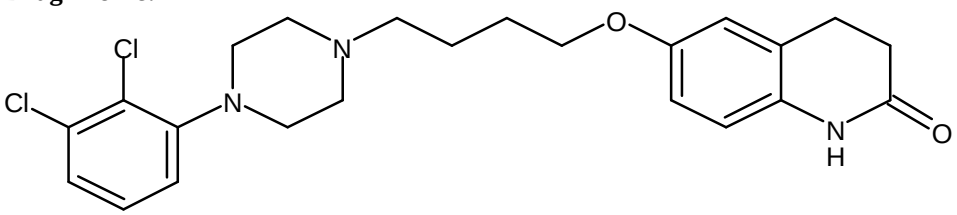
I. Literature Review:

M. V. V. N. Murali Krishna, et.al. 2017. Has developed a RP-HPLC method for the quantification of Aripiprazole and its impurities. Developed by using ACE C18 (4.6 x 250nm, 5µm) column. Method developed by gradient elution of 0.1 % Trifluoroacetic acid in water and ACN as mobile phase at flow rate of 1.0ml/min with 30^{o} C column temperature Detected at 254nm by PDA Detector. By forced degradation study he found ARI was degrade in oxidation, acid hydrolysis, heat and stable at remaining conditions. Degradation impurities formed during stress study were identified using LCMS. The present method was useful for determining the content of all the three main analytes present in the oral solution without interference from degradation impurities. The method was robust under the deliberately modified conditions.

Drug Profile:



IUPAC Name **:** 7-[4-[4-(2, 3-dichlorophenyl)-1- piperazinyl] butoxy]-3, 4 dihydrocarbostyril

Molecular Formula **:** $C_{23}H_{27}Cl_2N_3O_2$

Molecular Mass **:** 448.38538

Solubility **:** Soluble in Methanol, Ethanol, Water and Acetonitrile
Category **:** Antipsychotic Drug
Instruments:

- **UV-1700Schimadzu** with **UV probe** software UV-Visible spectrophotometer with 1cm quartz cells.
- **SCHIMADZU- Prominence liquid chromatography, Model LC-20AD**Photo diode array (PDA) Detector, with an automated sample injector. The output signal was monitored and integrated using **LC-solution software.**

II. Results & Discussion:

Chromatographic conditions:

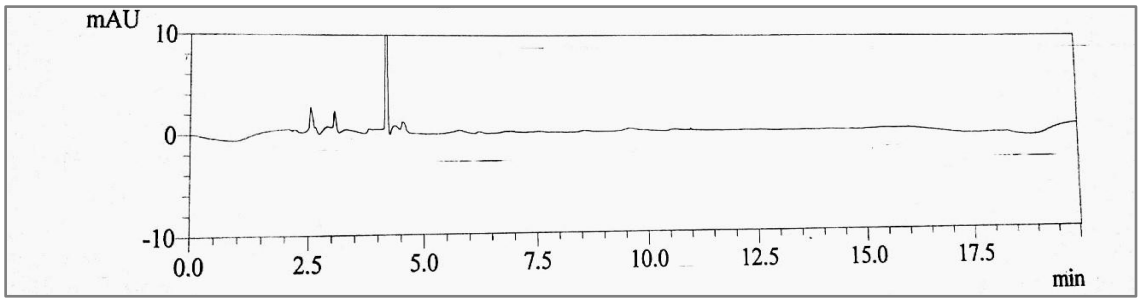
Column	Zorbax Eclipse XDB C18 (250×4.6 mm), 5 µ	There is good resolution between the Aripiprazole, Methyl and propyl paraben peak
Mobile phase composition	Phosphate buffer : Acetonitrile with Gradient elution	
Flow and injection volume	1.0mL/min, 15µL	
Column temperature	30 Ċ	

- **VALIDATION OF THE DEVELOPED METHOD**

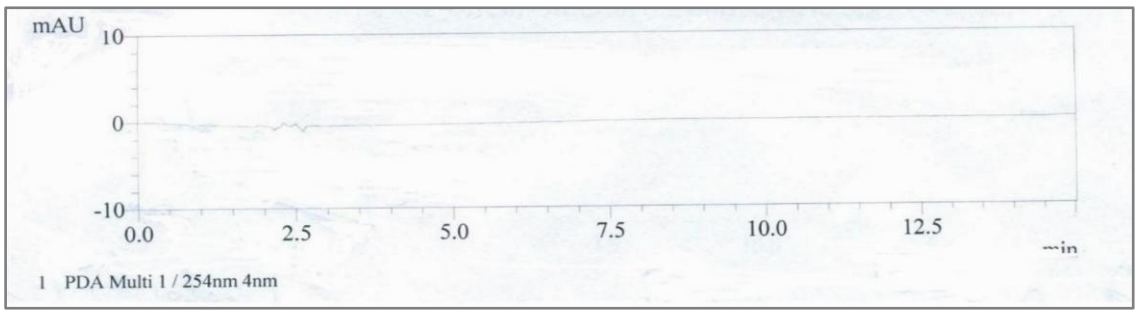
SPECIFICITY

In Specificity solutions were prepared by taking each 4ml of the Aripiprazole, methyl and Propyl Paraben from stock solutions and makeup with diluent in 50ml volumetric flask. By this six different samples of Blank, Placebo and standard are prepared and injected to check the interference
If there is no interference from Blank, with analyte and meeting the acceptance Criteria of NMT 0.2 % of target concentration then method is said to be specific.

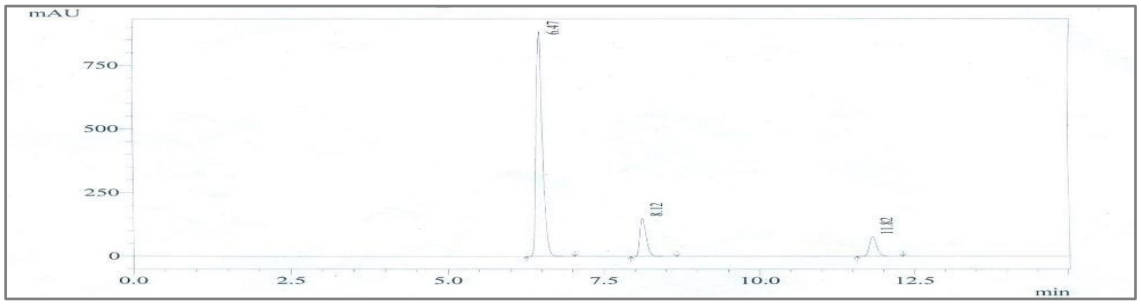
SPECIFICITY



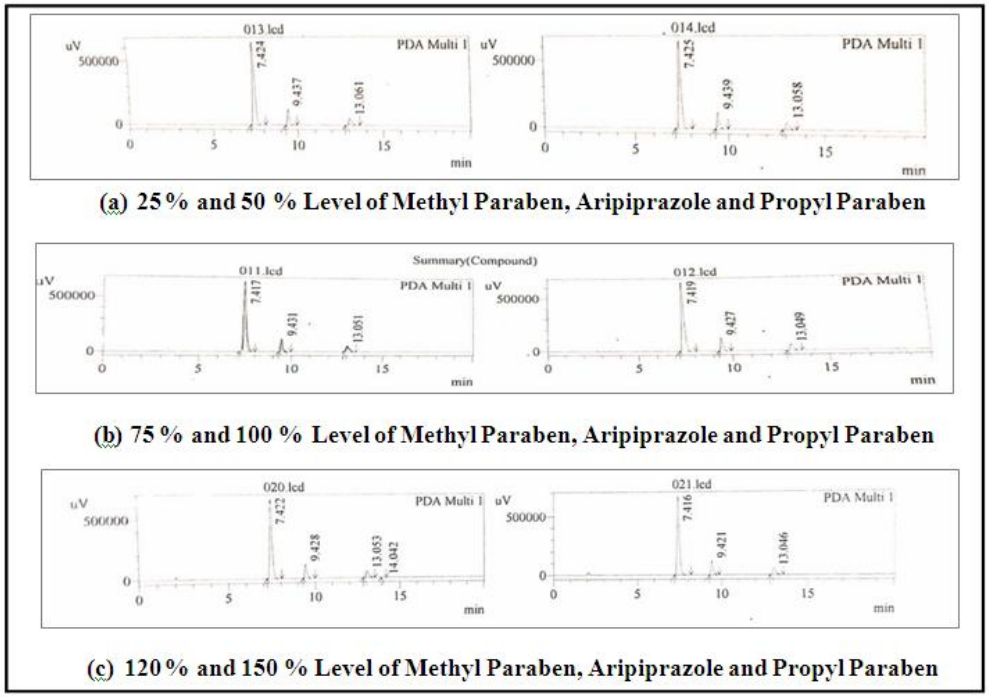
(a) Blank Chromatogram



(b) Placebo Chromatogram



(c) Standard Chromatogram



(a) 25 % and 50 % Level of Methyl Paraben, Aripiprazole and Propyl Paraben

(b) 75 % and 100 % Level of Methyl Paraben, Aripiprazole and Propyl Paraben

(c) 120 % and 150 % Level of Methyl Paraben, Aripiprazole and Propyl Paraben

Title	**Sample name**	**Sample ID**	**Ret. Time**	**Area**	**Theoretical Plate**
1	Aripiprazole os	Blank	0.000	0	0.000
2	Aripiprazole os	Placebo	0.000	0	0.000
3	Aripiprazole os (Standard)	Aripiprazole Methyl paraben Propyl paraben	8.12 6.47 11.32	1036538 620384 607869	9869 10171 16302
4	%RSD	Aripiprazole Methyl paraben Propyl paraben	0.27 0.02 0.09	------	------

LINEARITY

A series of standard drug solutions were prepared in the concentration range using diluent from 25% to 150 % of test concentration. The calibration curve of analytical method was assessed by plotting concentration versus standard peak area and represented graphically.

PRECISION

In this six samples are injected in spare into HPLC and analyzed. Average weight of the samples was taken in 50 ml volumetric flask and gets stirred for 45 minutes using magnetic stirrer bead for 45 min and makeup with diluent.

- In System precision the retention time and area of six determinations was measured and relative standard deviation was calculated.
- In Method precision three different sample concentrations was prepared and triplicate of each concentration in linearity range were taken for intra and inter day precision studies.
- Obtained %RSD for Aripiprazole, Methyl Paraben & Propyl Paraben is 0.05, 0.02 & 0.11
- Intermediate Precision: Obtained %RSD for Aripiprazole, Methyl Paraben & Propyl Paraben is 0.19, 0.28 & 0.81

ACCURACY (% RECOVERY)

In percentage recovery the recognized amount of standard drug was spiked in triplicate to the placebo samples and the recovery of the drug was calculated. Sample solutions are prepared by taking the known quantity of

stock solutions of drugs to the Placebo at 50 %, 100 % and 150 % of test concentration and injected duplicate injections into HPLC and analyzed for the recovery of the amount added of API.

Recovery level (%)	**Sample**	**Amount of stock added**	**Theoretical content(µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**SD**
50%	ARI	2	20	20.14	100.7	886.711
	MP	2	35	35.27	100.7	263.75
	PP	2	4	3.99	99.75	200.81
100%	ARI	4	40	40.11	100.27	560.73
	MP	4	35	35.27	100.77	6136.98
	PP	4	8	7.98	99.75	353.55
150%	ARI	6	60	60.09	100.15	1031.66
	MP	6	105	104.94	99.94	3015.81
	PP	6	12	12.00	100	320.31

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

From the linearity plot the LOD and LOQ were calculated by the following formula:

$$\text{LOD} = \frac{3.3 \times \sigma}{S} \qquad \text{LOD} = \frac{10 \times \sigma}{S}$$

Where,

σ = Standard deviation of areas from the calibration curve

S= Slope of the calibration curve

LOD = 0.0044 **LOQ** = 0.013

ROBUSTNESS

Robustness of an analytical procedure is a measure of its capacity to remain unaffected by small deliberate variation in method parameters. Prepared drug solution is injected into the HPLC at finalized condition and variable conditions like Flow variation, pH variation of buffer, column oven temperature variation, wave length variation and it is concluded that Tailing factor and Theoretical plates are within the acceptance limits in Flow variation, pH variation of buffer and column oven temperature variation for Aripiprazole, Methyl and Propyl Paraben

DEGRADATION CONDITIONS:

A. ACID DEGRADATION: 5N HCl, 2.5mL for 30 minutes at 60˚C temperature.
B. BASE DEGRADATION: 5N NaOH, 2.5mL for 30 minutes at 60˚C temperature.
C. PEROXIDE DEGRADATION: 30% H_2O_2, 2.5mL for 15 minutes at room temperature.
D. THERMAL DEGRADATION: Heated at 60˚C for 24 hrs.
E. HUMIDITY DEGRADATION: 75%RH for 12 hrs.

PEAK PURITY DATA OF ARIPIPRAZOLE

Degradation mechanism	**Degradation Condition**	**Aripiprazole (%)**	**% Degradation**	**Minimum Peak Purity Index**
Control	Undegraded	100.8	NA	0.05
Acid	5 N HCl, 2.5 mL for 30 min at 60 ˚C temp.	94.2	6.6	0.03
Base	5 N NaOH, 2.5 mL for 30 min at 60 ˚C temp.	94.2	6.6	0.01
Peroxide	30 % H_2O_2, 2.5 mL for 15 min at room temp.	96.6	4.2	0.06
Thermal	60 ˚C for 24 hours	98.6	2.2	0.05
Humidity	75 % RH for 24 hours	97.8	3.0	0.06

Aripiprazole was found to be degraded by 6.6 %, 6.6 %, 4.2 %, 2.2 % and 3.0 % in Acid, Base, Peroxide, Thermal and Humidity

PEAK PURITY DATA OF METHYL PARABEN

Degradation Mechanism	**Degradation Condition**	**Methyl Paraben (%)**	**Degradation (%)**	**Minimum Peak Purity Index**
Control	Undegraded	101.6	NA	0.06
Acid	5 N HCl, 2.5 mL for 30 min at 60 ˚C temp.	96.2	5.4	0.05
Base	5 N NaOH, 2.5 mL for 30 min at 60 ˚C temp.	96.2	5.4	0.05
Peroxide	30 % H_2O_2, 2.5 mL for 15 min at room temp.	97.3	4.3	0.04
Thermal	60 ˚C for 24 hours	99.2	2.4	0.01
Humidity	75 % RH for 24 hours	99.6	2.0	0.03

Methyl Paraben was found to be degraded by 5.4 %, 5.4 %, 4.3 %, 2.4 % and 2.0 % in Acid, Base, Peroxide, Thermal and Humidity

PEAK PURITY DATA OF PROPYL PARABEN

Degradation Mechanism	**Degradation Condition**	**Propyl Paraben (%)**	**% Degradation**	**Minimum Peak Purity Index**
Control	Undegraded	101.0	NA	1
Acid	5 N HCl, 2.5 mL for 30 min at 60 ˚C temp.	95.5	5.5	0.05
Base	5 N NaOH, 2.5 mL for 30 min at 60 ˚C temp.	95.5	5.5	0.02
Peroxide	30 % H_2O_2, 2.5 mL for 15 min at room temp.	97.0	4.0	0.03
Thermal	60 ˚C for 24 hours	99.0	2.0	0.05
Humidity	75 % RH for 24 hours	99.5	1.5	0.02

Propyl Paraben was found to be degraded by 5.5 %, 5.5 %, 4.0 %, 2.0 % and 1.5 % in Acid, Base, Peroxide, Thermal and Humidity

III. Summary And Conclusion

The developed method for Aripiprazole in oral solutions complies with Linearity; the linearity of the method was tested using the calibration solutions described in the results above. The plot of concentrations against responses was linear in the range of 10 – 60 µg/ mL. The mean regression equation was Y= 26615x – 2864. The correlation coefficient was 0.999.

The results obtained indicate that recovery was excellent, not less than 99% and that relative standard deviation also less than 2%.

The method was used for determination of Aripiprazole in an oral solution formulation. The results obtained 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.

This RP-HPLC method for analysis of Aripiprazole in oral solution 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.

With the above findings the developed method was found to be linear, précised, accurate and reproducible in nature. So, this method is stable and suitable method to establish identity and quantum of the Aripiprazole in oral liquid dosage form and in as active pharmaceutical ingredient.

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