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Development and Validation of Novel UPLC Method for Identification of Other Product in Aripiprazole

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Abstract: This study presents the development and validation of a cost-effective ultra-high-performance liquid chromatography (UPLC) method, for the accurate quantification of impurities in Aripiprazole. The method employs an advanced UPLC system, utilizing a novel chromatographic column and an optimized mobile phase composition. The development process involved systematic optimization of chromatographic parameters to achieve high levels of separation, resolution, and precision in detecting acetate impurities. The method demonstrated linearity within the range of LOQ% to 150% for impurities (Aripiprazole Related Compound G and F) and Aripiprazole, with an exceptional correlation coefficient of 1.000. Accuracy and precision were confirmed, with average recovery rates of 96.7% for Aripiprazole Related Compound G and 92.9% for Compound F. Robustness and ruggedness tests yielded favourable results, affirming the method's reliability. Notably, no interference from excipients was observed, highlighting its precision and accuracy. This novel UPLC method, aligned with International Conference on Harmonization (ICH) standards, provides a practical and economical solution for determining acetate content in Aripiprazole, making it highly suitable for routine analysis in bulk and pharmaceutical formulations.

Keywords: Aripiprazole, International Conference Harmonization, Ultra-high-performance liquid chromatography, Validation, Impurity

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Introduction

Aripiprazole, chemically identified as 7-(4-(4-(2,3-dichlorophenyl) – 1 -piperazinyl)butoxy) - 3, 4 -dihydrocarbostyril, appears as a white or almost white crystalline powder with a melting point of 139–141°C. As an atypical antipsychotic agent, aripiprazole functions primarily as a partial agonist at dopamine receptors in the mesolimbic dopaminergic pathway, reducing dopaminergic activity and alleviating positive symptoms of schizophrenia while minimizing extra-pyramidal side effects. Its pharmacological activity is attributed to aripiprazole and its metabolite,

dehydro-aripiprazole. It influences neurotransmitter release and addressing negative symptoms and cognitive impairments associated with schizophrenia (Matsul-Sakata *et al*., 2005; Kim *et al*., 2007).

Ensuring the quality of pharmaceutical formulations requires the accurate determination and quantification of impurities in pharmaceutical products. Method like high-performance liquid chromatography (HPLC) has been widely employed, but advancements in ultra-performance liquid chromatography (UPLC) offer significant benefits. UPLC enhances resolution and sensitivity, reduces analysis time, and improves peak shapes by utilizing smaller particle sizes and higher operating pressures. This study aims to develop and validate a robust UPLC method for impurity quantification in Aripiprazole, aligning with International Conference on Harmonization (ICH) guidelines. The validated method demonstrated accuracy, precision, ruggedness, and sensitivity, ensuring its suitability for routine quality control in pharmaceutical formulations (International Conference Harmonization, 2005; Branch, 2005; Bhavyasri *et al*., 2019).

Materials and Methods

The gift sample of Aripiprazole was acquired from Indeus Life Sciences Pvt. Ltd, located in Mumbai, India. Analytical grade solvents and reagents were procured from Merck Pvt. Ltd.

Chromatographic Conditions:

The UPLC method for the analysis of impurities present in Aripiprazole was developed. The sample separation was achieved on Waters Acquity UPLC H-Class, Ultisil UPLC XB-C18 (100X 2.1 mm) and 1.8 µm column. Gradient mobile phase A (Buffer pH 2.1: Acetonitrile, 920:80) and mobile phase B (Acetonitrile: Water 950:50) was run with flow rate of 0.474 ml/min and injection volume 1 µl.

Preparation of Mobile phase

Preparation of Buffer pH 2.1:

Dissolved 3.9 g of Sodium dihydrogen phosphate dihydrate in 1 L water, added 5 ml of Triethylamine and adjusted the pH to 2.1 with ortho-phosphoric acid.

Mobile phase A: Prepared a mixture of Buffer pH 2.1: Acetonitrile in the ratio of 920:80 v/v and mixed well.

Mobile Phase B: Prepared a mixture of Acetonitrile: Water in the ratio of 950:50 v/v and mixed well.

Preparation of Standard and Sample Solutions

Standard Solution

Weighed accurately about 15.0 mg of Aripiprazole standard and transferred into a 50 ml volumetric flask. Added about 35 ml of diluents and dissolved to volume with diluents and mixed well.

Sample Preparation

Tablets were weighed, powdered and transferred into a 250 ml volumetric flask. Solvent (175 ml) was added and dissolved. Filtered the sample solution through 0.22 µm syringe filter and discarded the first 5 ml of filtrate.

Validation

The proposed UPLC method was validated as per guideline using various parameters as mentioned below (Breaux *et al*., 2003; Sharma *et al*., 2018; Swartz and Krull, 2018; Sahu *et al*., 2018; Bhavyasri *et al*., 2019):

Specificity

The specificity of the method was established in which no interference was observed at retention time of all known impurities and sample.

Linearity

A series of different concentrations of Aripiprazole and related compounds were prepared and injected into the UPLC column. The calibration curve was established by plotting a graph of concentration versus area of solutions and determining the correlation coefficient.

Accuracy

The per cent recoveries were carried out for Aripiprazole related compound G and Aripiprazole related compound F at LOQ, 100% and 150% level, in triplicate.

Precision

The precision of the method was evaluated as inter-day by carrying out six independent samples (spiked and unspike) related compounds of test sample against a qualified reference standard and the %RSD of assay was calculated.

Limit of Detection (*LOD*)

The LOD of the method was evaluated injecting six injections of LOD where S/N ratio as well as % RSD or each impurity and Aripiprazole found below 33 %.

Limit of Quantitation (*LOQ*)

The LOQ of the method was evaluated injecting six injections of LOQ where S/N ratio as well as % RSD or each impurity and Aripiprazole found below 10 %.

Robustness

The robustness of a method is the ability of the method to remain unaffected by making slight deliberate changes in chromatographic conditions, such as change in ratio of the mobile phase and small changes in flow rate, column oven temperature and pH of buffer solution.

Results and Discussion

In the present investigation optimum results were achieved using described column specification and solvent system. Aripiprazole and its degradant impurities did not interfere in analysis. The chromatograms of standard, sample and related compounds are given in Figures 1, 2, 3 and 4.

Validation

The objective of this validation study was to demonstrate the in-house developed UPLC method is suitable for Related Compounds Test for Aripiprazole Tablets.

Specificity

Specificity has been evaluated by assuring no interference observed from solvent at the retention time of Aripiprazole and its known impurities peaks. All the peaks are well separated and well resolved from each other.

Limit of Detection (*LOD*)

LOD was determined at different concentrations 0.3 µg/ml and 0.2 µg/ml for Aripiprazole Related Compound G and Aripiprazole Related Compound F, respectively (Table 1). Similarly it was done at 0.06 µg/ml for Aripiprazole itself.

Limit of Quantitation (*LOQ*)

The LOQ solution is injected in six replicates to establish its precision. The concentration of the Limit of Quantitation (LOQ); was determined to be 0.8 µg/ml for Aripiprazole Related Compound G, 0.7 µg/mL for Aripiprazole Related Compound F, and 0.17 µg/ml for Aripiprazole. The results of the Limit of Detection (LOD) are illustrated in Table 2.

Linearity

The linearity of Aripiprazole Related Compound G, Aripiprazole Related Compound F and Aripiprazole was established by analyzing linearity solutions at various concentrations ranging from the LOQ to 150% of their respective specification levels. A linearity curve was plotted for peak area versus concentration. The results of the linearity analysis are summarized in Tables 3, 4 and 5. The linearity graphs for Aripiprazole Related Compound G, Aripiprazole Related Compound F, and Aripiprazole are presented in Figures 5, 6, and 7. The linearity data meets the acceptance criteria indicates that the method was linear within the concentration range from LOQ to 150% of test concentration.

Precision (*Method Precision*)

To evaluate method precision, samples and spiked samples (spiked with all known impurities at their respective specification levels) were prepared and analyzed following the prescribed analytical procedure. The results are summarized in Tables 6 and 7.

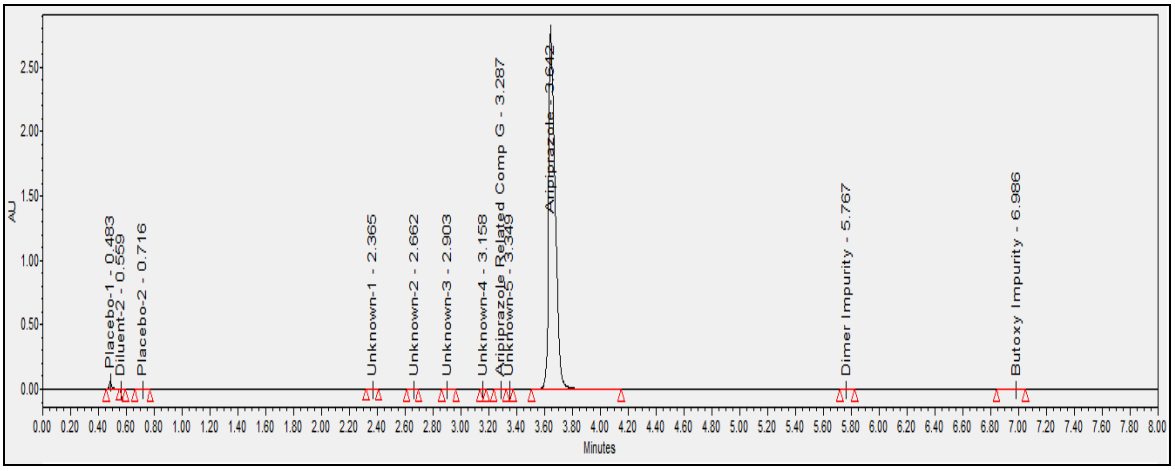

Fig. 1: Chromatogram of Sample.

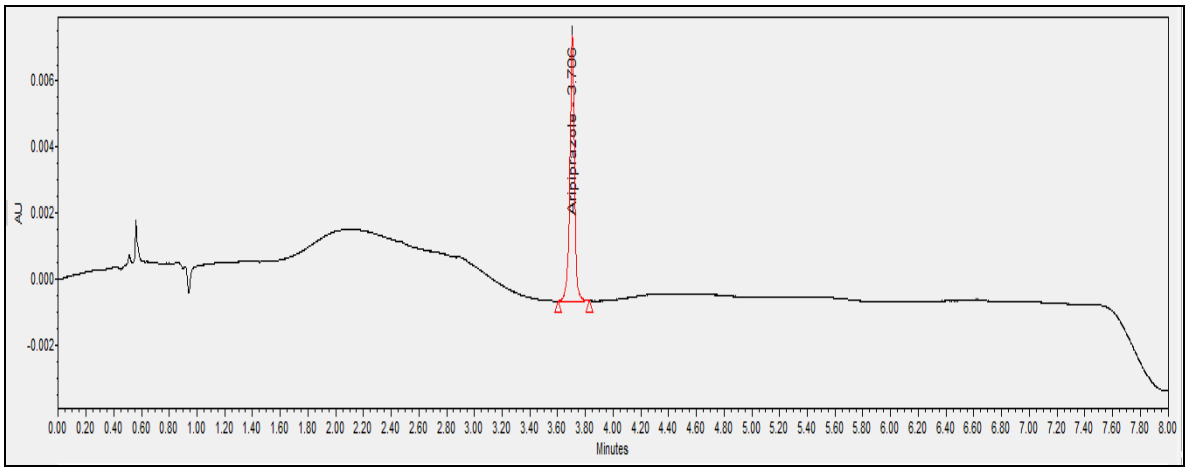

Fig. 2: Chromatogram of Standard.

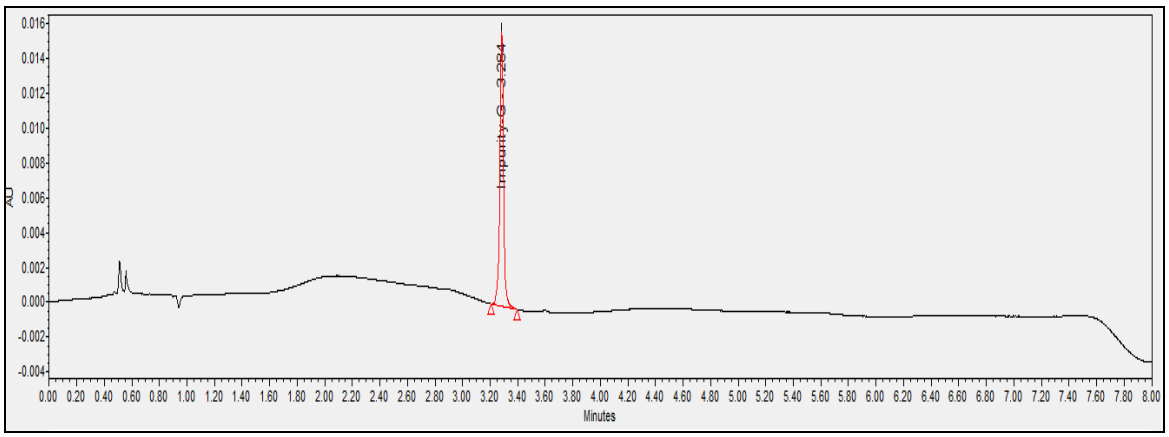

Fig. 3: Chromatogram of Aripiprazole Related Compound-G (Dehydro Impurity).

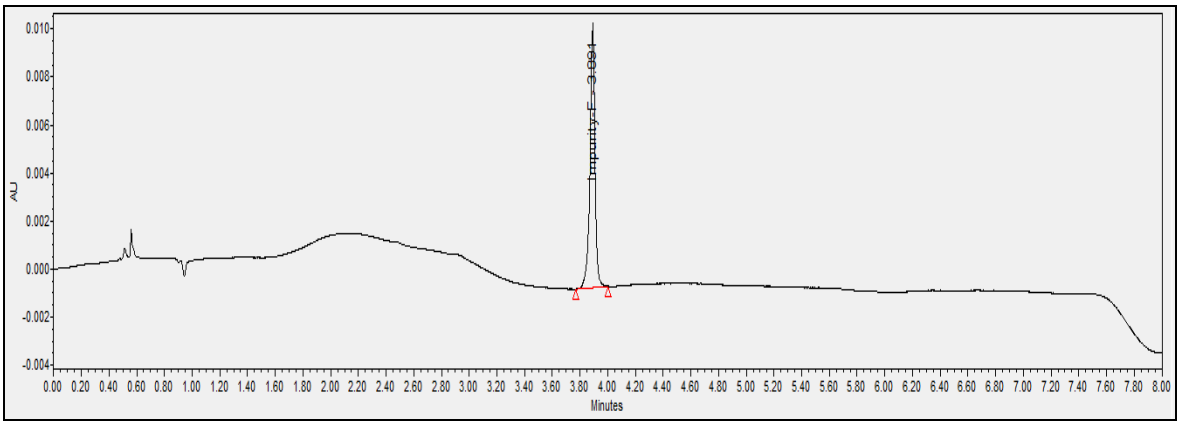

Fig. 4: Chromatogram of Aripiprazole Related Compound-F (N-oxide impurity).

Table 1: Result of Limit of Detection

Injection Replicates	Aripiprazole Related Compound G		Aripiprazole Related Compound F		Aripiprazole	
	Peak Area	S/ N ratio	Peak Area	S/ N ratio	Peak Area	S/ N ratio
1	209	7	1679	21	503	6
2	215	7	1667	24	510	4
3	220	8	1683	20	490	5
4	198	6	1672	22	515	7
5	211	6	1680	26	509	5
6	207	7	1665	25	514	6
Mean	210	NA	1674	NA	507	NA
% RSD	4	NA	0	NA	2	NA

Table 2: Results of Limit of Quantitation

Injection	Aripiprazole Related Compound G		Aripiprazole Related Compound F		Aripiprazole	
	Peak Area	S/ N ratio	Peak Area	S/ N ratio	Peak Area	S/ N ratio
1	532	12	2575	35	1586	23
2	540	16	2673	38	1600	28
3	530	18	2489	40	1590	25
4	545	15	2601	30	1578	27
5	535	20	2587	39	1610	22
6	547	16	2590	41	1589	29
Mean	538	NA	2586	NA	1592	NA
% RSD	1	NA	2	NA	1	NA

Table 3: Result of Linearity of Aripiprazole Related Compound G

Linearity level	Concentration (µg/ml)	Area
LOQ	0.0393	532
50 %	0.886	13704
80 %	1.4176	22565
100 %	1.7720	28154
120 %	2.1264	34215
150 %	2.6580	42797
Correlation Coefficient	1.000	
Slope	16187.8350	
Y-Intercept	348.9000	
Y-intercept bias at 100% level	-1.2	

Table 4: Result of Linearity of Aripiprazole Related Compound F

Linearity level	Concentration (µg/ml)	Area
LOQ	0.1833	2621
50 %	0.9168	12779
80 %	1.4668	20854
100 %	1.8336	26118
120 %	2.2003	31438
150 %	2.7504	39444
Correlation Coefficient	1.000	
Slope	14374.3924	
Y-Intercept	-193.9698	
Y-intercept bias at 100% level	-0.7	

Table 5: Result of Linearity of Aripiprazole

Linearity level	Concentration (µg/ml)	Area
LOQ	0.1081	1522
50 %	0.6144	9044
80 %	0.9831	14546
100 %	1.2289	18178
120 %	1.4746	21803
150 %	1.8433	27517
Correlation Coefficient	1.000	
Slope	14941.6069	
Y-Intercept	-135.1505	
Y-intercept bias at 100% level	-0.7	

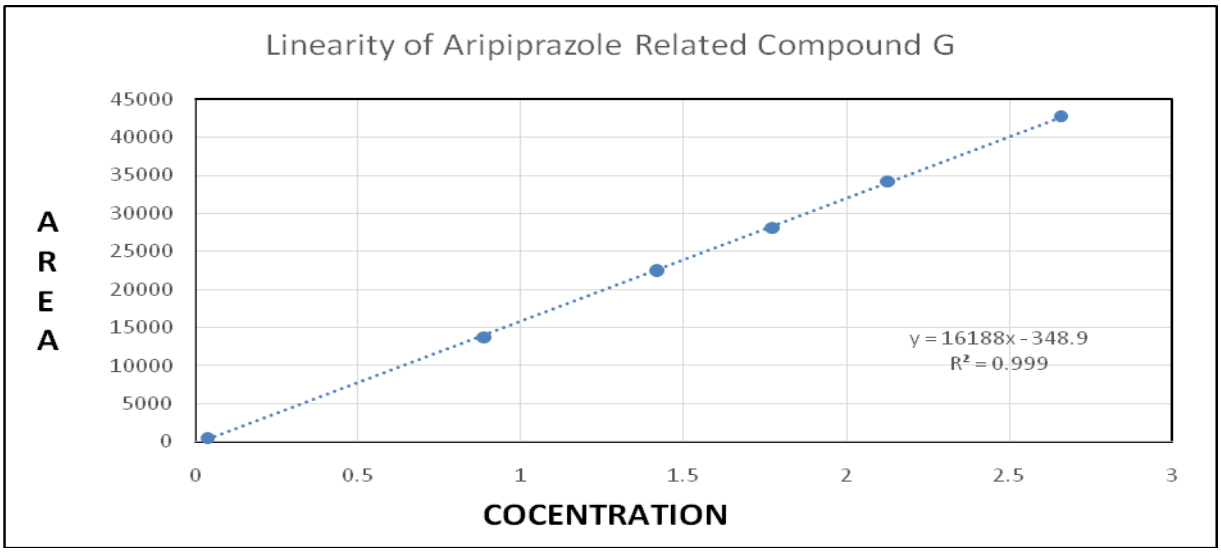

Fig. 5: Linearity Graph of Aripiprazole Related Compound G.

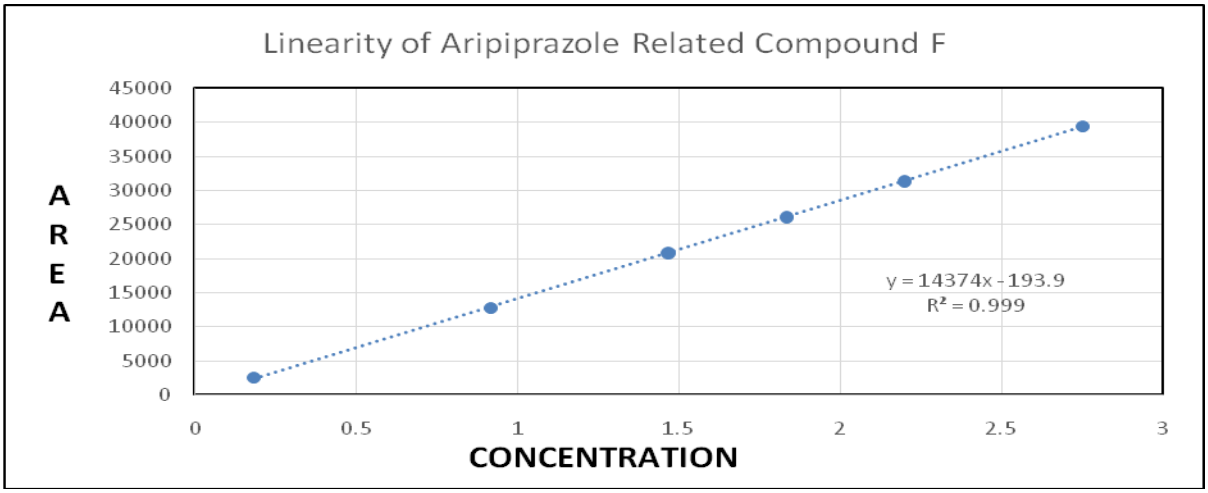

Fig. 6: Linearity Graph of Aripiprazole Related Compound F.

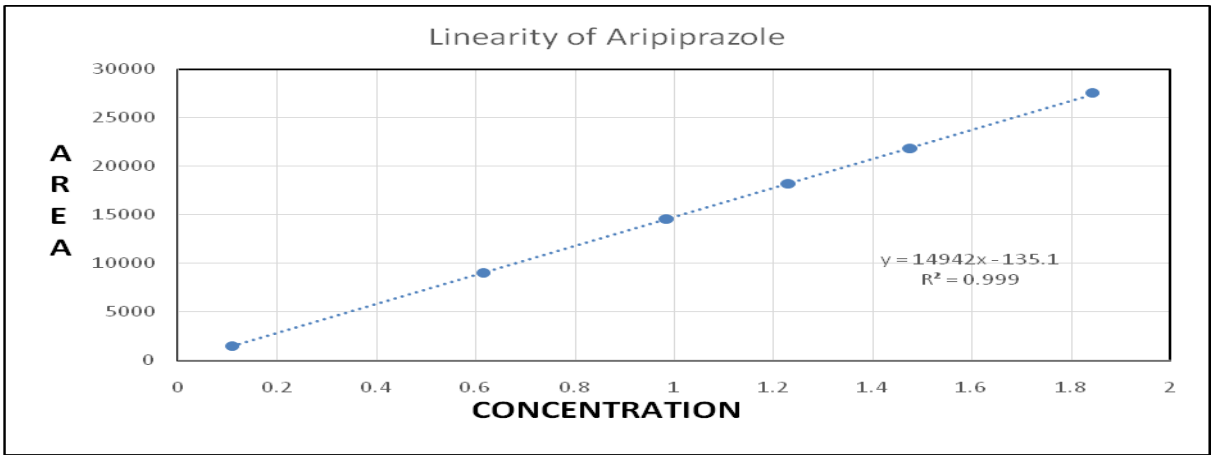

Fig. 7: Linearity Graph of Aripiprazole.

Table 6: Results of Method Precision (Sample without spiking)

Injections	**Aripiprazole Impurity-F (N-Oxide) in %**	**Aripiprazole Impurity-G (Dehydro) in %**	**Any individual unspecified degradation impurity in %**	**Total degradation impurities in %**
1	Not Detected	0.033	0.004	0.037
2	Not Detected	0.032	0.003	0.035
3	Not Detected	0.034	0.004	0.038
4	Not Detected	0.031	0.004	0.035
5	Not Detected	0.035	0.003	0.038
6	Not Detected	0.030	0.005	0.035
Mean	Not applicable	0.033	0.004	0.036

Table 7: Results of Method Precision with Spiked Sample

Injections	Aripiprazole Impurity-F (N-Oxide) in%	Aripiprazole Impurity-G (Dehydro) in %
1	0.279	0.284
2	0.275	0.288
3	0.280	0.280
4	0.276	0.282
5	0.281	0.285
6	0.278	0.287
Mean	0.278	0.284
% RSD	0.8	1.1

In this study, it was concluded that there was no significant difference in the "as such" results. Furthermore, the Relative Standard Deviation (RSD) of the results for each individual known impurity, obtained from six sample preparations, did not exceed 15.0%.

Accuracy

The accuracy of the method demonstrated by spiking known impurities (Aripiprazole Related Compound G and Aripiprazole Related Compound F) into the sample solution from LOQ % to 150 % of impurity level as per procedure. The accuracy results are given in Tables 8 and 9.

The average percentage recovery at each level, as well as the overall average percentage recovery of Aripiprazole from the process equipment surface, falls well within the acceptance criteria of 70.0% to 120.0%. This confirms that the analytical test method is accurate for its intended use.

Robustness

Robustness study was conducted by analyzing the

Table 8: Results of Accuracy for Aripiprazole Related Compound G

Recovery level	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean % Recovery	% RSD
LOQ Set-1	0.039	0.037	94.9	94.9	2.0
LOQ Set-2		0.038	97.4		
LOQ Set-3		0.036	92.3		
100% Set-1	1.772	1.713	96.7	97.0	2.7
100% Set-2		1.725	97.3		
100% Set-3		1.719	97.0		
150% Set-1	2.658	2.606	98.0	98.1	0.3
150% Set-2		2.598	97.7		
150% Set-3		2.621	98.6		
Overall average % recovery				96.7	

Table 9: Results of Accuracy for Aripiprazole Related Compound F

Recovery level	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean % Recovery	% RSD
LOQ Set-1	0.183	0.169	92.3	92.3	1.8
LOQ Set-2		0.172	94.0		
LOQ Set-3		0.166	90.7		
100% Set-1	1.833	1.690	92.2	91.8	1.4
100% Set-2		1.656	90.3		
100% Set-3		1.701	92.8		
150% Set-1	2.750	2.552	92.8	94.5	1.8
150% Set-2		2.601	94.6		
150% Set-3		2.645	96.2		
Overall average % recovery				92.9	

standard and test solutions under varying conditions. The results obtained from these altered conditions were compared with those obtained under standard chromatographic conditions. The robustness evaluation involved deliberate changes to various chromatographic parameters compared to the standard conditions. The corresponding data are presented in Tables 10 and 11.

The results of the robustness study were well within the acceptance criteria, confirming the reliability of the method. The findings

Table 10: Results of Robustness (System Precision)

Change in Parameters	Value	Asymmetry Factor	Area	% RSD
Actual	As Such	1.0	18666	0.4
Flow rate (± 10 %)	0.427 ml/ min	1.1	18744	0.2
	0.521 ml/ min	1.2	18802	0.6
Column Oven Temperature (±5 °C)	Temperature 30°C	1.3	18929	0.3
	Temperature 40°C	1.2	18745	0.2
Buffer pH	+ 0.2 pH	1.3	18599	0.1
	- 0.2 pH	1.1	18601	0.3

Table 11: Results of Robustness

Change in Parameters	Retention time	Aripiprazole Related Compound G	Aripiprazole	Aripiprazole Related Compound F
Actual	As Such	3.30	3.65	3.90
Flow rate (± 10 %)	0.427 ml/ min	3.12	3.48	3.72
	0.521 ml/ min	3.45	3.76	4.1
Column Oven Temperature (±5 °C)	Temperature 30°C	3.41	3.78	3.99
	Temperature 40°C	3.19	3.54	3.80
Buffer pH	+ 0.2 pH	3.37	3.74	3.97
	- 0.2 pH	3.25	3.59	3.83

demonstrated that the test method was robust, even when key parameters such as flow rate, column oven temperature, and buffer pH were deliberately altered (Pathuri *et* al., 2013; Moein *et al*., 2017; Sanap *et al*., 2017).

Conclusion

The developed analytical method validation for related compounds in Aripiprazole Tablets (30 mg) demonstrated compliance with all acceptance criteria. Specificity was confirmed, with no interference observed at the retention times of Aripiprazole and its known impurities, peaks were well-separated, and the purity angle was less than the purity threshold. The method was sensitive, with LOD and LOQ sufficient to detect and quantify process-related and degradation impurities, and it exhibited linearity from LOQ to 150%. Accuracy was demonstrated within the specified range. Robustness testing showed that results remained within defined limits despite changes in flow rate, column oven temperature, and buffer pH. Overall, the validation data confirm that the developed analytical method is reliable, robust and suitable for determining related compounds in Aripiprazole Tablets (30 mg).

Author Contributions

Each author has equally contributed in planning the study, performing the analysis, writing and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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