# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE QUANTITATIVE DETERMINATION OF ARIPIPRAZOLE IN API FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM

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***ABSTRACT:** A new analytical, precise, accurate and rapid high performance liquid chromatographic method has been developed and validated for the estimation of Aripiprazole in bulk form and marketed pharmaceutical dosage form. A Symmetry ODS ($C_{18}$) RP Column, 250 mm x 4.6 mm, 5µm in isocratic mode, with mobile phase containing a mixture of Phosphate Buffer (0.02M): Acetonitrile in the ratio of 48:52 v/v (pH was adjusted to 2.80 with orthophosphoric acid) was used. The mobile phase was pumped at a flow rate of 1.0 ml/min and the eluents were monitored at 248 nm. The selected chromatographic conditions were found to effectively separate Aripiprazole (RT: 3.645min). The method was validated in terms of linearity, accuracy, precision, and specificity, limit of detection and limit of quantitation. Linearity for Aripiprazole was found in the range of 30-70µg/ml. The percentage recoveries for Aripiprazole ranged from 98%-120%. The limit of detection and the limit of quantitation for Aripiprazole were found to be 0.09µg/ml and 0.027µg/ml respectively. The method was found to be robust and can be successfully used to determine the drug content of marketed formulations.*

***Key words:** Aripiprazole, RP-HPLC, Method Development, Validation, Precision, Accuracy.*

## I. INTRODUCTION

Aripiprazole is an atypical antipsychotic used in the treatment of schizophrenia and bipolar illness. Aripiprazole therapy has not been associated consistently with serum aminotransferase elevations and has yet to be linked to cases of clinically apparent acute liver injury. Aripiprazole is a quinoline derivate and atypical anti-psychotic agent. Aripiprazole has partial agonistic activity at dopamine D2 receptors and serotonin 5-HT1A receptors, as well as potent antagonistic activity on serotonin 5-HT2A receptors. This drug stabilizes dopamine and serotonin activity in the limbic and cortical system. Aripiprazole is used in managing symptoms of schizophrenia and of acute manic and mixed episodes associated with bipolar I disorders. Aripiprazole is an atypical antipsychotic orally indicated for treatment of schizophrenia, bipolar I, major depressive disorder, irritability associated with autism, and Tourette's. It is also indicated as an injection for agitation associated with schizophrenia or bipolar mania. Aripiprazole exerts its effects through agonism of dopaminic and 5-HT1A receptors and antagonism of alpha adrenergic and 5-HT2A receptors. The IUPAC Name of Aripiprazole is 7-[4-[4-(2, 3-dichloro phenyl) piperazin-1-yl] butoxy]-3, 4-dihydro-1H-quinolin-2-one. The Chemical Structure of Aripiprazole as follows

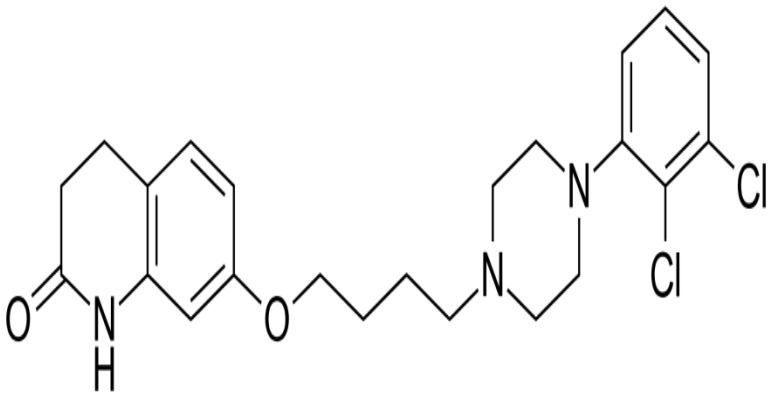


Fig.1. Chemical Structure of Aripiprazole

The purpose of the present study is to establish a simple, sensitive, validated and inexpensive RP-HPLC method[4] for the determination of Aripiprazole in pure form and in pharmaceutical dosage form.

## II. EXPERIMENTAL

**Table-1: List of Instrument used**

| S. No. | Instruments/Equipments/Apparatus |
|---|---|
| 1. | Waters HPLC with Empower2 Software with Isocratic with UV-Visible Detector. |
| 2. | ELICO SL-159 UV – Vis spectrophotometer |
| 3. | Electronic Balance (SHIMADZU ATY224) |
| 4. | Ultra Sonicator (Wensar wuc-2L) |
| 5. | Thermal Oven |
| 6. | Symmetry RP $C_{18}$, 5µm, 250mm x 4.6mm i.d. |
| 7. | $P^H$ Analyzer (ELICO) |
| 8. | Vacuum filtration kit (BOROSIL) |

**Table-2: List of Chemicals used**

| S.No. | Name | Specifications | | Manufacturer/Supplier |
|---|---|---|---|---|
| | | Purity | Grade | |
| 1. | Doubled distilled water | 99.9% | HPLC | Sd fine-Chem ltd; Mumbai |
| 2. | Methanol | 99.9% | HPLC | Loba Chem; Mumbai. |
| 3. | Dipotassium hydrogen orthophosphate | 96% | L.R. | Sd fine-Chem ltd; Mumbai |
| 4. | Acetonitrile | 99.9% | HPLC | Loba Chem; Mumbai. |
| 5. | Potassium dihydrogen orthophosphate | 99.9% | L.R. | Sd fine-Chem ltd; Mumbai |
| 6. | Sodium hydroxide | 99.9% | L.R. | Sd fine-Chem ltd; Mumbai |
| 7. | Hydrochloric acid | 96% | A.R. | Sd fine-Chem ltd; Mumbai |
| 8. | 3% Hydrogen Peroxide | 96% | A.R. | Sd fine-Chem ltd; Mumbai |

**Selection of Wavelength:**

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Aripiprazole, so that the same wave number can be utilized in HPLC UV detector for estimating the Aripiprazole.

**Sample & Standard Preparation for the Analysis:**

25 mg of Aripiprazole standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution[5] was done by transferring 0.3 ml of the above solution into a 10 ml volumetric flask and make up to volume with mobile phase.

**Preparation of 0.02M Potassium dihydrogen orthophosphate Solution:**

About 2.72172grams of Potassium dihydrogen orthophosphate was weighed and transferred into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC Grade water. The pH was adjusted to 2.80 with diluted

orthophosphoric acid Solution.

**Preparation of Mobile Phase:**

480mL (48%) of above Phosphate buffer solution and 520mL of HPLC Grade Acetonitrile (52%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The resulted solution was filtered through 0.45 µm filter under vacuum filtration.

**Method Validation**

Validation[6,7] is a process of documenting and proving, analytical method provides analytical data, for the intended use. There are many reasons for the need to validate analytical procedures. To assuming the quality and achieving the quality control requirements, to achieve acceptance of the product by international agencies.

**Accuracy:**

**Recovery study:**

To determine the accuracy[8] of the planned technique, recovery studies were distributed by adds completely different amounts (80%, 100%, and 120%) of pure drug of Aripiprazole were taken and extra to the pre-analysed formulation of concentration 30µg/ml. From that proportion recovery values[9] were calculated. The results were shown in table-4.

**Precision:**

**1. Repeatability**

The precision[10] of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Various precision levels are system or instrument precision, intermediate precision, repeatability, reproducibility.

**2. Intermediate Precision:**

**2.1 Intra-assay & inter-assay:**

The intra & inter day variation[11] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Aripiprazole revealed that the proposed method is precise.

**Linearity & Range:**

The calibration curve[12] showed good linearity in the range of 0-70µg/ml, for Aripiprazole (API) with correlation coefficient ($r^2$) of 0.999 (Fig-4). A typical calibration curve[13] has the regression equation of y = 11266.x + 50416 for Aripiprazole.

**Robustness:**

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±$2^0$C), Wavelength of detection (±2nm) & Acetonitrile content in mobile part (±2%) studied to work out the strength of the tactic also are in favour of (Table-8, nada RSD < 2%) the developed RP-HPLC technique[14] for the analysis of Aripiprazole (API).

**Limit of Detection (LOD) and Limit of Quantitation (LOQ):**

Limit of detection: is the lowest amount of an analyte in a sample which can be detected[15] but not necessarily quantified as an exact value. Limit of quantitation is the lowest concentration of an analyte in a sample which can be quantitatively[16] determined with suitable precision and accuracy.

**System Suitability Parameter:**

System quality testing[17] is associate degree integral a part of several analytical procedures. The tests square measure supported the idea that the instrumentation, physics, associate degree analytical operations and samples to be analyzed represent an integral system[18] that may be evaluated intrinsically. Following system quality check parameters were established. The info square measure shown in Table-9.

**Assay:**

Twenty pharmaceutical dosage forms were taken and the I.P. method was followed to work out the typical weight. On top of weighed tablets were finally pulverized and triturated well. A amount of powder cherish twenty five mg of medicine were transferred to twenty five cc meter flask, build and resolution[19] was sonicated for quarter-hour, there once volume was created up to twenty five cc with same solvent. Then ten cc of the on top of resolution was diluted to a hundred cc with mobile part. The answer was filtered through a membrane filter (0.45±m) and sonicated to remove. The answer ready was injected in 5 replicates into the HPLC system and therefore the observations were recorded.

A duplicate injection of the quality resolution was conjointly injected into the HPLC system and therefore the peak areas were recorded. The info square measure shown in Table-10.

**ASSAY:**

Assay[15] % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{\text{a hundred}} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak space of drug obtained with check preparation
AS = Peak space of drug obtained with normal preparation
WS = Weight of operating normal taken in mg
WT = Weight of sample taken in mg
DS = Dilution of normal resolution
DT = Dilution of sample resolution
P = proportion purity of operating normal

**Stability Studies**

**Acid Degradation:**

A precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base flagon. 30 ml of 0.1 N HCl was added to it and it was refluxed[20] in a water shower at 600C for 4 hours. Permitted to cool to room temperature. The sample was then neutralized using dilute NaOH solution & final volume of the sample was made up to 100ml with water to prepare 100 µg/ml solutions. It was infused into the HPLC[21] framework against a clear of portable stage (subsequent to advancing the versatile stage pieces). This experiment was repeated several times using same concentration of HCl (0.1N) and observed its degradation profile. The typical chromatogram shown below is the degradation profile of Rebamipide in 0.1N HCl.

**Basic Hydrolysis:**

A precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base flagon. 30 ml of 0.1N NaOH was added to it. & it was refluxed in a water bath at $60^0$C for 4 hours. Allowed to cool to room temperature. The example was then killed utilizing 2N HCl arrangement and last volume of the example was made up to 100ml to plan 100 µg/ml arrangements. It was infused into the HPLC framework against a clear of portable stage in the wake of enhancing the versatile stage arrangements. This experiment was repeated several times using same concentration of NaOH such as 0.1N to observe its degradation[22] profile. The chromatogram shown below is the degradation profile of Aripiprazole in 0.1N NaOH.

**Thermal Degradation:**

Precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base carafe. 30 ml of HPLC water was added to it. Then, it was refluxed in a water bath at $60^0$ c for 6 hours uninterruptedly. After the reflux was over, the drug became soluble and the mixture of drug & water was allowed to cool to room temperature. Last volume was made up to 100 ml with HPLC water to plan 100 µg/ml arrangements. It was infused into the HPLC framework against a clear of versatile stage/mobile phase.

**Photolytic Degradation:**

Around 10 mg of unadulterated medication was taken in a clean and dry Petri dish. It was kept in an UV bureau at 254 nm wavelength for 24 hours without interference. Precisely measured 1 mg of the UV uncovered medication was exchanged to a clean and dry 10 ml volumetric cup. First the UV exposed drug was dissolved in methanol & made up to the mark with mobile phase to get 100 µg/ml solution. At long last this arrangement was infused into the HPLC framework against a clear of portable stage and chromatogram was gotten.

**Oxidation with (3%) H2O2:**

Precisely measured 10 mg. of unadulterated medication was taken in a clean and dry 100 ml volumetric jar. 30 ml of 3% $H_2O_2$ and a little methanol was added to it to make it dissolvable and then kept in that capacity in dim for 24 hours. Last volume was made up to 100 ml. utilizing water to get ready 100 µg/ml arrangement. The above example was infused into the HPLC framework.

## III. RESULTS AND DISCUSSION

**Method Development**

**Selection of Wavelength:**

While scanning the Aripiprazole solution we observed the maxima at 248 nm. The UV spectrum[23] has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450. The scanned UV spectrum is attached in the following page,


Fig.2. UV Spectrum for Aripiprazole at 248 nm

**Summary of Optimised Chromatographic Conditions:**

**Table-3: Summary of Optimised Chromatographic Conditions**

| | |
|---|---|
| Mobile phase | Phosphate Buffer (0.02M): Acetonitrile = 48:52 (pH-2.80) |
| Column | Symmetry ODS ($C_{18}$) RP Column, 250 mm x 4.6 mm, 5µm |
| Column Temperature | Ambient |
| Detection Wavelength | 248 nm |
| Flow rate | 1.0 ml/ min. |
| Run time | 08 min. |
| Temperature of Auto sampler | Ambient |
| Diluent | Mobile Phase |
| Injection Volume | 20µl |
| Mode of Elution | Isocratic |
| Retention time | 3.645 minutes |

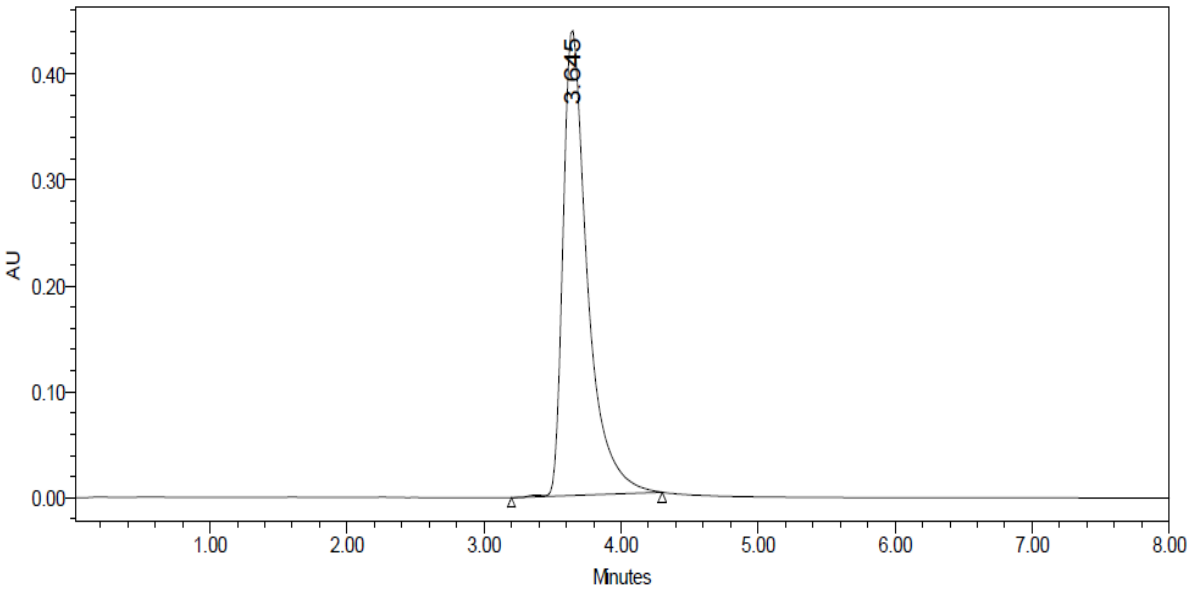


Fig.3. Optimized Chromatographic Condition

**Method Validation**

**Accuracy:**

Accuracy[24] results listed in Table 4 were found to be 100.230 ± 0.47 % w/w for Aripiprazole, which indicate high recovery of the method.

**Table-4: Accuracy Readings**

| Sample ID | Concentration (µg/ml) | | Peak Area | % Recovery of Pure drug | Statistical Analysis |
|---|---|---|---|---|---|
| | Amount Added | Amount Found | | | |
| $S_1$ : 80 % | 40 | 40.141 | 502647 | 100.352 | Mean= 100.3947%<br>S.D. = 0.071319<br>% R.S.D.= 0.071038 |
| $S_2$ : 80 % | 40 | 40.191 | 503214 | 100.477 | |
| $S_3$ : 80 % | 40 | 40.142 | 502656 | 100.355 | |
| $S_4$ : 100 % | 50 | 50.044 | 614215 | 100.088 | Mean= 99.98533%<br>S.D. = 0.183045<br>% R.S.D.= 0.183071 |
| $S_5$ : 100 % | 50 | 49.887 | 612451 | 99.774 | |
| $S_6$ : 100 % | 50 | 50.047 | 614254 | 100.094 | |
| $S_7$ : 120 % | 60 | 60.192 | 728547 | 100.32 | Mean= 100.311%<br>S.D. = 0.408574<br>% R.S.D.= 0.407308 |
| $S_8$ : 120 % | 60 | 59.939 | 725698 | 99.898 | |
| $S_9$ : 120 % | 60 | 60.429 | 731211 | 100.715 | |

**Precision:**

**1. Repeatability:**

The exactitude[25] of every technique was determined one by one from the height areas & retention times obtained by actual determination of six replicates of a set quantity of drug. Aripiprazole (API). The % relative variance was calculated for Aripiprazole square measure bestowed within the table-5.

**Table-5: Repeatability Readings**

| HPLC Injection Replicates of Aripiprazole | Retention Time (Minutes) | Peak Area |
|---|---|---|
| Replicate – 1 | 3.649 | 5674158 |
| Replicate – 2 | 3.684 | 5654715 |
| Replicate – 3 | 3.687 | 5665841 |
| Replicate – 4 | 3.688 | 5654578 |
| Replicate – 5 | 3.688 | 5652284 |
| Replicate – 6 | 3.687 | 5641487 |
| **Average** | | **5657177** |
| **Standard Deviation** | | **11369.72** |
| **% RSD** | | **0.200979** |

**Intermediate Precision:**

**Intra-assay & Inter-assay:**

The intra & inter day variation[26] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Aripiprazole revealed that the proposed method is precise.

**Table-6: Results of intra-assay & inter-assay**

| Conc. of Aripiprazole (API) (µg/ml) | Observed Conc. of Aripiprazole (µg/ml) by the proposed method | | | |
|---|---|---|---|---|
| | Intra-Day | | Inter-Day | |
| | Mean (n=6) | % RSD | Mean (n=6) | % RSD |
| 40 | 40.05 | 1.09 | 39.89 | 1.08 |

| | | | | |
|---|---|---|---|---|
| 50 | 50.08 | 0.95 | 49.54 | 0.76 |
| 60 | 60.09 | 0.97 | 59.86 | 0.94 |

**Linearity & Range:**

Calibration curve[27] was constructed by injecting five different concentrations of Aripiprazole. Results of the regression analysis and the coefficient of determination (r2) are listed in Table 7. The high coefficient[28] of determination values i.e. 0.9997 for indicated good linearity between their peak areas (y) and standard drug concentrations (x, µg/ml) in the range 30-70µg/ml for Aripiprazole and the obtained results are shown in Fig 4.

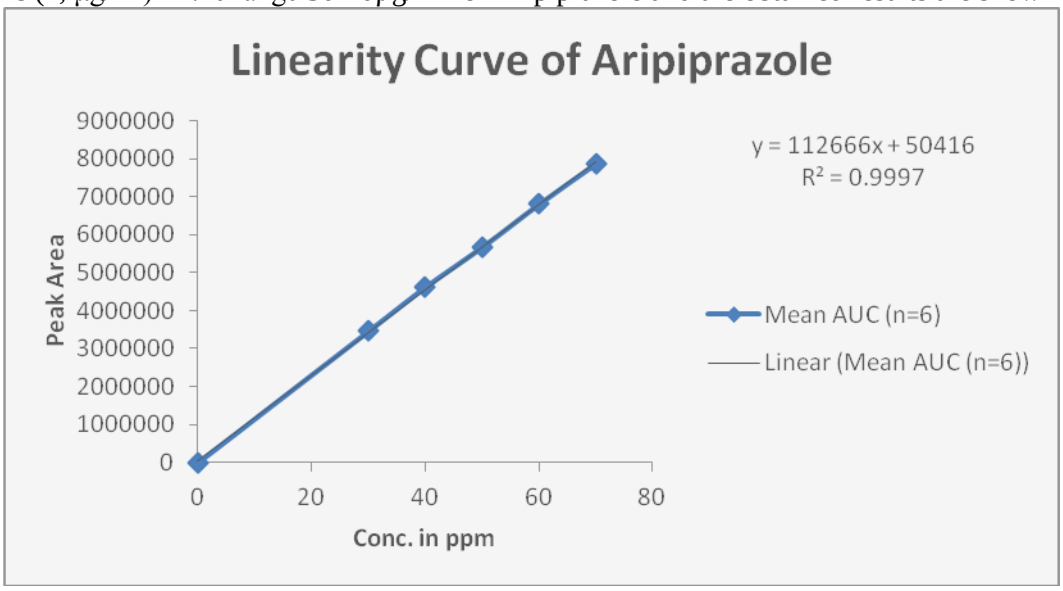


Fig.4. Calibration Curve of Aripiprazole (API)

**Table-7: Linearity Results**

| Conc.(µg/Ml) | Mean AUC (n=6) |
|---|---|
| 0 | 0 |
| 30 | 3465974 |
| 40 | 4626478 |
| 50 | 5682284 |
| 60 | 6815478 |
| 70 | 7878721 |

**Robustness:**

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine analysis. The robustness[29] of the method was evaluated by assaying the same sample under different analytical conditions deliberately changing from the original condition. Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2$^0$C), Wavelength of detection (±2nm) & Acetonitrile content in mobile part (±2%) studied to work out the strength of the tactic also are in favour of (Table-8, nada RSD < 2%) the developed RP-HPLC technique for the analysis of Aripiprazole (API). The %RSD value of assay determined for

the same sample under original conditions[30] and robustness conditions was less than 2.0% indicating that the developed method was robust.

**Table-8: Result of Method Robustness Test**

| Change in parameter | % RSD |
|---|---|
| Flow (1.1 ml/min) | 0.56 |
| Flow (0.9 ml/min) | 0.87 |
| Temperature ($27^0$C) | 0.72 |
| Temperature ($23^0$C) | 0.53 |
| Wavelength of Detection (257 nm) | 0.61 |
| Wavelength of detection (253 nm) | 0.96 |

**LOD & LOQ:**

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.027 µg/ml respectively.

**System Suitability Parameter:**

The system suitability test[31] was performed to ensure that the complete testing system was suitable for the intended application. The parameters measured were peak area, retention time, tailing factor and theoretical plates. In all measurements the peak area varied less than 2.0, the average retention time was 3.86 min, theoretical plates were 4765 (more than 2000) and tailing factor was 1.42 (less than 2) for the Aripiprazole peaks as shown in Table 9 respectively. The proposed method offers high sensitivity[32] and both the peaks can be detected accurately. In all the cases, the Aripiprazole peaks were well separated from the excipients.

**Table-9: Knowledge of System quality Parameter**

| S.No. | Parameter | Limit | Result |
|---|---|---|---|
| 1 | Resolution | Rs > 2 | 8.54 |
| 2 | Asymmetry | T ≤ 2 | Aripiprazole =0.98 |
| 3 | Theoretical plate | N > 2000 | Aripiprazole =4782 |
| 4 | Tailing Factor | T<2 | Aripiprazole =1.49 |

**Assay:**

**Table-10: Recovery Data for estimation Aripiprazole**

| Brand Name of Aripiprazole | Labelled amount of Drug (mg) | Mean (± SD) amount (mg) found by the proposed method (n=6) | Assay % (± SD) |
|---|---|---|---|
| Aripiprazole Tablets (Arpizol 10) (Sun Pharmaceutical Industries Ltd.) (10mg) | 10mg | 9.86 (± 0.682) | 99.53 (± 0.364) |

**Result & Discussion**: The amount of drugs in Aripiprazole Tablet was found to be 9.86 (± 0.682) mg/tab for Aripiprazole & % assay was 99.364 %.

**Stability Studies**

The results of the stress studies[33] indicated the specificity of the method that has been developed. Aripiprazole was stable in photolytic and peroxide stress conditions. The result of forced degradation studies are given in the following table-11.

**Table-11: Results of Forced Degradation Studies of Aripiprazole**

| Stress condition | Time | Assay of active substance | Assay of degraded products | Mass Balance (%) |
|---|---|---|---|---|
| Acid Hydrolysis (0.1 M HCl) | 24Hrs. | 98.76 | 1.24 | 100.0 |
| Basic Hydrolysis (0.1 M NaOH) | 24Hrs. | 98.63 | 1.37 | 100.0 |
| Thermal Degradation (50 $^0$C) | 24Hrs. | 93.98 | 6.02 | 100.0 |
| UV (248nm) | 24Hrs. | 98.84 | 1.16 | 100.0 |
| 3 % Hydrogen Peroxide | 24Hrs. | 94.61 | 5.39 | 100.0 |

## IV CONCLUSION

The proposed HPLC method was validated as per ICH guidelines and applied for the determination of Aripiprazole in bulk form and marketed pharmaceutical formulations. The method was found to be accurate, precise, robust and specific. At the same time the chromatographic elution step is undertaken in a short time (< 5 min). No interference was seen from any components of pharmaceutical dosage form. In conclusion, the high sensitivity, good selectivity, accuracy and reproducibility of the proposed method are suitable for determination of Aripiprazole in bulk form and marketed pharmaceutical formulations.

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