*Heterocyclic Letters*
*Vol. 15| No.1|167-182|Nov-Jan|2025*
*ISSN : (print) 2231–3087 / (online) 2230-9632*
*CODEN: HLEEAI*
***http://heteroletters.org***

# RAPID STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF ORGANIC IMPURITIES FOR ARIPIPRAZOLE IN ARIPIPRAZOLE TABLETS AND DRUG SUBSTANCES

**U. Chandra Sekhar[a], T. Veera Reddy[b], and P. Sanjeeva[c]**

[a]*Department of chemistry, Vikrama simhapuri University, Nellore-524 324.*
[b]*Department of chemistry Sri Krishnadevaraya University, Anantapur-51 5003.*
[*]***E-mail:*** *chandhu.viveka@gmail.com*

.

**ABSTRACT:**

A rapid, simple and validated reversed-phase high-performance liquid chromatographic method has been developed for analysis of aripiprazole in tablet dosage form. Aripiprazole was separated on an inertsil ODS analytical column with mobile phase-A in the composition of buffer pH 3.0 and mobile phase-B at a flow rate of 1.5 mL/min in gradient elution (MP-A: 0min, 85%;10min,70%; 15min, 65%; 18min, 65%; 24min, 50%; 34min, 40%, 35min, 85% & 45min, 85%). The effluent was monitored by UV detection at 252 nm. Calibration plots were linear in the range of LOQ to 125% and the LOD, LOQ were 0.03 and 0.15 µg mL-1, respectively. The high recovery and low relative standard deviation confirm the suitability of the method for routine quality control determination of aripiprazole in tablets.

**KEYWORDS:** Aripiprazole, organic impurities, stress degradation, HPLC.

## INTRODUCTION:

Aripiprazole1 is an atypical antipsychotic agent. 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 5HT1A receptor and like the other atypical antipsychotics, aripiprazole displays an antagonist profile at the 5HT2A receptor [i-ii]. Aripiprazole is 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydrocarbostyril. The empirical formula is $C_{23}H_{27}Cl_2N_3O_2$ and its molecular weight is 448.38. Aripiprazole is a white to off white crystalline powder that is soluble in chloroform and slightly soluble in methanol, practically insoluble in n-Hexane. Aripiprazole is formulated as injection for infusion administration. Aripiprazole injection contains 2 mg/mL and the following inactive ingredients: lactose monohydrate, corn starch, hydroxypropyl cellulose, microcrystalline cellulose, sodium starch glycolate, and magnesium stearate. The film coating for the 14 mg tablet is made of hypromellose, titanium dioxide, talc, polyethylene glycol and indigo carmine aluminum lake. In addition to these, the 7 mg tablet film coating includes iron oxide yellow [iii-vi].

As per the literature survey several methods have been reported for the estimation of Aripiprazole and its organic impurities. The present proposed method estimates Aripiprazole and its organic impurities in a simple and economical process. The main aim of this method was to determine and validate the Aripiprazole based on International Conference on Harmonization[-ix] guidelines. This method was made use of a reproducible procedure for the quantitative analysis of drug samples as injection dosage form and drug substances. Hence in the present study we attempted to develop a simple method for the estimation of Aripiprazole and its related impurities in pharmaceutical formulation of injection and drug substances[-xi]. The general information, molecular structure of Aripiprazole and its organic impurities in the study are given in Tables 1 and 2.

## MATERIALS AND METHODS:

### Instrumentation:

The separation and estimation of Aripiprazole with organic impurities on a HPLC. The inertsil pH 3.0 and mobile phase-B at a flow rate of 1.5 mL/min in gradient elution (MP-A: 0min, 85%;10min,70%; 15min, 65%; 18min, 65%; 24min, 50%; 34min, 40%, 35min, 85% & 35min, 85%). A 10-µL fixed volume sample was injected for the analysis using a with an auto injector and PDA detector. An analytical balance was used for weighing the standards and samples. pH of the mobile phase was adjusted using a digital pH meter. A photo stability chamber used for the sample stress. A water system was (Make: Millipore) used for the preparing the diluent and mobile phase preparations.

### Materials (Chemicals, reagents, standards and samples):

The active pharmaceutical ingredient Aripiprazole with 99.1% purity and its organic impurities were obtained from Sakam private limited. The marketed formulation of Aripiprazole injection was purchased in a local pharmacy. Analytical laboratory reagent grade Potassium dihydrogen phosphate ($KH_2PO_4$) and Triethylamine ($C_6H_{15}N$) were purchased from SD Fine Chem. Limited, Mumbai. HPLC grade acetonitrile, Methanol and water were purchased from Merck Chemicals, Mumbai, and 0.45-µm PVDF filters were used for filtration of samples and purchased from Millipore (India).

### Chromatographic Conditions:

A mobile phase system consisting of buffer, triethylamine and acetonitrile and separation was achieved with gradient elution mode. The flow rate was 1.5 mL/min. The injection volume was 10 µL. The eluent was monitored by the photo diode array detector (PDA) from 200 to 400 nm, and chromatograms were extracted at the wavelengths of 252 nm. The total run time was 45minutes.

### Preparation of solutions for Method Development:

Optimized separation was achieved on a inertsil ODS column (150 mm×4.6 mm; 5 µm) using mobile phase-A in the composition of buffer pH 3.0 and mobile phase-B at a flow rate of 1.5 mL/min in gradient elution (MP-A: 0min, 85%;10min,70%; 15min, 65%; 18min, 65%; 24min, 50%; 34min, 40%, 35min, 85% & 45min, 85%). UV detection was carried out at a wavelength of 252 nm.

### Preparation of buffer:

Weigh and dissolve 2.72 g of potassium dihydrogen phosphate in 1000 mL of water and add 10 mL of triethylamine and mix, adjust the pH of the solution to 3.0 with orthophaspharic acid. Filter the solution through 0.45µm membrane filter and degas.

**Preparation of Mobile phase-A:**
100% buffer use as mobile phase-A.

**Preparation of Mobile phase-B:**
100% acetonitrile use as mobile phase-B.

**Preparation of Blank solution:**
Mix the buffer and acetonitrile in the ratio of 62:38(%v/v) respectively.

**Preparation of standard solution:**
First 25 mg of standard drug Aripiprazole was weighed accurately and then transferred into a 25-mL volumetric flask. The drug was dissolved in diluent. Then the final volume was made up to 25 mL with diluent. Further 5 mL of this solution transferred to 50 mL of volumetric flask. Aripiprazole standard stock solution of 10.0 µg/mL.

**Preparation of sample solution:**
Taken equivalent 25 mg of Aripiprazole from Aripiprazole tablet, transferred into 50 mL volumetric flask, added 75% of diluent, sonicated for 30 min with intermediate shaking and diluted to volume with diluent and mixed well. Sample solution containing 500 µg/mL Aripiprazole
.

**FOR METHOD VALIDATION:**

**System suitability and system precision:**
System suitability tests were carried out on a freshly prepared standard solution (10 µg/mL) of the Aripiprazole and organic impurities to scrutinize the various optimized parameters such as retention time, % of relative standard deviation, tailing factor and USP plate count.

**Specificity: (Blank, Placebo and Impurity Interference)**
Specificity tests were carried out on a freshly prepared blank, placebo, individual impurities and spiked sample with organic impurities (500 µg/mL) of the Aripiprazole and to optimize the parameters such as blank, placebo and individual interference at retention time of Aripiprazole.

**Precision:**
Precision was determined using six spiked sample solution containing (500 µg/mL) of Aripiprazole and its organic impurities that were prepared and analyzed in the optimized method conditions. For intraday precision the solutions were prepared and analyzed six times on the same day. Peak area responses of six replicate analyses were calculated in terms of relative standard deviation (RSD).

**Ruggedness:**
Ruggedness was determined using six spiked sample solution containing (500 µg/mL) of Aripiprazole and its organic impurities that were prepared and analyzed in the optimized method conditions. For intraday precision the solutions were prepared and analyzed six times on the different day, different instrument, different analyst and different column. Peak area responses of six replicate analyses were calculated in terms of relative standard deviation (RSD).

**Limit of Detection, Limit of Quantification and Limit of Quantification Precision:**
Determined the limit of detection and quantification for Aripiprazole and its organic impurities by deriving the concentration which will give signal to noise ratio between 2.0 to 3.0 for limit of detection and which will give signal to noise ratio between 10 to 30 for limit of quantification. Determined precision by preparing the solution having impurities and Aripiprazole at about limit of quantification level and injected six times into the chromatographic system and calculated in terms of relative standard deviation (RSD).

**Linearity and Range:**
Linearity study was determined with calibration curves were prepared with six concentration range of LOQ to 150% level for Aripiprazole and its organic impurities. The solutions were analyzed in optimized conditions. The data of peak area vs concentration were analyzed using linear least square regression.

**Recovery:**
The standard addition for Aripiprazole and its organic impurities method was carried out for determining the accuracy of the method. For this LOQ, 100% and 120% level concentrations were spiked into a known concentration. Accuracy was determined by comparing the difference between the spiked value and the actual found value.

**Robustness:**
Robustness of the proposed method was tested by slight variation in optimized method conditions. Change in ±5 mL variation in mobile phase organic, ±0.2 pH modifier, ±0.1 flow rate, and ±5°C column oven temperature was studied. In each of the changed conditions, standard solutions containing 10 µg/mL Aripiprazole and spiked sample containing 500 µg/mL of Aripiprazole and organic impurities were analyzed in optimized method conditions. % relative standard deviation, tailing factor, theoretical plate count value.

**For Force degradation Studies:**

**Acid Degradation studies:**
Weighed and transferred equivalent to 25 mg of Aripiprazole tablet powder into 50 mL of volumetric flask, added 75% volume of diluent, sonicated to 30 min, added 10 ml of 1N Hydrochloric acid (HCl) solution. The solutions were kept at 80°C for 24 hours. After degradation the sample solution was neutralized with equal volume of 1N of Sodium hydroxide (NaOH) solution. For HPLC study, the resultant solution was diluted to obtain Aripiprazole solution was injected into the system and chromatograms were recorded to assess the stability of sample.

**Base Degradation Studies:**
Weighed and transferred equivalent to 25 mg of Aripiprazole tablet powder into 50 mL of volumetric flask, added 75% volume of diluent, sonicated to 30 min, added 10 ml of 1N Sodium hydroxide. The solutions were kept at 80°C for 24 hours and cooled the solution at room temperature. After degradation neutralized with equal volume of 1N of Hydrochloric acid (HCl). For HPLC study, the resultant solution was diluted to obtain Aripiprazole solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

**Oxidation:**
Weighed and transferred equivalent to 25 mg of Aripiprazole tablet powder into 50 mL of volumetric flask, added 75% volume of diluent, sonicated to 30 min, add 10% hydrogen peroxide ($H_2O_2$) and the solution was heated on water bath for 1 hour at 40°C and cooled the solution at room temperature. For HPLC study, the resultant solution was diluted to obtain Aripiprazole solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

**Photo Stability studies (For Visible – 1.2 million lux hours and UV – 200 watt/hours/$m^2$):**
Weighed and transferred equivalent to 25 mg of Aripiprazole tablet powder into 50 mL of volumetric flask, added 75% volume of diluent, sonicated to 30 min and diluted the solution. For HPLC study, the resultant solution was diluted to obtain Aripiprazole solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

**Thermal degradation studies (105°C for 24 hours):**
Weighed and transferred equivalent to 25 mg of Aripiprazole tablet powder into 50 mL of volumetric flask, added 75% volume of diluent, sonicated to 30 min and diluted the solution. For HPLC study, the resultant solution was diluted to obtain Aripiprazole solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

**Humidity Degradation Studies (90% RH for 7 days):**
Weighed and transferred equivalent to 25 mg of Aripiprazole tablet powder into 50 mL of volumetric flask, added 75% volume of diluent, sonicated to 30 min and diluted the solution. For HPLC study, the resultant solution was diluted to obtain Aripiprazole solution was injected into the system and the chromatograms were recorded to assess the stability of sample.

**Method Validation:**
The method was validated as per ICH guideline Q2 (R2) "Validation of Analytical Procedures".

**Results and Discussion:**
**For Method Development:**
The aim of the present work is to develop a simple, specific, precise and accurate reverse phase-HPLC-UV method for the quantification of Aripiprazole and its organic impurities in pharmaceutical formulations of tablets and drug substances. A literature survey reveals that no method was reported previously for the separation, qualitative and quantitative analysis of Aripiprazole and its organic impurities. Hence the attempt made here is novel and has significant importance in simultaneous detection and quantification of Aripiprazole and its organic impurities.

The mobile phase was confirmed by change in different solvent ratios, expected peak shape, and resolution achieved using the mobile phase compositions (buffer pH 3.0 and mobile phase-B at a flow rate of 1.5 mL/min in gradient elution (MP-A: 0min, 85%;10min,70%; 15min, 65%; 18min, 65%; 24min, 50%; 34min, 40%, 35min, 85% & 45min, 85%).

The mobile phase was pumped at a flow rate of 1.5 mL/min in isocratic elution. UV detection was carried out at a wavelength of 252 nm and separation was achieved on an inertsil ODS column (150 mm×4.6 mm; 5 µm). In the optimized conditions, well retained, resolved, and symmetric peaks are observed in the standard chromatogram containing 10 µg/mL Aripiprazole and its organic impurities. The standard chromatogram obtained in the optimized conditions is given in Figure 1. The blank analysis was performed by analyzing the mobile phase and it confirmed that no detection was observed in the blank chromatogram in Figure 2. The placebo analysis was performed by analyzing the mobile phase and it confirmed that no detection was observed in the placebo chromatogram in Figure 3. The spiked sample analysis was performed by analyzing the mobile phase and it confirmed that well retained and resolution was observed in the spiked chromatogram in Figure 4. This proved that the method developed was specific and no blank and placebo interference was observed in the chromatogram.

**For Method Validation: System suitability and system precision:**
Prior to validation of the developed method, repeatability and system suitability were determined at standard solution concentrations of 10 µg/mL of Aripiprazole. The solutions were injected and were analyzed in the developed method conditions in six. The system suitability conditions like plate count, tailing factor, % relative standard deviations were determined and found to be within the acceptance limits. Hence the developed method was found to be reproducible and all the system suitable parameters were within the acceptable limits and results given below in Table (3).

**Specificity: (Blank, Placebo and Impurity Interference)**
Specificity of blank, placebo, individual impurities and spiked sample solutions injected and analyzed in developed method conditions. The specificity conditions like blank, placebo interference at retention of Aripiprazole and its organic impurities was determined and it was demonstrated the blank, placebo, individual impurities are well separated from each other. Hence the developed method was found to be specific.

**Precision:**
Precision study was demonstrated by preparing six spiking test sample solutions with organic impurities at concentrations 500 µg/mL of Aripiprazole. The solution was injected and analyzed in developed method conditions. Determined the related substances of these samples and evaluated the precision of the method by computing the % of relative standard deviation of the Aripiprazole and organic impurities and found to be within the acceptance limits. Hence the developed method was found to be precise and results given below in Table (4).

**Ruggedness:**
Ruggedness study was demonstrated for different analyst, different day, different instrument and different column by preparing six spiking test sample solutions with organic impurities at concentrations 500 µg/mL of Aripiprazole. The solution was injected and analyzed in developed method conditions. Determined the organic impurities of these samples and evaluated the ruggedness of the method by computing the % of relative standard deviation of the Aripiprazole in system suitability and resolution for organic impurities. All the obtained results are well within the limits. Hence the developed method was found to be ruggedness results given below in Table (5).

**Limit of Detection, Limit of Quantification and Limit of Quantification Precision:**
Determined the limit of detection and limit of quantification for Aripiprazole and its organic impurities by deriving concentration which will give signal to noise ratio between 2.0 to 3.0 for limit of detection and which will give signal to noise ratio not less than 10 for limit of quantification. Determined the precision by preparing solution having Aripiprazole and its organic impurities about limit of quantification level and injected in developed method conditions. Calculated the % of relative standard deviation of the Aripiprazole and its organic impurities and found to be within the acceptance limits. Hence the developed method was found to be capable to detect the concentration level as well as quantification concentration level in tables (6, 7).

**Linearity and Range:**
Demonstrated the linearity of detector response of organic impurities method, prepared the linearity solutions for Aripiprazole and its organic impurities with concentration range from LOQ to 150% level and injected in developed method conditions. Plotted graph to concentration versus peak area. Hence the method is found to be linear and range within the concentration range studied. The linearity results are given below in the table 8 and calibration curves shown in Figure 4, 5, 6.

**Accuracy:**
Accuracy of the method was determined by spiked recovery studies. For this accuracy of the test method by preparing recovery sample solutions (i.e spiking test sample with organic impurities and Aripiprazole at the levels of LOQ%, 100% and 150%. The accuracy sample solutions were injected in developed method conditions. The percentage recovery was found to be acceptance limit of 50%-125%. Hence the method is accurate and results are given below in table 9.

**Robustness:**
Robustness was tested by analyzing the standard solution and spiked sample solution in the optimized conditions that were changed deliberately (i.e mobile phase composition, pH variation, and column oven temperature and flow rate). The percentage change in each changed condition was calculated complete system suitability and RRT for organic impurities. This confirms that a small change in the analytical conditions did not influence the chromatographic separation and detection of Aripiprazole and its organic impurities. Hence the method was found to be robust and results given below in table 10.

**Forced degradation Studies:**
The drug product was exposed to different stress conditions and was analyzed in the optimized conditions and the results were compared with those of an unstressed sample vs stressed condition samples. The percentage degradation was found in oxidation degradation study about 27.140 and no degradation was observed in base hydrolysis, photolytic, thermal and humidity. These additional compounds were not observed in the drug product sample of unstressed chromatogram. Both the impurities and the degradation products were successfully separated in the optimized conditions and hence the method is stability indicating and can separate and quantify the potential impurities in Aripiprazole tablets. The forced degradation results are given in table 11 and degradation chromatograms given below in figures, 12, 13, 14.

**Table-1: General Information for Aripiprazole**

| | | |
|---|---|---|
| Recommended International Non-proprietary Name | : | Aripiprazole |
| Chemical Name (s) | : | 7-[4-{4-(2,3-dichlorophenyl) piperazin-1-yl} butoxy] 3,4-dihydro 1H-quinolin-2-one. |
| Solubility | : | Freely Soluble in dichloromethane, N, N-dimethylformamide, N, N-dimethylacetamide. |
| Physical Characteristics | : | White to off white powder. |
| Hygroscopicity | : | Non- Hygroscopic |

**Table-2: Molecular Structures for Aripiprazole and Organic Impurities**

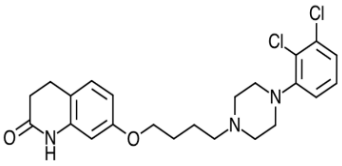
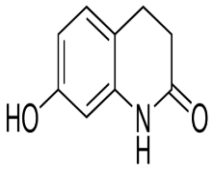
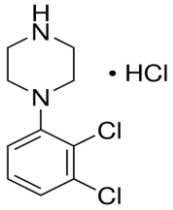
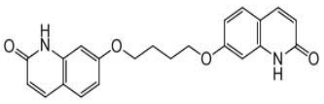
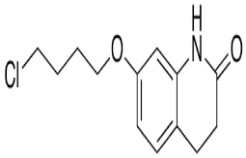
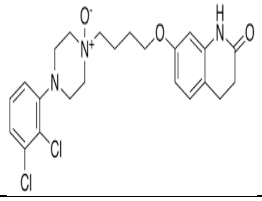
| **Name** | **Aripiprazole** | **Impurity-A** | **Impurity-B** |
|---|---|---|---|
| Structure |  |  |  |
| Chemical name | 7-[4-{4-(2,3-dichlorophenyl) piperazin-1-yl} butoxy] 3,4-dihydro 1H-quinolin-2-one. | 7-hydroxy-3,4-dihydroquinolin-2(1H)-one | 1-(2,3-dichlorophenyl) piperazine hydrochloride |
| Molecular formula | $C_{23}H_{27}Cl_2N_3O_2$ | $C_9H_9NO_2$ | $C_{10}H_{13}Cl_3N_2$ |
| Molecular weight | 448.38 | 163.17 | 267.58 |
| **Name** | **Impurity-C** | **Impurity-D** | **N-Oxide** |
| Structure |  |  |  |
| Chemical name | 7,7-[butane1,4-diylbis(oxy)] bis(3,4-dihydroquinolin-2(H)-one | 7-(4-Chlorobutoxy)-3,4-dihydroquinolin-2(1H)-one | 7-[4-[4-(2,3-Dichlorophenyl)-1-oxido-1-piperazinyl] butoxy]-3,4-dihydro-2(1H)-quinolinone |
| Molecular formula | $C_{16}H_{20}N_3O_4$ | $C_{13}H_{16}Cl_2NO_2$ | $C_{23}H_{27}Cl_2N_3O_3$ |
| Molecular weight | 380.44 | 253.72 | 464.4 |

**Table-3: System suitability and system precision results for Aripiprazole**

| **Injection No.** | **Peak Area** | **% RSD NMT 2.0** | **Tailing Factor NMT 2.0** | **Theoretical Plates NLT 2000** |
|---|---|---|---|---|
| 1 | 225091 | | | |
| 2 | 221145 | | | |

| | | | | |
|---|---|---|---|---|
| 3 | 223410 | 0.9 | 1.1 | 15452 |
| 4 | 225841 | | | |
| 5 | 223539 | | | |
| 6 | 226993 | | | |

% RSD – Relative standard deviation

**Table-4. Method Precision results**

| S.No | Sample | Impurity-A | Impurity-B | Impurity-C | Impurity-D | N-oxide |
|---|---|---|---|---|---|---|
| 1 | Preparation-1 | 0.515 | 0.499 | 0.498 | 0.515 | 0.491 |
| 2 | Preparation-2 | 0.509 | 0.495 | 0.496 | 0.51 | 0.495 |
| 3 | Preparation-3 | 0.506 | 0.498 | 0.508 | 0.506 | 0.496 |
| 4 | Preparation-4 | 0.499 | 0.491 | 0.505 | 0.509 | 0.489 |
| 5 | Preparation-5 | 0.496 | 0.501 | 0.512 | 0.512 | 0.499 |
| 6 | Preparation-6 | 0.511 | 0.497 | 0.511 | 0.504 | 0.493 |
| Average | | 0.506 | 0.497 | 0.505 | 0.509 | 0.494 |
| % RSD | | 0.9 | 0.7 | 1.3 | 0.8 | 0.7 |

**Table-5: Ruggedness results for Aripiprazole & its impurities**

| S.No | Name of the condition | Resolution | %RSD | Acceptance criteria |
|---|---|---|---|---|
| 1 | Normal condition | 3.0 | 0.5 | The Resolution between the Aripiprazole & N-oxide should be not less than 1.5<br>The % of Relative standard deviation should be Not more than 10.0% |
| 2 | Low Flow rate | 2.9 | 0.6 | |
| 3 | High Flow rate | 2.6 | 0.3 | |
| 4 | Low column Temperature | 2.9 | 0.4 | |
| 5 | High column Temperature | 2.6 | 0.9 | |
| 6 | Low pH | 2.8 | 0.8 | |
| 7 | High pH | 2.7 | 0.6 | |

Variation in Flow rate:± 0.2 mL/min, Variation in temperature:±5°C, Variation in pH : ±0.2

**Table-6: Limit of detection results for Aripiprazole and its organic impurities**

| S. No | Name of the component | Concentration | S/N ratio value | Acceptance criteria |
|---|---|---|---|---|
| 1 | Aripiprazole | 0.03 µg/mL | 3 | Signal to noise ratio between 2.0 to 3.0 |
| 2 | Impurity-A | 0.02 µg/mL | 3 | |
| 3 | Impurity-B | 0.03 µg/mL | 3 | |
| 4 | Impurity-C | 0.03 µg/mL | 3 | |
| 5 | Impurity-D | 0.03 µg/mL | 3 | |
| 6 | N-oxide | 0.03 µg/mL | 3 | |

**Table-7: Limit of quantification results for Aripiprazole and its organic impurities**

| S. No | Name of the component | Concentration | S/N ratio value | Acceptance criteria |
|---|---|---|---|---|
| 1 | Aripiprazole | 0.15 µg/mL | 20 | Signal to noise ratio should be not less than 10 |
| 2 | Impurity-A | 0.08 µg/mL | 21 | |
| 3 | Impurity-B | 0.15 µg/mL | 15 | |

| | | | | |
|---|---|---|---|---|
| 4 | Impurity-C | 0.14 µg/mL | 19 | |
| 5 | Impurity-D | 0.13 µg/mL | 18 | |
| 6 | N-oxide | 0.12 µg/mL | 21 | |

**Table-8: Linearity results for Aripiprazole and its organic impurities**

| **S. No** | **Aripiprazole** | | | **Impurity-A** | | **Acceptance criteria** |
|---|---|---|---|---|---|---|
| | **Linearity Level (%)** | **Concentration (µg/mL)** | **Peak area** | **Concentration (µg/mL)** | **Peak area** | |
| 1 | LOQ | 0.150 | 2545 | 0.080 | 3994 | The correlation coefficient derived from the least square fit of the data should not be less than 0.99 |
| 2 | 10 | 0.250 | 5015 | 0.250 | 9601 | |
| 3 | 20 | 0.500 | 11378 | 0.500 | 18458 | |
| 4 | 50 | 1.250 | 28158 | 1.250 | 49935 | |
| 5 | 100 | 2.500 | 56364 | 2.500 | 89118 | |
| 6 | 150 | 3.750 | 84697 | 3.750 | 136164 | |
| **Correlation Coefficient** | | | **0.99** | **NA** | **0.99** | |
| **Intercept** | | | **-475.56** | **NA** | **1422.72** | |
| **Slope** | | | **22739.33** | **NA** | **35862.69** | |

| **S. No** | **Impurity-B** | | | **Impurity-C** | | **Acceptance criteria** |
|---|---|---|---|---|---|---|
| | **Linearity Level (%)** | **Concentration (µg/mL)** | **Peak area** | **Concentration (µg/mL)** | **Peak area** | |
| 1 | LOQ | 0.150 | 2845 | 0.140 | 3458 | The correlation coefficient derived from the least square fit of the data should not be less than 0.99 |
| 2 | 10 | 0.251 | 4501 | 0.250 | 8105 | |
| 3 | 20 | 0.499 | 8595 | 0.500 | 15594 | |
| 4 | 50 | 1.262 | 21399 | 1.250 | 39145 | |
| 5 | 100 | 2.510 | 43547 | 2.500 | 77478 | |
| 6 | 150 | 3.778 | 64807 | 3.750 | 114151 | |
| **Correlation Coefficient** | | | **0.99** | **NA** | **0.99** | |
| **Intercept** | | | **127.14** | **NA** | **227.70** | |
| **Slope** | | | **17151.61** | **NA** | **30579.84** | |

| **S. No.** | **Impurity-D** | | | **N-Oxide** | | **Acceptance criteria** |
|---|---|---|---|---|---|---|
| | **Linearity Level (%)** | **Concentration (µg/mL)** | **Peak area** | **Concentration (µg/mL)** | **Peak area** | |
| 1 | LOQ | 0.130 | 2968 | 0.120 | 3385 | The correlation coefficient derived from the least square fit of the data should not be less than 0.99 |
| 2 | 10 | 0.248 | 5594 | 0.249 | 5410 | |
| 3 | 20 | 0.498 | 11098 | 0.501 | 10457 | |
| 4 | 50 | 1.245 | 27494 | 1.248 | 25198 | |
| 5 | 100 | 2.451 | 56001 | 2.503 | 51348 | |
| 6 | 150 | 3.735 | 82989 | 3.741 | 79102 | |
| **Correlation Coefficient** | | | **0.99** | **NA** | **0.99** | |
| **Intercept** | | | **67.96** | **NA** | **40.10** | |
| **Slope** | | | **22359.00** | **NA** | **20887.28** | |

**Table-9: Accuracy results for Aripiprazole & its organic impurities**

| S.No | Spiking Level | % Recovery | | | | | Acceptance criteria |
|---|---|---|---|---|---|---|---|
| | | Impurity-A | Impurity-B | Impurity-C | Impurity-D | N-Oxide | |
| 1 | LOQ-1 | 105.4 | 101.5 | 104.1 | 99.5 | 106.4 | The Individual recovery should be between 80.0 – 120.0 % |
| 2 | LOQ-2 | 106.4 | 100.1 | 103.5 | 98.4 | 107.2 | |
| 3 | LOQ-3 | 107.1 | 102.5 | 102.9 | 97.9 | 108.4 | |
| 4 | 50%-1 | 106.0 | 102.5 | 101.1 | 109.5 | 99.7 | |
| 5 | 50%-2 | 101.5 | 106.6 | 101.9 | 103.4 | 100.1 | |
| 6 | 50%-3 | 102.4 | 108.1 | 106.5 | 105.1 | 99.2 | |
| 7 | 100% -1 | 100.2 | 100.8 | 96.5 | 99.6 | 99.7 | |
| 8 | 100% -2 | 99.4 | 99.3 | 97.3 | 99.9 | 100.5 | |
| 9 | 100% -3 | 98.9 | 101.2 | 95.6 | 100.8 | 100.9 | |
| 10 | 125% -1 | 98.6 | 96.2 | 100.1 | 99.3 | 99.9 | |
| 11 | 125% -2 | 98.6 | 98.9 | 98.2 | 98.7 | 100.1 | |
| 12 | 125% -3 | 99.2 | 99.7 | 97.6 | 98.5 | 100.9 | |

**Table-10: Robustness results for system suitability**

| S. No. | Condition | System Suitability for Aripiprazole | | |
|---|---|---|---|---|
| | | % RSD | Tailing factor | Theoretical plate count |
| 1 | Optimized | 0.1 | 1.1 | 19012 |
| 2 | MP 1 | 0.2 | 1.1 | 18458 |
| 3 | MP 2 | 0.1 | 1.1 | 19487 |
| 4 | COT 1 | 0.1 | 1.0 | 18987 |
| 5 | COT 2 | 0.4 | 1.0 | 19258 |
| 6 | FV 1 | 0.2 | 1.0 | 18871 |
| 7 | FV 2 | 0.2 | 1.0 | 19478 |
| 8 | pH 1 | 0.4 | 1.1 | 19871 |
| 9 | pH 2 | 0.1 | 1.1 | 20781 |

MP 1: Mobile phase composition variation -5%, MP 2: Mobile phase composition variation +5%, COT 1: Column Oven Temperature -5%, COT 2: Column Oven Temperature +5%, FV 1: Flow Variation – 0.1 mL, FV 2: Flow Variation – 0.2 mL, pH 1: pH variation – 0.2 and pH 1: pH variation + 0.2

**Table 11: Forced degradation results**

| S. No | Stress name and conditions | % Amount remaining | % Amount degraded | Peak Purity | |
|---|---|---|---|---|---|
| | | | | Purity Angle | Purity Threshold |
| 1 | Undegraded | 99.5 | 0.5 | 0.218 | 0.316 |
| 2 | Acid hydrolysis | 83.1 | 15.1 | 2.944 | 3.525 |
| 3 | Base Hydrolysis | 91.9 | 8.3 | 1.969 | 2.839 |
| 4 | Oxidation | 73.1 | 25.6 | 0.267 | 1.409 |
| 5 | Photolytic Visible | 99.2 | 0.6 | 0.210 | 0.325 |
| 6 | Photolytic UV | 99.1 | 0.6 | 0.221 | 0.341 |
| 7 | Thermal | 97.5 | 1.8 | 0.215 | 0.378 |
| 8 | Humidity | 100.4 | 0.5 | 0.150 | 0.312 |

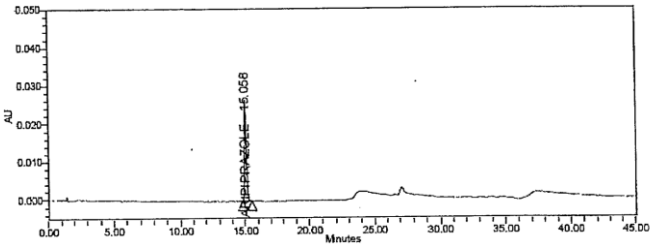



**Fig.1: Typical Chromatogram for standard solution**

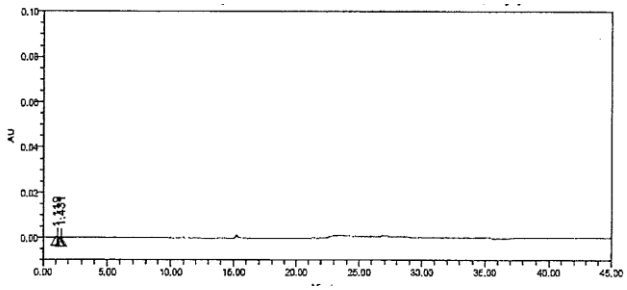



**Fig.2.** Blank chromatogram

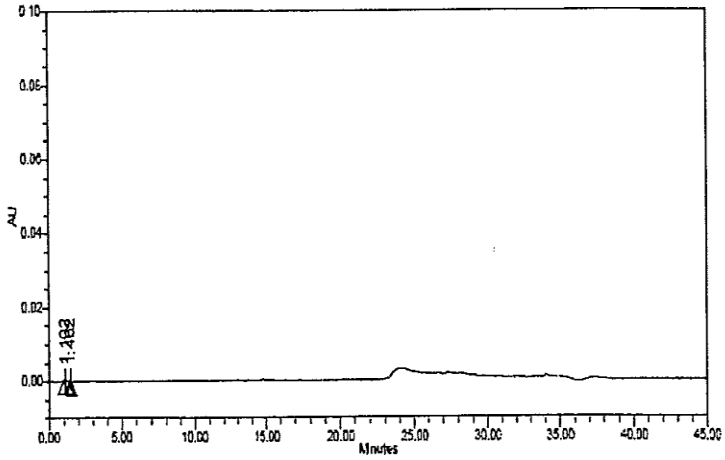



**Fig.4.** Placebo chromatogram

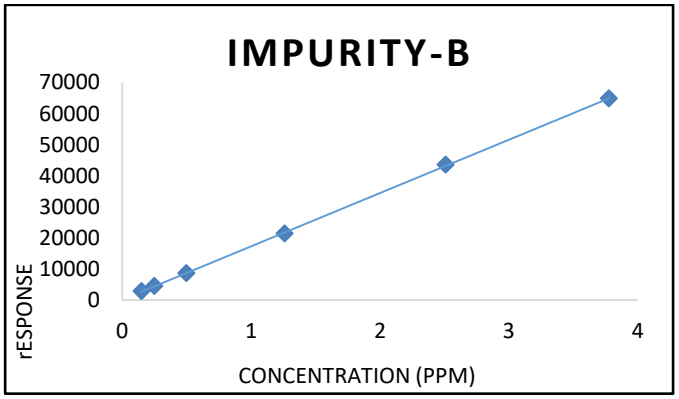



**Fig-5.** Calibration curve for Aripiprazole

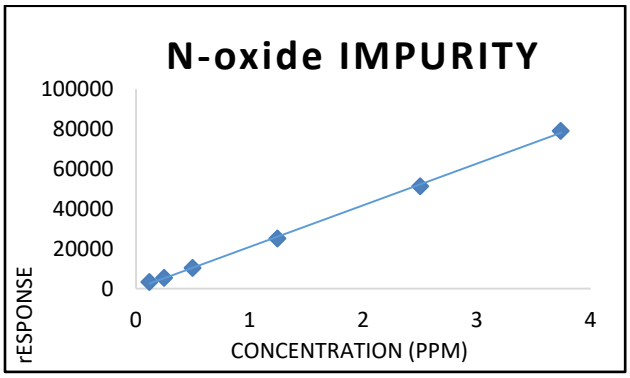



**Fig-6.** Calibration curve for Impurity-A

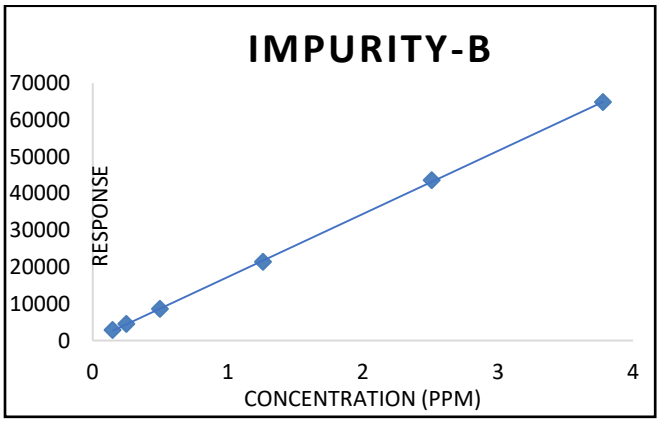



**Fig-7.** Calibration curve for Impurity-B

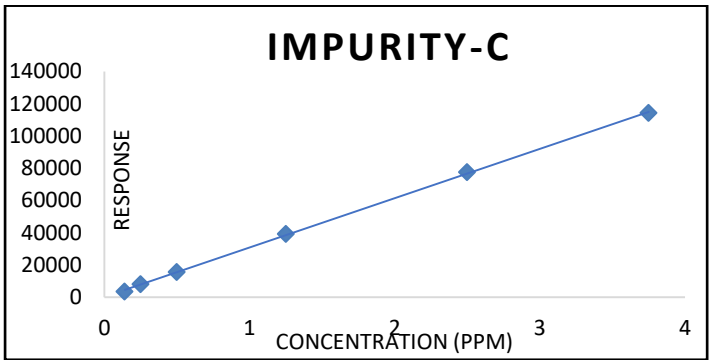



**Fig-8.** Calibration curve for Impurity-C

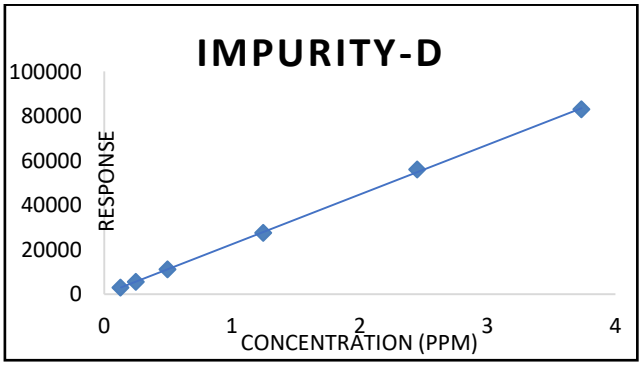


**Fig-9.** Calibration curve for Impurity-D

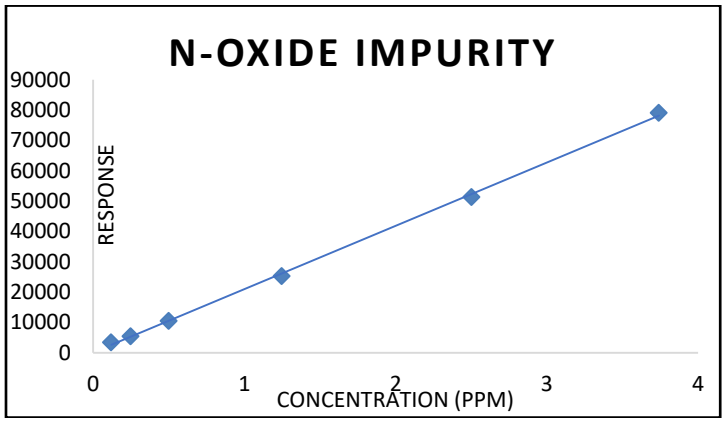



**Fig-10.** Calibration curve for N-Oxide Impurity

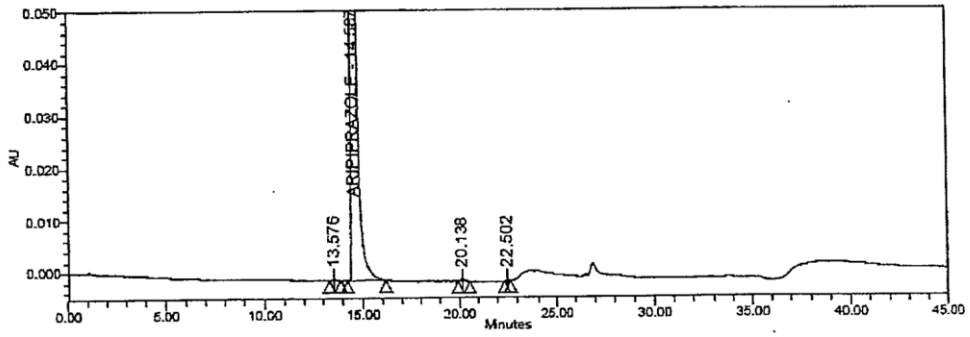



**Figure-11: Typical chromatogram for Undegraded sample**

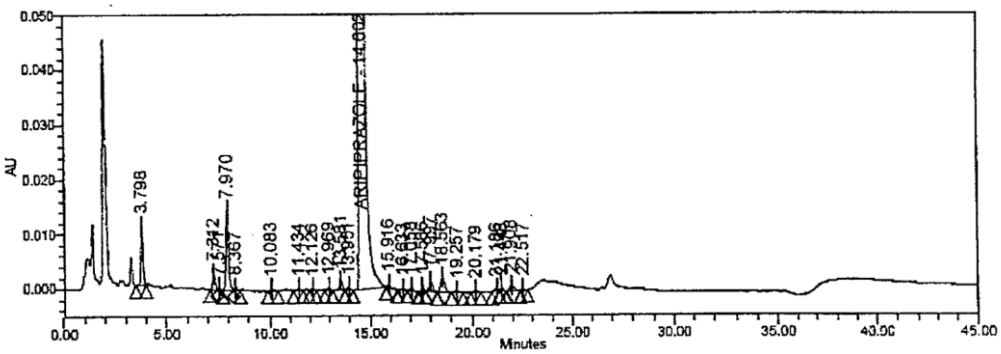



**Figure-12: Typical chromatogram for Acid stresses sample**

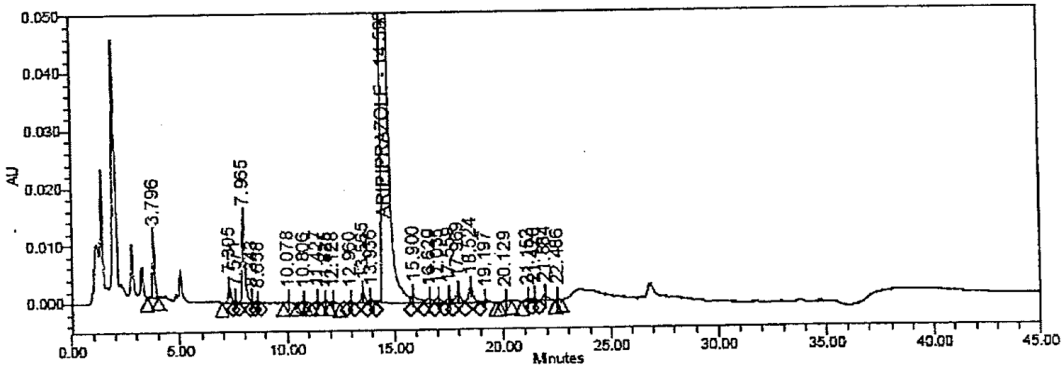



**Figure-13: Typical chromatogram for Base stresses sample**

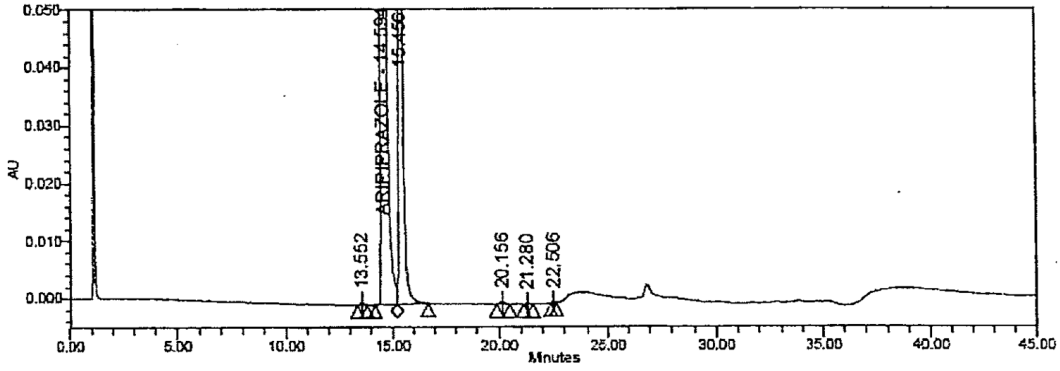



**Figure-14: Typical chromatogram for Peroxide stresses sample**

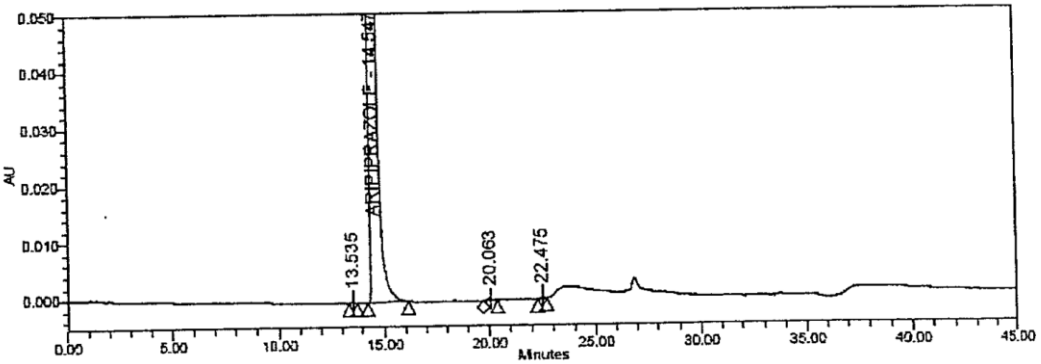



**Figure-15: Typical chromatogram for Photolytic Visible stresses sample**

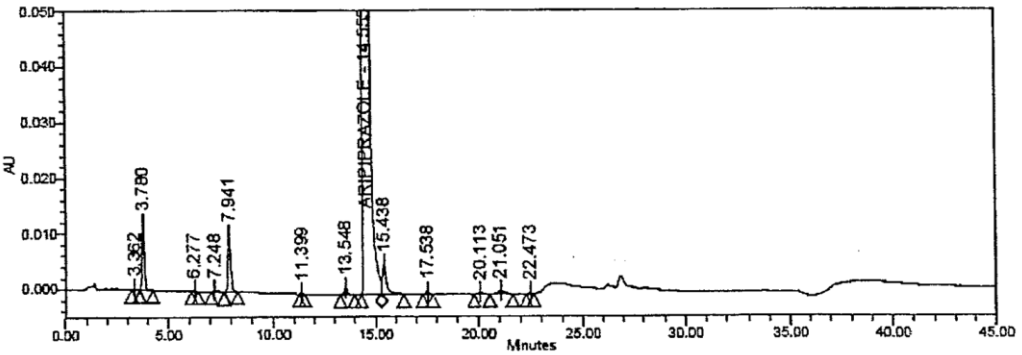



**Figure-16: Typical chromatogram for Thermal stresses sample**

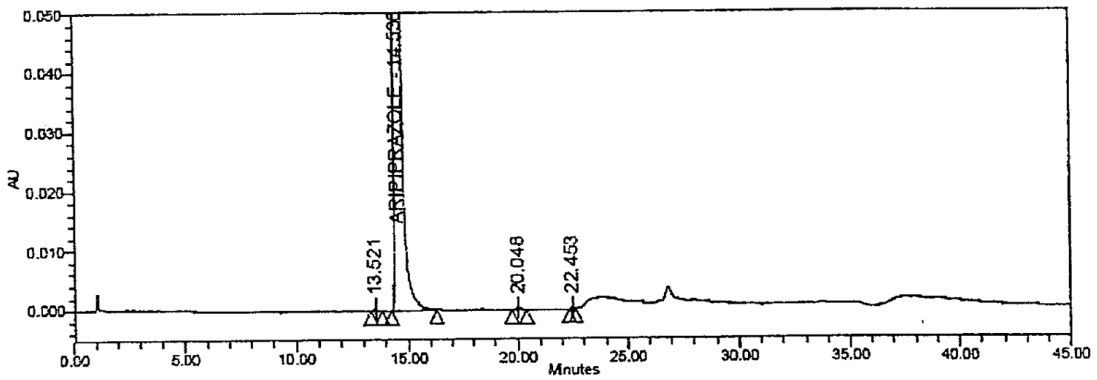



**Figure 17: Typical chromatogram for Humidity stresses sample**

**Conclusion:**

A simple, validated, and fast stability indicating HPLC method is established for quantification of Aripiprazole and its organic impurities. In the literature no method was found to be established for the simultaneous quantification of Aripiprazole and its organic impurities. Hence the method represents the first report about a stability indicating method for the determination of Aripiprazole and its organic impurities. The proposed method achieves satisfactory separation of Aripiprazole from impurities and the degradation products, an extended linear range, and rapid analysis time. A high recovery of Aripiprazole in formulation was obtained. The proposed method ensured precise and accurate determination of Aripiprazole in pharmaceutical formulations. The excipients present in the formulation were not interfering in the method. Hence the method is simple, convenient, and suitable for analyzing Aripiprazole and its organic impurities in pharmaceutical formulations of infusion and drug substances in the presence.

**REFERENCES:**


i. Nerkar P.; Gide P.; Chitnis A.; Mahajan H. and Gattani S.; Development of stability indicating reverse phase HPLC method for aripiprazole from solid dosage form. *International Journal of Pharmaceutical Sciences and Nanotechnology;* 2009**, 2(2),** 572-81.

ii. Murali Krishna M.V.; Rao S.V, and Venugopal N.V.; Identification of degradation impurities in aripiprazole oral solution using LC–MS and development of validated stability indicating method for assay and content of two preservatives by RP-HPLC. *Journal of Liquid Chromatography & Related Technologies;* 2017, **40(14),** 741-50.

iii. Cong Z.X.; Wang F.; Xu P.; Rong-Hua Z, and Huan-De L.; LC-ESI-MS for rapid and sensitive determination of aripiprazole in human plasma. *Chromatographia;* **2006, 64(1-2),** 387-391,

iv. Kubo M.; Mizooku Y.; Hirao Y, and Osumi T.; Development and validation of an LCMS/MS method for the quantitative determination of aripiprazole and its main metabolite, OPC-14857. *in human plasma.J.Chromatogr. B.* 2005, **822,** 294-299.

v. Musenga A.; Saracino M.A.; Spinelli D.; Boncompagni E.G.; Kenndler E, and Raggiv M. A,; Analysis of the recent antipsychotic aripiprazole in human plasma by capillary electrophoresis and high-performance liquid chromatography with diode array detection. *Anal. Chimica Acta,;* 2008, **612(2),** 204-211.

vi. Suganthi A.; Ravimathi P.; Shrikumar S. and Ravi T.K.; HPTLC Method for the Estimation of Aripiprazole from Tablet Formulation.Pharmainfo.net, **2006.**

vii. Huang H.; Liu C.; Lan T.; Hu T.; Chiu H.; Wu Y. and Tseng Y.; Detection and quantification of aripiprazole and its metabolite, dehydroaripiprazole, by g a s aripiprazole in human plasma by capillary chromatography mass spectrometry in blood samples of psychhiatric patients. *J. Chromatogr. B.;* 2007, **856(1-2),** 57-61**.**

viii. Shimokawa Y.; Akiyama H.; Kashiyama E.; Koga T. and Miyamoto G.; High performance liquid chromatographic methods for the determination of aripiprazole with ultraviolet detection in rat plasma and brain: *Application to the pharmacokinetic study. J. Chromatogr. B.,* 2005, **82(1),** 18-14**.**

ix. International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) Q2B. Validation of Analytical Procedures, Methodology, 1996, 1-8.

x. FDA: Guidance for Industry, Analytical Procedures and Methods Validation, **2000.**

xi. US Pharmacopeial Convention, United States Pharmacopoeia, 28th Edn, US Pharmacopeial Convention, Rockville, MD, 2005,1196 -1198.


Received on September 2, 2024.