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# A Green Analytical Stability Indicating RP HPLC Method for Determination of Related Impurities in Istradefylline Dosage Forms. Robustness Study by Quality by Design Approach

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## Abstract

Istradefylline, a selective adenosine A2A receptor antagonist used as an add-on therapy to levodopa therapy for Parkinson's disease, requires reliable impurity profiling to ensure pharmaceutical quality and safety. In this study, a novel, stability-indicating, Quality by Design (QbD)-based and eco-friendly RP-HPLC method was developed and validated for the determination of Istradefylline and its related impurities in tablet dosage forms. Method robustness was systematically evaluated using a three-level, two-factor factorial design. Chromatographic separation was achieved on an XBridge C18 column (250 × 4.6 mm, 5 µm) using 0.01M ammonium formate buffer (pH 4.0) and acetonitrile at a flow rate of 0.8mL $min^{-1}$, with detection at 280 and 360nm. The method demonstrated excellent specificity, effectively separating Istradefylline from its impurities (IST-4, IST-5, IST-CIS, IST-10, and IST-40) without interference from excipients and degradation products. Linearity was established over the concentration range of 0.200–4.000µg$mL^{-1}$ ($R^2 > 0.999$), while accuracy, precision, and sensitivity met ICH acceptance criteria. Forced degradation studies confirmed the stability-indicating capability of the method. The method also complies with Green Analytical Chemistry principles through reduced solvent consumption and efficient chromatographic performance. Validation results demonstrated that the method is robust, reliable, and suitable for routine quality control, impurity profiling, and stability assessment of Istradefylline tablet formulations.

**Keywords:** Istradefylline tablets, HPLC, method development, Validation.



## 1. INTRODUCTION

Istradefylline is a selective adenosine A2A receptor antagonist used as an adjunctive therapy for the treatment of Parkinson's disease (Mizuno *et al.,* 2013). It is slightly soluble in water and freely soluble in alcohol. The chemical structure of Istradefylline is shown in Figure 1.

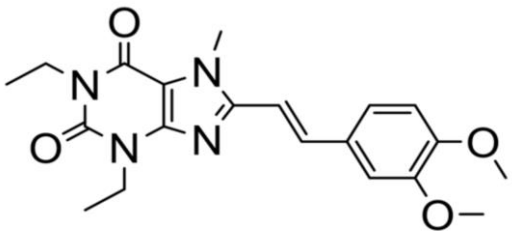


**Figure 1: Structure of Istrdefylline**

Several analytical methods have been reported for the determination of Istradefylline and its known impurities in pharmaceutical formulations using chromatographic techniques, particularly HPLC (Adireddy *et al.,* 2021; Pathan *et al.,* 2019; Xu *et al.,* 2020; Wang *et al.,* 2021). However, the reported methods are primarily focused on assay determination or individual impurity analysis and do not adequately achieve the simultaneous separation of Istradefylline, its related impurities, degradation products, and formulation excipients with satisfactory specificity and robustness. Furthermore, no published method has employed a systematic Analytical Quality by Design (AQbD) framework combined with Green Analytical Chemistry (GAC) principles for impurity profiling of Istradefylline in tablet dosage forms.To the best of our knowledge, the present study is the first to report a green, stability-indicating, and AQbD-based RP-HPLC method for the simultaneous quantification of Istradefylline and its related impurities in tablet dosage forms. The method was developed using a structured AQbD approach involving risk assessment, design of experiments (DoE), and establishment of a design space to ensure method robustness and lifecycle performance. In addition, Green Analytical Chemistry principles were integrated during method development by employing environmentally benign chromatographic conditions, minimizing organic solvent consumption through a low flow rate, and reducing analytical waste generation. The developed method is cost-effective, robust, and highly specific, demonstrating complete separation of Istradefylline, its impurities, degradation products, and excipients without interference. Consequently, the proposed method provides a reliable and environmentally sustainable analytical tool for routine quality control and stability studies of Istradefylline tablet formulations.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

The AR grade Ammonium format and formic acid were procured from Ankit Raj organo chemicals LTD, India. The HPLC grade of Acetonitrile (J.T. beaker) Methanol (J.T. beaker) with certified purity of 99.9% were purchased from Avantor performance materials, Mumbaei. High-quality In-House purity water was used for the experiments (TOC <500ppb, pH about 7.0, Conductivity < 1.0 µs/cm, finally exposed to UV radiation and followed filtered through 0.2 µm filter). Istradefylline and impurities were procured from Simson- pharma private limited, Hyderabad, India.

### 2.2. Instruments

Waters HPLC system Alliance e2695 separation module with auto injector, temperature controller for sample storage, and column were used for current analysis. The signal output was observed through Empower 3. The LC column XBridge C18 column (250 × 4.6 mm, 5.0 µm particle size, is manufactured by Waters. Analytical balance model AX205 (make: Mettler Toledo), sonicator (make: ENERTECH), Rotary shaker (make: REMI; model: RS-24BL) were employed in this work.

### 2.3. Preparation of diluent

Mixed 50% methanol and 50% water as a diluent.

### 2.4. Preparation of Istradefylline standard stock solution

Weighed and transferred about 40 mg of Istradefylline working standard into 100mL volumetric flask and add 60 mL of diluent. Sonicate to dissolve the material completely and dilute up the volume with diluent, Istradefylline concentration was about 400µg/mL.

### 2.5. Preparation of Istradefylline standard solution

Transferred 1.0 mL of Istradefylline standard stock solution into 200 mL volumetric flask and diluted the volume with diluent and mixed well. The concentration was about 2µg/mL

### 2.6. Preparation of Placebo solution

Accurately weighed and transferred Istradefylline placebo powder equivalent to 40mg of placebo powder into 100mL volumetric flask, added about 50mL of diluent and sonicated for 30 minutes with intermittent shaking and dilute to volume with diluent, and mixed well and centrifuge some portion of solution at 3500 rpm for 10 minutes.

### 2.7. Preparation of sample solution (About 400 ppm)

weighed and crushed the tablet into smooth powder and transferred 40mg of equivalent to Istradefylline powder into a100ml volumetric flask added about 70mL of diluent and sonicated for 30 min with intermittent shaking and dilute to volume with diluent and mixed well and centrifuge some portion of solution at 3500 rpm for 10 minutes. The Istradefylline was about 400µg/mL

## 3. RESULTS AND DISCUSSIONS

### 3.1. Method Development and Optimization

The primary objective of the method development was to establish a stability-indicating RP-HPLC method capable of achieving baseline separation of Istradefylline, its degradation-related impurities (IST-4, IST-5, IST-CIS, IST-10, and IST-40), and placebo components present in the finished dosage form. As specified in the DMF, IST-4, IST-CIS, and IST-10 impurities were quantified at 280nm, whereas Istradefylline, IST-5, and IST-40 impurities were monitored at 360nm to ensure optimal sensitivity and specificity. All investigated impurities were classified as degradation products according to the DMF. Initially, chromatographic separation was attempted under isocratic conditions using a mobile phase composed of 0.02M potassium dihydrogen phosphate and acetonitrile 70:30 (v/v). Separation was performed on a Hypersil BDS column (250 × 4.6 mm, 5 µm) at a flow rate of

1.0mLmin$^{-1}$ and a column temperature of 30°C. Detection was carried out using a photodiode array (PDA) detector with dual-wavelength monitoring at 280nm and 360 nm.

A spiked sample containing Istradefylline and its impurities was prepared and analyzed under the optimized conditions; however, all impurity peaks co-eluted at nearly identical retention times. To enhance chromatographic selectivity and improve peak resolution, the mobile phase composition was modified to 0.02M potassium dihydrogen phosphate adjusted to pH 3.0 and acetonitrile 70:30(v/v), while maintaining all other chromatographic parameters constant. Despite this modification, satisfactory separation was not achieved, and the analyte and impurity peaks exhibited poor resolution with unresolved peak profiles. There were no peak shapes that were observed. To achieve complete peak separation with improved resolution and reduced run time, a gradient elution program was evaluated. The mobile phase consisted of 0.02M potassium dihydrogen phosphate buffer (pH adjusted to 3.0 with orthophosphoric acid) as Mobile Phase A and acetonitrile as Mobile Phase B. However, the peaks were not adequately separated under these conditions. To improve peak separation, a C18 XBridge column was evaluated; however, not all impurity peaks were eluted. Therefore, the buffer salt was modified to 0.02M ammonium phosphate, adjusted to pH 4.0 with orthophosphoric acid. A gradient system was applied using Mobile Phase A composed of 80% buffer and 20% acetonitrile (v/v), and Mobile Phase B composed of 80% acetonitrile and 20% buffer (v/v). However, the impurity peak shapes were still unsatisfactory. To further enhance peak shape, resolution, and reduce run time, another buffer system was attempted by dissolving 0.01M ammonium formate in 1000mL of water and adjusting the pH to 4.0 with formic acid. Used 100% of buffer as a mobile phase-A, and 100% acetonitrile as a mobile phase-B, and remaining impurities were unchanged. All impurity peaks were eluted but resolution was not satisfied. Hence flow rate was changed to 0.8mLmint$^{-1}$. All peaks, i.e. Istradefylline, IST-4 impurity, IST-5 impurity, IST CIS impurity and IST-10 impurity and IST-40 impurity eluted. But the resolution between Istradefylline and IST impurity-10 peak resolution was not satisfied. To separate for better resolution with less run time an attempt was made with mobile phase-B composition. For mobile phase-B mixed 90% Acetonitrile and 10% of 0.01M buffer (pH 4.00). All known impurities were successfully separated, and the resolution between all peaks was satisfactory. Based on these experiments and optimized conditions. X-bridge C18, 250 x 4.6 mm, 5.0µm was used as the stationary phase. The column temperature was maintained at 30°C and sample cooler maintained at 10°C detection was monitored at 280nm and 360nm. The flowrate was 0.8mLmin$^{-1}$ and the injection volume was 20µL.

A series of aqueous and organic modifiers used as a diluent, finally decided to 50% methanol and 50% water as a diluent. The typical retention time of impurity IST-4 impurity, IST-5 impurity, IST CIS impurity, Istradefylline and IST-10 impurity and IST-40 impurity were eluted about 13.71,19.45,20.55,22.30,23.21,23.78min respectively. The impurities of Istradefylline, blank and placebo interference were not observed at retention time of Istradefylline and impurities. So, the method was found specifically for determination of impurities. See Table 1 for individual impurity all peaks retention time.

### 3.2. Chromatographic conditions

The chromatographic separation was performed using a gradient program with 0.01M ammonium format buffer (pH 4.00), adjusted with formic acid as buffer and 100% buffer as mobile phase A, for mobile phase-B used 90% of acetonitrile and 10% of 0.01M of ammonium format. The flow rate, column temperature, and sample cooler were set to 0.8 mLmin$^{-1}$, 30℃, and 10℃, respectively. X-bridge C18 column (250mm × 4.6mm, 5.0µm particle size) column was used. Gradient elution was employed, with an injection volume of 20µL, and component detection was carried out at 280nm for IST-4, IST CIS and IST-10 impurities and 360nm for Istradefylline, IST-5, IST-40 impurities.

**Gradient programme**

| Time (mins) | Mobile Phase - A (%) | Mobile Phase - B (%) |
|---|---|---|
| 0 | 90 | 10 |
| 2 | 90 | 10 |
| 20 | 30 | 70 |
| 30 | 30 | 70 |
| 31 | 10 | 90 |
| 38 | 10 | 90 |
| 39 | 90 | 10 |
| 50 | 90 | 10 |

### 3.3. Method Validation

The method was validated based on International Conference on Harmonization (ICH) Q2(R2) Guidelines. Validation parameters included system suitability, Specificity, Linearity, Precision, Accuracy, Robustness and forced degradation (Lankalapalli *et al.,* 2025, Myneni *et al.,* 2025, Teja Kami Reddy *et al.,* 2024, Kaja *et al.,* 2026).

### 3.4. System suitability

System suitability parameters (tailing factor, number of theoretical plates) were assessed by injecting a blank diluent followed by standard (2.0µg/mL) and shown in Table 2.

**Table 2: System suitability results**

| Name of the parameter | Observed Value | Acceptance criteria |
|---|---|---|
| USP tailing factor for Istradefylline peak from standard solution | 1.0 | NMT 2.0 |
| USP Plate count for Istradefylline peak from standard solution | 22752 | NLT 2500 |
| % RSD of Istradefylline peak areas for three replicate injections of standard solution | 0.3 | NMT 10.0% |

### 3.5. Specificity

Prepared blank, known impurities spec level and placebo as per the optimized test procedure and verified the interference of peaks at retention time of active. The results showed no interference at the active peak. All chromatograms showed no interference at retention time of Istradefylline as with known impurities (see Figure 2-4).

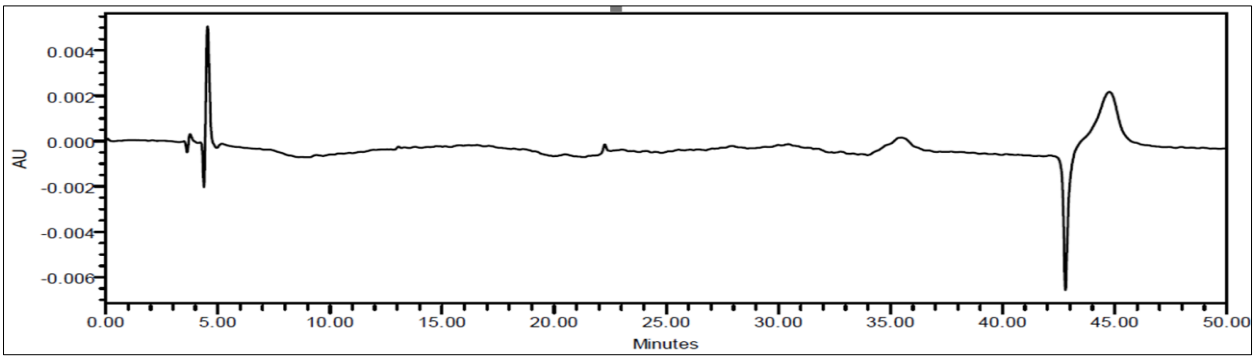



**Figure 2: Chromatogram of Blank solution**

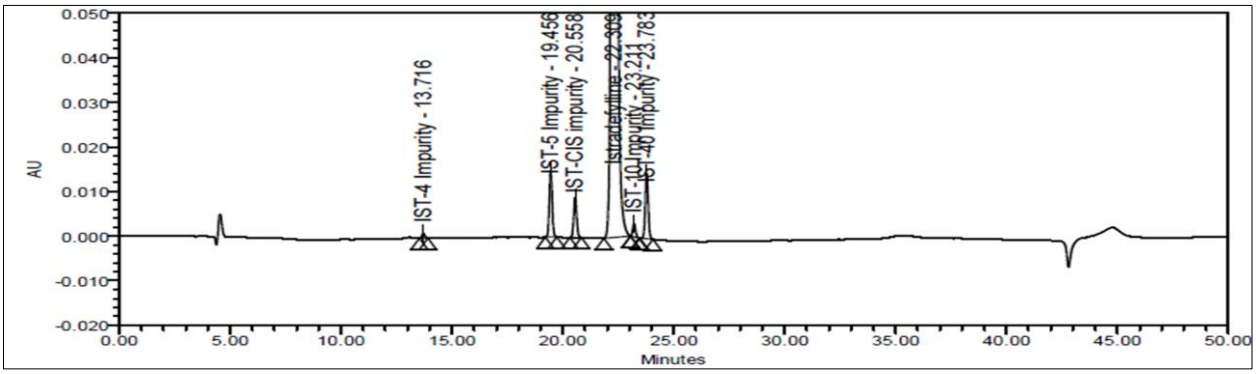



**Figure 3: Chromatogram of Spiked sample 360nm**

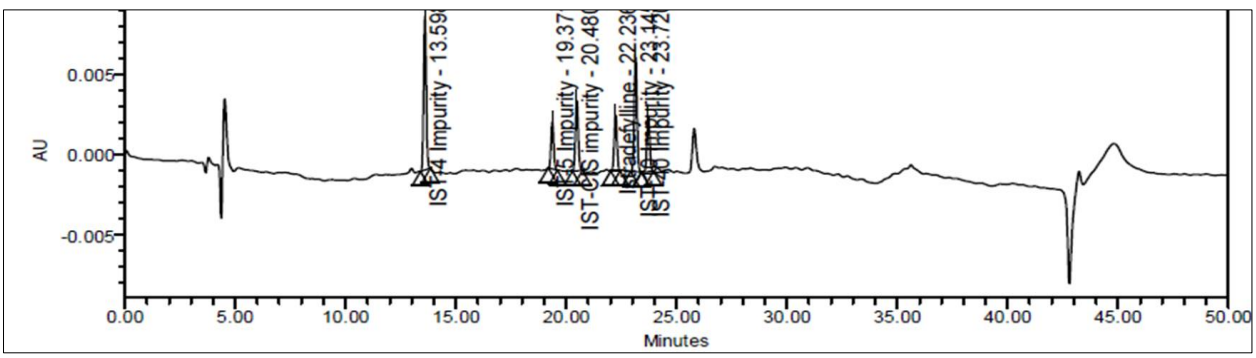



**Figure 4: Chromatogram of Spiked sample 280nm**

### 3.6. Linearity

Linearity of the optimized method was evaluated by preparing a series of concentrations of for

Istradefylline, IST-5, IST-40, IST-4, IST CIS and IST-10 (0.2000µg/mL4.0000µg/mL) from the concentration range by using suitable amounts of the stock solutions. A curve was created by mapping the peak response and concentration. The components showed correlation coefficient > 0.999. The results were shown in Table 3.

### 3.7. Sensitivity

The limit of detection (LOD) and Limit of quantification (LOQ) values for Istradefylline, IST-5, IST-40, IST-4, IST CIS and IST-10 were established by signal to noise ratio(S/N) method. The LOD and LOQ results were shown in Table 3.

### 3.8. Precision

Precision provides a degree of agreement between the individual test results by applying the method to the homogeneous sample (H.Divadari *et al.,* 2024, N.Maddukuri et al.,2025, P.K. Lankalapalli *et al.,* 2024). Typically, it is expressed as variance, SD. In normal conditions, it is a measure of the degree of repeatability or reproducibility. Six individual samples were taken from a homogenous mixture of samples and were spiked with them at the 100% level to check the reproducibility. The precision of the analysis was calculated on the RSD values of the six impurity-spiked samples results. To check the ruggedness (intermediate precision) of the method, the analysis was repeated on different days. The precisions for the analysis of each individual impurity at 100% concentration level were <2.0%. The results were shown in Table 3.

### 3.9. Accuracy

They prove the accuracy of optimized method prepared four levels (LOQ,50%, 100% & 150%). Prepared each sample in triplicate preparation range from 50% level and 150% level and the concentration of Istradefylline, IST-5, IST-40, IST-4, IST CIS and IST-10 were 1.0000µg/mL (50%), 3.0000µg/mL (150%) these six-sample preparation ranges from LOQ and 100% level and concentration of Istradefylline, IST-5, IST-40, IST-4, IST CIS and IST-10 were 0.2000µg/mL (LOQ) and 2.0000µg/mL (100%). The recovery was calculated in terms of the amount estimated to the amount spiked. All the method validation parameters results were shown in Table 4. According to the acceptance criteria, the mean recovery should be within the range of 85.0 % -115.0 % found to be within the range. The results were shown in Table 3.

**Table 3: Validation summary results**

| Validation parameters | Istradefylline | IST-4 | IST-5 | IST-CIS | IST-10 | IST-40 |
|---|---|---|---|---|---|---|
| RRT w.r.to Istradefylline | 1.00 | 0.61 | 0.87 | 0.97 | 1.04 | 1.07 |
| Linearity range(µg/ml) | 0.2009-4.0079 | 0.2004-4.0014 | 0.2001-4.0019 | 0.2002-4.0011 | 0.2009-4.0005 | 0.2001-4.0017 |
| Coefficient($r^2$) | 0.99999 | 1.00000 | 0.99999 | 1.00000 | 1.00000 | 1.00000 |
| Slope | 76973.892 | 40784.197 | 76584.579 | 19836.499 | 31035.120 | 73433.504 |
| Intercept | 106.094 | -1924.735 | 1778.757883 | -677.967 | -1402.835 | -1955.149 |
| (%) Y-intercept | 0.1 | -2.4 | -1.4 | -1.7 | -2.2 | -1.3 |
| Bias | 0.8 | -0.2 | -1.9 | -0.6 | -0.9 | -0.5 |
| RRF | 1.00 | 1.00 | 0.99 | 0.49 | 0.76 | 0.95 |
| LOQ concentration(ppm) | 0.2004 | 0.2008 | 0.2006 | 0.2001 | 0.2007 | 0.2003 |
| LOD concentration(ppm) | 0.0662 | 0.0667 | 0.0666 | 0.0664 | 0.0661 | 0.0664 |
| ACC-LOQmean %RSD(n=6) | 101.4,1.8 | 102.6,1.2 | 100.6,2.1 | 103.6,2.5 | 101.8,1.5 | 102.7,1.8 |
| ACC-50% mean, %RSD(n=3) | 99.1,0.7 | 101.4, 1.2 | 101.6,1.6 | 102.6,1.8 | 100.8,2.8 | 102.8,1.2 |
| ACC-100% mean %RSD(n=6) | 101.2,1.2 | 100.8 ,1.9 | 99.8 ,2.5 | 101.8,1.5 | 101.7,1.2 | 103.8,1.5 |
| ACC-150% mean RSD(n=3) | 102.3,0.5 | 102.8,1.1 | 101.8,2.4 | 100.3,1.2 | 102.8,1.8 | 101.9,1.3 |
| Method precision %RSD(n=6) | 1.2 | 1.6 | 1.8 | 1.3 | 0.8 | 1.5 |
| Intermediate precision % RSD(n=6) | 1.6 | 1.2 | 1.4 | 1.7 | 1.3 | 1.1 |

### 3.10. Robustness

Robustness was performed using a Qbd approach under varying chromatographic conditions in and around the optimized parameters.

### QbD experiments

The robustness of the method was evaluated using Istradefylline spiked sample. The experiment employed a three level, two factorial design under the response surface methodology. A quadratic model was found to be adequate for each factor at three levels. The DOE software suggested 13 experimental runs for this study, as shown in Table 4. Changes in flow rate (0.6-0.8-1.0mL/min) and column temperature (25-30-35°C) were selected as the experimental parameters. After applying the experimental design, the model terms for resolution between IST-5 and IST CIS, resolution between IST and IST-10, and resolution between IST-10 and IST-40 showed statistical significance, with p-values < 0.005 and "Prob > F" indicating model adequacy. The significance of the p-values and the ANOVA summary were presented in Table 5 and Figure 5 and 6.

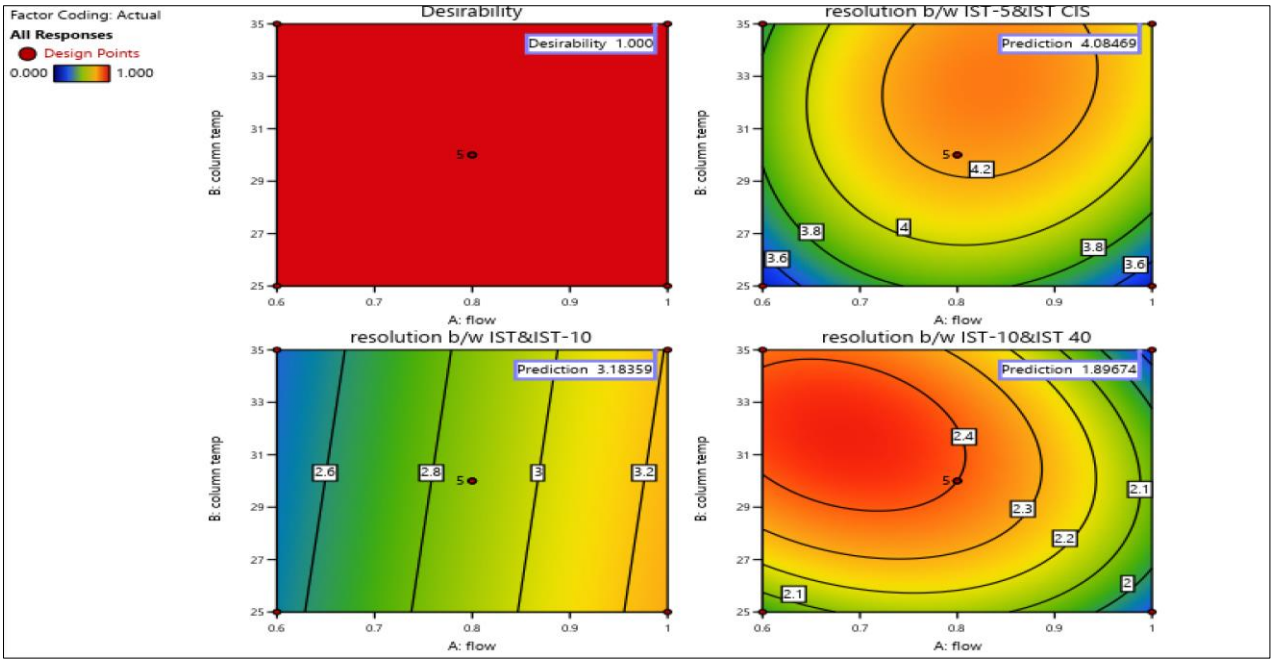



**Figure 5: The contour response surface plots**

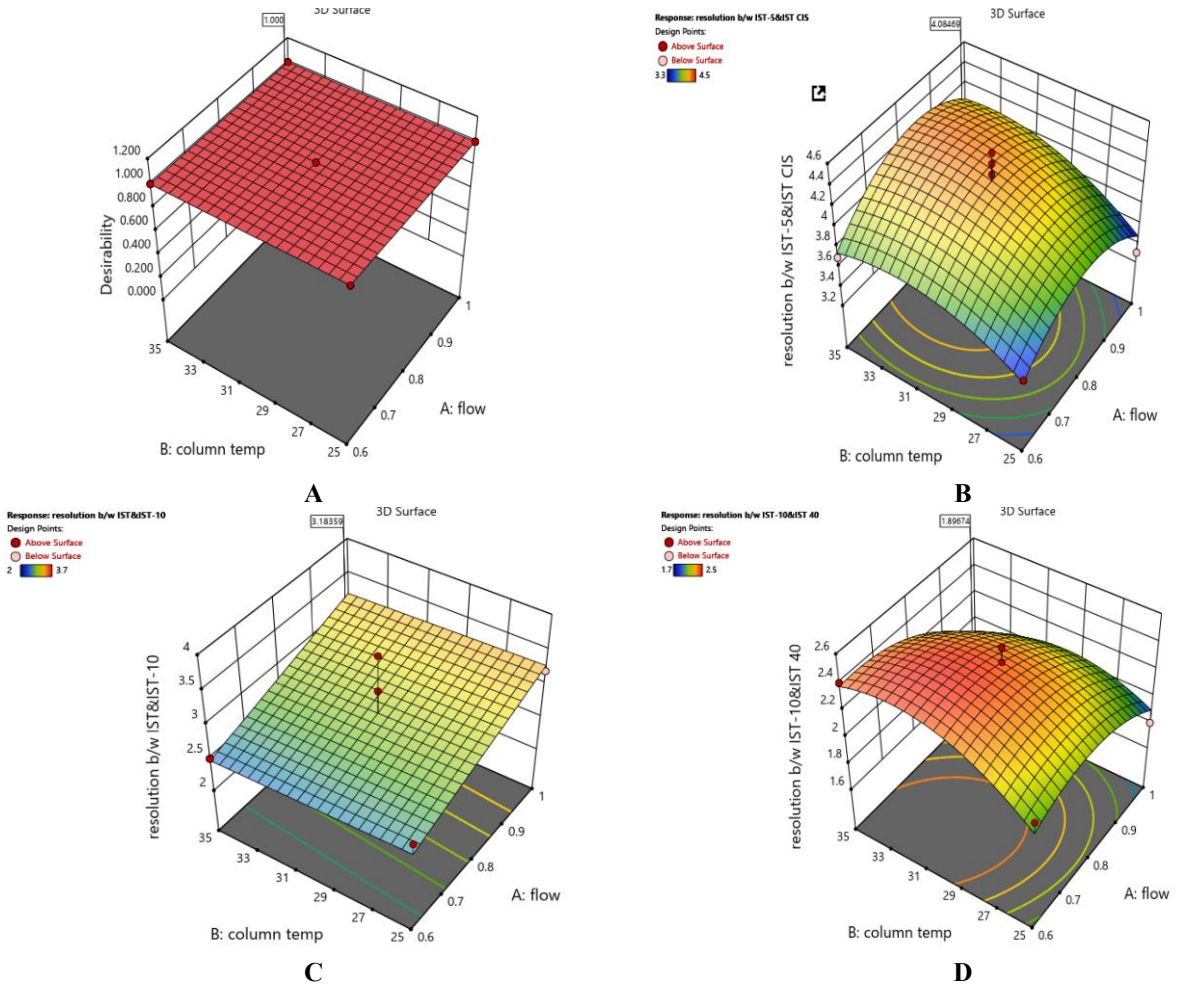



**Figure 6: 3D plots A) Desirability of IST B) Resolution b/w IST-5&IST-CIS C) Resolution b/w IST&IST-10 D) Resolution b/w IST-10&IST-40 with effect of flow and column temperature**

**Table 4: Results of robust ness study**

| Sr.no. | Flow rate | Column temp | Resolution b/w IST-5&IST CIS | Resolution b/w IST&IST-10 | Resolution b/w IST-5&IST CIS |
|---|---|---|---|---|---|
| 1 | 0.5 | 30 | 3.4 | 2 | 2.2 |
| 2 | 0.8 | 30 | 3.8 | 2.8 | 2.4 |
| 3 | 1.0 | 25 | 3.3 | 3.2 | 1.8 |
| 4 | 1.0 | 30 | 3.9 | 3.3 | 2 |
| 5 | 0.8 | 37 | 4.3 | 2.8 | 2.2 |
| 6 | 0.6 | 25 | 3.5 | 2.7 | 2.1 |
| 7 | 0.8 | 30 | 4.5 | 3.7 | 2.5 |
| 8 | 0.6 | 35 | 3.7 | 2.5 | 2.4 |
| 9 | 0.8 | 30 | 4.2 | 3.2 | 2.3 |
| 10 | 0.8 | 30 | 4.3 | 2.7 | 2.5 |
| 11 | 0.8 | 30 | 4.4 | 2.6 | 2.3 |
| 12 | 0.8 | 22 | 3.6 | 2.8 | 1.9 |
| 13 | 1 | 35 | 3.8 | 3.1 | 1.7 |

**Table 5: ANOVA summary report**

| S.NO | Model terms | p-value | F-value | $R^2$ | Pred.$R^2$ | Adj.$R^2$ |
|---|---|---|---|---|---|---|
| 1 | Resolution b/w IST-5&IST-CIS | 0.0439 | 4.20 | 0.5666 | 0.4150 | 0.4799 |
| 2 | Resolution b/w IST&IST-10 | 0.0314 | 4.99 | 0.8658 | 0.6948 | 0.7700 |
| 3 | Resolution b/w IST-10&IST-40 | 0.0109 | 2.45 | 0.5012 | 0.1584 | 0.4014 |

### 3.11. Degradation studies

Degradation studies were conducted to determine the specificity and stability-indicating properties of the proposed method. The stress conditions applied included acidic, alkaline, oxidative, and aqueous environments.

An amount equivalent to 40 mg of Istradefylline tablet powder was weighed and transferred into a 100 mL volumetric flask. Then, 5 mL of 1N HCl or 1N NaOH was added, the mixture was thoroughly mixed, and the flask was placed in a water bath at 60°C for 1 hour. Afterward, the solution was neutralized with 5 mL of 1N NaOH or 1N HCl. Following neutralization, about 50 mL of diluent was added, and the sample was sonicated for 30 minutes. Finally, the volume was made up to volume with diluent and mixed well.

For the peroxide degradation sample, an amount equivalent to 40mg of Istradefylline tablet powder was weighed and transferred into a 100mL volumetric flask. Then, 5mL of 10% hydrogen peroxide solution was added, the mixture was thoroughly mixed, and the flask was placed in a water bath at 60°C for 1 hour. Afterward, about 50mL of diluent was added, and the sample was sonicated for 30 minutes. Finally, the volume was made up to volume with diluent and mixed well.

For the water degradation sample, an amount equivalent to 40mg of Istradefylline tablet powder was weighed and transferred into a 100mL volumetric flask. Then, 5mL of water was added, the mixture was thoroughly mixed, and the flask was placed in a water bath at 60°C for 1 hour. Afterward, about 50 mL of diluent was added, and the sample was sonicated for 30 minutes. Finally, the volume was made up to volume with diluent and mixed well. Centrifuged above all degradation solutions at 3500 rpm in 10 minutes. Supernatant solution was injected into HPLC system. The results were shown in table 6.

**Table 6: Forced degradation conditions and results**

| Nature of Stress | % of total impurities | % assay of stressed Sertraline | Mass Balance | PA* | PT** |
|---|---|---|---|---|---|
| Unstressed | 0.023 | 98.3 | NA | 2.079 | 4.053 |
| 1N HCl@60°C, for 1hrs | 1.3 | 96.5 | 99.4 | 0.079 | 0.562 |
| 1 N NaoH@60°C for 1hrs | 2.1 | 95.3 | 99.0 | 0.078 | 0.609 |
| 10%Peroxide@60°C for 1hrs | 0.04 | 98.1 | 99.8 | 0.180 | 0.468 |
| 5 mL water@60°C for 1hrs | 0.04 | 97.7 | 99.4 | 0.319 | 0.764 |

***- indicates purity angle**

****- indicates purity threshold**

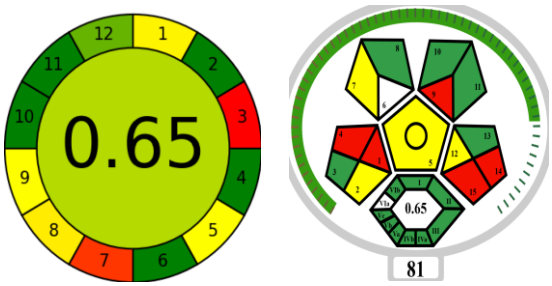
### 3.12. Stability of Mobile Phase and Sample Solutions

The solution and mobile phase stability was established for control sample solution, spiked sample solution, and dilute standard solutions for impurity determination level at room temperature condition. The solution stability was evaluated using freshly diluted

standard solution, and % recoveries were compared from the initial to as stable for 72 hours at 10℃ temperature storage conditions.

**Assessment of GREEnness**

The greenness of the proposed method was evaluated using AGREE and GAPI tools based on the 12 principles of Green Analytical Chemistry. The corresponding pictograms are presented in Figure 7, with detailed assessments provided in table 7. The method achieved AGREE and GAPI scores of 0.65 and 81, respectively, demonstrating high environmental sustainability and strong compliance with Green Analytical Chemistry principles. Favorable ratings for sample consumption, sample preparation, automation, derivatization avoidance, waste generation, and energy efficiency further confirmed the method as an eco-friendly analytical approach.




A B

**Figure 7: Greenness evaluation (a) AGREE Analytical GREEnness Metric Approach (b) GAPI Green Analytical Procedure Index**

**Table 7: Point of the Agree 12 principles**

| Category | Result |
|---|---|
| 1. Select the sampling procedure | off-line |
| 2.The amount of sample in g | 0.04g |
| 3.position of analytical device | off-line |
| 4.How many major distincts are there in the sample procedure | Sonication |
| 5.Degree of automation | Not miniaturized |
| 6.Select derivatization agent | No |
| 7.Amount of waste in ml | 64ml |
| 8.Number of analytes determine in single run | 6 |
| 9.Select the most energy used in technique used in the method | LC |
| 10.Select the type of reagent | No |
| 11.Does the method involve toxic reagent | No |
| 12.Select the threats which are not avoided | Corrosive |

## 4. CONCLUSION

A green, stability-indicating RP-HPLC method for the quantification of Istradefylline-related impurities in tablet dosage forms was successfully developed using an Analytical Quality by Design (AQbD) approach. The method was validated according to ICH Q2(R2) guidelines and demonstrated acceptable specificity, linearity, precision, accuracy, and robustness. The AQbD strategy ensured method robustness, while the greenness assessment confirmed its favorable environmental performance. The developed method is suitable for routine quality control, stability studies, and impurity profiling of Istradefylline tablet dosage forms.

**Ethical approval:** This work did not contain any studies with human participants performed by any of the authors.

**Conflict of interest:** The authors declare no conflict of interest.

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