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Original Article

QbD-Based Development, Greenness and Blueness Evaluation of a Stability-Indicating HPTLC Method for Ivacaftor with Mass Spectrometric Identification of Degradation Products

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ABSTRACT

Aim/Background: Quality by Design (QbD) is a structured approach to pharmaceutical development that emphasizes a deep understanding of processes and control of variability to ensure consistent quality. This study aims to develop and validate a robust, eco-friendly HPTLC-based analytical method for the quantification of Ivacaftor in bulk drug and pharmaceutical formulations, in alignment with QbD principles. **Materials and Methods:** The method development incorporated QbD tools such as risk assessment, method optimization, and robustness testing. HPTLC analysis was performed on silica gel pre-coated plates using a mobile phase composed of toluene, ethyl acetate, and methanol (6:2:2 v/v/v). Detection was carried out at 312 nm using UV spectroscopy. The method was validated as per ICH guidelines for specificity, linearity, accuracy, precision, and robustness. Forced degradation studies were conducted under various stress conditions (acidic, alkaline, oxidative, thermal, photolytic, and neutral), and degradation products were identified using Mass Spectrometry (MS). The method's environmental sustainability was assessed using AGREE, GAPI, and BAGI tools. **Results:** The developed HPTLC method demonstrated excellent linearity with a correlation coefficient (r^2) of 0.9995. The recovery values ranged from 99.71% to 100.22%, indicating high accuracy. The method also exhibited good specificity, precision, and robustness. Stress degradation studies revealed distinct degradation products, which were successfully identified through MS, providing valuable insights into Ivacaftor's stability profile. AGREE, GAPI, and BAGI assessments confirmed the method's greenness and eco-friendliness. **Conclusion:** The proposed HPTLC method, developed under QbD principles and validated as per ICH guidelines, is accurate, robust, and suitable for routine analysis of Ivacaftor. The incorporation of degradation product identification and green analytical metrics enhances its applicability for stability studies and quality control in pharmaceutical settings.

Keywords: Ivacaftor, QbD, HPTLC, MS, Forced Degradation, Validation, Greenness and Blueness Assessment.

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INTRODUCTION

An effective substitute for GC and HPLC is HPTLC, which is a sophisticated and automated version of TLC with improved and increased separation efficiency and detection limits. HPTLC and MS are both analytical techniques widely used in scientific research and industry for the identification and quantification of chemical compounds.[1]

Ivacaftor binds to the CFTR protein on the cell surface, boosting its activity. It increases the likelihood that the chloride channel stays open, enabling the movement of chloride ions across cell membranes. This is crucial for patients with cystic fibrosis as it aids in hydrating and clearing mucus from the airways, leading to improved lung function and reduced respiratory complications. Ivacaftor chemically is, N-(2,4-D i-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide.[2,3]

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Literature review reveals analysis of Ivacaftor by UV spectroscopy and HPLC; simultaneous estimation of Ivacaftor with other drugs by HPLC is also reported.[4-8] No HPTLC method is documented for the quantification of Ivacaftor, nor is there any method for the characterization of their degradation products by MS. Therefore, the goal of this study is to develop and validate a Quality by Design-based analytical method for quantifying Ivacaftor using HPTLC and degradation products identification using Mass Spectrometry. Additionally, the environmental friendliness of the method was assessed using the AGREE tool.

MATERIALS AND METHODS

Lupin Ltd., (Pune, India) generously provided a pure Ivacaftor drug sample with a purity of 99.30%. Fixed-dose granules containing 25 mg of Ivacaftor were formulated in an in-house laboratory. Camag Linomat V sample applicator and Camag 100 µL sample syringe was used for sample application.

Preparation of stock solution

Ivacaftor API was precisely weighed to 7.5 mg was prepared in 10 mL of methanol (750 µg/mL).

Selection of Analytical Wavelength

To achieve a concentration of 300 ng/band, 0.4 µL of standard stock solution of Ivacaftor (750 µg/mL) was applied to the HPTLC plate and was scanned within the UV range of 200-400 nm.

Background on Fishbone Diagram

A visual aid for arranging and identifying possible causes of a particular issue or result is a fishbone diagram (Figure 1), also referred to as an Ishikawa or cause-and-effect diagram. This diagram helps systematically analyze complex processes by categorizing factors to uncover root causes and areas for improvement.

The fishbone diagram outlines a structured approach for method development in analytical chemistry, focusing on three main categories: Materials, Method Parameters, and Instrumentation. It starts with the assessment of "Greenness and Blueness," evaluating the sustainability and environmental impact of the materials used, such as solvents and reagents. The method parameters include the optimization of chromatographic conditions to ensure robustness and accuracy. Instrumentation, featuring techniques like HPLC systems and mass spectrometry, is then applied for effective analysis.

A forced degradation study was performed to assess the analyte's stability under different stress conditions. The final step involves meeting regulatory requirements through method validation and adherence to ICH guidelines, ensuring the method's suitability for routine analysis. This systematic approach integrates environmental considerations with method optimization and regulatory compliance.

Method development as per experimental design

HPTLC method development by Analytical QbD was as follows:

Factorial Design

In order to develop a method for Ivacaftor, the Analytical Target Profile (ATP) had to be established. Three key Critical Method Parameters (CMPs) were chosen: the solvent front (development distance), the mobile phase, and saturation time. It was determined that Critical Analytical Attributes (CAA) was the retardation factor (R_f). Three factors and one response of Central Composite Design (CCD) were used for the optimization. Seventeen runs were carried out using Design Expert® version 13.01.11.0 (Stat-Ease Inc. Minneapolis). Response surface methodology was utilized to analyze the relationship between independent and dependent variables.

The Coefficient of Variation (% CV), Standard Deviation (SD), adequate precision, and R^2 value were taken into account while estimating the model's appropriateness. The importance of the model was validated using ANOVA, or variance analysis. Estimates were made for the model *p* value, F value, and lack of fit test to assess the model's significance. A model coefficient of statistical significance < 0.05 was taken into consideration for polynomial equations. The purpose of the initial experiments was to identify the independent and dependent variables. 3D response surface plots were taken into consideration for response surface analysis. The CCD's design matrix, which allows for 17 runs, is explained in Table 1.

Chromatographic Conditions

In a ratio of 6: 2: 2 v/v/v, methanol, ethanol, and toluene made up the mobile phase. The development of mobile phase was done by a trial-and-error approach, which was optimized by Central Composite Design (CCD). Each chromatographic run required 10 mL of this mixture. The plate was developed after 15 min of the chamber being saturated with mobile phase. Each chromatogram had a development distance of 80 mm (optimized with CCD), and the plates were dried after development. The plates were scanned using a Camag TLC scanner III at a wavelength of 312 nm. Concentrations were evaluated based on peak areas, using linear regression for the calculations.

Method Validation

Linearity

Ivacaftor standard solutions were made at concentrations between 75 and 450 µg/mL. A TLC plate was spotted with 1 µL of each solution, yielding a final concentration ranging from 75 to 450 ng/band. Each concentration was applied in triplicate and developed in an optimized mobile phase and the calibration curve was plotted.[9]

Precision

Repeatability and intermediate precision experiments validated the method's precision. Analyzing the drug at three distinct concentrations (150, 300, and 450 ng/band) six times in a single day allowed for the assessment of repeatability. The studies were performed on three distinct days in order to assess intermediate precision.

Limit of Detection and Limit of Quantification

Using the formula below, the least quantity of analyte that can be Detected (LOD) and Quantified (LOQ) with sufficient precision and accuracy was determined.

$$LOD = \frac{3.3 \times \text{Standard Deviation}}{\text{Slope}} \qquad LOQ = \frac{10 \times \text{Standard Deviation}}{\text{Slope}}$$

Accuracy

A recovery study was conducted using a 25 mg tablet formulation, and a known amount of Ivacaftor API equivalent to 80, 100, and 120% of the label claim was added. Methanol was used to extract the powder, and densitometric analysis was performed. Samples were developed after being spotted on TLC plates.

Robustness

The effects of small adjustments to the mobile phase composition were investogated. Chromatograms were recorded for a variety of mobile phase compositions, including toluene, ethyl acetate, and methanol in the volumetric ratios of 6.1: 2: 2, 6: 2.1: 2, 6: 1.9: 2, and 6: 2: 2.1. The volume of the mobile phase was varied within a tolerance of ±5%. Prior to chromatography, the plates underwent a methanol prewash and were activated for two, five, and 7 min at 60°C. There was a variation of ±10 min in the time intervals between chromatography and scanning, between spotting and chromatography.

Specificity

Ivacaftor samples from dosage forms and standard Ivacaftor were analyzed to ascertain the specificity of the procedure. By contrasting the sample's bands with the standard's reflectance absorbance spectra and R_f values, the bands were verified. Furthermore, at the R_f value that corresponded to the standard, the possible interference from the blank, degradation products, contaminants, and excipients was evaluated.

SIAM Development

Both pure and stability solutions were applied on TLC plates and testing them with different solvent systems allowed refining the TLC process for creating a stability-indicating assay method. Following multiple experiments, the ideal mobile phase was determined to be 6: 2: 2 v/v/v with toluene, ethyl acetate, and methanol and was allowed to ascend to a distance of 8 cm.

Stress Studies

Ivacaftor API was subjected to the following environments for conducting a forced degradation study:

Acidic Condition

Methanol (3 mL) was added to 7.5 mg of Ivacaftor to facilitate its dissolution. The mixture was mixed with 3 mL of 0.1 N HCl and left to remain at room temperature for 4 hr. After neutralizing the solution with 0.1 N NaOH, it was diluted with methanol (750 µg/mL) to the mark.

Alkaline Condition

Methanol (3 mL) was added to 7.5 mg of Ivacaftor to facilitate its dissolution. The mixture was mixed with 3 mL of 0.1 N NaOH and left to remain at room temperature for 4 hr. After neutralizing the solution with 0.1 HCl, it was diluted with methanol (750 µg/mL) to the mark.

Oxidative Condition

Methanol (3 mL) was added to 7.5 mg of Ivacaftor to facilitate its dissolution. After adding 3 mL of 3% H_2O_2, the mixture was left at room temperature for 4 hr and was diluted up to mark with methanol (750 µg/mL).

Thermal Condition

Methanol (10 mL) was added to 7.5 mg of Ivacaftor to facilitate its dissolution. The mixture was exposed to heat in an oven at 40°C for 45 min (750 µg/mL).

Photolytic Condition

Methanol (10 mL) was added to 7.5 mg of Ivacaftor to facilitate its dissolution. The mixture was exposed to direct sunlight for 24 hr (750 µg/mL).

Neutral condition

Methanol (3 mL) was added to 7.5 mg of Ivacaftor to facilitate its dissolution. The mixture was mixed with 3 mL of water and left to stand at room temperature for 4 hr. The solution was diluted up to mark with methanol (750 µg/mL).

From the above prepared samples, 0.4 µL was applied on HPTLC pre-coated plates to achieve a final concentration of 300 ng/band.

Preparation of marketed formulation

Ivacaftor granules were prepared in-house for performing further studies on the finished dosage form. The base granules were prepared, and the solution of the active ingredient, i.e. Ivacaftor was sprayed on the granules to prepare granules of 25 mg. Based on physical property evaluation, the formula was selected. The formula for granule preparation is given in Table 2.

Procedure for granules preparation is as follows

Dry mixing: Lactose monohydrate, microcrystalline cellulose, and croscarmellose sodium were mixed together.

Wet mixing: Starch paste (0.1%) was added slowly with consistent mixing to get wet masses.

Wet granules: Wet mass was pressed through 20# to get uniform granules.

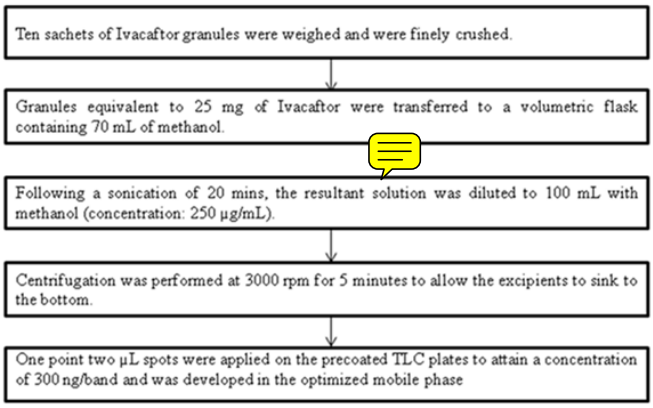
Drying: Granules were dried under controlled temperature at 60°C to get free flowing dried granules.

Drug loading: The equivalent amount of sifted base granules was taken in a rotating coating pan, and an ethanolic solution of Ivacaftor was sprayed under the current of warm air.

Lubrication: Drug loaded granules were mixed with silicon dioxide, sodium lauryl sulfate, and magnesium stearate to get the final product.

Packing: Granules equivalent to 25 mg were packed in a sachet and sealed.

Marketed Formulation Assay



Identification and Characterization of Degradation Products

The stress study samples were analyzed using a mass spectrophotometer in positive ESI mode. The acquired m/z values were then cross-referenced with the molecular weights of known degradation products. Subsequently, the fragmentation pattern was scrutinized to confirm the presence of degradation products, and the potential molecular structures were proposed. Based on these findings, the degradation pathway was defined, drawing conclusions from the molecular weights and fragmentation patterns observed.

Method's Greenness Evaluation

Greenness Assessment using Analytical Greenness Metric Approach and Software (AGREE) tool

AGREE software uses the 12 Principles of Green Analytical Chemistry to assess how analytical techniques affect the environment. It assigns scores to factors like use of safer solvents, waste prevention, energy efficiency, and chemical hazards. Each principle is rated on a scale of 0 (non-green) to 1 (green), and these ratings are combined to produce an overall "greenness" score. The result is displayed in a radial graphic or as a numerical value, helping users identify eco-friendly practices and areas for improvement. This tool enables comparison of methods to promote more sustainable and environmentally responsible processes. These principles help evaluate how eco-friendly an analytical method is.

Twelve green chemistry principles-direct analysis, minimal sample use, *in situ* measurements, integrated and automated processes, avoidance of derivatization, low waste and energy use, multi-analyte capability, use of safe and renewable reagents, and operator safety-are used by the AGREE tool to evaluate analytical methods.

These principles guide the software's evaluation of a method's environmental impact, leading to a comprehensive "greenness" assessment.

Greenness Assessment Using Green Analytical Procedure Index tool (GAPI)

A tool for evaluating the environmental impact of analytical techniques is GAPI. Using a color-coded approach, it assesses many steps of a process, including sample preparation, reagent usage, and energy consumption. Green denotes aspects that are environmentally friendly, yellow denotes moderate impact, and red shows regions that have a major negative influence on the environment. In laboratory practices, GAPI aids in the development and selection of more environmentally friendly, sustainable techniques.

Method's Blueness Evaluation

Blue Applicability Grade Index (BAGI) makes it easier to compare the performance of blue spaces to environmental objectives, enabling insightful comparisons with the current data. Analytical chemistry methods' practicality is evaluated using the BAGI metric, which assigns values between 25 and 100. Higher scores denote better practicality. The performance of several analytical procedures can be compared using this tool, which also makes it possible to quickly determine a method's applicability strengths and shortcomings.

Table 1: Optimization of parameters for analysis of Ivacafter using Central Composite Design.

Runs	Factor Mobile phase content (mL)	Factor Saturation Time (min)	Factor Solvent front (cm)	Response R_f Values
1	3	15	90	0.45
2	1	20	80	0.49
3	2	15	80	0.45
4	2	10	90	0.45
5	2	15	80	0.46
6	2	15	90	0.45
7	2	20	90	0.48
8	1	15	90	0.48
9	3	15	70	0.44
10	3	10	80	0.43
11	1	10	80	0.46
12	1	15	70	0.47
13	2	20	70	0.47
14	2	20	80	0.46
15	2	15	80	0.45
16	3	20	80	0.45
17	2	10	70	0.44

Table 2: Formula of Granules.

Category	Ingredients	Percent	Weight
Main	Lactose	80%	4 g
Filler	Microcrystalline cellulose	10%	0.5 g
Disintegrant	Croscarmellose sodium	5%	0.25 g
Glidant	Silicon dioxide	1%	0.05 g
Lubricant	Sodium lauryl sulphate	1%	0.05 g
Flow agent	Magnesium stearate	1%	0.05 g
Binder	Starch	0.1%	0.005 g

RESULTS

Wavelength Selection

The UV spectra of Ivacaftor (20 µg/mL in methanol) showed maximum absorbance at 312 nm.

HPTLC Method Development by QbD Approach

Optimization was performed using a QbD approach, where mobile phase ratio, saturation time, and solvent front were identified as critical parameters. The optimized conditions included Toluene: Ethyl Acetate: Methanol (6:2:2 v/v/v), band width of 6 mm, development distance of 80 mm, and chamber saturation time of 15 min. The final R_f value obtained was 0.46 with good resolution.

Response Surface Analysis and Model Development

The 3D response surface plots (Figure 2) indicated that R_f value increased with saturation time and solvent front but decreased with an increase in the mobile phase ratio. The polynomial equation describing the R_f value was:

$$Y = 0.454 - 0.01625\ A + 0.01375\ B + 0.005\ C - 0.0025\ AB + 0.00175\ A^2 + 0.00175\ B^2 + 0.00425\ C^2$$

The ANOVA analysis confirmed that the model's predictions were statistically significant which is shown in Table 3.

Validation Studies

Linearity

The method exhibited linearity within the concentration range of 75 to 450 ng/band.

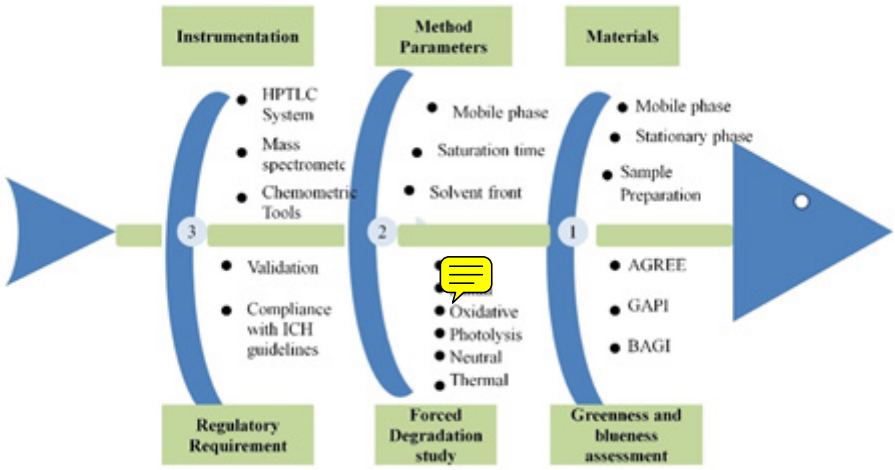


Figure 1: Fishbone or Ishikawa diagram.

Table 3: Summary of results of ANOVA.

Source	Sum of Squares	d_f	Mean Square	F-value	*p*-value	
Model	0.0040	9	0.0004	21.25	0.0003	significant
A-Mobile phase	0.0021	1	0.0021	101.98	< 0.0001	
B-Saturation time	0.0015	1	0.0015	73.02	< 0.0001	
C-Solvent front	0.0002	1	0.0002	9.66	0.0171	
AB	0.0000	1	0.0000	1.21	0.3083	
AC	0.0000	1	0.0000	0.0000	1.0000	
BC	0.0000	1	0.0000	0.0000	1.0000	
A^2	0.0000	1	0.0000	0.6225	0.4560	
B^2	0.0000	1	0.0000	0.6225	0.4560	
C^2	0.0001	1	0.0001	3.67	0.0969	
Residual	0.0001	7	0.0000			
Lack of Fit	0.0000	3	8.333E-06	0.2778	0.8395	not significant
Pure Error	0.0001	4	0.0000			
Cor Total	0.0041	16				

Precision

Both intra-day and inter-day precision results had RSD less than 2%.

LOD & LOQ

The LOD and LOQ were determined as 22.7 ng/band and 68.9 ng/band, respectively.

Robustness

The method remained robust despite minor variations.

Accuracy

Recovery ranged from 99.71% to 100.22%, confirming accuracy.

Specificity

No interference was observed, confirming method specificity.

Assay of Ivacaftor in Tablet Formulation

The assay results showed 98.94% to 100.70% recovery, confirming method suitability for commercial formulations.

Summary of the validation study is shown in Table 4.

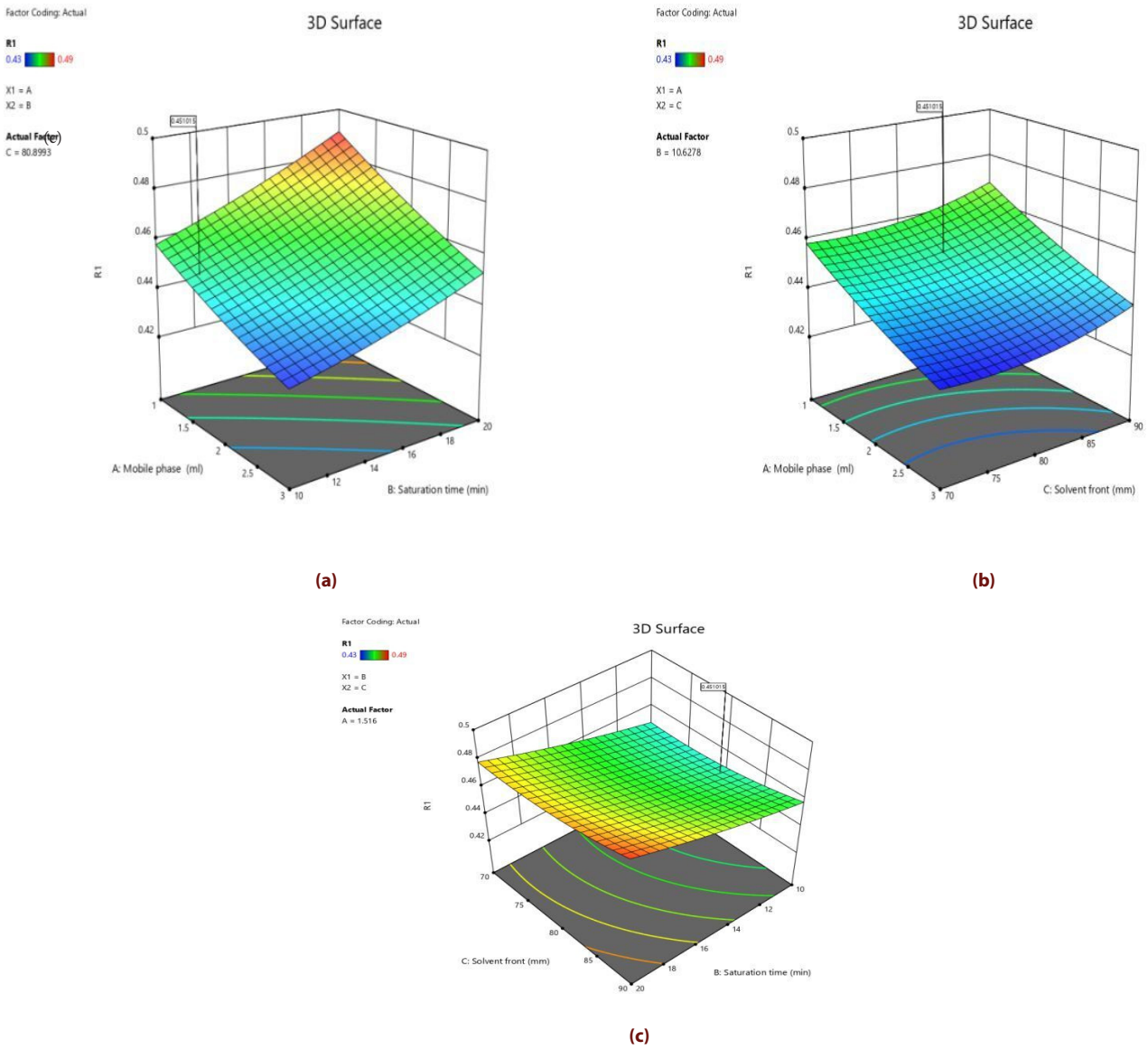


Figure 2: 3D response surface plots. (a) Impact of mobile phase and saturation time on the RF value. (b) Impact of saturation time and solvent front on the Rf value. (c) Impact of mobile phase and solvent front on the RF value.

Table 4: Summary of Validation study.

Sl. No.	Parameters	Observation
1.	Linearity range Regression equation Coefficient (R^2)	75-450 ng/band y=15.06x+2150 0.9997
2.	Precision	<2%
3.	Robustness	Robust
4.	Recovery	99.71-100.22%
5.	Assay	99.74-101.07%
6.	Specificity	Specific

Stability-Indicating Assay Method (SIAM) Development

Ivacaftor was subjected to acid, base, oxidative, thermal, photolytic, and neutral degradation (Table 5). The densitograms for standard and stress studies are shown in Figure 3.

Degradation Product Characterization

MS analysis revealed major degradation products identified via m/z values. Acidic, oxidative, and neutral degradation produced fragments at m/z 337 (McLafferty rearrangement) and m/z 415 (formation of a phenolate moiety). Base degradation led to fragmentation at m/z 337 (tert-butyl group loss) and m/z 227 (aromatic ring cleavage). The sodium adduct (m/z 415) was also detected (Figures 4 and 5). Summary of HR-MS data of Ivacaftor is shown in Table 6.

Method's Greenness and Blueness Evaluation

For AGREE tool, the overall greenness score was 0.71 (Figure 6a). For GAPI tool, a color-coded assessment identified areas needing improvement (Figure 6b) and for BAGI tool, the Blueness score was 80.0, indicating high sustainability (Figure 6c).

DISCUSSION

HPTLC Method Development and Optimization

A QbD-based approach was used to systematically optimize HPTLC parameters. The selected mobile phase composition (Toluene: Ethyl Acetate: Methanol 6:2:2 v/v/v) provided a well-resolved peak at R_f = 0.46. The 3D response surface plots were generated and demonstrated that saturation time and solvent front positively impacted R_f, while an increase in mobile phase content led to a decrease in R_f value.

Validation and Suitability of the Developed Method

The method showed excellent linearity (R^2>0.99) in the tested range, with high precision and low %RSD. LOD (22.7 ng/band) and LOQ (68.9 ng/band) demonstrated good sensitivity. The high recovery (99.71-100.22%) confirmed the method's accuracy. Specificity tests showed no interference, validating the method's ability to distinguish Ivacaftor from other components.

Stability Studies and Degradation Profiling

Stress degradation studies indicated that Ivacaftor is susceptible to acidic, oxidative, and base-induced degradation. McLafferty rearrangement and tert-butyl cleavage were identified as primary fragmentation pathways. The identified degradation products (m/z 337, 415, 227) provide insights into Ivacaftor's stability profile, which is crucial for regulatory and quality control purposes.

Sustainability and Environmental Considerations

The AGREE score (0.71) confirmed the method's compliance with Green Analytical Chemistry principles. The GAPI assessment highlighted areas for improvement, particularly in reagent selection and waste reduction. The BAGI score (80.0) indicated high efficiency and sustainability, confirming the method's eco-friendly nature.

Practical Implications

The developed HPTLC method is precise, robust, and environmentally friendly, making it a suitable tool for routine quality control and regulatory applications. The ability to detect degradation products enhances the method's role in stability studies and pharmaceutical quality assurance. The sustainability evaluation supports the adoption of greener analytical practices in the pharmaceutical industry.

Table 5: Percentage Degradation in Stress Study.

Sl. No.	Stress condition	Exposure conditions	R_f value of Ivacaftor	R_f value of degradation products	% degradation
1.	Acid hydrolysis	0.1N HCl, 4 hr, room temperature.	0.49	0.75, 0.85	17.22
2.	Alkaline hydrolysis	0.1 N NaOH, 4 hr, room temperature.	0.47	0.84	11.81
3.	Oxidation	3% H_2O_2, 4hr, room temperature.	0.48	0.28	10.72
4.	Thermal	45°C in oven, 45 min	0.47	0.69	30.72
5.	Photolytic	Exposure to UV light, 24 hr.	0.48	0.82	9.54
6.	Neutral	H_2O, 4 hr, room temperature.	0.46	0.87	23.03

Table 6: Summary of HR-MS data of Ivacaftor.

Sl. No.	Compound	Molecular Weight (g/mol)	Molecular formula	IUPAC Name
1.	Ivacaftor	392.21	$C_{24}H_{28}N_2O_3$	N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide.
2.	Ivacaftor DP I	337	$C_{20}H_{21}N_2O_{3+}$	4-(tert-butyl)-3-(4-oxo-1,4-dihydroquinoline-3-carboxamido) cyclohexa- 2,4-dien-1-ylidene) oxonium.
3.	Ivacaftor DP II	336	$C_{20}H_{20}N_2O_{3+}$	N-(2-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3- carboxamide.
4.	Ivacaftor DP III	227	$C_{13}H_{10}N_2O_{2+}$	1-methyl-3-(1-oxoethylidene) quinoxalin-2(1H)-one.
5.	Ivacaftor DP IV	415	$C_{19}H_{15}NaO_4$	sodium 2,4-di-terr-butyl-5-(4-oxo-1,4-dihydroquinoline-3- carboxamido) phenolate.

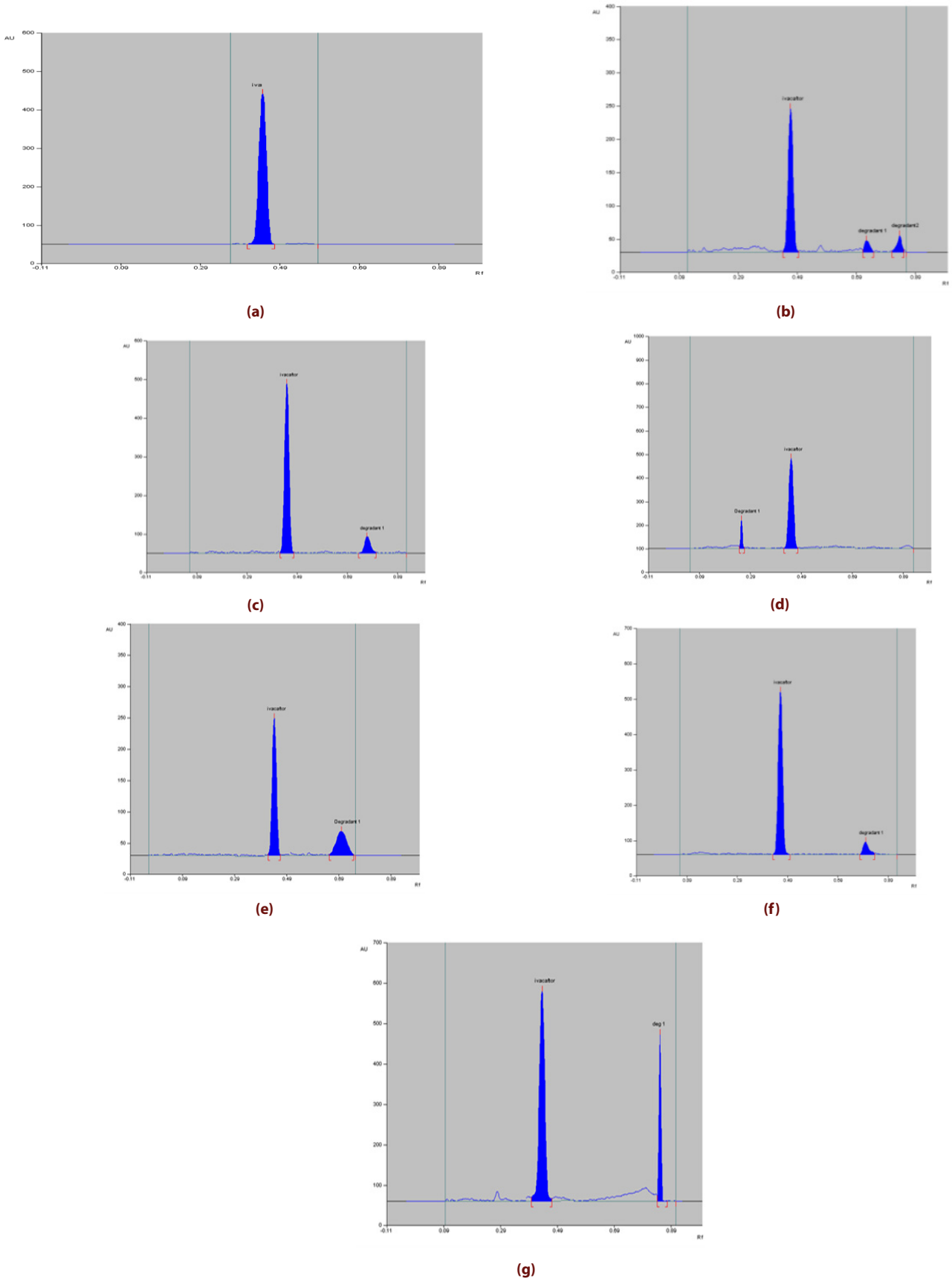


Figure 3: Degradation in (a) Ivacafter Standard (b) Acidic condition (c) Alkaline condition (d) Thermal (e) Oxidation (f) Photolytic (g) Neutral conditions.

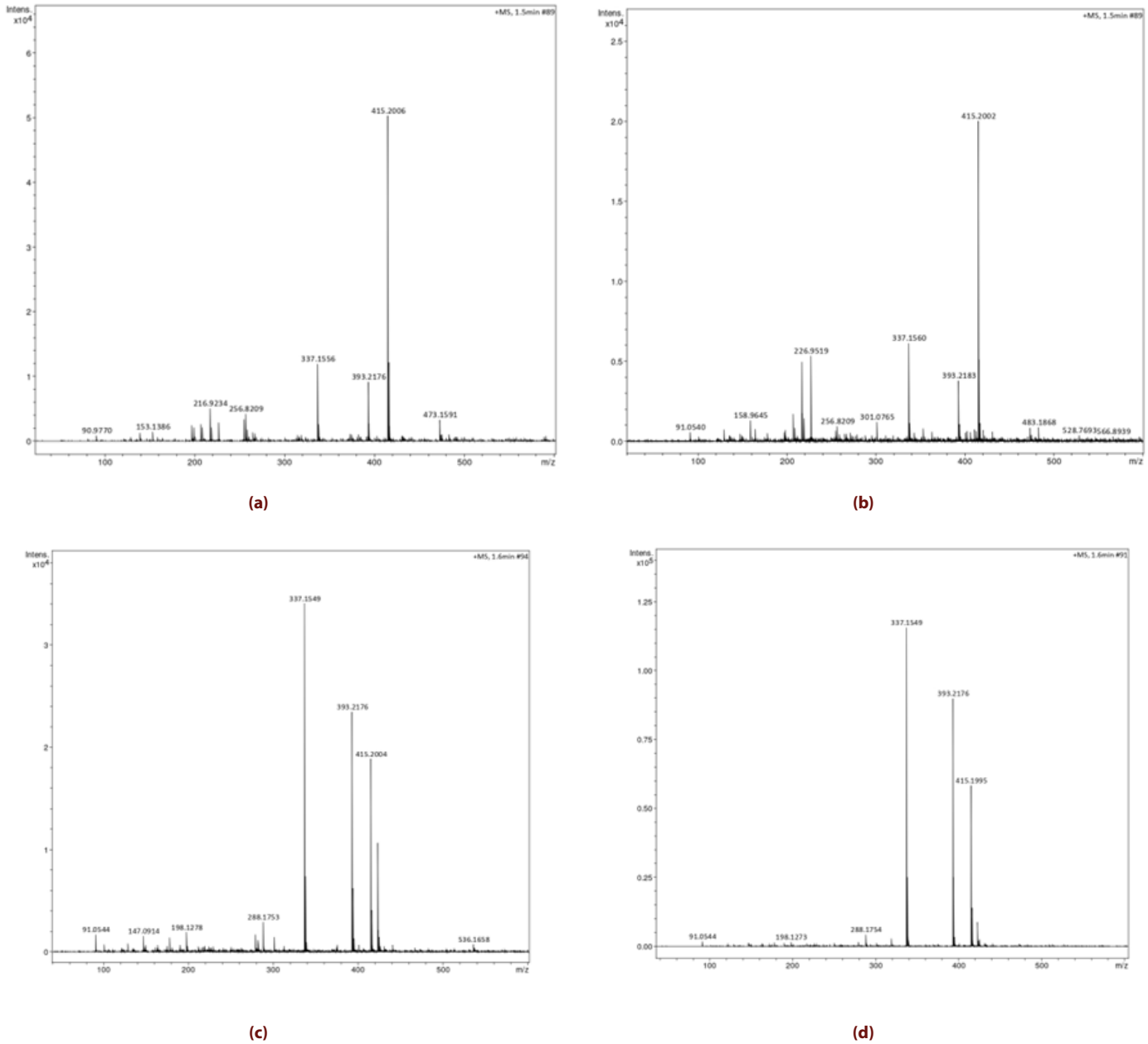


Figure 4: Mass spectra of Ivacaftor in various stress conditions (a) Acid Degradation spectra (b) Alkaline Degradation Spectra (c) Oxidative Degradation Spectra (d) Neutral Degradation spectra.

CONCLUSION

The implementation of QbD principles in developing and validating HPTLC-based analytical method for estimating Ivacaftor in bulk and dosage forms was highly efficient and effective. The systematic approach facilitated the identification of critical method parameters, optimization of chromatographic conditions, and enhancement of method robustness, thus ensuring precise and reproducible quantification of Ivacaftor. The developed method was rigorously validated in accordance with ICH guidelines, demonstrating satisfactory results for all parameters.

Furthermore, the study successfully identified the degradation products of Ivacaftor through mass spectrometry. This demonstrates the method's stability-indicating capabilities, which enable it to successfully separate the active pharmaceutical ingredient from its degradation products. These findings provide a reliable analytical tool for the routine quality control of Ivacaftor in pharmaceutical formulations and support the overall stability assessment of the drug substance.

The developed method offers a significant advancement in the analytical assessment of Ivacaftor and can be employed in both regulatory and industrial settings for ensuring the quality, safety, and efficacy of Ivacaftor-containing products.

Overall, this study presents a reliable, eco-friendly analytical approach for pharmaceutical stability testing, contributing to sustainable practices in drug development and quality assurance.

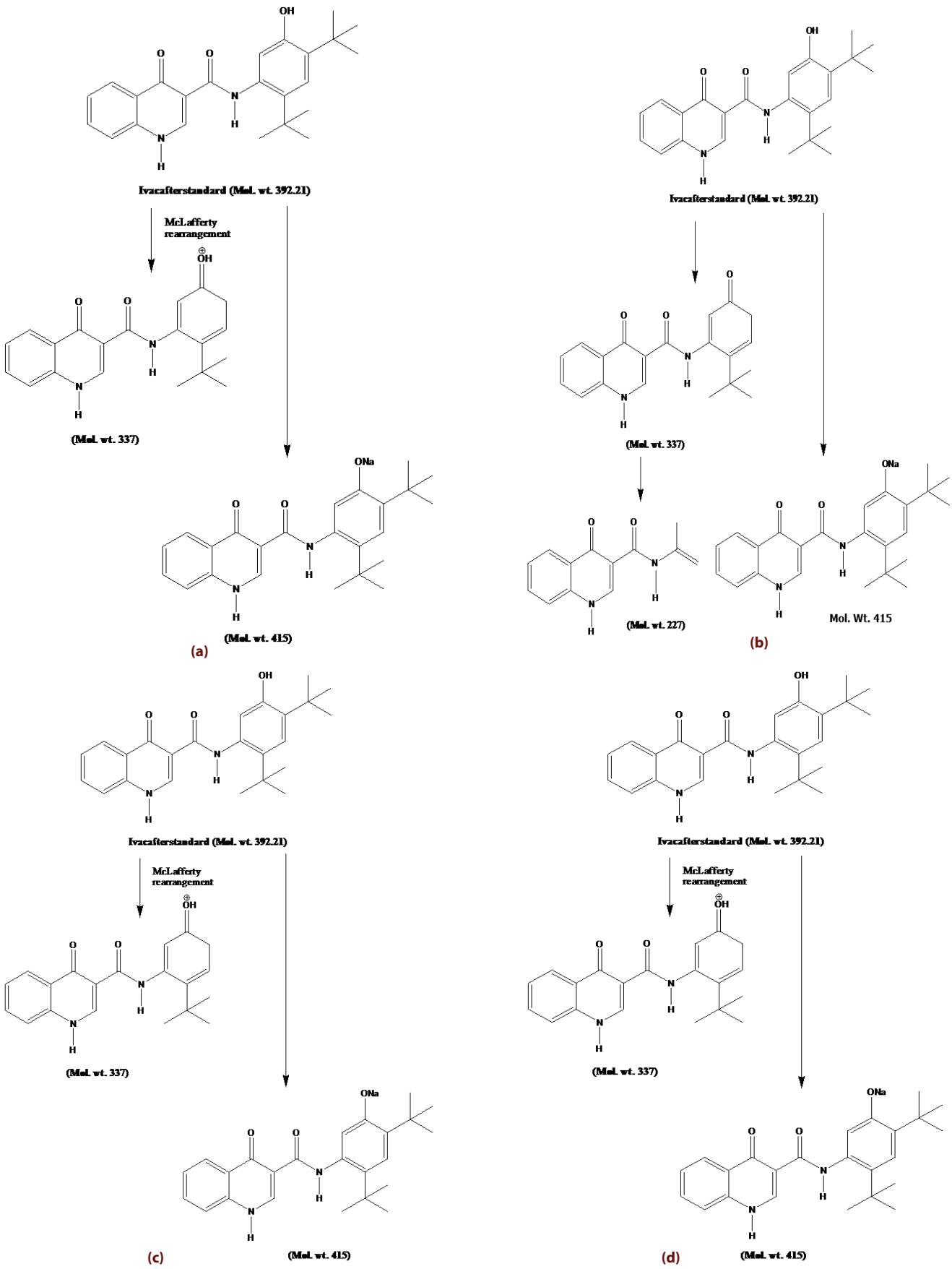
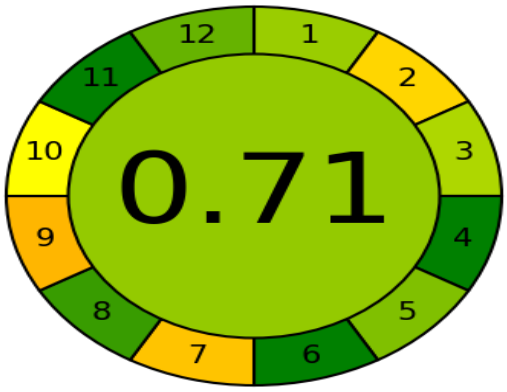
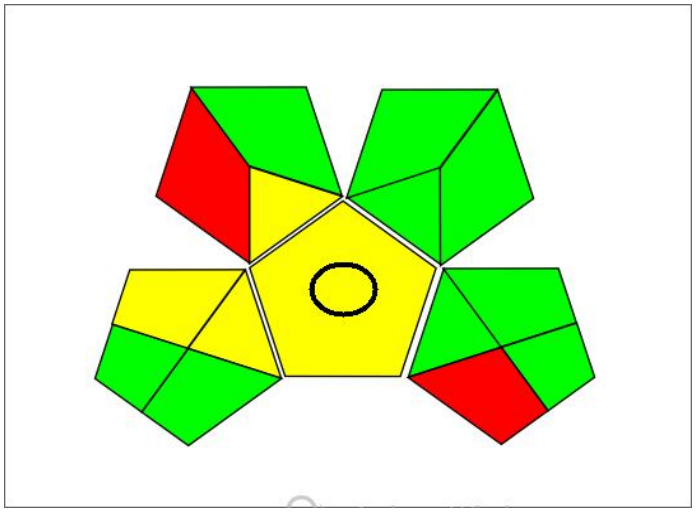


Figure 5: Possible degradation pathways in all stress condition (a) Acidic condition (b) Alkaline (c) Oxidation (d) Neutral conditions.



(a)



(b)

(c)

Figure 6: Greenness and Blueness assessment using a) AGREE tool b) GAPI tool c) BAGI tool.

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ABBREVIATIONS

HPTLC: High Performance Thin Layer Chromatography; **HRMS:** High Resolution Mass Spectrometry; **TLC:** Thin Layer Chromatography; **GC:** Gas Chromatography; **MS:** Mass Spectrometry; **SIAM:** Stability Indicating Assay Method; **ICH:** International Conference on Harmonization; **CMP:** Critical Method Parameters; **CCD:** Central Composite Design; **RSD:** Relative standard deviation; **UV:** Ultraviolet; **R_f:** Retardation factor.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Not Applicable.

ETHICAL APPROVAL

Not Applicable.

SUMMARY

This study applies Quality by Design (QbD) principles to develop and validate a High-Performance Thin-Layer Chromatography (HPTLC) method for Ivacaftor estimation in bulk and dosage forms. The systematic approach optimized chromatographic conditions, enhancing method robustness, precision, and

reproducibility. Validation per ICH guidelines confirmed accuracy, linearity, specificity, and sensitivity. Additionally, mass spectrometry identified Ivacaftor's degradation products, establishing the method's stability-indicating capability. This eco-friendly analytical approach supports routine quality control and stability assessment, aligning with sustainable pharmaceutical practices.

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