

Stability Indicating Eco Friendly UV Spectrophotometric Determination of Berberine Hydrochloride Using Quality by Design and AGREE Approach

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Research Article

Keywords: Berberine Hydrochloride, UV Spectrophotometry, Method validation, forced degradation, stability-indicating method, Greenness assessment, AGREE matrices, Validation, ICH, QBD

Posted Date: June 16th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9679254/v1>

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Additional Declarations: No competing interests reported.

Stability Indicating Eco Friendly UV Spectrophotometric Determination of Berberine Hydrochloride Using Quality by Design and AGREE Approach

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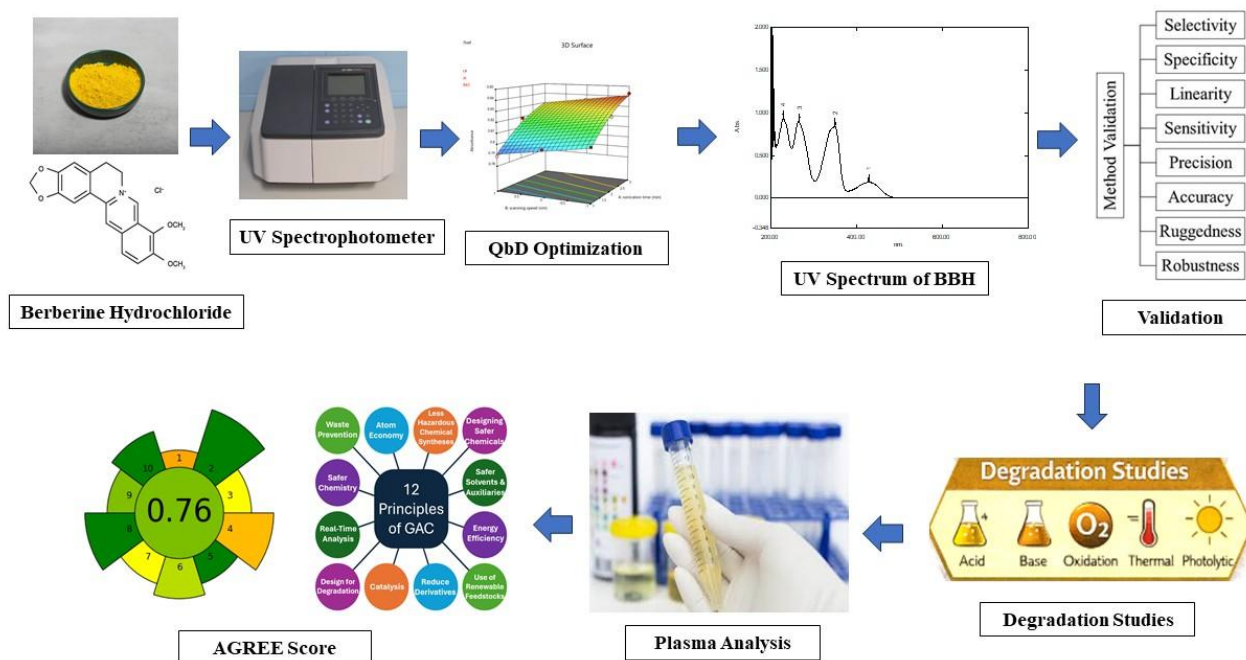
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Abstract

A Quality by Design (QbD) approach provides a structured framework to ensure product quality by identifying critical factors, process variables, and their interactions through systematic experimentation and controlled conditions. In this study, key analytical parameters—namely mobile phase composition, column temperature, and flow rate—were optimized using a Box–Behnken design for factor screening. A stability-indicating analytical method was developed and validated for the quantification of Berberine Hydrochloride in bulk drug and pharmaceutical formulations. The method demonstrated strong robustness and environmental compatibility, reflected by an AGREEprep score of 0.76. Methanol was selected as the solvent due to its superior solubilizing capability. The method produced a well-defined and symmetrical spectrum at 428nm. Validation, conducted in accordance with ICH Q2(R1) guidelines, confirmed excellent linearity over the concentration range of 2–10 µg/mL ($R^2 = 0.9989$), along with high sensitivity (LOD: 0.462 µg/ml; LOQ: 1.400 µg/ml) and accuracy, with recovery values between 98.5% and 101.5% (%RSD < 2%). Forced degradation studies under acidic, alkaline, oxidative, photolytic, and thermal conditions established the method's stability-indicating capability by effectively Berberine Hydrochloride resolving from its degradation products. The novelty of the method lies in its simplified solvent system, eliminating the need for buffers and hazardous reagents, and its successful application to an in-house tablet formulation demonstrates its suitability for routine quality control and stability assessment.

Keywords: Berberine Hydrochloride, UV Spectrophotometry, Method validation, forced degradation, stability-indicating method, Greenness assessment, AGREE matrices, Validation, ICH, QBD.

Graphical Abstract



INTRODUCTION

Berberine hydrochloride (BBH) is a naturally occurring isoquinoline alkaloid found in various medicinal plants, particularly those belonging to the *Berberis* genus and Berberidaceae family [1]. Characterized by its vivid yellow hue and bitter flavor, BBH's chemical designation is 9,10-dimethoxy-5,6-dihydro[1,3]dioxolo[4,5-g]isoquino[3,2-a]isoquinolin-7-ium. It has a molecular formula- $C_{20}H_{19}NO_5$ and molecular weight- 353.36 g/mol [2]. The chemical structure of BBH is shown in figure 1. This compound is present in several botanicals such as *Guduchi*, *Berberis vulgaris*, goldenseal, goldthread, Oregon grape, and some rosid dicot species [3]. BBH is known for its broad pharmacological profile, including antibacterial, antiviral, anti-inflammatory, antihypertensive, hypoglycemic, and anticancer properties [4].

Given its therapeutic value, multiple analytical techniques have been established for its quantification, each varying in terms of sensitivity, specificity, and operational complexity. Among these, UV-spectrophotometry stands out due to its straightforward application, cost effectiveness, and reliability for quantitative assessments. This technique functions based on absorption of ultraviolet or visible light by a solution, adhering to Beer-Lambert law. When used for BBH, UV-spectrophotometry offers high sensitivity, broad applicability, and minimal sample preparation requirements [5,6].

To ensure accurate and dependable results, method must undergo rigorous development and validation, addressing critical parameters such as accuracy, precision, specificity, ruggedness, robustness, and linearity. This study aims to develop and validate UV-spectrophotometric method specifically for the determination of BBH. The goal is to provide reliable and standardized approach suitable for the quality control of pharmaceutical and herbal preparations containing BBH. Furthermore, this validated method may find application in

pharmacokinetic evaluations, formulation development, and therapeutic monitoring, thereby contributing to the advancement of research in natural products and phytotherapeutics [6,7].

The AGREE (Analytical Greenness) metric is modern, holistic tool designed to quantitatively assess the greenness of analytical procedures based on 12 principles of Green Analytical Chemistry (GAC). Developed to address limitations of earlier greenness assessment methods, AGREE provides a visual and numerical evaluation of how environmentally sustainable given analytical method is. It uses circular, color-coded graphical interface to display scores for each principle, ranging from 0 (least green) to 1 (most green), alongside an overall average score. This tool not only enhances transparency in method assessment but also guides analysts in optimizing procedures to minimize environmental impact, resource consumption, and health risks. By incorporating AGREE into routine method validation and development, laboratories can align analytical practices with global sustainability goals. [15,16].

2. MATERIAL AND METHODS

2.1 Materials: Drug Sample Berberine Hydrochloride was purchased from Yucca Enterprises, Mumbai. Reagents and All chemicals and reagents used were also of analytical grade and obtained from KAHER Dr Prabhakar Kore Basic Science Research Centre, Belagavi.

2.2 Instrumentation: For analytical method development of BBH, Shimadzu UV-1800 with LabSolutions UV Probe software system was utilized. Optimization was carried out using Design-Expert software version 13.0 by Stat-Ease Inc. Minneapolis, MN, USA.

2.3 Selection of Solvents: A range of analytical solvents were examined through trial-and-error experiments to establish the ideal solvent. Solvents such as water, ethanol, methanol, and DMSO were examined. Based on the drug profile and experimental findings, water was selected as the solvent for method development.

2.4 Analytical method validation

As per guidelines set forth by ICH (International Conference on Harmonization), validation is defined as process of establishing documented proof that provides a high degree of assurance regarding the ability of a selected activity to consistently yield the intended outcome or a product that meets its predetermined specifications and quality characteristics. The following factors were evaluated in order to validate the method being used [6].

2.5 Preparation of Standard Stock Solution

A precisely weighed 10mg of BBH was dissolved in methanol in a clean, dry 10 mL volumetric flask and subjected to bath sonication for 10 minutes. This solution was designated as the standard stock solution (Stock Solution 1) with a concentration of 1000 µg/mL. Subsequent dilutions were prepared from this stock. To prepare Stock Solution 2 (100 µg/mL), 1 mL of Stock Solution 1 was transferred into another clean, dry 10 mL volumetric flask, diluted with methanol, and bath-sonicated for 10 minutes. Similarly, Stock Solution 3 (10 µg/mL) was prepared by taking 1 mL of Stock Solution 2 and diluting it to 10 mL with methanol in a clean, dry volumetric flask, followed by bath sonication for 10 minutes [6].

2.6 Selection of Wavelength

A working standard solution of BBH (10 µg/mL) was prepared in methanol and scanned using a UV spectrophotometer between 800 and 200 nm, with the highest absorption at 428 nm [7].

2.7 DoE Based Method Development and Optimization

The optimization of the analytical method was carried out using a Design of Experiments (DoE) approach to systematically evaluate the influence of critical method parameters on the selected response. A full factorial design was employed to study the interaction effects of independent variables on the Critical Analytical Attribute (CAA). Specifically, a 3^2 full factorial design, comprising two independent factors at three levels each, was utilized, resulting in nine experimental runs. The selected factors, namely scanning speed and sonication time,

were varied systematically to evaluate their impact on the absorbance of berberine hydrochloride. Scanning speed was investigated due to its influence on spectral resolution and measurement accuracy, while sonication time was studied for its role in ensuring complete dissolution and extraction efficiency. The experimental design, data analysis, and optimization were performed using Design-Expert software (version 13.0; Stat-Ease Inc., Minneapolis, MN, USA). Statistical tools, including analysis of variance (ANOVA), response surface methodology, and desirability functions, were applied to identify optimal conditions that ensure robust method performance while maintaining green analytical characteristics.

2.7 Preparation of Calibration Curve

Serial dilutions of 2, 4, 6, 8 and 10 µg/mL were made from the stock solution 2. The absorbance of the solution was measured at 428 nm, a standardization curve was produced with concentration on the X-axis and absorbance on the Y-axis, and a linear regression equation was calculated [8].

2.8 Linearity and range

To evaluate the linearity of the method, 10 mg of BBH was precisely measured and placed into a 10 mL volumetric flask. Methanol was added to dissolve the compound, and the solution was diluted to the mark to produce a stock solution with a concentration of 1000 µg/mL. From this primary stock, a secondary working solution was prepared by transferring 1 mL into another 10 mL volumetric flask and diluting to volume with methanol, resulting in a 100 µg/mL solution [9].

From the working stock, a series of standard solutions were prepared with concentrations of 2, 4, 6, 8, and 10 µg/mL through serial dilution. Each concentration was prepared in triplicate to ensure consistency. The absorbance of these solutions was then recorded at a wavelength of 428 nm using a UV spectrophotometer [9,10].

2.9 Limit of Detection and Limit of Quantification

In UV-Vis spectrophotometry, the Limit of Detection (LOD) and Limit of Quantification (LOQ) are key parameters that reflect the method's sensitivity and dependability. LOD denotes the smallest amount of a substance that can be detected, though not necessarily measured accurately, while LOQ indicates the lowest concentration that can be quantified with acceptable levels of precision and accuracy.

LOD and LOQ was calculated by using statistical calculations using formulas:

$$\text{LOD} = 3.3 \times \text{standard deviation of regression} / \text{Slope}$$

$$\text{LOQ} = 10 \times \text{standard deviation of regression} / \text{Slope} [11].$$

2.10 Specificity and Selectivity

A blank solution was prepared using the solvent (e.g., methanol) without the addition of BBH. This blank was scanned across the same wavelength range to check for any notable absorbance at the wavelength corresponding to BBH's maximum absorbance. To confirm that the absorbance at $\lambda_{\text{max}} = 428 \text{ nm}$ in the sample solution was specifically due to BBH, an overlay spectrum was generated, verifying that no interference occurred from the solvent or other components [12-15].

2.11 Precision

To assess system precision, three replicate solutions of BBH at concentrations of 2 µg/ml, 6 µg/ml, and 10 µg/ml were prepared, and their absorbance was measured at 428 nm. The

percentage Relative Standard Deviation (%RSD) was then calculated to evaluate consistency. Method precision was determined through both intraday and interday analyses. For intraday precision, three replicates at each concentration (2 µg/ml, 6 µg/ml, and 10µg/ml) were analysed at different times within the same day, and the %RSD was calculated. For interday precision, the same concentration levels were tested over three consecutive days, and the %RSD values were computed to determine day-to-day reproducibility [16-19].

2.12 Accuracy

Accuracy was confirmed by doing recovery experiments in which the percent mean recovery of the sample was calculated using a standardization approach at three distinct levels: 50, 100, and 150% of the sample solutions. Any dilutions are made from the above solution. Three replicates of the concentration solution were prepared for each level, and a recovery study was conducted [20].

2.13 Ruggedness

To determine the ruggedness normally three replicates of solutions containing BBH were prepared and absorbance of each replicate was measured by different analyst also by using different instruments and %RSD as calculated for absorbance [21].

2.14 Robustness

Robustness was measured using two completely distinct wavelengths, 426nm and 430nm, to analyze concentration solution in methanol at 1 µg/mL. % RSD was used to indicate the results [22].

2.15 Forced Degradation Studies of Berberine Hydrochloride

Forced degradation studies were carried out to evaluate the stability of Berberine Hydrochloride under various stress conditions in accordance with ICH guidelines. The

degradation behavior was assessed using UV–Visible spectrophotometry by comparing stressed samples with unstressed drug solutions [23-25].

2.15 a. Acid Degradation Studies

For acid degradation studies, 1 mL of the stock solution of Berberine Hydrochloride was mixed with 1mL of 1N Hydrochloric acid and the mixture was refluxed for 2hour at 60 °C. After completion of degradation, the solution was allowed to cool to room temperature and neutralized with 1N Sodium hydroxide. The resultant solution was suitably diluted with the solvent to obtain a final concentration of 10µg/mL.

The degraded sample was analyzed at the λ_{max} of Berberine Hydrochloride using a UV–Visible spectrophotometer. The UV spectrum was compared with that of the unstressed sample to assess the extent of acid-induced degradation.

2.15 b. Alkali Degradation Studies

For alkali degradation studies, 1mL of the stock solution of Berberine Hydrochloride was treated with 1mL of 1N Sodium hydroxide and refluxed for 2 hours at 60 °C. After degradation, the solution was cooled and neutralized with 1N Hydrochloric acid. The solution was then diluted to obtain a 10µg/mL working concentration.

The UV absorbance of the degraded sample was recorded at the selected λ_{max} and compared with that of the standard drug solution.

2.15 c. Oxidative Degradation Studies

Oxidative degradation was performed by adding 1mL of 3% v/v hydrogen peroxide to 1mL of the stock solution of Berberine Hydrochloride. The mixture was kept at room temperature for 2 hours. After completion of the reaction, the solution was diluted with the solvent to obtain a 10 µg/mL concentration.

The UV spectrum of the oxidatively degraded sample was recorded and compared with the untreated drug solution to evaluate oxidative stability.

2.15 d. Thermal Degradation Studies

Thermal degradation studies were conducted by exposing drug sample of Berberine Hydrochloride to 70 °C in a hot air oven for 6 hours. After thermal exposure, an accurately weighed quantity of the drug was dissolved and diluted with the solvent to prepare a 10µg/mL solution.

The UV absorbance of the thermally stressed sample was measured at λ_{max} and compared with that of the standard solution.

2.15 e. Photolytic Degradation Studies

Photolytic degradation studies were carried out by exposing the Berberine Hydrochloride solution to UV Sunlight for 6 hours. After exposure, the solution was diluted to achieve a 10 µg/mL concentration.

The UV spectrum of the photodegraded sample was recorded and compared with the unstressed sample to assess photostability [14].

2.16 Plasma Sample Preparation

For plasma analysis, 1mL of the stock solution of Berberine Hydrochloride was transferred into a centrifuge tube containing 0.5mL of blank plasma and vortex-mixed to ensure uniform distribution of the drug within the plasma matrix. Protein precipitation was carried out by the addition of 0.5mL of acetonitrile, followed by thorough vortex mixing. The mixture was then centrifuged at 3000 rpm for 20 minutes to achieve effective separation of precipitated plasma proteins.

After centrifugation, the clear supernatant was carefully collected and suitably diluted with the solvent to obtain the required concentration. The absorbance of the processed plasma sample was measured using a UV–Visible spectrophotometer at the predetermined λ_{max} of Berberine Hydrochloride, employing a plasma blank prepared under identical conditions as the reference. The resulting UV spectrum was evaluated to assess the detectability of Berberine Hydrochloride in plasma [15].

2.17 Methodology for AGREE Greenness Evaluation

The AGREE (Analytical GREENness Metric) tool is a comprehensive, semi-quantitative software based on the 12 Principles of Green Analytical Chemistry (GAC). These principles were developed to promote safer, more sustainable, and eco-friendly analytical techniques. Each principle is individually scored from 0 (non-green) to 1 (ideal greenness) depending on the method's compliance. The overall greenness is represented by the average of these twelve scores. In this study, AGREE was applied to assess a validated UV-spectrophotometric method for Berberine Hydrochloride (BBH) quantification. Information such as solvent type, energy usage, instrumentation, reagent consumption, and safety measures was analyzed to assign scores. The scoring followed guidance from the AGREE tool developers and regulatory validation practices [7-10].

3 RESULT AND DISCUSSION

3.1 Method Development

Using the UV-1800 equipment and methanol as a solvent, a UV-spectrophotometric technique was devised.

3.2 Selection of Wavelength

The maximum absorbance wavelength (λ_{max}) of berberine hydrochloride was determined by scanning a standard solution (10 $\mu\text{g/mL}$) over the wavelength range of 200–400 nm using an appropriate blank. Berberine hydrochloride exhibited a distinct absorption maximum at **428 nm**, which was selected for further analytical studies due to its optimal sensitivity and specificity.

3.3 Optimization of Method

Extensive optimizations were conducted to pinpoint the optimal conditions within the given design space. Furthermore, the effect of variables such as sonication time and scanning speed on the response (absorbance) was assessed. The optimization process involved overlaying the contour of the critical response with contour plots using **Design-Expert software**.

Table 1 summarizes the design of experiments (DoE) runs and the corresponding responses and

Table 2 specifies the ANOVA analysis of the response, i.e., absorbance of berberine hydrochloride, with a significant p-value (**p = 0.0047**). The model F-value of **47.09** indicates that the model is statistically significant. The probability that such a high F-value could occur due to noise is only **0.47%**, confirming model reliability.

Among the studied factors, **sonication time (A)** and **scanning speed (B)** were found to be significant model terms with p-values of **0.0007** and **0.0196**, respectively. This indicates that both parameters significantly influence the absorbance response. However, the interaction term (**AB**) and quadratic terms (**A² and B²**) were found to be insignificant ($p > 0.05$), suggesting minimal interaction and curvature effects within the studied range.

Figure 1 represents the 3D response surface plot, while Figure 2 shows the 2D contour plot illustrating the correlation between the selected variables, i.e., sonication time and scanning speed, and the response. Figure 3 illustrates the overlay plot of the optimized method.

The optimum conditions were determined based on the overlay plot and desirability criteria, indicating that **moderate sonication time and intermediate scanning speed** provide optimal absorbance with robust analytical performance.

3.4 Method Validation

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment. The developed method was validated in terms of linearity and range, specificity, selectivity, precision, accuracy robustness, and ruggedness. Chemical Structure of Berberine Hydrochloride is shown in Figure 4.

3.3 Linearity and Range

Linearity and range were resolute by plotting standard calibration curve using concentration UV absorbance's obtained by linear dilution of BBH. The absorbance range was between the concentration of 2, 4, 6, 8 and 10 μ g/mL. The regression equation of BBH was 0.9989. Linearity data as signified in Table 3. UV Spectrum of methanal is shown in Figure 5. Spectra of BBH is represented in Figure 6. Overlay spectrum of linearity of BBH is shown in Figure 7 with standard calibration curve was presented in Figure 8.

3.4 Limit of Detection and Limit of Quantification

In UV vis spectrophotometry, the Limit of Detection (LOD) and Limit of Quantification (LOQ) are key parameters that reflect the method's sensitivity and dependability. LOD denotes the smallest amount of a substance that can be detected, though not necessarily measured accurately, while LOQ indicates the lowest concentration that can be quantified with acceptable

levels of precision and accuracy. By statistical calculation LOD and LOQ was found to be 0.462 µg/ml and 1.400 µg/ml respectively.

3.5 Specificity and Selectivity

BBH has the highest absorbance at 428nm, indicating that the technique is specific and selective as represented in Table 4.

3.6 Precision

As stated in the procedure, three replicates of a solution containing 2µg/ml, 6µg/ml, and 10 µg/ml of BBH were created and the absorbance of each solution was measured at 428nm to determine system precision. The percent RSD was calculated, and it was found to be less than 2%.

3.6a Intraday precision

For intraday precision, three replicates of solution containing concentration 2µg/ml, 6µg/ml, 10 µg/ml of BBH analysed and %RSD was calculated at different time intervals on same day and %RSD was found to be less than 2% as given in Table 5.

3.6b Interday precision

Three replicates of a solution comprising concentrations of 2µg/ml, 6µg/ml, and 10 µg/ml of BBH were evaluated for Interday precision, and percent RSD was calculated on three consecutive days. Furthermore, the %RSD was determined to be less than 2% as represented in Table 6.

3.7 Accuracy

The accuracy of the analytical method was assessed through recovery studies conducted at three concentration levels: 50%, 100%, and 150% of the target sample amount. At each level,

sample solutions were prepared and analysed using a standardization technique. The measured amount of BBH in the samples aligned with the labelled content of the formulation. The average percent recovery was calculated to be 99.07% with individual recoveries ranging from 97.59% to 99.78% as shown in Table 7, demonstrating the high accuracy of the method.

However, it was observed that the %RSD exceeded 2%, indicating a degree of variability in the recovery values. Despite this, the method showed consistent quantification of BBH across different concentrations, confirming its reliability. These results support the effectiveness of the UV spectrophotometric method for the accurate and precise estimation of BBH in pharmaceutical products.

3.8 Ruggedness

Ruggedness of the proposed method was examined by having different analysts perform the procedure to test its reproducibility. The percentage Relative Standard Deviation (%RSD) for repeatability remained below 2% as shown in Table 8, confirming the method's robustness. The uniformity of results across different individuals indicates that slight variations in operating conditions or personnel do not significantly impact the method's performance. This demonstrates that the method is rugged and dependable, making it appropriate for routine quantitative analysis of BBH.

3.9 Robustness

The results of the robustness study are summarized in Table 9. The evaluation involved testing the method at two different wavelengths -426 nm and 430 nm- to assess its stability under slight variations in analytical conditions.

3.10 Force degradation studies

Forced degradation studies of Berberine Hydrochloride were carried out under oxidative, acidic, alkaline, thermal, and photolytic stress conditions and analyzed using UV–Visible spectrophotometry at predetermined wavelength of 428nm. Acidic degradation with hydrochloric acid showed an absorbance value of 0.089 as shown in Figure 9(a), while alkaline degradation using sodium hydroxide resulted in an absorbance of 0.082 as shown in figure (b), suggesting moderate degradation under both acidic and basic conditions. Under oxidative stress using hydrogen peroxide, absorbance was found to be 0.087, indicating degradation of drug as shown in figure(c). Thermal degradation studies conducted in hot air oven showed significantly higher absorbance of 1.516, indicating pronounced degradation due to heat exposure as shown in figure (d). Similarly, photolytic degradation under UV sunlight resulted in an absorbance of 1.457, confirming substantial degradation upon light exposure as shown in figure (e). Overall, results demonstrate that Berberine Hydrochloride undergoes degradation under all applied stress conditions, with maximum degradation observed under thermal and photolytic stress, while comparatively lower degradation was observed under oxidative, acidic, and alkaline conditions as shown in table 10 and overlay of degradation studies is represented in Figure 10.

3.11 Plasma Analysis

Plasma analysis of Berberine Hydrochloride was performed using UV–Visible spectrophotometry after protein precipitation. The treated plasma sample showed distinct absorbance value of 0.686 at 428nm, corresponding to predetermined λ_{max} of Berberine Hydrochloride, thereby confirming presence of drug in plasma. Very low absorbance (0.006) was recorded at 744 nm, indicating minimal interference from plasma constituents in higher wavelength region. Additionally, an absorption peak of 2.086 at 344 nm was observed, which

can be attributed to plasma matrix components or secondary electronic transitions as shown in Figure 11 . Overall, findings confirm that Berberine Hydrochloride can be effectively detected in plasma using UV–Visible spectrophotometry at 428 nm following suitable sample preparation.

3.12 AGREEprep Score for developed UV method

The AGREEprep evaluation and pictogram score (0.76) shown in Fig. 12 confirmed the green and eco-friendly nature of the developed UV spectrophotometric method for Berberine Hydrochloride (BBH). The method involved on-site analysis with minimal solvent consumption (0.01) and low reagent volume (5.0 mL), while 50–75% of the reagents and materials used were sustainable or renewable. Only 0.01 g of sample was required, and the analysis was completed within 15 minutes using a simple semi-automatic procedure with two or fewer steps. The method consumed low energy (1 kWh) and employed spectrophotometry, which is considered an environmentally safer analytical technique due to low waste generation and minimal chemical usage. No significant hazards were associated with the procedure. The higher green segments observed in the AGREEprep diagram indicated strong compliance with green analytical chemistry principles, as presented in Table 11. Overall, the developed method was found to be rapid, safe, sustainable, and suitable for routine analysis of BBH in bulk, formulation, and plasma samples.

DISCUSSION

The present study focused on development and validation of simple, cost-effective, and environmentally sustainable UV-spectrophotometric method for quantitative estimation of Berberine Hydrochloride (BBH). The method was systematically validated in accordance with ICH Q2(R1) guidelines, confirming its specificity, linearity, precision, accuracy, ruggedness, and robustness. A strong linear relationship between absorbance and concentration was

observed over the studied range, with high correlation coefficient ($R^2 = 0.9989$), demonstrating excellent linearity. The low percentage relative standard deviation (%RSD) values obtained from intraday and interday precision studies, along with satisfactory recovery results, further confirm the method's reliability, repeatability, and accuracy.

In comparison with previously reported analytical methods, proposed approach offers significant advantages in terms of simplicity, accessibility, and environmental sustainability. Earlier UV-spectrophotometric methods described by Chauhan et al. (2024) and Tavade et al. (2022) primarily focused on the estimation of BBH in complex matrices such as nanoparticles and polyherbal formulations [6,7]. Although effective, these methods often required additional sample preparation steps and the use of multiple solvents or reagents, thereby increasing analytical complexity, solvent consumption, and environmental burden. Similarly, chromatographic methods such as HPLC, reported by Wang et al. (2013) and Kamal et al. (2011), provide high sensitivity but involve costly instrumentation, larger solvent volumes, higher energy consumption, and greater chemical waste generation, which limits their routine use in resource-limited laboratory settings [8,9].

In contrast, method developed in present study employs methanol as sole solvent, involves minimal sample preparation, and relies on direct absorbance measurement at 428nm without need for derivatization or chromatographic separation. These characteristics inherently reduce solvent usage, analysis time, and waste generation. The greenness of method was quantitatively evaluated using the AGREEprep metric, yielding a score of 0.76, indicating good compliance with 12 principles of Green Analytical Chemistry. High AGREEprep scores were obtained for parameters such as direct analysis, avoidance of derivatization, minimal instrumentation, low energy consumption, and reduced waste production. Although methanol is not considered greenest solvent, its limited use and absence of additional hazardous reagents contribute to an overall favorable environmental profile. Importantly, many previously reported UV methods

did not assess greenness quantitatively, making present study more aligned with contemporary sustainability-focused analytical practices.

Forced degradation studies conducted under acidic, alkaline, oxidative, thermal, and photolytic stress conditions demonstrated that Berberine Hydrochloride undergoes degradation under all applied conditions, with pronounced degradation observed under thermal and photolytic stress. These results highlight sensitivity of BBH to heat and light, providing critical information for formulation development, storage, and stability evaluation. The ability of the method to detect degradation-induced changes without additional separation steps further reinforces its effectiveness as a stability-indicating and environmentally responsible analytical tool.

The applicability of developed method was further extended to biological matrices through plasma analysis following a simple protein precipitation procedure. The clear detection of BBH at its characteristic wavelength in plasma, with minimal interference from endogenous components, demonstrates the method's potential use in preliminary bioanalytical and pharmacokinetic studies. Notably, the plasma analysis was achieved without complex extraction techniques or excessive solvent use, thereby maintaining greenness of overall analytical process.

Method robustness was confirmed by evaluating minor variations in analytical conditions, including wavelength changes, as well as reproducibility across different analysts and instruments. These findings indicate that method remains stable, consistent, and reliable under varied laboratory conditions. The established limits of detection ($LOD = 0.462\mu\text{g/mL}$) and quantification ($LOQ = 1.400\mu\text{g/mL}$) further demonstrate adequate sensitivity for routine quality control analysis of BBH in bulk drug and pharmaceutical formulations.

Overall, this study addresses a validated, user-friendly, and environmentally responsible UV-spectrophotometric method that integrates analytical performance, stability assessment,

biological applicability, and greenness evaluation. The incorporation of AGREEprep metric strengthens sustainability assessment and supports growing emphasis on green analytical chemistry.

CONCLUSION

A UV-spectrophotometric method was successfully developed for estimation of Berberine Hydrochloride (BBH) and was found to be simple, sensitive, precise, and reproducible. Validation carried out in accordance with ICH guidelines demonstrated that method meets regulatory requirements in terms of accuracy, precision, and reliability.

The stability-indicating nature of method was established through forced degradation studies performed under acidic, alkaline, oxidative, thermal, and photolytic conditions. The method effectively detected degradation-induced changes, with greater susceptibility of BBH observed under thermal and light exposure. Furthermore, successful detection of BBH in plasma samples following simple protein precipitation step highlights the method's potential application in preliminary bioanalytical and pharmacokinetic studies.

Assessment of environmental impact using the AGREEprep metric yielded a greenness score of 0.76, indicating good conformity with principles of Green Analytical Chemistry. Minimal solvent usage, low energy requirements, and reduced waste generation contribute to the eco-friendly nature of the method. Overall, developed UV-spectrophotometric approach offers robust, economical, and environmentally responsible alternative for quantitative analysis of Berberine Hydrochloride in pharmaceutical and biological samples.

Acknowledgment

The authors would like to extend their gratitude to Yucca enterprise, Mumbai for generously providing of drug. We are also thankful to KAHER Dr. Prabhakar Kore Basic Science Research Center, Nehru Nagar, Belagavi, for their invaluable support and facilities.

Authors contributions

All authors contributed equally for a Green and Stability-Indicating UV-Spectrophotometric Method for Berberine Hydrochloride with Validation, Forced Degradation and Plasma Analysis., Tejaswini P. Maroor, Swati M karanda and S. Yuvraj developed and validated the method. Priya P. Shetti provided guidance for research work and assisted in reviewing literature. Tejaswini P. Maroor, Swati M karanda and S. Yuvraj contributed to writing and reviewing the manuscript. Tejaswini P. Maroor performed calculations presented in results section., Tejaswini P. Maroor, Priya P. Shetti, Swati M karanda, S. Yuvraj and Dr. A.M. Patel contributed to drafting manuscript. All authors read and approved final version of manuscript.

FUNDING

The study was carried out without financial assistance from any funding body.

Data availability

Data will be made available on request

DECLARATIONS

Ethical approval and consent to participate

This is not applicable, because this article does not contain any studies with human or animal subjects.

Consent for publication

Not applicable

Conflict of Interest

The authors declare no conflict of interest.

ABBREVIATIONS

BBH: Berberine hydrochloride; **µg:** Microgram; **mL:** Millilitre; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **UV:** Ultra violet spectrophotometer; **RSD:** Relative Standard Deviation; **ICH:** International Council of Harmonization.

Reference

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Factor Coding: Actual

3D Surface

Absorbance

Design Points:

● Above Surface
○ Below Surface
0.789 0.843

X1 = A

X2 = B

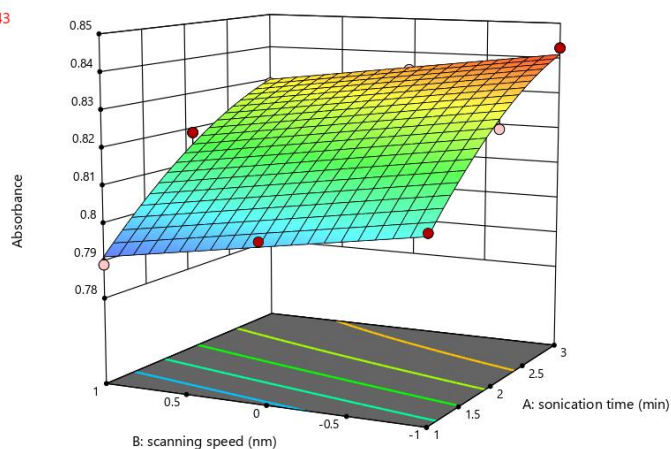


Figure 1. 3D plot of response of Berberine Hydrochloride

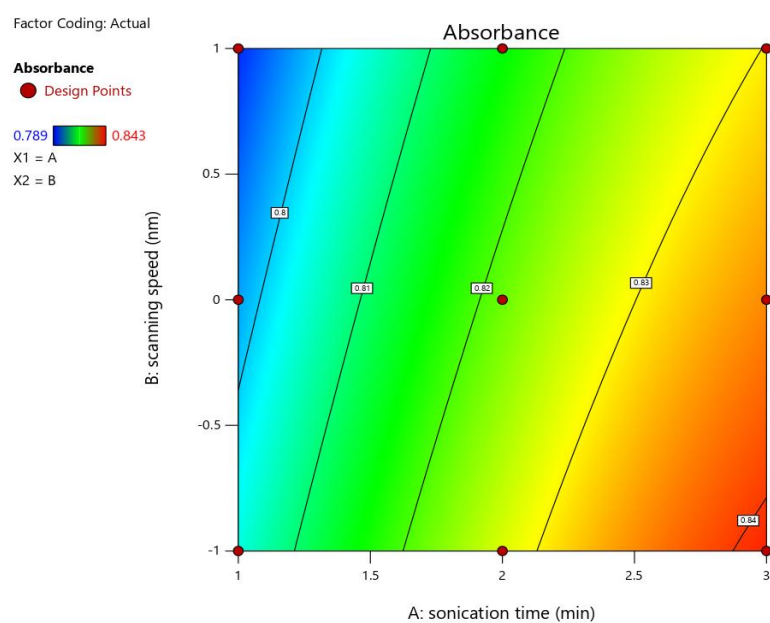


Figure 2. 2D contour plot showing the correlation between the selected variables i.e., sonication time and scanning interval with absorbance of Berberine Hydrochloride

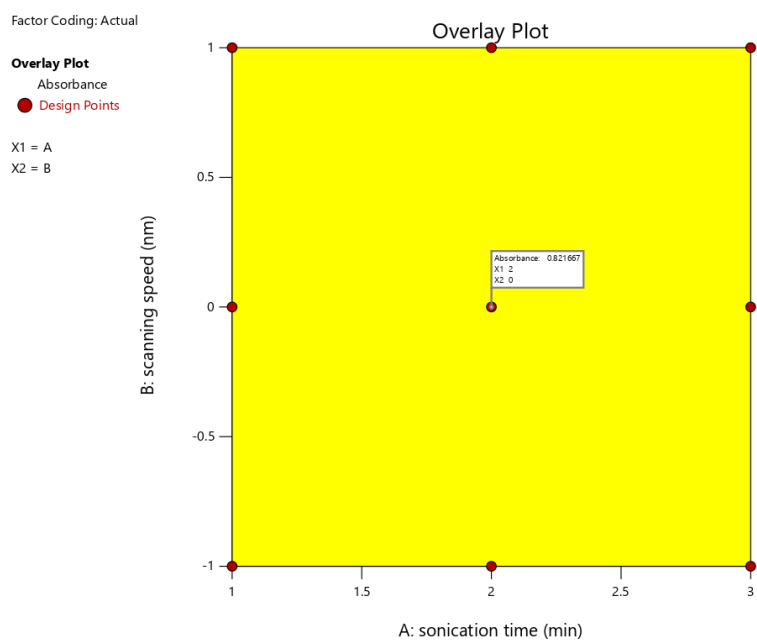


Figure 3. Overlay plot showing the design space from experimental area

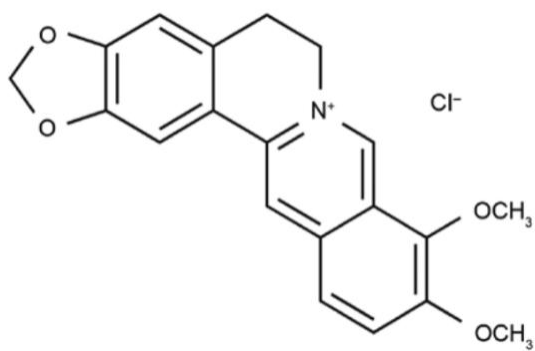


Figure 4: Chemical Structure of Berberine Hydrochloride

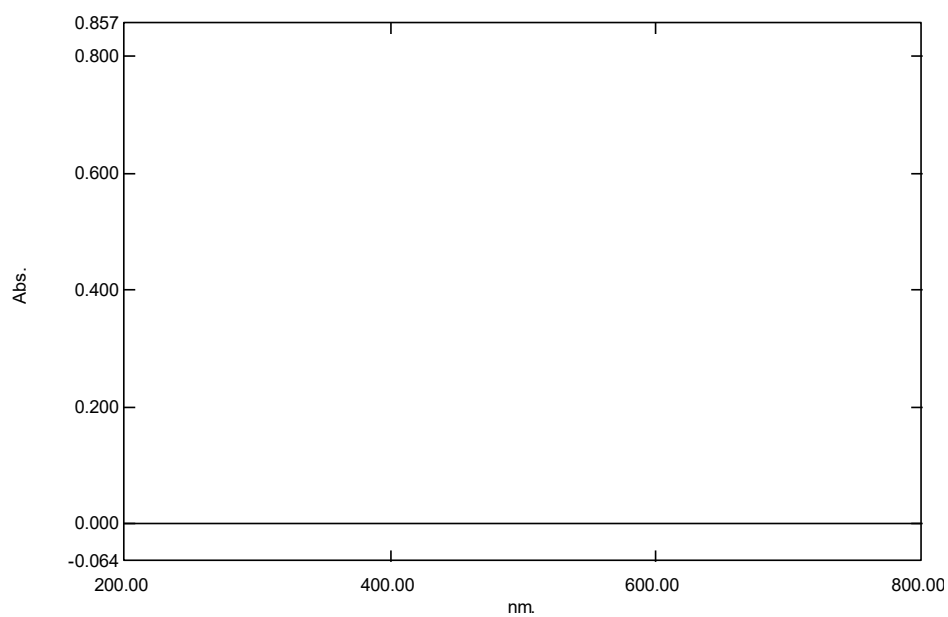


Figure 5: UV Spectrum of Methanol

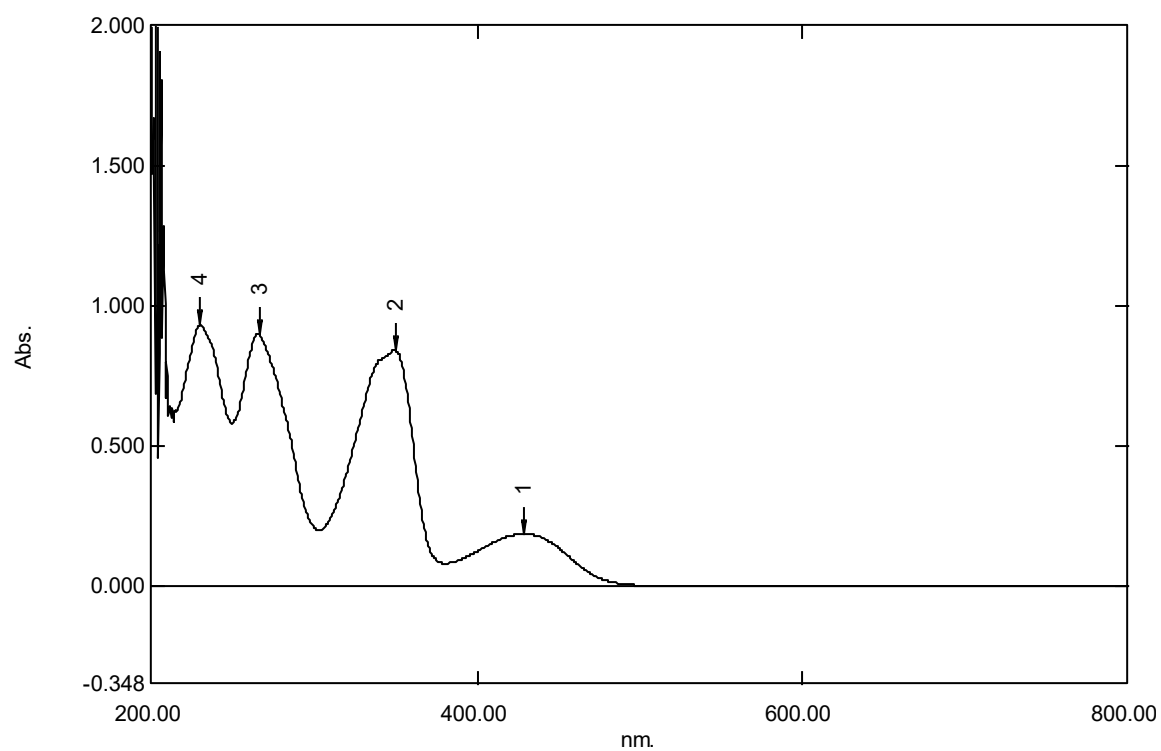


Figure 6: Spectra of Berberine Hydrochloride

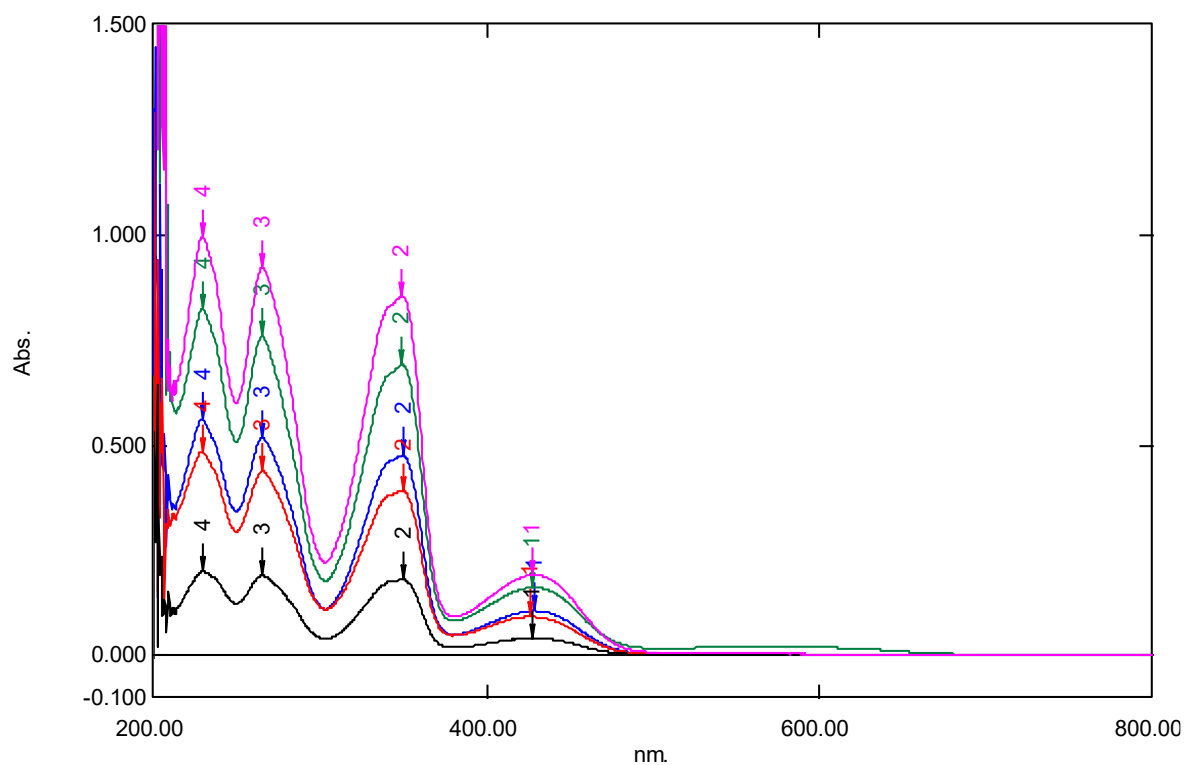


Figure 7: Overlay of Berberine Hydrochloride

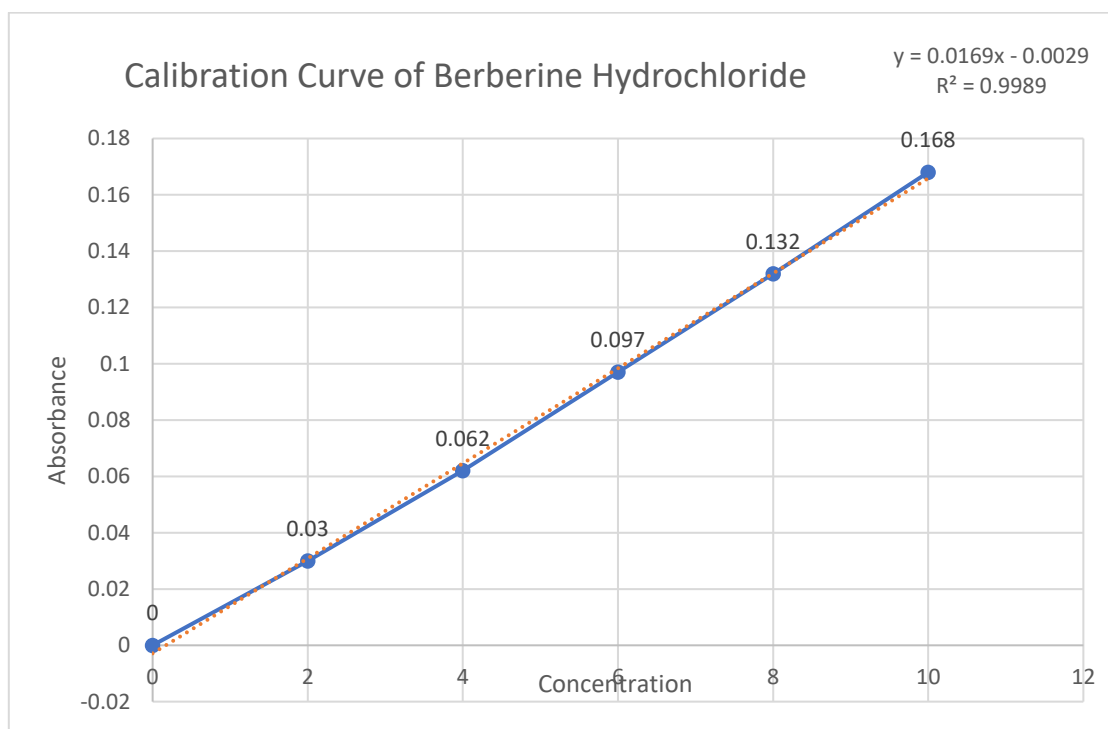


Figure 8: Linearity of Berberine Hydrochloride

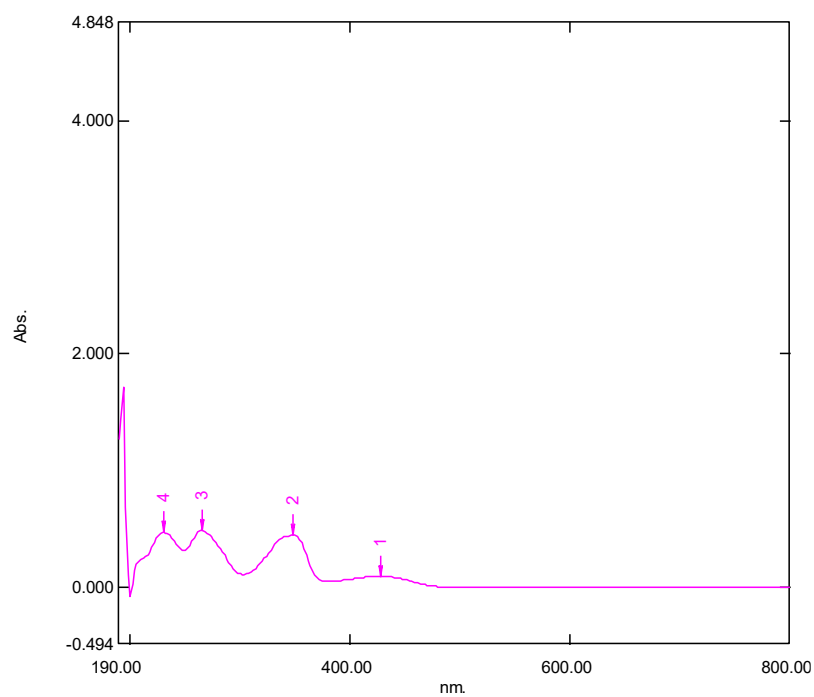
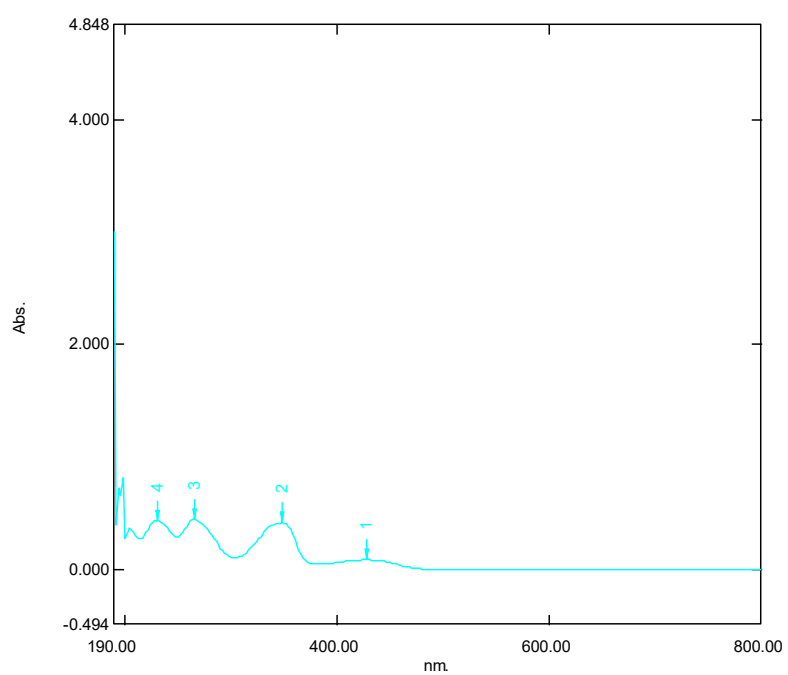
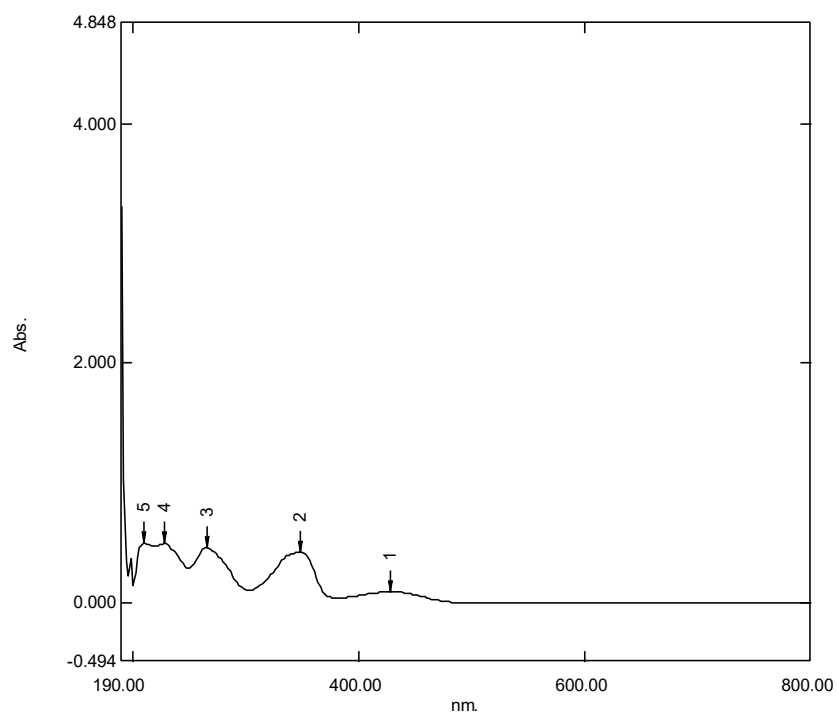


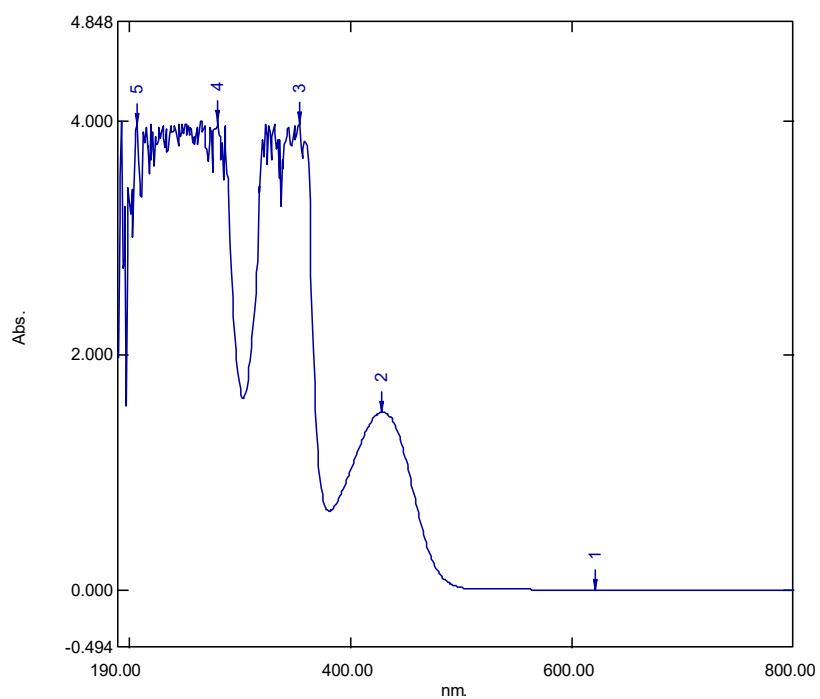
Figure 9: Force Degradation studies a) Acid degradation



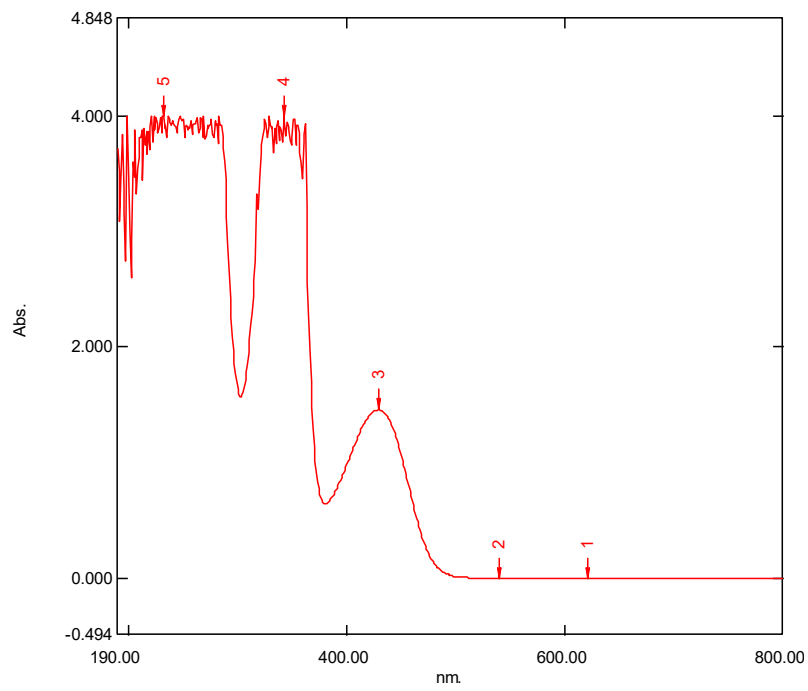
b) Alkaline degradation



c) Oxidative degradation



d) Thermal degradation



e) Photolytic degradation

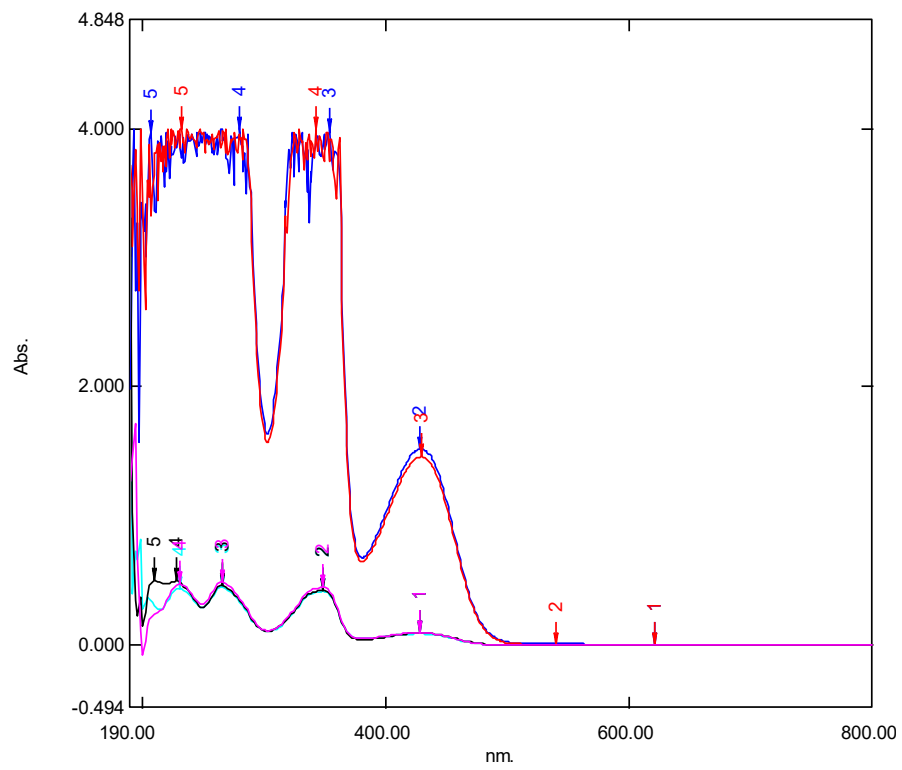


Figure 10: Overlay of force degradation studies

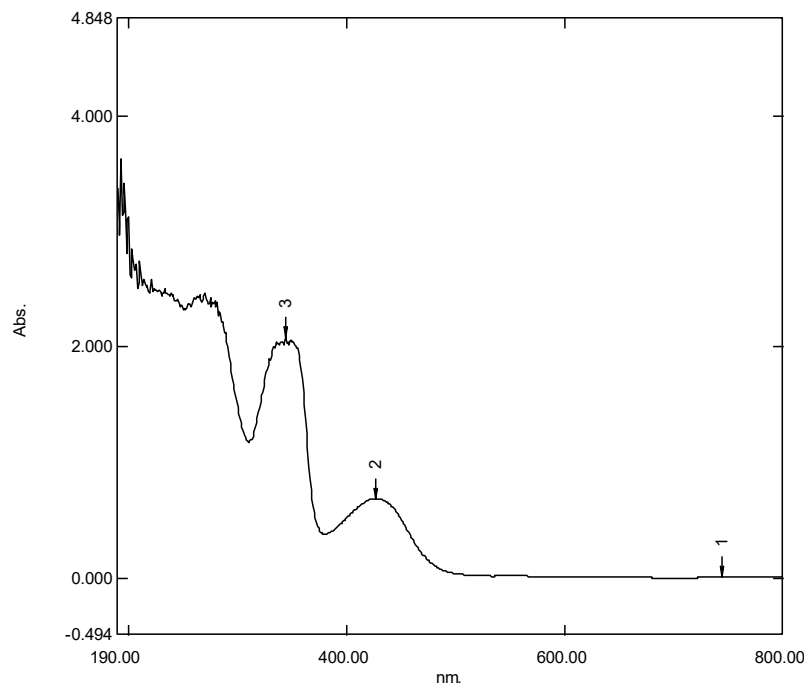


Figure 11: Plasma Analysis

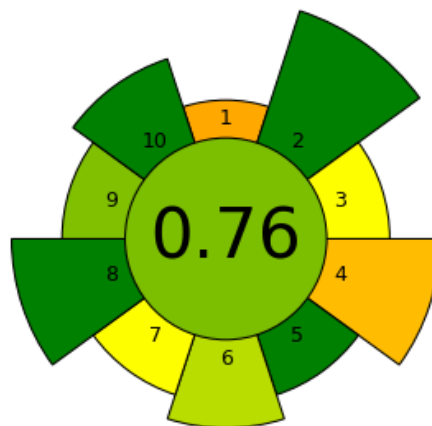


Figure 12: AGREEprep Analysis Graph for UV Method of BBH

Table 1 summarizes the design of experiments (DoE) runs and the corresponding responses

		Factor 1	Factor 2	Response 1
Std	Run	A: sonication time (min)	B: scanning speed (nm)	Absorbance
6	1	3	0	0.835
7	2	2	-1	0.825
9	3	2	0	0.821
8	4	2	1	0.819
3	5	1	1	0.789
2	6	3	-1	0.843
1	7	1	-1	0.805
4	8	3	1	0.829
5	9	1	0	0.799

Table 2 ANOVA analysis of response

Source	F-value	p-value	Remarks
Model	47.09	0.0047	Significant
A – Sonication time	209.61	0.0007	
B – Scanning speed	20.90	0.0196	
AB	0.096	0.7761	
A ²	4.83	0.1152	
B ²	0	1.000	

Table 3: Linearity of Berberine Hydrochloride

Concentration(µg/ml)	Absorbance
2	0.030
4	0.062
6	0.097
8	0.132
10	0.168

Table 4: Optical Characteristics

Statistical	Results
Absorption maxima	428nm
Correlation coefficient	0.9989
Regression equation	0.0169x - 0.0029
Slope	0.0168
Intercept	0.00236
LOD µg/ml	0.462 µg/ml
LOQ µg/ml	1.400 µg/ml

Table 5: Precision Intraday of Berberine Hydrochloride

Concentration(µg/ml)	Morning		Afternoon		Evening	
	Absorbance	%RSD	Absorbance	%RSD	Absorbance	%RSD
2	0.064	1.814	0.077	1.299	0.081	0.710
6	0.119	1.456	0.101	1.517	0.152	1.316
10	0.220	1.146	0.213	0.271	0.284	0.932

Table 6: Precision Interday of Berberine Hydrochloride

Concentration($\mu\text{g/ml}$)	Day 1		Day 2		Day 3	
	Absorbance	%RSD	Absorbance	%RSD	Absorbance	%RSD
2	0.074	1.351	0.068	0.845	0.057	1.754
6	0.117	0.984	0.116	1.313	0.123	0.471
10	0.171	1.170	0.224	0.683	0.258	0.224

Table 7: Accuracy of Berberine Hydrochloride

Concentration($\mu\text{g/ml}$)	level	Absorbance	% recovery
3	50%	0.058	97.5969238
6	100%	0.116	99.8309103
9	150%	0.172	99.7889451

Table 8: Ruggedness of Berberine Hydrochloride

Concentration($\mu\text{g/ml}$)	analyst	Absorbance	%RSD
1	I	0.1437	0.402
1	II	0.1777	0.860

Table 9: Robustness of Berberine Hydrochloride

Concentration($\mu\text{g/ml}$)	Wavelength(nm)	Absorbance	%RSD
1	426	0.132	1.154
1	428	0.141	1.418
1	430	0.140	1.646

Table 10: Force Degradation Studies

Stress Condition	Absorbance at 428 nm	Change in Absorbance (ΔA) *	Degradation Behavior
Control (Unstressed)	0.188	-	-
Acidic (HCl)	0.089	-0.099	Moderate Degradation
Alkaline (NaOH)	0.082	-0.106	Moderate Degradation
Oxidative (H ₂ O ₂)	0.087	-0.101	Moderate Degradation
Thermal (Hot Air Oven)	1.516	+1.328	Severe Degradation
Photolytic (UV Sunlight)	1.457	+1.269	Severe Degradation

* $\Delta A = A_t - A_0$

Table 11: AGREEprep Score Table for UV Method of BBH

Codes	Chosen
S1	On-site
S2	0.01
S3	50-75% of reagents and materials are sustainable or renewable
S4	5.0mL
S5	0.01g
S6	15
S7	2 step or fewer, Semiautomatic
S8	1kWh
S9	Spectrophotometry
S10	No hazard