RESEARCH PAPER

# Development And Validation Of A Uv Spectrophotometric Method For The Simultaneous Estimation Of Berberine Hydrochloride And Amphotericin B Loaded Mesoporous Silica Nanoparticles In Combination Therapy

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**Abstract**

Combination therapy has emerged as an effective strategy for enhancing therapeutic efficacy in microbial and parasitic infections. The present study aimed to develop and validate a simple, rapid, and economical uv spectrophotometric method for the simultaneous estimation of berberine hydrochloride (bbr) and amphotericin b (amb) loaded in mesoporous silica nanoparticles (msns). The method employed a simultaneous equation approach using absorbance measurements at 349.5 nm and 406 nm. Beer–Lambert's law was obeyed in the concentration range of 2–12 µg/ml with correlation coefficients greater than 0.999. The method was validated as per ich guidelines and showed excellent accuracy, precision, specificity, and sensitivity. The developed method was successfully applied to nanoparticle formulations and is suitable for routine quality control analysis.

**Keywords:** Uv Spectrophotometry, Berberine Hcl, Amphotericin B, Msns, Validation.

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## INTRODUCTION

Nanotechnology-based drug delivery systems have revolutionized pharmaceutical research by improving drug solubility, bioavailability, and targeted delivery. Among these systems, mesoporous silica nanoparticles (MSNs) are widely explored because of their high surface area, tunable pore size, and excellent drug loading capacity. These characteristics enable efficient encapsulation and controlled release of therapeutic agents.[1]

Berberine hydrochloride is a naturally occurring isoquinoline alkaloid obtained from several medicinal plants such as *Berberi's* species. It exhibits a wide range of pharmacological activities including antimicrobial, anti-inflammatory, antidiabetic, and antiprotozoal effects.[2] Because of its broad therapeutic potential, berberine hydrochloride has been investigated as an adjunct drug in various combination therapies. However, its clinical utility is limited by poor bioavailability.[3]

Amphotericin B is a polyene macrolide antifungal antibiotic widely used to traet systemic fungal infections and visceral leishmaniasis.[4] The drug acts by binding to ergosterol present in fungal cell membranes, resulting in pore formation and leakage of intracellular components, ultimately leading to cell death. Despite its efficacy, its use is associated with toxicity.[5]

Combination therapy with BBR and AMB has shown potential synergistic effects, improving therapeutic outcomes while reducing toxicity. Therefore, accurate and simultaneous quantification of both drugs in combined formulations is essential.[6]

Several analytical techniques have been reported for the individual determination of berberine hydrochloride and amphotericin B.[7-8] However, very few analytical methods are available for their simultaneous estimation in combined dosage forms. UV spectrophotometry is a simple, economical, and rapid analytical technique suitable for routine pharmaceutical analysis. Therefore, the present study aimed to develop and validate a UV spectrophotometric method based on the simultaneous equation approach for the quantitative estimation of berberine hydrochloride and amphotericin B in combined formulations.

## MATERIALS AND METHODS

**Materials**

Berberine hydrochloride was purchased from TCI Mumbai, India, and amphotericin B was a generous gift sample from a Sumar Biotech, LLP, Mehsana, Gujarat. Mesoporous silica nanoparticles were synthesized using a sol-gel method. DMSO, methanol and distilled water were used as analytical grade solvents for the preparation of the solutions. The prepared MSNP-BBR-AMB was used.

**Instruments**

Analysis was carried out using a UV-Visible spectrophotometer (Shimadzu, Pharma Spec-1700) equipped with matched quartz cells (1 cm path length). Analytical weighing was performed using a Denver, digital analytical balance. Ultrasonication was used to dissolve the drugs completely.

**PREPARATION OF STANDARD SOLUTIONS**

**Berberine Hydrochloride Stock Solution**

Accurately weighed 10 mg of berberine hydrochloride was transferred into a 10 mL volumetric flask and dissolved in a minimal quantity of DMSO. The volume was adjusted with methanol to obtain a stock solution of 10 mg/10 ml.

**Amphotericin B Stock Solution**

Similarly, 10 mg of amphotericin B was dissolved in a minimal quantity of DMSO, and the volume was subsequently adjusted with methanol to obtain a stock solution of 10 mg/10 ml.

Working standard solutions were prepared by appropriate dilution with methanol to obtain concentrations ranging from 2–20 µg/mL.

**SELECTION OF WAVELENGTH**

Individual standard solutions of berberine HCl and amphotericin B were scanned over the 200–500 nm range. In the overlay spectrum, the individual spectra of amphotericin B and berberine HCl were overlapped to identify all points of intersection. The observed intersection point in the overlay spectrum was at 359.0 nm. The highest intersection point was considered the isosbestic point for the analysis of drugs in the combined formulation. The UV spectra were superimposed to determine two wavelengths with a minimal overlap of 349.5 nm for berberine HCl and 406 nm for AMB. These wavelengths were selected for subsequent analysis using the simultaneous equation method.[9]

**PREPARATION OF CALIBRATION CURVE**

The absorbances of the prepared dilutions (2-12 µg/ml) were measured by using a UV-Visible spectrophotometer at the λmax of each drug. The absorbance obtained in tabulated form and the calibration curve were plotted. The calibration curve obeyed Beers-Lambert law in the concentration range 2-12 µl.[10]

**DETERMINATION OF ISOBEISTIC POINT**

To determine the isobeistic point, a stock solution (1 mg/ml) of pure amphotericin B, and berberine hydrochloride was prepared. The stock solutions were further diluted separately with methanol to obtain 2, 4, 6, 8, and 10 µg/ml solutions. The spectra of amphotericin B at 10 µg/ml and berberine HCl 10 µg/ml were measured at 406 and 349.5 respectively, and the data were saved. The isobeistic point of the two drugs was determined from the overlay curve obtained by using a UV-Visible spectrophotometer.[11]

**SIMULTANEOUS EQUATION METHOD**

The simultaneous equation method[12] allows the estimation of two components using their absorbances at two selected wavelengths:

$$C_{BBR} = \frac{(A_2 \cdot a_{AMB,\lambda 1}) - (A_1 \cdot a_{AMB,\lambda 2})}{(a_{BBR,\lambda 2} \cdot a_{AMB,\lambda 1}) - (a_{BBR,\lambda 1} \cdot a_{AMB,\lambda 2})}$$

$$C_{AMB} = \frac{(A_1 \cdot a_{BBR,\lambda 2}) - (A_2 \cdot a_{BBR,\lambda 1})}{(a_{BBR,\lambda 2} \cdot a_{AMB,\lambda 1}) - (a_{BBR,\lambda 1} \cdot a_{AMB,\lambda 2})}$$

where:

- $C_{BBR}$and $C_{AMB}$= are the concentrations of BBR HCl and AMB, µg/ml
- $A_1$and $A_2$= are the absorbances of the sample at 249.5 and 406 nm, respectively.
- $a_{BBR,\lambda 1}$, $a_{BBR,\lambda 2}$, $a_{AMB,\lambda 1}$, and $a_{AMB,\lambda 2}$= are the absorptivities of berberine HCl and amphotericin B at the respective wavelengths

**METHOD VALIDATION**

The developed UV spectrophotometric method was validated in accordance with the ICH Q2(R1) guidelines for linearity, accuracy, precision, specificity, and sensitivity.[13]

**LINEARITY**

Linearity was evaluated by preparing standard solutions of berberine hydrochloride (BBR) and amphotericin B (AMB) in the concentration range of 2–12 µg/mL. Absorbances were measured at 349.5 nm (BBR) and 406 nm (AMB). Calibration curves were constructed by plotting the absorbance versus the concentration.[13]

**ACCURACY (RECOVERY STUDY)**

The accuracy was determined using the standard addition method at three levels 80%, 100%, and 120%. Known amounts of standard drugs were added to the preanalyzed samples and the % recovery was calculated.[13]

**PRECISION**

The Precision was evaluated follows: repeatability (intraday), and intermediate precision (interday). Three concentrations (4, 6, and 8 µg/mL) were analyzed three times.[13]

**SPECIFICITY**

Specificity was assessed by analyzing pure drug solutions, combined drug formulations (MSNs), blanks (solvent). Spectral overlap and interference were evaluated.[13]

**LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)**

LOD and LOQ were calculated as follows:[14]

$$LOD = 3.3 \times \frac{\sigma}{S}$$

$$LOQ = 10 \times \frac{\sigma}{S}$$

where:

- σ = standard deviation of the intercept
- S = slope of the calibration curve

**RESULTS AND DISCUSSION**

**Linearity**

Linearity was established over the concentration range of 2–12 µg/mL for both drugs. The calibration curves exhibited excellent linear relationships with correlation coefficients greater than 0.999. The regression equations confirmed the proportionality between the absorbance and concentration. Both drugs showed excellent linearity in the range of 2–12 µg/mL, with correlation coefficients greater than 0.999.[15]

**Table: 1. Linearity data for amphotericin B and berberine HCl.**

| Conc. (µg/ml) | Amphotericin B (Absorbance) | Berberine HCl (Absorbance) |
|---|---|---|
| 0 | 0 | 0 |
| 2 | 0.12 | 0.199 |
| 4 | 0.261 | 0.39 |
| 6 | 0.404 | 0.57 |
| 8 | 0.526 | 0.723 |
| 10 | 0.648 | 0.906 |

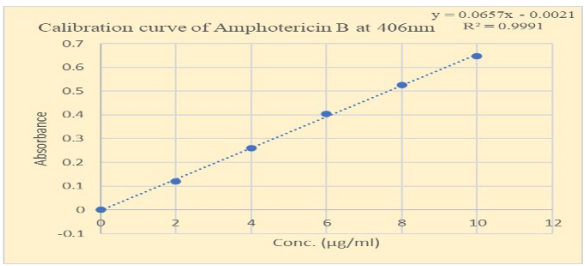


**Figure 1: Calibration Curve of Amphotericin B**

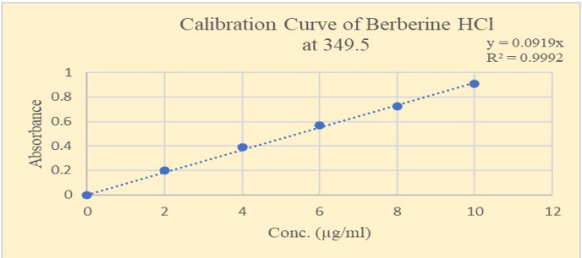


**Figure 2: Calibration Curve of Berberine Hydrochloride**

**Estimation of the content of drugs in the supernatant by simultaneous equations for berberine HCL and amphotericin B- loaded mesoporous silica nanoparticles:**

**Table: 2: Observations for Simultaneous estimation for Formulation B**

| Sr. no. | Drug | Conc. (µg/ml) | Absorbance λ1 406 nm | λ2 349.5 nm | Absorptivity (abs/conc.) λ1 nm | | λ2 nm |
|---|---|---|---|---|---|---|---|
| 1 | AMB | 0 | 0 | 0 | 0 | | 0 |
| | | 4 | 0.12 | 0.056 | 0.03 | | 0.014 |
| | | 6 | 0.261 | 0.123 | 0.0435 | | 0.0205 |
| | | 8 | 0.404 | 0.119 | 0.0505 | | 0.0149 |
| | | 10 | 0.526 | 0.301 | 0.0526 | | 0.0301 |
| | | Mean | | ax1 | 0.0353 | ax2 | 0.0795 |
| 2 | BBR | 0 | 0 | 0 | 0 | | 0 |
| | | 4 | 0.024 | 0.199 | 0.006 | | 0.0498 |
| | | 6 | 0.034 | 0.39 | 0.01 | | 0.065 |
| | | 8 | 0.055 | 0.57 | 0.00687 5 | | 0.0713 |
| | | 10 | 0.085 | 0.723 | 0.0085 | | 0.0723 |
| | | Mean | | ay1 | 0.0054 | ay2 | 0.0517 |
| 3 | Mixture | | A1 0.49 | A2 0.276 | | | |

**ax1 absorptivity of AMB at λ1**
**ax2 absorptivity of AMB at λ2**
**ay1 absorptivity of BBR at λ1**
**ay2 absorptivity of BBR at λ2**

$$\mathbf{Cx\ (AMB)} = \frac{\mathbf{A2ay1 - A1ay2}}{\mathbf{ax2ay1 - ax1ay2}}$$

$$\mathbf{Cy\ (BBR)} = \frac{\mathbf{A1ax2 - A2ax1}}{\mathbf{ax2ay1 - ax1ay2}}$$

**Cx** = 18.21257 µg

**Cy** = 7.052123 µg

Initial weight of MSNP taken = 500 µg

Amount of AMB taken initially= 100 µg

Amount of BBR taken initially = 200 µg

**Overlay Spectrum of Amphotericin B and Berberine HCL for Simultaneous Estimation of Drugs.**

In the overlay spectrum, the individual spectra of amphotericin B and berberine HCl were overlapped to identify all points of intersection. The observed intersection point in the overlay

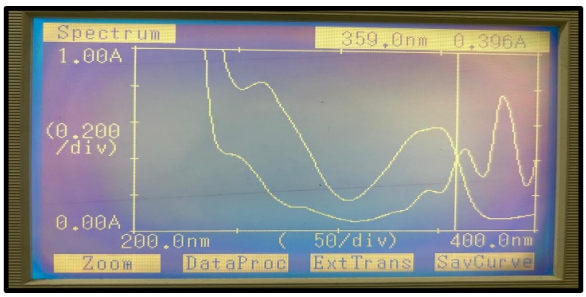


**Figure. 3: Overlay spectra of BBR and AMB with an intersection point at 359.0 nm**

spectrum was at 359.0 nm with an absorbance of 0.396 A, as shown in Figure 3. The highest intersection point was considered the isosbestic point for the analysis of drugs in the combined formulation.

**Accuracy**

The accuracy was evaluated by recovery studies using the standard addition method at 80%, 100%, and 120% levels. The percentage recovery for both drugs ranged between 98.50% and 100.66%, indicating high accuracy and absence of interference. Recovery values close to 100% indicate high accuracy.[16]

**Table 3. Accuracy Data for Amphotericin B**

| Level (%) | Amount Added (µg) | Amount Found (µg) | % Recovery |
|---|---|---|---|
| 80 | 8 | 7.92 | 99.00 |
| 100 | 10 | 9.95 | 99.50 |
| 120 | 12 | 12.05 | 100.41 |

**Mean Recovery = 99.63%**

**Table 4. Accuracy Data for Berberine HCl**

| Level (%) | Amount Added (µg) | Amount Found (µg) | % Recovery |
|---|---|---|---|
| 80 | 8 | 7.88 | 98.50 |
| 100 | 10 | 9.96 | 99.60 |
| 120 | 12 | 12.08 | 100.66 |

**Mean Recovery = 99.59%**

**Precision**

The results of precision studies demonstrated that the %RSD values for both the intraday and interday analysis were less than 2%, confirming that the method is precise and reproducible. The %RSD values were less than 2%, indicating good precision. %RSD values < 2% confirm that the method is precise and reproducible.[16]

**Table 5. Precision Data for Amphotericin B**

| Conc. (µg/mL) | Mean Absorbance | SD | %RSD |
|---|---|---|---|
| 4 | 0.261 | 0.003 | 1.15 |
| 6 | 0.404 | 0.004 | 0.99 |
| 8 | 0.526 | 0.005 | 0.95 |

**Table 6. Precision Data for Berberine HCl**

| Conc. (µg/mL) | Mean Absorbance | SD | %RSD |
|---|---|---|---|
| 4 | 0.390 | 0.004 | 1.02 |
| 6 | 0.570 | 0.006 | 1.05 |
| 8 | 0.723 | 0.007 | 0.96 |

**SPECIFICITY**

The method was found to be specific as no interference from excipients, solvents, or other components was observed at selected wavelengths. Distinct absorbance maxima allowed accurate simultaneous estimation.[17]

**Table 7: Specificity Observations**

| Sample Type | Observation |
|---|---|
| Blank | No absorbance at selected wavelengths |
| BBR | Peak at 349.5 nm only |
| AMB | Peak at 406 nm only |
| Mixture | No interference; both drugs quantified accurately |

**LOD and LOQ**

The low LOD and LOQ values indicate the high sensitivity of the method, making it suitable for detection and quantification at low concentrations. Low detection and quantification limits confirmed the sensitivity of the methods.[18]

**Table 8. LOD and LOQ**

| Drug | Slope (S) | SD (σ) | LOD (µg/mL) | LOQ (µg/mL) |
|---|---|---|---|---|
| AMB | 0.064 | 0.0021 | 0.108 | 0.327 |
| BBR | 0.089 | 0.0025 | 0.092 | 0.278 |

**Statistical Analysis**

**Regression Statistics**

**Table 9. Regression Statistics**

| Parameter | AMB | BBR |
|---|---|---|
| Slope | 0.064 | 0.089 |
| Intercept | 0.002 | 0.015 |
| $R^2$ | 0.9992 | 0.9995 |

**ANOVA (Linearity Validation)**

**Table 10. ANOVA for Calibration Curves**

| Source | SS | df | MS | F |
|---|---|---|---|---|
| **Regression** | High | 1 | High | >>100 |
| **Residual** | Low | 3 | Low | — |

The results indicate that very high F-values confirm a significant linear relationship and the residual error is minimal indicating excellent fit.

The developed UV spectrophotometric method is simple, accurate, precise, and economical for simultaneous estimation of berberine hydrochloride and amphotericin B. This method can be effectively used for routine quality control of nanoparticle-based formulations. The method was successfully applied to MSNs, confirming its practical applicability.

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