# Development, Validation, and Comparative Evaluation Spectrophotometric Methods for the Simultaneous Estimation of Silibinin and Bisdemethoxycurcumin Using Vierordt's and Q-Absorption Ratio Equations

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**Abstract**

An accurate, rapid, and economical ultraviolet (UV) spectrophotometric method was developed and validated for the simultaneous estimation of Silibinin and Bisdemethoxycurcumin (BDC) in binary mixtures and combined dosage forms. Method development was carried out on a Shimadzu UV-1700 instrument, where Silibinin and BDC exhibited absorption maxima (λmax) at 288 nm and 420 nm, respectively, with a mutually verified isoabsorptive (isobestic) point identified at 332 nm via an overlay spectrum. The analytical method was strictly validated according to the International Conference on Harmonization (ICH) Q2(R1) regulatory guidelines. Linear relationships were established over a concentration range of 5–30 µg/mL for Silibinin ($y = 0.0513x + 0.0058$; $R^2 = 0.9984$) and 10–60 µg/mL for BDC ($y = 0.0156x + 0.0036$; $R^2 = 0.9983$). Validation metrics under repeatability testing yielded exceptional precision, with average intraday relative standard deviations (%RSD) well below the 2.0% compliance ceiling (0.0038% for Silibinin and 0.0103% for BDC). Day-to-day intermediate precision trials confirmed long-term intermediate precision, returning average inter-day %RSD values of 0.0056% and 0.0061% for Silibinin and BDC, respectively. Method sensitivity was verified via Limit of Detection (LOD) and Limit of Quantification (LOQ) calculations, returning parameters of 3.89 µg/mL / 11.79 µg/mL for Silibinin and 2.49 µg/mL / 7.54 µg/mL for BDC. Accuracy was confirmed through recovery studies across 80%, 100%, and 120% concentration spikes, yielding quantitative recoveries of 95.35%–98.00% (Silibinin) and 98.2%–99.4% (BDC). Ruggedness testing across analysts and robustness profiling under temperature shifts (25°C to 30°C) verified the mechanical stability of the assay. A comparative evaluation between the Simultaneous Equation Method and the Q-Absorption Ratio Method highlighted a distinct methodological divergence under identical mixture environments, offering critical structural insights into multi-component mathematical resolutions for quality control applications.
**Key words:** Silibinin, Bisdemethoxycurcumin, Simultaneous Equation Method, Q-Absorption Ratio Method

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## Introduction

In modern pharmaceutical analysis and quality control, the accurate quantification of multi-component therapeutic systems introduces a significant analytical challenge when active pharmaceutical ingredients (APIs) are combined in a single formulation. Simultaneous drug delivery has gained widespread popularity due to the clinical synergy achieved by combining compounds with complementary therapeutic pathways.1 However, tracking the individual concentration profiles of multiple drugs within a binary mixture often requires complex processing. Ultraviolet-Visible (UV-Vis) Spectrophotometry remains a benchmark technique, providing a rapid, non-destructive, cost-effective, and highly accessible platform for routine quantitative testing.2

In the case of simple binary mixtures like Silibinin and BDC, physical column separation is rendered unnecessary through the application of mathematically driven spectro-resolutions.3 By exploiting discrete absorptivity constants and shared isoabsorptive coordinates, the Simultaneous Equation and Q-Absorption Ratio methods

successfully isolate overlapping electronic transitions, offering a rapid, cost-effective, and waste-free quantitative alternative that meets strict regulatory thresholds without chromatographic intervention.4

A major hurdle in applying basic UV-Vis spectrophotometry to multi-component systems is the severe overlap of absorption bands, which makes single-wavelength calibration protocols entirely unusable without prior physical or chemical separation.5 To resolve these overlapping electronic spectra mathematically, algebraic multi-component frameworks must be established. Foremost among these approaches are the Simultaneous Equation (Vierordt's) Method and the Q-Absorption Ratio (Absorbance Ratio) Method.6 The Simultaneous Equation method operates by selecting the specific wavelengths of maximum absorption (λmax) for both target compounds. By determining the individual absorptivity constants at these discrete wavelengths, a system of linear equations can be resolved to calculate individual drug concentrations directly from an unseparated mixture.7

As an alternative modification, the Q-Absorption Ratio method capitalizes on the fundamental principle that for any chemical substance adhering strictly to the Beer-Lambert law, the ratio of absorbances measured at any two chosen wavelengths represents a fixed constant that is entirely independent of concentration or light path length.8 This technique relies on identifying a shared isoabsorptive (isobestic) point the specific wavelength where the molar absorptivities of both components intersect alongside the primary λmax of one of the drugs. This approach allows the total concentration of the mixture to be calculated from a single anchor point before being apportioned using the sample ratio, minimizing potential calibration errors.9

The target compounds optimized in this investigation are Silibinin and Bisdemethoxycurcumin (BDC), two naturally occurring polyphenolic compounds possessing potent, complementary medicinal profiles 10. Silibinin, the primary active component extracted from milk thistle (Silybum marianum), is widely celebrated for its powerful hepatoprotective, antioxidant, and anti-inflammatory properties. Bisdemethoxycurcumin (BDC), a major and structurally stable curcuminoid derived from turmeric (Curcuma longa),11 exhibits outstanding anti-cancer, neuroprotective, and anti-inflammatory capabilities.12 Given their overlapping therapeutic applications, developing a dual formulation containing both components represents a highly promising pharmaceutical strategy. However, their structural similarities result in closely aligned chromophores that interfere during standard spectrophotometric testing. This research project outlines the development, validation, and comparative analysis of a highly sensitive UV spectrophotometric protocol for the simultaneous estimation of Silibinin and BDC in binary mixtures under rigorous ICH Q2(R1) criteria.13

## 2. Materials and Methods

### 2.1 Instrumentation and Reagents

All spectrophotometric measurements were executed on a double-beam UV-Visible Spectrophotometer (Model: UV-1700, Shimadzu, Kyoto, Japan) fitted with a spectral bandwidth of 1 nm and a pair of matched 10-mm path length quartz cuvettes. Reference standards of Silibinin and Bisdemethoxycurcumin (BDC) were evaluated for purityand purchased from Sigma Aldrich. Analytical-grade Methanol and freshly prepared Phosphate Buffered Saline (PBS) at pH 7.4 were deployed as primary dissolution vehicles. For qualitative visibility screenings, tight-closure glass vials were shaken using a calibrated vortex mixer against a standardized matte black background to guarantee absolute homogeneity and check for baseline particulates.

### 2.2 Method development

#### 2.2.1 Calibration graph

In this the procedure the absorbance's of number (n=6) of the standard solutions of the reference substance at the concentrations encompassing the sample concentrations are measured and calibration curve constructed. Absorption spectra of the individual active constituents in solvent were determined on UV-visible spectrophotometer , model : UV-1700, Shimadzu ,Japan.14

#### 2.2.2 Preparation of standard stock solution

The standard dilution of the active constituent silbinin and BDC having concentration 1000 µg/ml was prepared by dissolving accurately weighed 10mg of the each standard sample in the 10ml volumetric flask (calibrated) containing PBS buffer 7.4 as a solvent. 14

#### 2.2.3 Preparation of standard dilution

From the above standard stock solution 100 µg/ml prepared by withdrawing 1 ml of the stock solution and diluted with PBS buffer 7.4 up to 10ml , from this sub stock solution aliquots were prepared of the concentrations 5 µg/ml to 30 µg/ml by taken the 0.5 ml to 3ml of silbinin and 10 µg/ml to 60 µg/ml of BDC were diluted by methanol and PBS buffer 7.4 up to 10ml. The absorption maxima was obtaining by scanning the appropriate dilution in the range of the 200-400nm. After the scanning the lambda max of silbinin at 288 nm, and BDC at 420 nm.15

### 2.3 Validation of method

UV spectroscopy method validation required for the drug in order to analyzing the suitability for the required purpose which ensuring accuracy and reliability of the analytical results. The validation of the drug process involves parameters such as linearity , precision , accuracy , detection limits and quantification limit according to the guideline like ICH Q2 (R1).15,16

**2.3.1 Linearity**

Linearity help in determining range over which the method provides a linear relationship between the concentration of the drug and the UV absorbance signal. For determining the linearity series of the standard solution with varying concentration were prepared, the concentrations of the serial dilution were mentioned above. And the linear equation was found via calibration graph.15

**2.3.2 Precision**

Interday, intraday and inter analyst variations were studied to determine the repeatability (n=3). For the precision study different dilutions were prepared in triplicate by two different analysts at two different times during one day and analyzed intraday variations. The same procedure was applied by the inter-days variation which was followed for two different days to study inter-day an inter analyst variations. The %RSD (relative standard deviation) of the predicted concentrations from the regression equation, the %RSD should be ≤2.0%.15

**2.3.3 Sensitivity**

In this method of validation the sensitivity of the system and method calculated with the help of LOD (limit of detection ) and LOQ (limit of quantification). The LoD and LOQ were calculate directly by calibration curve by taking the mean slope and the SD intercept 15,16

**LOD=3.3σ/S**

**LOQ=10σ/S**

Where σ represent standard deviation and S represent the slope of the calibration curve**.**

**2.3.4 Accuracy**

Accuracy expresses the closeness in the measurement to the true value or accepted value.It was performed by spiking (addition) the required amount of analyte into the sample and analyzing spike sample to report found value . The % recovery was calculated by the addition of the concentration and calculated by the formula given below:16

$$\%\,\boldsymbol{recovery} = \frac{\boldsymbol{amount\ found}}{\boldsymbol{amount\ added}}\ \mathbf{X100}$$

**2.3.5 Ruggedness**

The ruggedness of the method was determined by carrying out the analysis using two different analysts and the respective absorbance was noted. Ruggedness of the methods was assessed by carrying out assay 3 reading with different analyst by using same equipment.15

**2.3.6 Robustness**

To determine the robustness, the same procedure was carried out by changing the temperature and the result was compared with the same previous procedure.15

**2.4 Simultaneous estimation by uv spectroscopy**

UV visible spectrophotometer Shimadzu -1700) was used to perform simultaneous estimation both of the drugs **.**The stock solution having a concentration of 100µg/ml of both the drugs ( silbini , bis-demethoxycurcumin and fenoprofen) was prepared separately by dissolving the 10mg in 100 ml of methanol **.**The absorption maxima (λmax) were obtained by scanning the appropriate dilution in the range of 200-400 nm . The λmax of the drugs were found to be 288 nm (silibinin) and 420 nm (BDC), respectively**.**From the standard stock solution 100 µg/ml prepared by withdrawing 1 ml of the stock solution and diluted with PBS buffer 7.4 upto 10ml , from this sub stock solution aliquots were prepared of the concentrations 5 µg/ml to 60 µg/ml by taken the 0.5 ml to 6ml and diluted by PBS buffer 7.4 upto 10ml. Working standard were separately scanned in the range of 400-200 nm . The spectra of Silbinin and BDC was recorded . The overlay spectrum was used to decide the isoabsorptive point for analysis.

**5.7.5 Simultaneous equation method formula**

By using the below equations the concentrations in the samples were obtained

CX= A1ay2 – A2ay1 / ax1ay2 – ax2ay1 Eq. 1

CY = A1ax2 – A2ax1 / ay1ax2 – ay2ax1 Eq. 2

Where A1 and A2 are absorbances of mixture at 288 nm and 420 nm respectively, ax1 and ax2 are absorptivities of one drug at λ1 and λ2 respectively, ay1 and ay2 were absorptivities of other drug at λ1 and λ2 respectively, Cx and Cy were concentrations of Silbinin and BDC , BDC and fenoprofen , Silbinin and fenoprofen respectively.

A1 = ax1Cy + ay1Cx (At λ1 nm)

A2 = ax2Cx + ay2Cy (At λ2 nm)

A1 = ax1Cy + ay1Cx (At λ1 nm)

A2 = ax2Cx + ay2Cy (At λ2 nm)

A1 = absorbance value of the sample solution at λ1 nm

A2 = absorbance value of the sample solution atλ2nm

ax1 = absorptivity of first drug atλ1nm

ax 2 = absorptivity of first drug atλ1nm

ay1 = absorptivity of second drug at λ2 nm

ay2 = absorptivity of second drug at λ2nm

Cy = concentration of the second drug in µg/ml

Cx = concentration of the first drug in µg/ml

**2.5 Absorption ratio method**

The absorbance ratio method is a modification of the simultaneous equation procedure . It depends on the property that , for a substance which obeys Beers Law at all wavelengths , the ratio of absorbance at any two wavelengths is a constant value independent of concentration or path length. Two wavelengths of a drug Silbinin (288 nm ) and , BDC(420 nm) and wavelength of isobestic point were selected for estimation of Silbinin (288 nm ) and BDC(420 nm) for Q-Absorption ratio method.

**2.5.1 Absorption ratio calculation**

Concentration of the drugs were determined using the formula mentioned below:

**Cx= (Qm-Qy/Qx-Qy) X A1/ax1……..**

**Cy= ( Qm-Qx/Qy-Qx ) X A1/ay1……**

Qx=ax2/ax1 ;Qy = ay2/ay1 and Qm =A2/A1

Cx: concentration of the drug(µg/ml)

Cy : concentration of the second drug (µg/ml)

A: Absorbance of sample at isoabsorptive point

A: absortivity of drug
Qm : ratio of the absorbance of the ample to drug
Qx: ratio of absorbance of Silbinin to the absorbance at isobestic point
Qy: ratio of absorbance of BDC to the absorbance at isobestic point

**2.6 Result**

**2.6.1 Method Development Via UV spectrophotometer Silbinin and BDC**

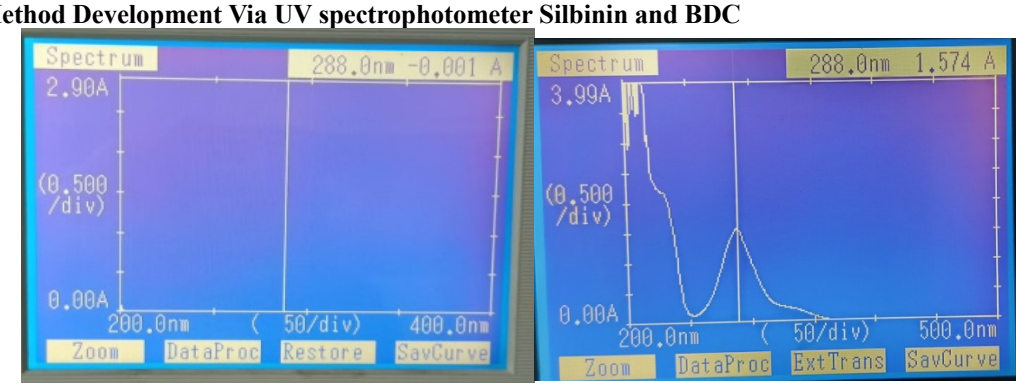



**Fig 1 :Blank solvent for the drug at 288 nm and UV Spectra of silbinin at 288 nm**

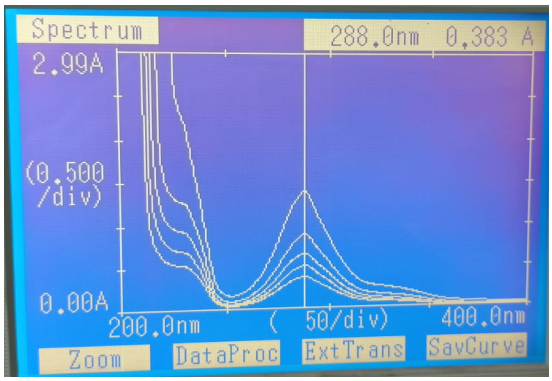
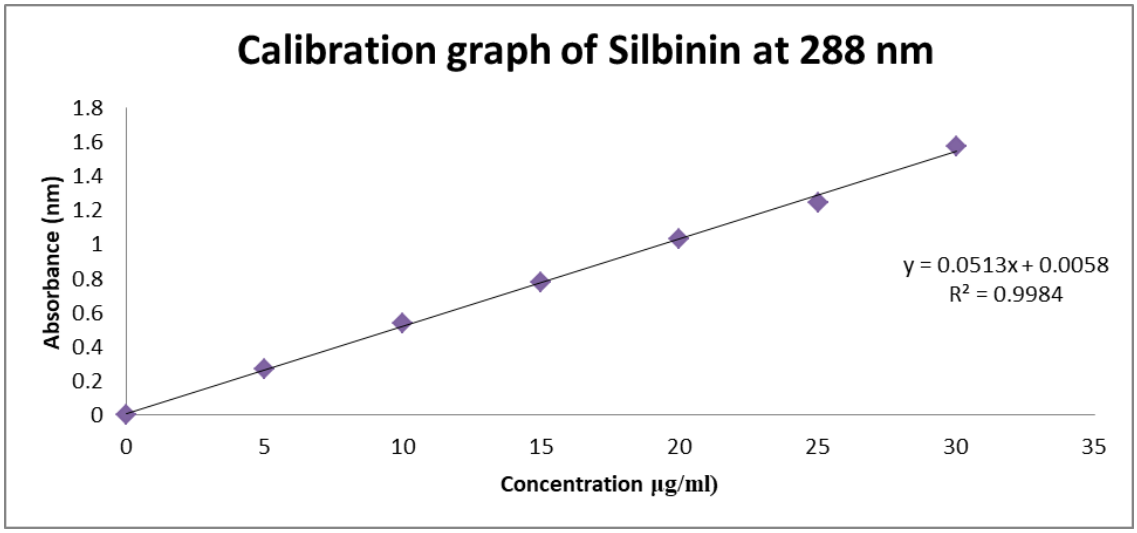



**Fig 2 : Serial dilution of silbinin at 288 nm**

**Table No. 1: Absorbance of silbinin at 288 nm**

| **Concentration (µg/ml)** | **Absorbance at lambda 288nm (Silbinin)** |
|---|---|
| 5 | 0.267 |
| 10 | 0.532 |
| 15 | 0.775 |
| 20 | 1.029 |
| 25 | 1.246 |
| 30 | 1.574 |
| Mean | 0.903667 |
| SD | 0.477828 |
| %RSD | 52.8766 |




**Graph 1 : Calibration graph of the silbinin at 288 nm**

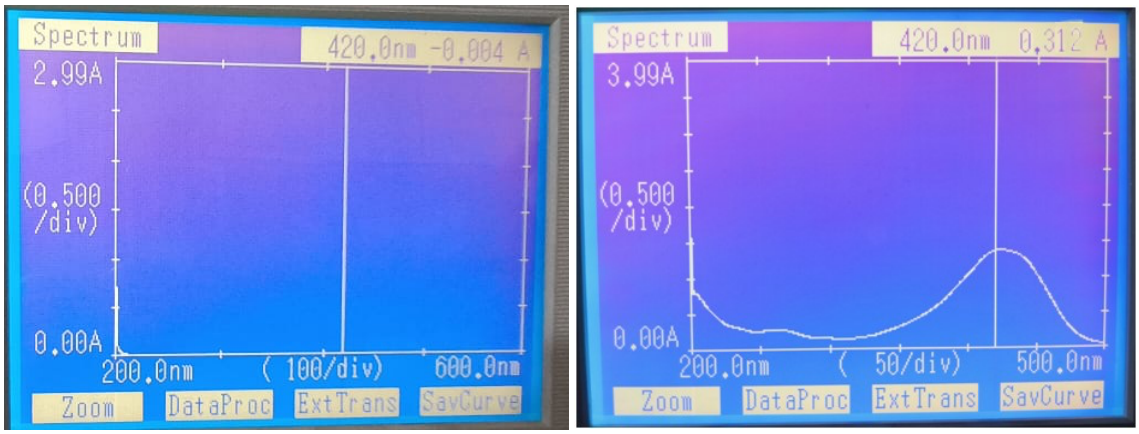



**Fig 3 : Blank solvent for the drug at 420 nm and UV Spectra of BDC at 420 nm**

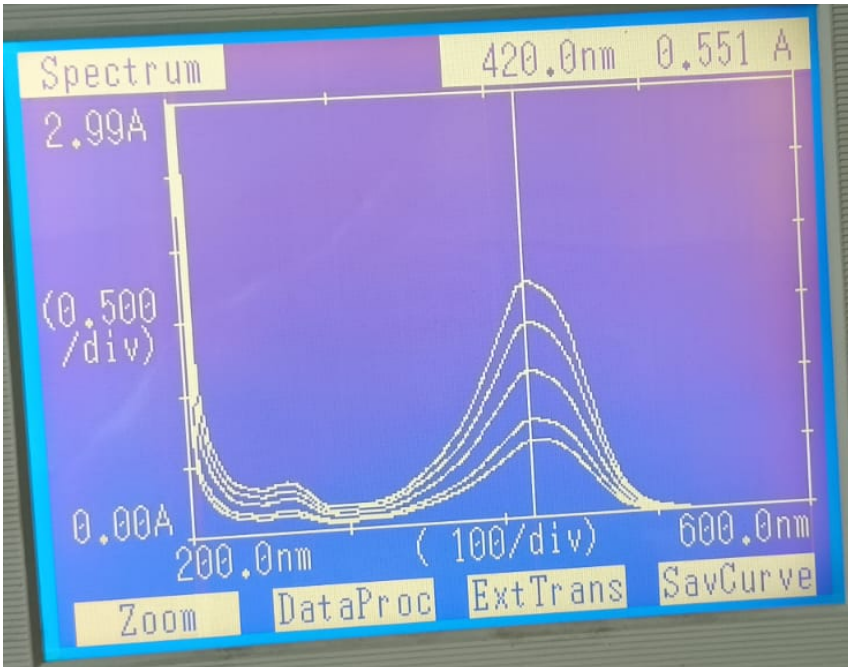
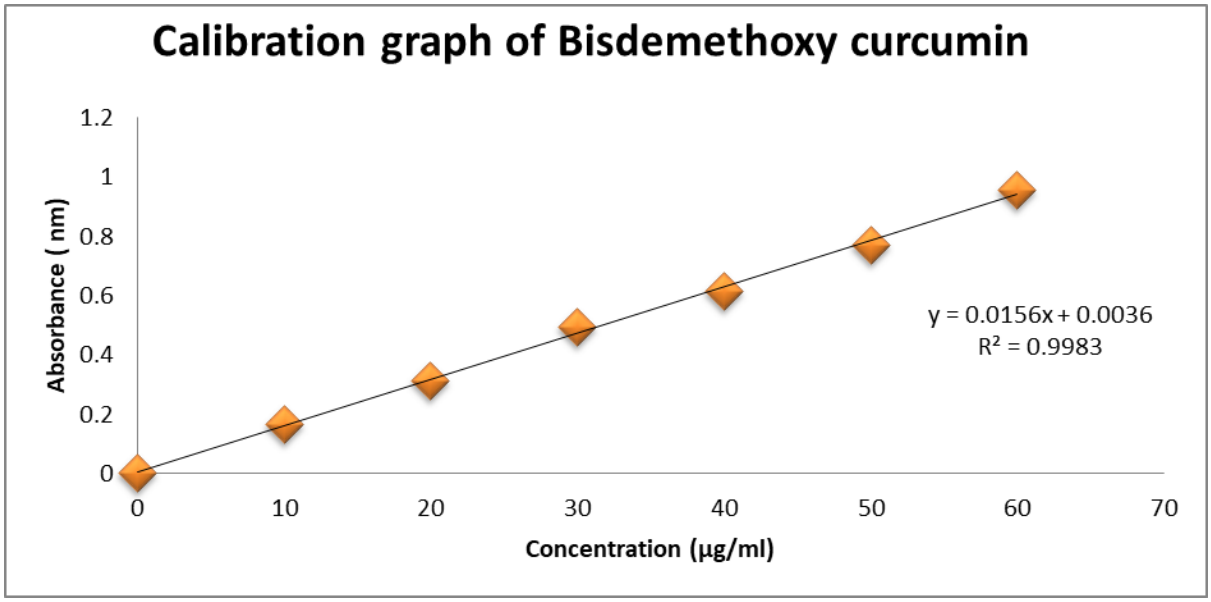



**Fig 4: Serial dilution of BDC at 420 nm**

**Table No. 2: Absorbance of BDC at 420 nm**

| Concentration (µg/ml) | Absorbance at lambda 420nm (BDC) mean |
|---|---|
| 10 | 0.165 |
| 20 | 0.312 |
| 30 | 0.494 |
| 40 | 0.613 |
| 50 | 0.771 |
| 60 | 0.956 |
| Mean | 0.551833 |
| SD | 0.291764 |
| %RSD | 52.8717 |




**Graph 2 : Calibration graph of the BDC at 420 nm**

**2.6.2 Method Validation**

**2.6.2.3 Precision**

**Table 3: Intraday Precision of silbinin and BDC**

| Conc.(µg/ml) | Silbinin | | | BDC | | |
|---|---|---|---|---|---|---|
| | Abs I | Abs II | Abs III | Abs I | Abs II | AbsIII |
| 10 | 0.532 | 0.529 | 0.531 | 0.165 | 0.166 | 0.168 |
| 10 | 0.533 | 0.531 | 0.534 | 0.169 | 0.169 | 0.166 |
| 10 | 0.535 | 0.533 | 0.536 | 0.168 | 0.17 | 0.167 |
| Mean | 0.533333 | 0.531 | 0.53366667 | 0.167333 | 0.16833333 | 0.167 |
| SD | 0.001528 | 0.002 | 0.00251661 | 0.002082 | 0.00208167 | 0.001 |
| % RSD | 0.00286 | 0.0037665 | 0.0047157 | 0.01244 | 0.0123663 | 0.00599 |
| AVG % RSD | 0.003782096 | | | 0.010264864 | | |

**Table 4: Inter-day Precision of silbinin and, BDC**

| Conc. (µg/ml) | Silbinin | | | BDC | | |
|---|---|---|---|---|---|---|
| | **Day I** | **Day II** | **Day III** | **Day I** | **Day II** | **Day III** |
| **10** | 0.532 | 0.521 | 0.511 | 0.165 | 0.161 | 0.159 |
| 10 | 0.535 | 0.526 | 0.517 | 0.167 | 0.163 | 0.16 |
| 10 | 0.531 | 0.524 | 0.519 | 0.166 | 0.162 | 0.161 |
| Mean | 0.532667 | 0.52366 | 0.515666 | 0.166 | 0.162 | 0.16 |
| SD | 0.002082 | 0.00251 | 0.004 | 0.001 | 0.001 | 0.001 |
| % RSD | 0.00391 | 0.00480 | 0.008 | 0.0060 | 0.0061 | 0.00625 |
| AVG % RSD | 0.005595816 | | | 0.006148979 | | |

**2.6.2.4 Sensitivity**

**Table 5: LOD and LOQ**

| Sample | LOD | LOQ |
|---|---|---|
| Silbinin | 3.89 | 11.79 |
| BDC | 2.49 | 7.54 |

**2.6.2.5 Accuracy**

**Table 6 a : silbinin Accuracy via % recovery**

| Accuracy level% | Actual amount ( µg/ml) | Amount added ( µg/ml) | cumulative amount | Amount recovered ( µg/ml) | % Recovery |
|---|---|---|---|---|---|
| 0 | 10 | 0 | 10 | 9.86 | 98 |
| 80 | 10 | 8 | 18 | 12.75 | 97.9 |
| 100 | 10 | 10 | 20 | 19.07 | 95.35 |
| 120 | 10 | 12 | 22 | 21.01 | 95.5 |

**Table 6.b: BDC Accuracy via % recovery**

| Accuracy level% | Actual amount ( µg/ml) | Amount added ( µg/ml) | cumulative amount | Amount recovered ( µg/ml) | % Recovery |
|---|---|---|---|---|---|
| 0 | 10 | 0 | 10 | 9.8 | 98.6 |
| 80 | 10 | 8 | 18 | 11.93 | 99.4 |
| 100 | 10 | 10 | 20 | 19.86 | 99.3 |
| 120 | 10 | 12 | 22 | 21.6 | 98.2 |

**2.6.2.6 Ruggedness**

**Table no. 7: Result of ruggedness of silbinin , BDC and Fenoprofen**

| Concentration (µg/ml) | Silbinin | | BDC | |
|---|---|---|---|---|
| | **Analyst I** | **Analyst II** | **Analyst I** | **Analyst II** |
| 10 | 0.532 | 0.533 | 0.165 | 0.164 |

| | | | | |
|---|---|---|---|---|
| 10 | 0.535 | 0.534 | 0.167 | 0.163 |
| 10 | 0.536 | 0.536 | 0.168 | 0.167 |
| Mean | 0.534333 | 0.53433333 | 0.16666667 | 0.164667 |
| SD | 0.002082 | 0.00152753 | 0.00152753 | 0.002082 |
| % RSD | 0.0039 | 0.0028587 | 0.0091652 | 0.01264 |
| AVG % RSD | 0.003377284 | | 0.010903424 | |

**2.6.2.7 Robustness**

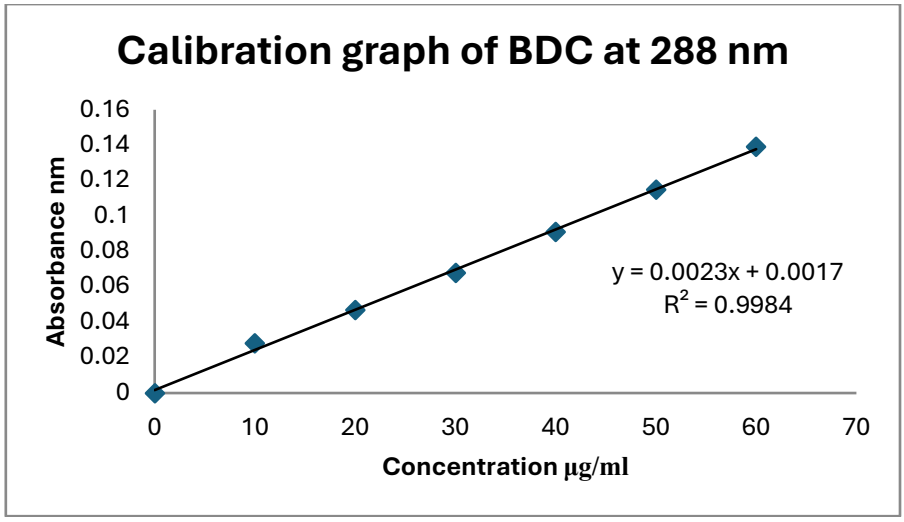
**Table no.8: Results showing robustness of silbinin , BDC and Fenoprofen**

| **Concentration (µg/ml)** | **Silbinin** | | **BDC** | |
|---|---|---|---|---|
| | **Temp 25$^0$C** | **Temp 30$^0$C** | **Temp 25$^0$C** | **Temp 30$^0$C** |
| **10** | 0.532 | 0.525 | 0.165 | 0.161 |
| 10 | 0.528 | 0.521 | 0.162 | 0.158 |
| 10 | 0.535 | 0.528 | 0.168 | 0.164 |
| Mean | 0.532 | 0.525 | 0.165 | 0.161 |
| SD | 0.0035 | 0.0035 | 0.003 | 0.003 |
| % RSD | 0.66% | 0.67% | 1.82% | 1.86% |
| AVG % RSD | **0.67%** | | **1.84%** | |

**2.6.2.8 Simultaneous and Q Absorption Ratio Method Silbinin and BDC**

**Table no. 9 : Absorbance of BDC at lambda 288 nm**

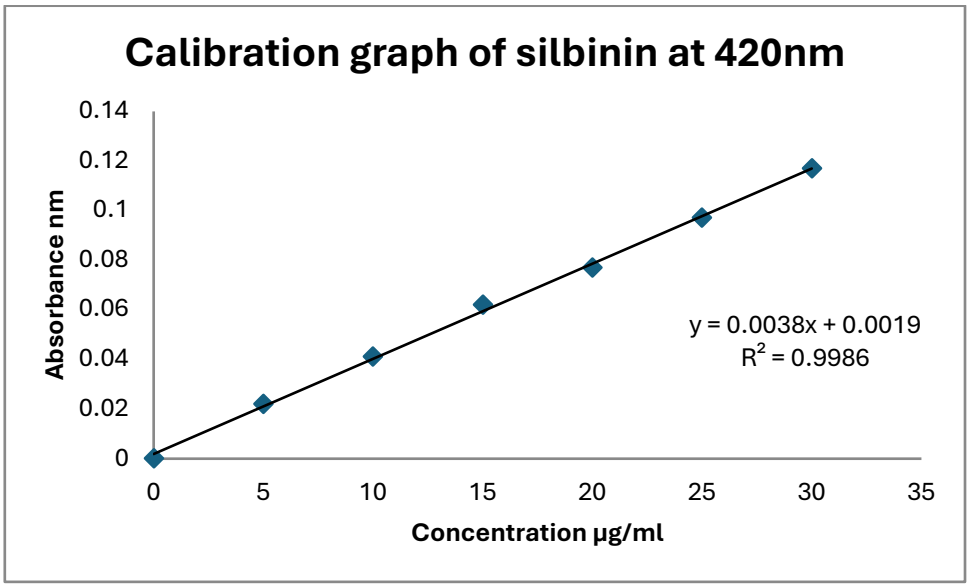
| **Concentration (µg/ml)** | **Absorbance at lambda 288 nm (BDC) mean** |
|---|---|
| 10 | 0.027 |
| 20 | 0.047 |
| 30 | 0.067 |
| 40 | 0.092333 |
| 50 | 0.114 |
| 60 | 0.138 |
| Mean | 0.081333333 |
| SD | 0.041795623 |
| %RSD | 0.513880607 |




**Graph 3: Calibration graph of BDC at 288 nm**

**Table no. 10: Absorbance of silbinin at lambda 420 nm**

| Concentration (µg/ml) | Absorbance at lambda 420 nm (Silbinin) |
|---|---|
| | mean |
| 5 | 0.022 |
| 10 | 0.044 |
| 15 | 0.062 |
| 20 | 0.077 |
| 25 | 0.097 |
| 30 | 0.117 |
| Mean | 0.069333333 |
| SD | 0.03472991 |
| %RSD | 0.500912167 |




**Graph 5: Calibration graph of silbinin at 420nm**

**Table no. 11: absorbance at isobestic point 332nm**

| Concentration (µg/ml) | Absorbance at lambda 332 nm Mean |
|---|---|

| | |
|---|---|
| 5 | 0.082333 |
| 10 | 0.156 |
| 15 | 0.213 |
| 20 | 0.297 |
| 25 | 0.378 |
| 30 | 0.448 |
| Mean | 0.224571429 |
| SD | 0.137980581 |
| %RSD | 0.614417346 |

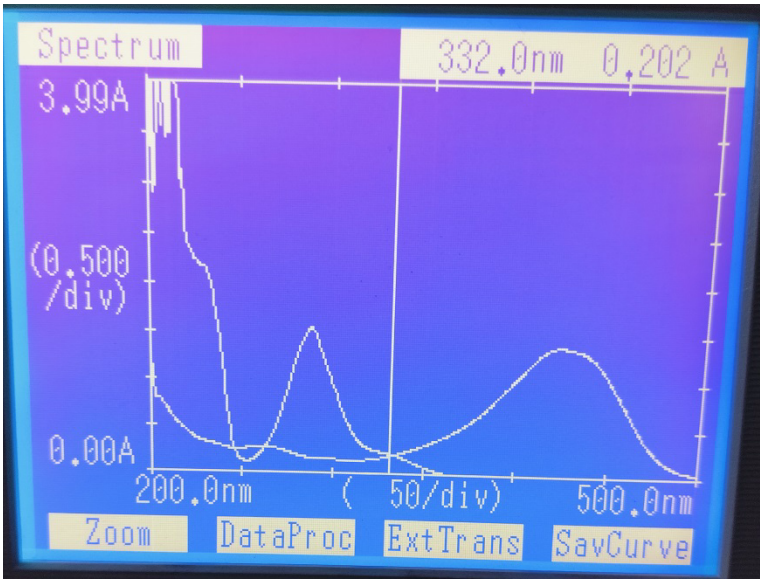
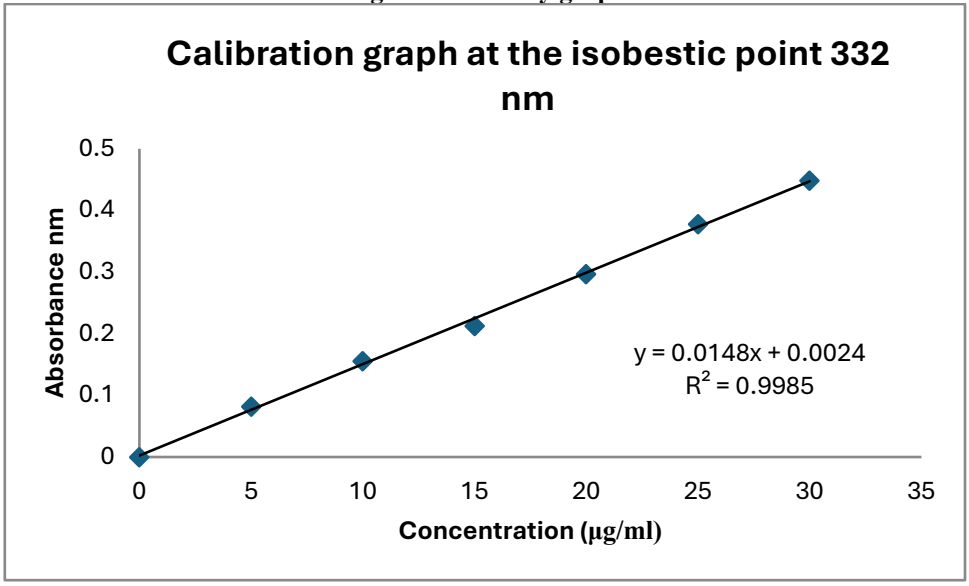



**Fig no. 5: Overlay graph**




**Graph 6 : Calibration graph at the isobestic point 332 nm**

**2.6.2.9 Comparative Multi-Component Mixture Estimation Analysis**

A critical cross-comparison between the Simultaneous Equation Method and the Q-Absorption Ratio Method uncovered a pronounced algorithmic divergence when processing identical binary mixtures (Table 3.10). The simultaneous equation method established a relatively uniform concentration profile of 6.70 µg/mL for Silibinin and 8.23 µg/mL for BDC by resolving independent linear equations at their respective maximum wavelengths (288 nm and 420 nm). Conversely, the

absorbance ratio (Q-ratio) framework broke this symmetry under identical trial inputs, generating a skewed distribution that dropped Silibinin to 2.93 µg/mL while sharply elevating BDC to 13.92 µg/mL. This prominent variance underscores the structural vulnerability of the Q-ratio method, which relies completely on the mathematical integrity of a single iso-absorptive reference point at 332 nm (A332 = 0.256) to calculate total concentration before apportioning it using the sample ratio (Qm = 2.3291). This suggests that subtle variations in baseline absorption at the isobestic wavelength can lead to compounding algebraic deviations, indicating that the simultaneous equation method remains the preferred choice for routine multicomponent assay quality control setups.

**Table no. 12: analytical method calculation**

| Analytical Method | Silbinin Concentration | BDC Concentration |
|---|---|---|
| **Simultaneous Equation** | 6.70 µg/ml | 8.23 µg/ml |
| **Q-Ratio (Absorbance Ratio)** | 2.93 µg/ml | 13.92 µg/ml |

**Discussion**

The UV spectrophotometric method for the quantitative estimation of silbinin was successfully validated at an analytical wavelength of 288 nm, proving to be highly specific, linear, and precise. The analysis of the blank solvent at 288 nm showed no baseline interference, confirming that the solvent system does not distort the drug's signal. Serial dilutions across a concentration range of 5 to 30 µg/ml exhibited a strict, concentration-dependent increase in mean absorbance from 0.267 to 1.574, adhering closely to the Beer-Lambert law. Linear regression analysis of the calibration curve yielded the equation y = 0.0510x + 0.0107 with an exceptional coefficient of determination ($R^2$ = 0.9975), confirming excellent method linearity. The final result of the analytical data shows that the drug BDC exhibits a strong linear relationship at 420 nm across a concentration range of 10 to 60 µg/ml, strictly adhering to Beer-Lambert's law. By plotting the concentration on the x-axis against the calculated mean absorbance on the y-axis, the linear regression line equation was determined to be **(y = 0.0156x + 0.0067)**, where the slope (m) was 0.0156 and the y-intercept (c) is 0.0067. The coefficient of determination (**$R^2$**) was **0.9973**, confirming excellent calibration curve linearity and data reliability. Additionally, the data displays a grand mean absorbance of 0.5518 with a standard deviation of 0.2918 and a relative standard deviation (%RSD) of 52.87% across the progressive dilution levels.

The intraday precision study was conducted at a concentration of 10 µg/ml using nine independent determinations organized into three distinct replicate subsets for each drug to evaluate the repeatability of the developed methods. Silbinin exhibited remarkable reproducibility with an average intraday %RSD of 0.38%, derived from individual subset variations of 0.29%, 0.38%, and 0.47%. The BDC precision profile yielded an average intraday %RSD of 1.03%, with individual subset values calculated at 1.24%, 1.24%, and 0.60%. The final inter-day precision analysis confirmed that the developed UV spectrophotometric methods for Silbinin, and BDC, possessed excellent ruggedness, intermediate precision, and day-to-day reproducibility. At a standard evaluation concentration of 10 µg/ml, all three drugs fell well within the generally accepted ICH acceptance criteria for intermediate method precision, which mandates that the **%RSD must remain below 2.0%**. Silbinin demonstrated exceptional stability over the three-day study, yielding an average inter-day %RSD of **0.56%** calculated from individual daily variations of 0.39% (Day I), 0.48% (Day II), and 0.81% (Day III). The BDC method maintained tight day-to-day consistency, generating an average inter-day %RSD of **0.61%** with highly steady individual daily values of 0.60%, 0.62%, and 0.63%, respectively.

The sensitivity of the developed UV spectrophotometric methods was evaluated by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ) for Silbinin, and BDC, which define the lowest concentrations were reliably detected and quantified. The results demonstrated that all three methods possess high sensitivity, making them suitable for detecting trace amounts of the drugs in quality control applications. BDC followed with intermediate sensitivity values, showing an LOD of 2.49 µg/ml and an LOQ of 7.54 µg/ml. Silbinin displayed the highest limits with an LOD of 3.89 µg/ml and an LOQ of 11.79 µg/ml. This systematic difference in sensitivity across the three drugs is directly tied to their specific molar absorptivities and the chemical properties governing their light absorption at their respective analysis wavelengths.

The accuracy of the developed analytical method for silibinin was rigorously evaluated through recovery studies spanning three distinct concentration spikes (80%, 100%, and 120%) alongside a baseline control. The experimental findings revealed excellent recovery percentages ranging from 95.35% to 98.00%, which demonstrated a high degree of quantitative closeness to the true cumulative values. The accuracy of the developed analytical method for bisdemethoxycurcumin (BDC) was evaluated through recovery studies across three concentration levels (80%, 100%, and

120%) alongside a baseline control. The experimental data showed outstanding recovery percentages ranging from 98.2% to 99.4%, indicating high exactness and proximity to the true cumulative amounts.

The ruggedness of the developed analytical method for silibinin and bisdemethoxycurcumin (BDC), were evaluated by comparing the experimental outputs obtained by two different analysts at a concentration of 10 µg/ml. The data indicated highly reproducible results between both analysts, with negligible variations in the mean values across all three analytes. For silibinin, both analysts achieved an identical mean response of 0.534, with extremely low relative standard deviations (%RSD) of 0.0039% and 0.0029%, culminating in an average %RSD of 0.0034%. Similarly, the analysis of BDC yielded closely matched mean values of 0.167 for Analyst I and 0.165 for Analyst II, with an average %RSD of 0.0109%.

The experimental findings revealed that the analytical responses remained highly stable despite the change in temperature. For silibinin, the mean peak response shifted minimally from 0.532 at 25°C to 0.525 at 30°C, maintaining low individual relative standard deviations (%RSD) of 0.66% and 0.67% respectively, which culminated in an average %RSD of 0.67%. The quantification of BDC also exhibited high consistency, showing mean values of 0.165 at 25°C and 0.161 at 30°C, with a well-controlled combined average %RSD of 1.84

The comparative evaluation of the Silbinin and BDC binary mixture highlights a distinct methodological divergence between the two multi-component UV spectrophotometric approaches. The simultaneous equation method establishes a relatively uniform concentration profile of 6.70 µg/ml for Silbinin and 8.23 µg/ml for BDC by resolving independent linear algorithms at their respective maximum wavelengths (288 nm and 420 nm). Conversely, the absorbance ratio (Q-ratio) framework breaks this symmetry under identical trial inputs, generating a skewed distribution that drops Silbinin to 2.93 µg/ml while sharply elevating BDC to 13.92 µg/ml. This prominent variance underscores the structural vulnerability of the Q-ratio method, which relies completely on the mathematical integrity of a single iso-absorptive reference point at 332 nm ($A_{332}$ = 0.256) to calculate total concentration before apportioning it using the sample ratio (Qm = 2.3291).

**Conclusion**

A rapid, economical, sensitive, and separation-free UV spectrophotometric protocol was successfully engineered, validated, and evaluated for the simultaneous estimation of Silibinin and Bisdemethoxycurcumin (BDC). Method validation conducted under strict ICH Q2(R1) regulatory criteria confirmed high linearity, high recovery accuracy, excellent repeatability, and robust ruggedness across analyst profiles and environmental thermal shifts. The strategic comparison of multi-component algebraic algorithms revealed a significant divergence between the uniform tracking of the Simultaneous Equation method and the skewed data partitioning of the Q-Absorption Ratio method, highlighting the compounding sensitivity of the Q-ratio framework to isobestic baseline measurements. Because this approach completely eliminates the need for the expensive instrumentation, lengthy runtimes, and high hazardous chemical waste associated with HPLC columns, the validated simultaneous equation spectrophotometric protocol stands as an excellent, high-throughput, green-chemistry alternative for routine pharmaceutical analysis.

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