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**Research Article**

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# Development and Validation of Q-Absorbance Ratio Spectrophotometric Method for Simultaneous Estimation of Mangiferin and Berberin HCL in Bulk and Synthetic Mixture

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## ABSTRACT

*Introduction: There are so many promising plant based chemical constituents are present which act as alternative therapy for the control of diabetes. But due to lack of its proper quality control parameters they are not widely used. The US FDA patent is approved for fixed dosage combination of Mangiferin (MF) and Berberin HCl (BER) as antidiabetic herbal formulation. Objective: The literature review suggested that no UV spectroscopic method been reported in the literature review for the combination formulation of MF and BER. Methodology: Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an iso-absorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that MF and BER show an iso-absorptive point at 317 nm. The second wavelength used is 257 nm, which is the λ-max of MF. Methanol was used as a solvent in this method. The method was validated with respect to linearity, accuracy, precision as per the International conference on harmonisation (ICH) guidelines. Results: The drug response with respect to absorbance was linear over the concentration range 5-30 µg/ml for MF and 10-60 µg/ml for BER. The percentage recovery of MF and BER as found to be 100.00% and 100.07% respectively. Conclusion: The method can be successfully employed for the simultaneous determination of MF and BER in pharmaceutical formulations. The developed method is validated as per ICH guideline Q2(R1).*

**Keywords:** Mangiferin; Berberin HCl; Simultaneous equation method; ICH guidelines; Quality control

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## INTRODUCTION

The number of drugs and drug formulations introduced into the market has been increasing at an alarming rate. These drugs or formulations may be either new entities or partial structural modification of the existing ones or novel dosage forms. The US FDA US 7867979 B2 patent is approved for fixed dosage combination of MF and BER as antidiabetic herbal formulation [1-3]. The literature review suggested that various HPLC, HPTLC and UV-visible Spectrophotometric methods have been reported for estimation of MF and BER individually or in combination with other drugs from pharmaceutical dosage form. It is also revealed that no UV spectroscopic method has been reported in the literature review for the combination formulation of Mangiferin and Berberin HCl. The condition thus, provides the scope of development of accurate, sensitive, reproducible and simple Spectroscopic method capable of estimating both the drugs from the formulation simultaneously [4-13].

## EXPERIMENTAL SECTION

### Apparatus and Instrument

A Double beam UV-Visible spectrophotometer (Shimadzu, model pharmaspec 1800) having two matched quartz cells with 1 cm light path and Electronic analytical balance, (Shimadzu AUX-220) was used. Corning volumetric flasks, pipettes of borosilicate glasses were used in the study.

### Spectophotometric Conditions

- Mode: Spectrum
- Scan speed: Fast
- Wavelength range: 400-200 nm
- Absorbance scale: 0.00A–2.00A
- Initial base line correction: Methanol

### Chemicals and Reagents

MF reference standard was purchased from Sigma Aldrich. BER reference standard was given as gift sample by Enovate life Mumbai.

### Preparation of Standard Solutions

To Prepare standard solution of MF (100 µg/ml) and BER (1000 µg/ml), accurately weigh 25 mg of each drugs were transferred in two different 100 and 10 ml volumetric flasks respectively, dissolve and diluted up to mark with methanol, from these stock solutions, 5 ml and 1 ml aliquots of MF and BER respectively were transferred in two different 10 ml volumetric flasks and were diluted up to mark with distilled water to get working standard solution having concentration of MF of 50 µg/ml and BER of 100 µg/ml.

### Methodology

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an iso-absorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that MF and BER show an iso-absorptive point at 317 nm. The second wavelength used is 257 nm, which is the λ-max of MF. Working standards were prepared in methanol and the absorbances at 317 nm (iso-absorptive point) and 257 nm (λ-max of MF) were measured and absorptivity coefficients were calculated using calibration curve. The concentration of two drugs in the mixture can be calculated using following equations.

$$C_X = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1 / ax_1 \quad \text{..... (1)}$$

$$C_Y = [(Q_M - Q_X) / (Q_Y - Q_X)] \times A_1 / ay_1 \quad \text{…….. (2)}$$

Where, $A_1$ and $A_2$ are absorbances of mixture at 257 nm and 317 nm; $ax_1$ and $ay_1$ are absorptivities of MF and BER at 257 nm; $ax_2$ and $ay_2$ are absorptivities of MF and BER respectively at 317 nm;

$$Q_M = A_2 / A_1,\ Q_X = ax_2 / ax_1,\ Q_Y = ay_2 / ay_1$$

### Calibration Curve for MF and BER

To check linearity of the method, working standard solution having concentration in range of 5-30 µg/ml of MF and 10-60 µg/ml of BER were prepared from the standard stock solutions of both drugs. The absorbance was measured at 257 nm (λmax of MF) and at 317 nm (λmax of BER). Calibration curves were constructed by plotting absorbance *vs*. concentration.

### Preparation of sample solution from laboratory prepared synthetic mixture:

Synthetic mixture of MF (25 mg) and BER (75 mg) was prepared by using common excipients like Corn Starch (50 mg), Lactose (113 mg) and Magnesium Stearate (2 mg) per tablet. Tablet powder was prepared by calculating formula for 10 Tablets having label claim for MF and BER 25 mg and 75 mg respectively. From this mixture, powder equivalent to 45 mg BER was dissolved in 250 ml methanol and then sonicated for 15 min. and filtered through Whatman filter paper. From this solution, 2.5 ml aliquot was taken in 10 ml volumetric flask and diluted up to the mark with methanol to make final concentration of MF and BER, 15 µg/ml and 45 µg/ml, respectively which was used for assay.

## RESULTS AND DISCUSSION

### Method Development

For this measurement, the solutions of MF and BER were prepared separately in methanol at a concentration of 15 µg/ml and 45 µg/ml respectively. They were scanned in the wavelength range of 200-400 nm. From the overlay spectra of two drugs, it is evident that MF and BER show an iso-absorptive point at 317 nm. MF showed 241 nm, 257 nm, 317 nm, 366 nm λmax from which 257 nm was selected as λmax of MF in this method due to good linearity (Figure 1).

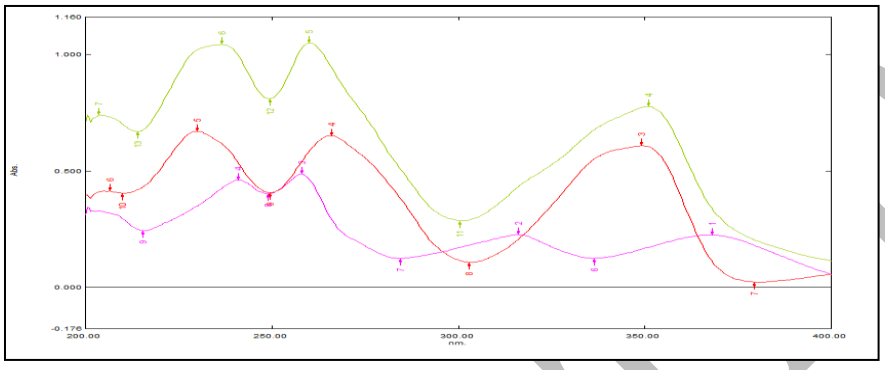

**Figure 1: Zero order overlain spectra of MF (10 µg/ml), BER (45 µg/ml) and combination (10 µg/ml MF and 45 µg/ml BER)**

### Validation of the Proposed Method

**Linearity:**

Linear correlation was obtained between absorbance Vs concentration of MF and BER in the concentration ranges of 5- 30 µg/ml and 10-60 µg/ml respectively and is shown in overlain chromatogram of MF and BER, Figures 2 and 3 respectively and calibration curve data of MF and BER shown in Tables 1 and 2. Regression parameters are mentioned in Table 3 and the linearity spectra and calibration curves of these two drugs at 257 nm and 317 nm are shown in Figures 4-7 respectively.

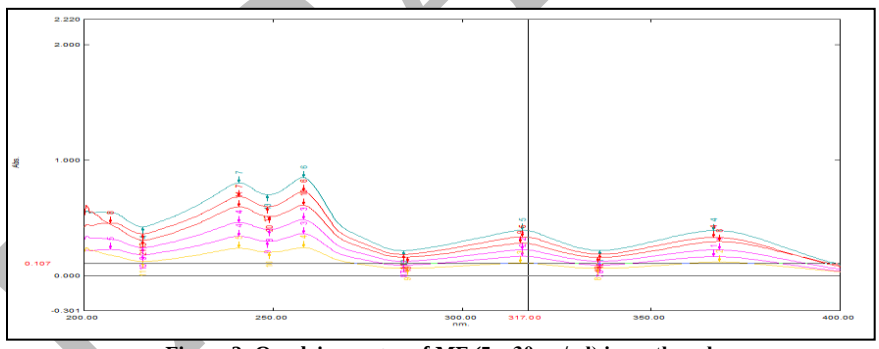

**Figure 2: Overlain spectra of MF (5 – 30 µg/ml) in methanol**

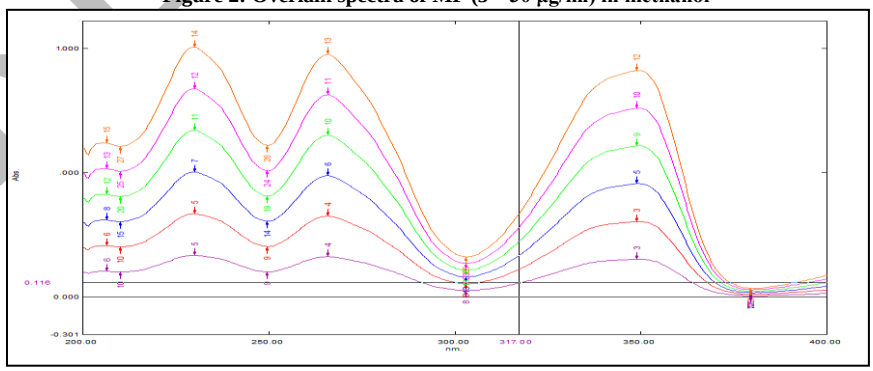

**Figure 3: Overlain spectra of BER (10 – 60 µg/ml) in methanol**

**Table 1: Linearity data for MF**

| Sr. no. | Concentration of MF (µg/ml) | At Wavelength 257 nm | | At Wavelength 317 nm | |
|---|---|---|---|---|---|
| | | Absorbance (Mean ± SD) | %RSD (%) | Absorbance (Mean ± SD) | %RSD (%) |
| **1** | 5 | 0.224 ± 0.004 | 1.61 | 0.221 ± 0.003 | 0.44 |
| **2** | 10 | 0.391 ± 0.002 | 0.62 | 0.293 ± 0.004 | 0.94 |
| **3** | 15 | 0.555 ± 0.002 | 0.59 | 0.345 ± 0.004 | 0.34 |
| **4** | 20 | 0.687 ± 0.003 | 0.26 | 0.434 ± 0.002 | 0.55 |
| **5** | 25 | 0.745 ± 0.004 | 0.36 | 0.500 ± 0.002 | 0.72 |
| **6** | 30 | 0.895 ± 0.003 | 0.34 | 0.586 ± 0.004 | 0.94 |

**Table 2: Linearity data for BER**

| Sr. no. | Concentration of BER (µg/ml) | At Wavelength 257 nm | | At Wavelength 317 nm | |
|---|---|---|---|---|---|
| | | Absorbance (Mean ± SD) | %RSD | Absorbance (Mean ± SD) | %RSD (%) |
| **1** | 10 | 0.305 ± 0.002 | 1 | 0.221 ± 0.005 | 1.05 |
| **2** | 20 | 0.415 ± 0.004 | 0.33 | 0.290 ± 0.004 | 1.01 |
| **3** | 30 | 0.525 ± 0.003 | 1.22 | 0.355 ± 0.004 | 0.75 |
| **4** | 40 | 0.637 ± 0.002 | 0.26 | 0.430 ± 0.002 | 0.45 |
| **5** | 50 | 0.745 ± 0.003 | 1.36 | 0.500 ± 0.005 | 0.71 |
| **6** | 60 | 0.860 ± 0.002 | 0.94 | 0.590 ± 0.005 | 1.4 |

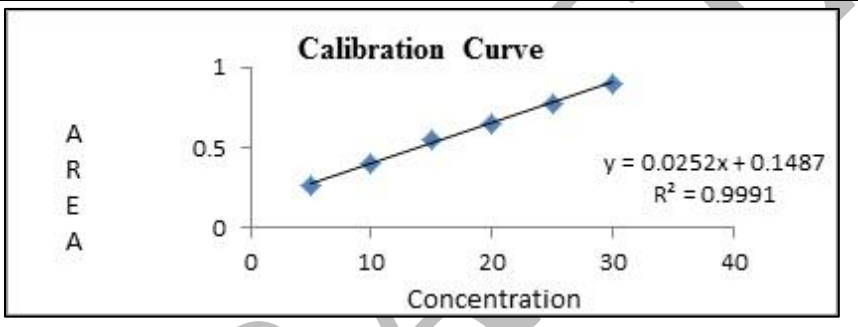


**Figure 4: Calibration curve of MF (5-30 µg/ml) at 257 nm**

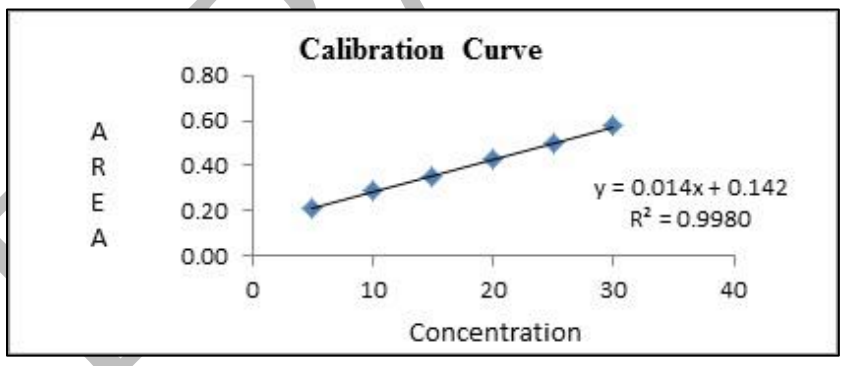


**Figure 5: Calibration curve of MF (5-30 µg/ml) at 317 nm**

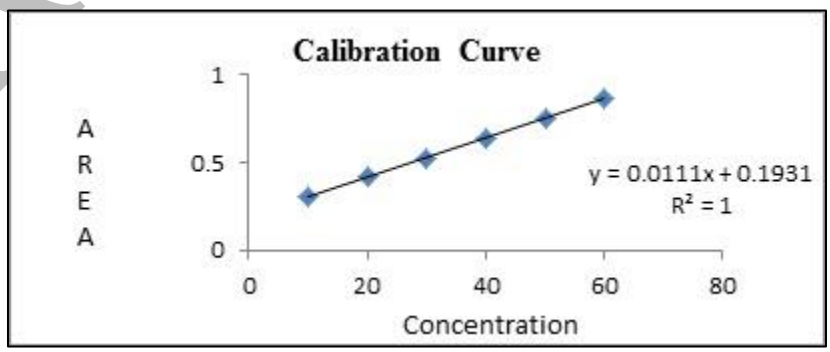


**Figure 6: Calibration curve of BER (10-60 µg/ml) at 257 nm**

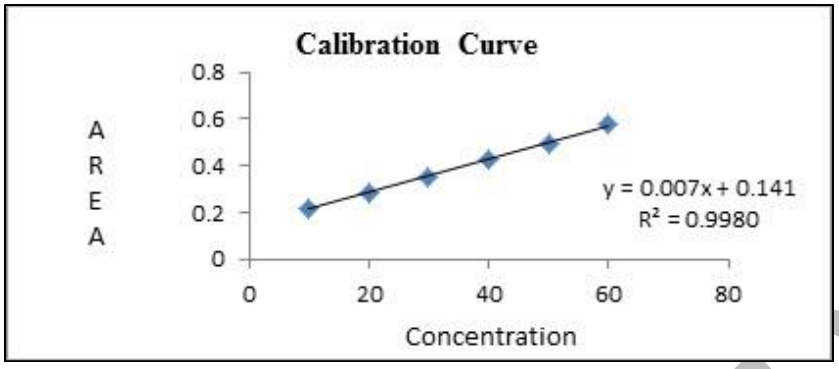


**Figure 7: Calibration curve of BER (10-60 µg/ml) at 317 nm**

**Table 3: Linearity Data**

| Parameter | MF At 257 nm | MF At 317 nm | BER At 257 nm | BER At 317 nm |
|---|---|---|---|---|
| Linearity range (µg/ml) | 5 – 30 | 5 – 30 | Oct-60 | Oct-60 |
| Regression equation | y = 0.0252x + 0.1487 | y = 0.014x + 0.142 | y = 0.0111x + 0.1931 | y = 0.007x + 0.141 |
| Correlation coefficient ($r^2$) | 0.9991 | 0.998 | 1 | 0.998 |
| y-intercept | 0.1487 | 0.142 | 0.1931 | 0.141 |
| Standard deviation of slope | 0.0056 | 0.0098 | 0.0054 | 0.0066 |

**Accuracy:**

Accuracy of the method was confirmed by recovery study from marketed formulation at three levels of standard additions (80%, 100% and 120%). Percentage recovery for MF was in the range of 99.99-100.07%**,** while for BER, it was found to be in range of 99.84-101.10%. The results are shown in Tables 4 and 5. Recovery greater than 98% with low SD justifies the accuracy of the method.

**Table 4: Recovery data for MF**

| % Level | Concentration of MF in sample (µg/ml) | Concentration of MF recovered (µg/ml) | % Recovery of MF (%Recovery + SD) | %RSD (%) |
|---|---|---|---|---|
| 80% | 12 | 11.9 | 99.99 ± 1.92 | 1.92 |
| | 12 | 11.67 | | |
| | 12 | 12.1 | | |
| 100% | 15 | 14.02 | 100.00 ± 0.65 | 0.65 |
| | 15 | 15.98 | | |
| | 15 | 15.1 | | |
| 120% | 18 | 18.09 | 100.07 ± 0.49 | 0.49 |
| | 18 | 18 | | |
| | 18 | 17.95 | | |

**Table 5: Recovery data for BER**

| % Level | Concentration of BER in sample (µg/ml) | Concentration of BER recovered (µg/ml) | % Recovery of BER (%) | %RSD (%) |
|---|---|---|---|---|
| 80% | 36 | 36.11 | 101.83 ± 1.19 | 1.18 |
| | 36 | 36.79 | | |
| | 36 | 36 | | |
| 100% | 45 | 45.02 | 100.07 ± 0.14 | 1.12 |
| | 45 | 44.98 | | |
| | 45 | 45.1 | | |
| 120% | 54 | 54.09 | 99.84 ± 0.30 | 0.3 |
| | 54 | 53.77 | | |
| | 54 | 53.88 | | |

**Precision:**

**Repeatability (n=6):** The repeatability was checked by scanning and measurement of the responses of solutions of MF (5-30 µg/ml) and BER (10-60 µg/ml) without changing the parameters of the proposed method. The procedure was repeated six times and %RSD was calculated. The data for repeatability for combined solution of MF and BER is presented in Table 6. %R.S.D was found to be 1.12% and 1.02% at 257 nm and 317 nm, respectively for MF and

1.03 and 0.90% at 257 nm and 317 nm respectively for BER. % R.S.D was less than 2% complied with the standard limits.

**Table 6: Repeatability data for MF and BER**

| Drug | Concentration (µg/ml) | Absorbance (Mean ± SD) At 257 nm | %RSD (%) | Absorbance (Mean ± SD) At 317 nm | %RSD (%) |
|---|---|---|---|---|---|
| MF | 15 | 0.555 ± 0.008 | 1.12 | 0.343 ± 0.006 | 1.02 |
| BER | 45 | 0.759 ± 0.007 | 1.03 | 0.353 ± 0.004 | 0.9 |

**Intra-day and inter day precision (n=3):** The data for intraday precision for MF and BER is shown in Table 7. The %R.S.D. for Intraday precision was found to be 0.22-0.40% for MF and 0.34-0.56% for BER at 257nm respectively and 0.45-1.26% for MF and 0.37-0.59% for BER at 317nm respectively. The data for intraday precision for MF and BER is shown in Table 8. The %R.S.D. for Interday precision was found to be 0.96-1.75% for MF and 1.58-1.89% for BER at 257nm respectively and 0.96-1.01% for MF and 0.89-1.89% for BER at 317nm respectively.

**Table 7: Intraday precision data for MF and BER**

| Drug | Sr. No. | Concentration (µg/ml) | Absorbance (Mean ± SD) At 257 nm | %RSD (%) | Absorbance (Mean ± SD) At 317 nm | %RSD (%) |
|---|---|---|---|---|---|---|
| MF | 1 | 10 | 0.391 ± 0.002 | 0.22 | 0.520 ± 0.005 | 0.45 |
| | 2 | 15 | 0.555 ± 0.002 | 0.23 | 0.630 ± 0.005 | 0.67 |
| | 3 | 20 | 0.687 ± 0.003 | 0.4 | 0.756 ± 0.008 | 1.26 |
| BER | 1 | 30 | 0.565± 0.004 | 0.44 | 0.522 ± 0.002 | 0.37 |
| | 2 | 40 | 0.672± 0.003 | 0.34 | 0.635 ± 0.005 | 0.59 |
| | 3 | 50 | 0.745±0.004 | 0.56 | 0.743 ± 0.005 | 0.54 |

**Table 8: Interday precision data for MF and BER**

| Drug | Sr. No. | Concentration (µg/ml) | Absorbance (Mean ± SD) At 257 nm | %RSD (%) | Absorbance (Mean ± SD) At 317 nm | %RSD (%) |
|---|---|---|---|---|---|---|
| MF | 1 | 5 | 0.391 ± 0.005 | 1.75 | 0.521 ± 0.006 | 1 |
| | 2 | 10 | 0.545 ± 0.007 | 1.02 | 0.631 ± 0.003 | 1.01 |
| | 3 | 15 | 0.685 ± 0.008 | 0.96 | 0.755 ± 0.008 | 0.96 |
| BER | 1 | 30 | 0.563± 0.008 | 1.58 | 0.523 ± 0.008 | 0.89 |
| | 2 | 40 | 0.672± 0.007 | 1.65 | 0.636 ± 0.007 | 1.36 |
| | 3 | 50 | 0.744±0.005 | 1.89 | 0.742 ± 0.007 | 1.85 |

**LOD and LOQ:**

The LOD was calculated by standard deviation of response and was found to be 2.33 µg/ml and 5.22 µg/ml for MF and BER respectively at 257 nm, be 2.30 µg/ml and 4.32 µg/ml for MF and BER respectively at 317 nm. The LOQ was calculated by standard deviation of response and was found to be 3.91 µg/ml and 6.69 µg/ml for MF and BER respectively at 257 nm, be 3.22 µg/ml and 7.22 µg/ml for MF and BER respectively at 317 nm.

**Analysis of Synthetic Mixture**

Here, 15 µg/ml solution of MF and 45 µg/ml solution of BER synthetic mixture were prepared in triplicate manner and analyzed. The assay was carried out as per regression equation. The result of assay is shown in Table 9.

**Table 9: Assay of Synthetic Mixture**

| Drug | Amount of drug (mg) | % Amount found (Mean% ± SD) |
|---|---|---|
| MF | 25 | 100.83 ± 1.22 |
| BER | 75 | 99.88 ± 0.70 |

## CONCLUSION

A new, simple, accurate, and precise UV spectroscopic method was developed for the simultaneous estimation of MF and BER in bulk drugs and in the presence of tablet excipients. The recovery studies suggested non-interference of formulation excipients in the estimation. Hence, the proposed method can be used for the quality control of the cited drugs and can be extended for routine analysis of the drugs in their pharmaceutical dosage forms.

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