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## DEVELOPMENT AND VALIDATION OF FIRST ORDER DERIVATIVE SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF MANGIFERIN AND BERBERIN HCL IN BULK AND SYNTHETIC MIXTURE

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### Keywords:

Mangiferin, Berberin HCl,  
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**ABSTRACT: Introduction:** There are so many promising plant-based chemical constituents are present which act as an alternative therapy for the control of diabetes. But due to the lack of its proper quality control parameters, they are not widely used. The USFDA patent is approved for a 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:** The method is based on a simultaneous method using two wavelengths, 257 nm ( $\lambda_{\max}$  of MF) and 265 nm ( $\lambda_{\max}$  of BER). Methanol was used as a solvent. **Results:** The drug response with respect to absorbance was linear over the concentration range 5-30  $\mu\text{g/ml}$  for MF and 10-60  $\mu\text{g/ml}$  for BER. The percentage recovery of MF and BER as found to be 100.00% and 99.48%, respectively. The % R. S. D. values for intra-day, and the inter-day precision study was less than 2.0%, confirming that the method was sufficiently precise. **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) for global acceptance of standardized herbal formulations.

**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 a fixed dosage combination of MF and BER as antidiabetic herbal formulation<sup>1-2</sup>.

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 the 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<sup>3, 4, 5, 6, 7, 8, 9, 10</sup>.

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**EXPERIMENTAL:**

**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.

**Spectrophotometric 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 and Methanol - AR Grade from Merck India Limited.

**Preparation of Standard Solutions:** To Prepare a standard solution of MF (100  $\mu\text{g/ml}$ ) and BER (1000  $\mu\text{g/ml}$ ), accurately weigh 25 mg of each drug were transferred in two different 250 and 25 ml volumetric flasks respectively, dissolve and diluted up to mark with methanol, from these stock solutions, 12.5 ml and 2.5 ml aliquots of MF and BER respectively were transferred in two different 25 ml volumetric flasks and were diluted up to mark with distilled water to get working standard solution having a concentration of MF of 50  $\mu\text{g/ml}$  and BER of 100  $\mu\text{g/ml}$ .

**METHODOLOGY:**

**Calibration Curve for MF and BER:** To check the linearity of the method, working standard

solution having a concentration in the range of 5-30  $\mu\text{g/ml}$  of MF and 10-60  $\mu\text{g/ml}$  of BER were prepared from the standard stock solutions of both drugs. 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 the formula for 10 Tablets having a 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 a 10 ml volumetric flask and diluted up to the mark with methanol to make the final concentration of MF and BER, 15  $\mu\text{g/ml}$ , and 45  $\mu\text{g/ml}$ , respectively which was used for the assay.

**RESULTS AND DISCUSSION:**

**Selection of Detection Wavelength:** The standard solutions of 5  $\mu\text{g/ml}$  of MF, 50  $\mu\text{g/ml}$  of BER and their mixture in methanol were scanned over 400 - 200 nm against methanol as a blank. It showed wavelength maxima at 305 nm for MF and 210 nm for BER.

The zero-order spectra of MF and BER were transformed to first derivative spectra with the aid of UV Probe software ( $\Delta\lambda = 5 \text{ nm}$ ). Both the phytomarkers showed ZCPs with considerable sensitivity for the estimation of respective drugs on each other's ZCPs.

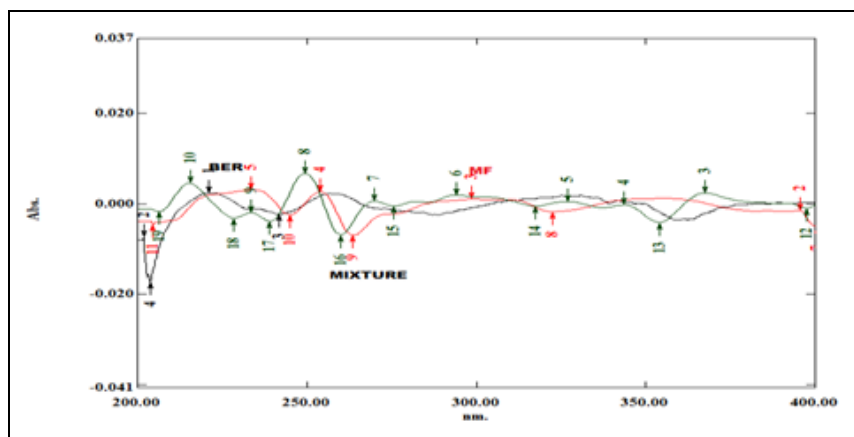


FIG. 1: FIRST ORDER OVERLAIN UV SPECTRA OF MF (5  $\mu\text{g/ml}$ ) AND BER (10  $\mu\text{g/ml}$ ) AND SYNTHETIC MIXTURE

Hence, first-order derivative method was used in the present work. I got 241 nm, 285 nm, 315 nm, 368 nm on ZCP for MF and 230 nm, 265 nm, 303 nm, 348 nm, 378 nm on ZCP of BER.

From this all, ZCP of MF 368 nm was selected for analysis of BER and ZCP of BER 265 nm was selected for analysis of MF **Fig. 1.** represents first-order overlain spectra of MF (5 µg/ml) and BER (10 µg/ml) and sample mix solution.

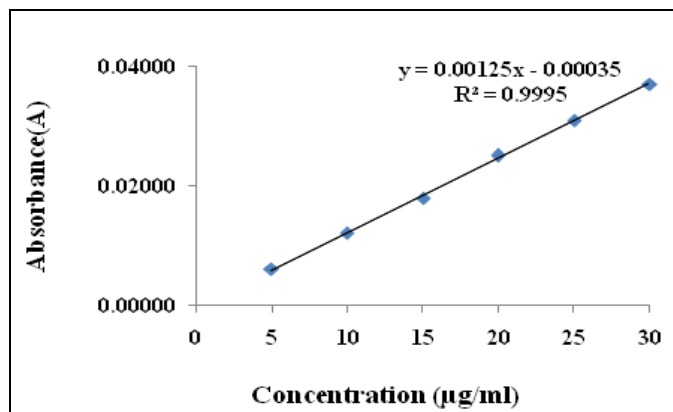
**Calibration Curve for MF and BER:** The solutions of each concentration levels were prepared as per methodology given in preparation of sample solution from laboratory prepared synthetic mixture of this method. The mean concentration and respective % RSD of calibration range for MF and BER are represented in **Table 1** and **Table 2.**, respectively. **Fig. 2** and **3** represent the calibration curve of MF (5-30 µg/ml) and BER (10-60 µg/ml).

**TABLE 1: RESULTS OF CALIBRATION CURVE FOR MF**

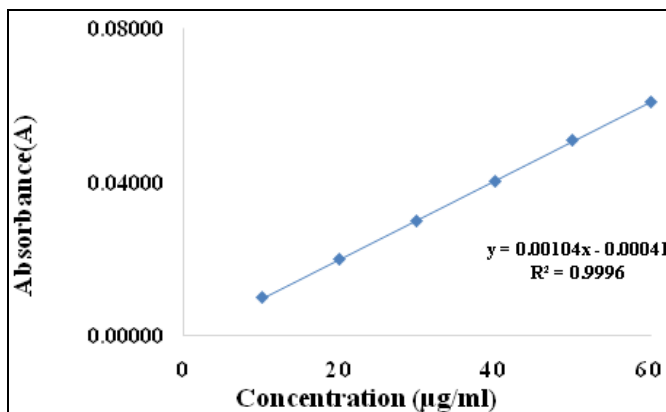
S. no.	Concentration (µg/ml)	Absorbance (Mean ± SD)	%RSD (%)
1	5	0.00609 ± 0.00004	0.73
2	10	0.01210 ± 0.00009	0.71
3	15	0.01804 ± 0.00005	0.27
4	20	0.02506 ± 0.00005	0.21
5	25	0.03111 ± 0.00009	0.30
6	30	0.03706 ± 0.00008	0.22

**TABLE 2: RESULTS OF CALIBRATION CURVE FOR BER**

S. no.	Conc. (µg/ml)	Absorbance (Mean ± SD)	%RSD (%)
1	10	0.01009 ± 0.00001	0.12
2	20	0.02007 ± 0.00002	0.12
3	30	0.03011 ± 0.00018	0.61
4	40	0.04015 ± 0.00007	0.18
5	50	0.05109 ± 0.00013	0.25
6	60	0.06106 ± 0.00018	0.30



**FIG. 2: CALIBRATION CURVE OF MF (5-30 µg/ml)**



**FIG. 3: CALIBRATION CURVE OF BER (10-60 µg/ml)**

### Validation of the Proposed Method:

**Linearity:** The linearity was determined by analyzing five independent calibration curve concentrations in the range of 5 - 30 µg/ml for MF and 10 - 60 µg/ml for BER.

Each concentration level of linearity was scanned at 400-200 nm. All the graphs of UV were transformed to first derivative spectra with aid of UV Probe software ( $\Delta\lambda = 5$  nm).

Then, the Mean absorbance vs. concentration graph was plotted, and the regression line equation and

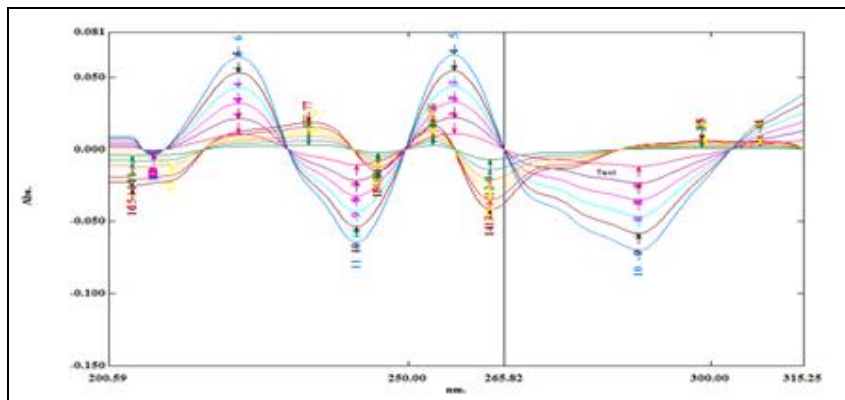
correlation coefficient for MF and BER at a selected wavelength was calculated.

The linear equations calculated were  $y = 0.00125x - 0.00035$  and  $y = 0.00104x - 0.00041$  for MF and BER respectively.

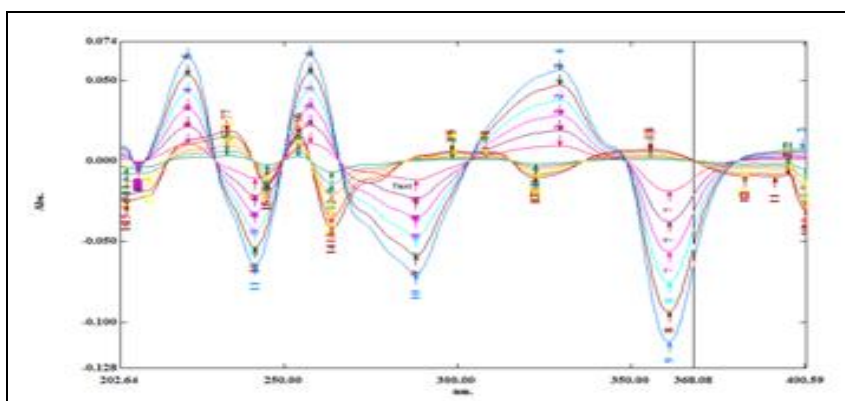
The correlation coefficient ( $r^2$ ) calculated was 0.9995 and 0.9996 for MF and BER, respectively. The first-order derivative overlay spectra of MF (5-30 µg/ml) and BER (10-60 µg/ml) are shown in **Fig. 4** and **5**, respectively. **Table 3** represents linear regression data for MF and BER.

**TABLE 3: LINEARITY DATA FOR MF AND BER**

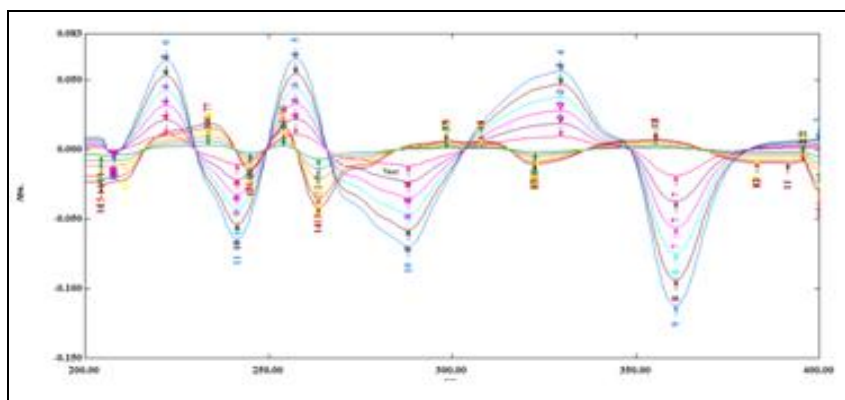
Parameter	MF	BER
Linearity range (µg/ml)	5 – 30	10 – 60
regression equation	$y = 0.00125x - 0.00035$	$y = 0.00104x - 0.00041$
Correlation coefficient (r <sup>2</sup> )	0.9995	0.9996
y-intercept	0.00125	0.00104
Slope	0.00035	0.00041



**FIG. 4: FIRST ORDER DERIVATIVE OVERLAIN UV SPECTRA OF MF (5-30 µg/ml) AND BER (10-60 µg/ml) ZCP OF BER 265 nm**



**FIG. 5: FIRST ORDER DERIVATIVE OVERLAIN UV SPECTRA OF MF (5-30 µg/ml) AND BER (10-60 µg/ml) ZCP OF MF 368 nm**



**FIG. 6: FIRST ORDER DERIVATIVE OVERLAIN UV SPECTRA OF MF (5-30 µg/ml) AND BER (10-60 µg/ml)**

**Accuracy:** The accuracy was carried out at three levels in the range of 80-120% of the working concentration of the sample. The calculated amount of MF and BER standards were added in placebo containing volumetric flasks

to prepare 80%, 100%, and 120% level of the working concentration of the sample. Each level was prepared in a triplicate manner, and each preparation was scanned in duplicate.

Amount recovered was found, and % recovery was calculated at each level using calibration curve

equations and recorded in **Table 4** and **Table 5**.

**TABLE 4: RECOVERY DATA FOR MF**

% Level	Conc. of MF Spiked (µg/ml)	Conc. of MF Recovered (µg/ml)	% Recovery (% Recovery ± SD)	%RSD (%)
80%	12	11.98	99.33 ± 0.52	0.52
	12	11.99		
	12	11.95		
100%	15	14.99	99.40 ± 1.06	1.06
	15	14.91		
	15	15.01		
120%	18	17.93	99.22 ± 0.54	0.54
	18	17.94		
	18	17.99		

**TABLE 5: RECOVERY DATA FOR BER**

% Level	Conc. of BER Spiked (µg/ml)	Conc. of BER Recovered (µg/ml)	% Recovery (%)	%RSD (%)
80%	36	36.01	99.24 + 1.34	1.35
	36	35.08		
	36	36.00		
100%	45	44.89	99.92 + 0.12	0.12
	45	44.99		
	45	45.00		
120%	54	53.34	99.76 + 1.09	1.09
	54	53.11		
	54	54.01		

**Precision:**

**Repeatability:** MF (20 µg/ml) and BER (50 µg/ml) were scanned in UV without changing any parameters. This same procedure was replicated six

times. % RSD was calculated were 0.21 and 0.25 for MF and BER, respectively. **Table 6** represents the repeatability data for MF and BER.

**TABLE 6: REPEATABILITY DATA FOR MF AND BER**

Phytomarkers	Conc. (µg/ml)	Area (Mean ± SD)	% RSD (%)
MF	20	0.02506 ± 0.00005	0.21
BER	50	0.05109 ± 0.00013	0.25

**Intra-day Precision:** Three different concentrations of standards MF (10, 15, 20 µg/ml) and BER (30, 40, 50 µg/ml) were analyzed in a triplicate manner at different times on the same day. % RSD at selected wavelengths for both

phytomarkers was calculated to check the intra-day precision. % R.S.D was ranging between 0.65–0.95 for MF and 0.71-0.86 for BER. **Table 7** represents the data for intra-day precision.

**TABLE 7: INTRA-DAY PRECISION DATA FOR MF AND BER**

Phytomarkers	S. no.	Conc. (µg/ml)	Area (Mean ± SD)	%RSD (%)
MF	1	10	0.01213 ± 0.00012	0.95
	2	15	0.01802 ± 0.00011	0.62
	3	20	0.02505 ± 0.00020	0.79
BER	1	30	0.03021 ± 0.00026	0.86
	2	40	0.04017 ± 0.00029	0.71
	3	50	0.05033 ± 0.00038	0.75

**Inter-day Precision:** Three different concentrations of standards MF (10, 15, 20 µg/ml) and BER (30, 40, 50 µg/ml) were analyzed in a triplicate manner at different times on different

days over a period of one week. %RSD at selected wavelengths for both phytomarkers was calculated to check the intra-day precision. % R.S.D was ranging between 0.92-1.24 for MF and 1.00–1.46



for BER. **Table 8** represents the data for inter-day precision.

**Sensitivity:** The sensitivity of the method was determined in terms of LOD and LOQ. LOD and LOQ was calculated from slope of the calibration

curve and S. D. of response values. The LOD was found to be for RES, and BER was found to be 0.26 µg/ml and 0.86, respectively. Furthermore, the LOQ was found to be 0.79 µg/ml and 2.60 µg/ml for MF and BER, respectively. **Table 9** represents the data of sensitivity

**TABLE 8: INTER-DAY PRECISION DATA FOR MF AND BER**

Phytomarkers	S. no.	Conc. (µg/ml)	Area (Mean ± SD)	%RSD (%)
MF	1	10	0.01211 ± 0.00015	1.24
	2	15	0.01799 ± 0.00016	0.92
	3	20	0.02501 ± 0.00025	1.00
BER	1	30	0.03084 ± 0.00045	1.46
	2	40	0.040707 ± 0.00041	1.00
	3	50	0.05084 ± 0.00054	1.07

**TABLE 9: RESULTS OF SENSITIVITY DATA FOR MF AND BER**

Parameter	MF	BER
LOD (µg/ml)	0.26	0.86
LOQ (µg/ml)	0.79	2.60

**Analysis of MF and BER Synthetic Mixture:** As per the sample mixture solution preparation as per 2. methodology for this method was used to calculate the percentage purity of MF and BER from prepared sample mix solution. The % purity was found to be 100.12 and 99.08 for MF and BER, respectively. The Result of the assay is shown in **Table 10**.

**TABLE 10: ASSAY OF SYNTHETIC MIXTURE**

Phytomarkers	Amount of drug (mg)	% Amount found (% Mean ± SD)
MF	25	100.12 ± 0.22
BER	75	99.08 ± 0.70

**CONCLUSION:** A new, simple and accurate 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 implied that there was no interference of formulation excipients in the estimation of phytomarkers. Hence, the proposed methods can be used for the quality control of the MF and BER. It can be extended for routine analysis of these phytomarkers in their pharmaceutical dosage forms.

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**CONFLICTS OF INTEREST:** Nil

#### REFERENCES:

- Chawla R: Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes: a Dreadful lifestyle disorder of 21<sup>st</sup> century. *JDMD* 2013; 12-35.
- Guang'ai Xu: Drug composition for treating 2 type diabetes and diabetic chronicity complications. *US* 2011; 7867979: 2.
- Jyotshna: Uni-dimensional double development HPTLC densitometry method for simultaneous analysis of mangiferin and lupeol content in *Mangifera indica* pulp and peel during storage. *Food chemistry* 2015; 176: 91-8.
- Sethiya NK, Trivedi A and Mishra SH: Rapid validated HPTLC method for simultaneous estimation of mangiferin and scopoletin in *Cassia decussata* extract. *Revista B D Pharmacognosia* 2015; 193-98.
- Khurana RK: Analytical Quality by design for developing a validated HPTLC densitometry method for estimating mangiferin in human plasma. *Curr Pharm Anal* 2015; 3(10).
- Kaliappan I: LC-MS quantification of mangiferin in hydroalcoholic extract of *Salacia oblonga*, *S. roxburghii* and Polyherbal formulations. *Int J Phytopharmacy* 4(1): 11-15.
- Padh H, Parmar S and Patel B: Stability indicating HPTLC method for estimation of mangiferin in bulk and dosage form. *IJPBS* 2017; 7: 71-77.
- Mandlik S, Ghadge A and Ranpise N: analytical method development and validation of phytoconstituent - berberine hydrochloride by RP-HPLC method. *Inventi Rapid Pham Ana Q A* 2017; 1-5.
- Wang J, Zhang Q and Cai D: Stability-Indicating Validated HPLC method for analysis of berberine hydrochloride and trimethoprim in pharmaceutical dosage form. *J of Chem* 2017; 1-9.
- Padh: development and validation of Q-absorbance ratio spectrophotometric method for simultaneous estimation of mangiferin and berberine HCL in bulk and synthetic mixture. *IJPSR* 2018; 9(8): 3355-59.

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