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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: 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. 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 phytochemicals, 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. 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 100.07% respectively. 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).

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 formulation¹⁻². 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^{3, 4, 5, 6, 7, 8, 9}.

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:

1. Mode: Spectrum
2. Scan speed: Fast

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3. Wavelength range: 400 - 200 nm
4. Absorbance scale: 0.00A - 2.00A
5. 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 methanol 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.

$$CX = [(Q_M - Q_Y) / (Q_X - Q_Y)] \times A_1 / a_{x1} \quad \dots (1)$$

$$CY = [(Q_M - Q_X) / (Q_Y - Q_X)] \times A_1 / a_{y1} \quad \dots (2)$$

Where, A_1 and A_2 are absorbances of mixture at 257 nm and 317 nm; a_{x1} and a_{y1} are absorptivities of MF and BER at 257 nm; a_{x2} and a_{y2} are absorptivities of MF and BER respectively at 317 nm;

$$Q_M = A_2 / A_1, Q_X = a_{x2} / a_{x1}, Q_Y = a_{y2} / a_{y1}$$

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 (λ_{\max} of MF) and at 317 nm (λ_{\max} 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 λ_{\max} from which 257 nm was selected as λ_{\max} of MF in this method due to good linearity **Fig. 1**.

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, **Fig. 2** and **3** respectively.

Calibration curve data of MF and BER shown in **Table 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 **Fig. 4, 5** and **6, 7** respectively.

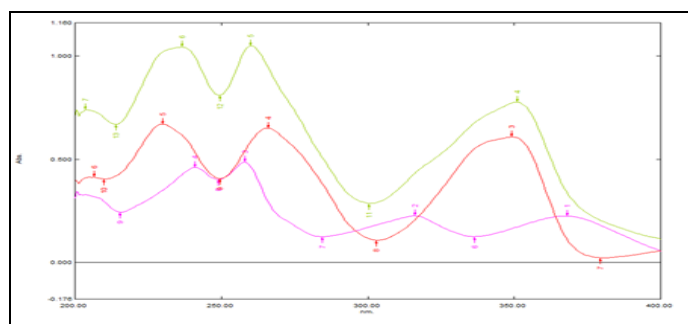


FIG. 1: ZERO ORDER OVERLAIN SPECTRA OF MF (10 µg/ml), BER (45 µg/ml) AND COMBINATION (10 µg/ml MF AND 45 µg/ml BER)

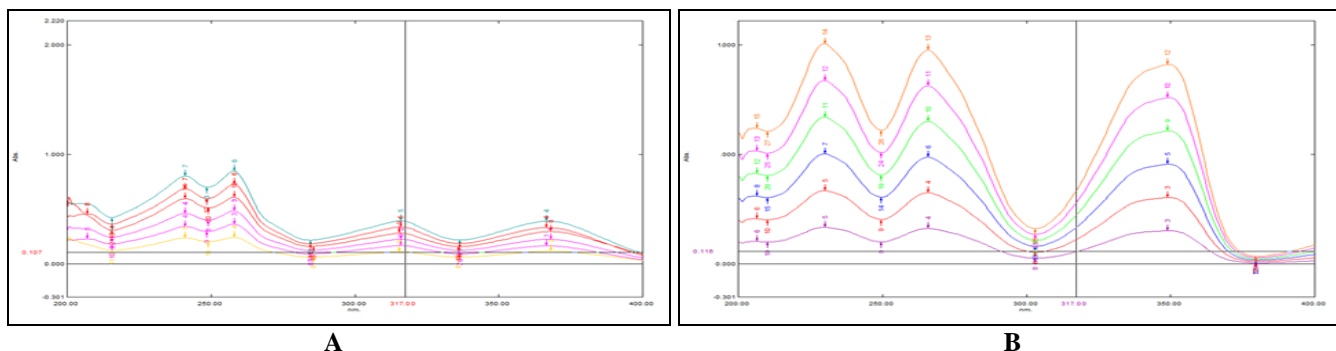


FIG. 2: (A) OVERLAIN SPECTRA OF MF (5 - 30 µg/ml) IN METHANOL (B) OVERLAIN SPECTRA OF BER (10 - 60 µg/ml) IN METHANOL

TABLE 1: LINEARITY DATA FOR MF

S. 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

S. 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	1.00	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	1.40

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	10 - 60	10 - 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	0.9980	1.000	0.9980
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 **Table 4** and **5**. Recovery greater than 98% with low SD justifies the accuracy of the method.

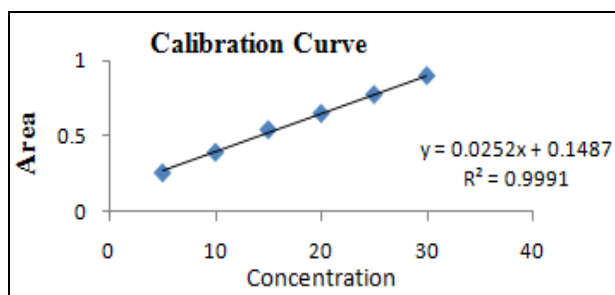


FIG. 4: CALIBRATION CURVE OF MF (5 - 30 µg/ml) AT 257 nm

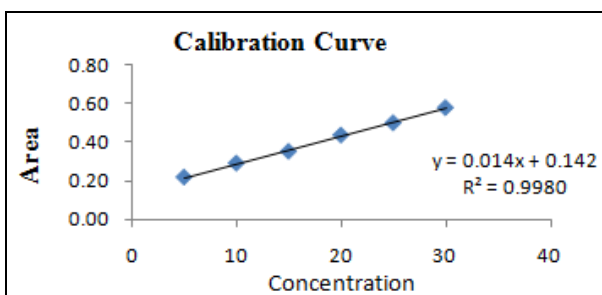


FIG. 5: CALIBRATION CURVE OF MF (5 - 30 µg/ml) AT 317 nm

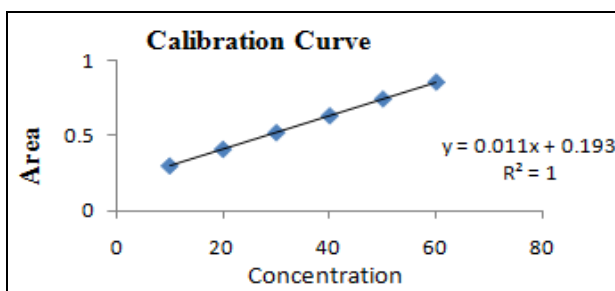


FIG. 6: CALIBRATION CURVE OF BER (10 - 60 µg/ml) AT 257nm

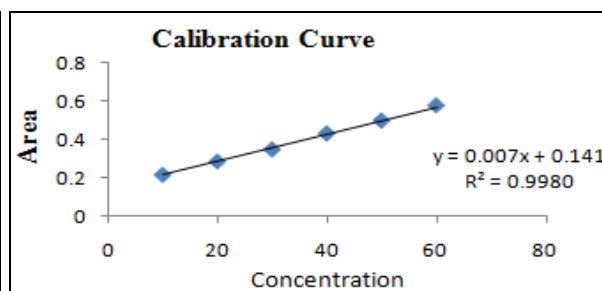


FIG. 7: CALIBRATION CURVE OF BER (10 - 60 µg/ml) AT 317nm

TABLE 4: RECOVERY DATA FOR MF

% Level	Conc. of MF in sample (µg/ml)	Conc. of MF recovered (µg/ml)	% Recovery of MF (%) Recovery ± SD)	% RSD
80%	12	11.90	99.99 ± 1.92	1.92
	12	11.67		
	12	12.10		
100%	15	14.02	100.00 ± 0.65	0.65
	15	15.98		
	15	15.10		
120%	18	18.09	100.07 ± 0.49	0.49
	18	18.00		
	18	17.95		

TABLE 5: RECOVERY DATA FOR BER

% Level	Conc. of BER in sample (µg/ml)	Conc. of BER recovered (µg/ml)	% Recovery of BER (%)	% RSD
80%	36	36.11	101.83 ± 1.19	1.18
	36	36.79		
	36	36.00		
100%	45	45.02	100.07 ± 0.14	1.12
	45	44.98		
	45	45.10		
120%	54	54.09	99.84 ± 0.30	0.30
	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	Conc. (µg/ml)	Abs. (Mean ± SD) at 257 nm	% RSD	Abs. (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	0.90

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 257 nm respectively and 0.45 - 1.26% for MF and 0.37 - 0.59% for BER at 317 nm 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 257 nm respectively and 0.96 - 1.01% for MF and 0.89 - 1.89% for BER at 317 nm respectively.

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 257nm, 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.

TABLE 7: INTRADAY PRECISION DATA FOR MF AND BER

S. no.	Drug	Concentration (µg/ml)	Absorbance (Mean ± SD) at 257 nm	% RSD	Absorbance (Mean ± SD) 317 nm	% RSD
1	MF	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	0.40	0.756 ± 0.008	1.26
1	BER	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

S. no.	Drug	Concentration (µg/ml)	Absorbance (Mean ± SD) at 257 nm	% RSD	Absorbance (Mean ± SD) at 317 nm	% RSD
1	MF	5	0.391 ± 0.005	1.75	0.521 ± 0.006	1.00
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
1	BER	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

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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CONFLICT OF INTEREST: Nil

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