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RESEARCH ARTICLE

Spectrophotometric Methods for the Analysis of Berberine Hydrochloride and Eugenol in Formulated Emulgel

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Abstract

The present work describes three spectrophotometric methods for determining two phytoconstituent berberine hydrochloride and eugenol in formulated gels: simultaneous equation method, absorbance correction, and zero-crossing derivative method. In the simultaneous equation method, the absorbance at 263 nm and 280 nm and the absorbance correction method at 345 nm and 280 nm were measured and applied to their respective equation for the estimation of berberine hydrochloride and eugenol in phosphate buffer and formulated emulgel. The amplitudes of the first derivative spectra were measured at 252.5 nm for berberine hydrochloride and 263.5 nm for eugenol in zero-crossing crossing derivative spectrophotometry. For berberine hydrochloride and eugenol, linearity was attained in the concentration ranges of 4–20 and 2–10 µg/ml, respectively. Validation shows the applicability of the above procedures for the quantitative determination of berberine hydrochloride and eugenol. As a result, the presented method sucessfully estimated the aforesaid active phytoconstituent in formulated emulgel, with no interference from excipients.

Keywords: Berberine Hydrochloride, Eugenol, Phytoconstituent, UV-spectrophotometric Method, UV Simultaneous Method, Zero-crossing Derivative Method

1. Introduction

Dermal problems are the most likely reason for patients to seek primary health care. Inflammatory diseases and pathogenic microorganisms are responsible for the majority of these skin ailments. Topical and combination therapy is beneficial in the management of skin illnesses because it directs medications to the diseased locations within the skin, reducing or eliminating systemic adverse effects[1,2].

Eugenol (EGN) is chemically named as 4-allyl-2-methoxyphenol is yellow viscous oil and belongs to the class of terpenes. Earlier studies have documented EGN's biological activities, which include antibacterial, antifungal, anti-inflammatory, antioxidant, and chemoprotective actions, which may be due to its phenolic group characteristics[3]. It was discovered that EGN lowered carrageenan-induced pleural volumes in mice. Also, COX-2, NF-B, IL-6, leukotriene C4, and 5-LOX is among the proinflammatory mediators inhibited by EGN [4,5].

Berberine hydrochloride (BRB), ($C_{20}H_{18}ClNO_4$), chemical name as 9,10-dimethoxy-5,6-dihydro-[1,3] dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-7-ium hydrochloride, an isoquinoline alkaloid, is one of the herbal and safe compounds which exhibit anti-oxidative, antimicrobial, anti-inflammatory effects, as

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well as its modulatory effects on a spectrum of enzymes, receptors, and cell signaling pathways[6]. Dermatological disorders were treated using Rasaut, the decoction of *B. aristatais* as ethnobotanical studies[7].

Novel herbal delivery technologies improve therapeutic effectiveness and bioavailability by decreasing toxicity. Emulgel is one of the recent technology in NDDS used topically having characteristics of dual control release, i.e., emulsion as well as gel. It can deliver the drug directly to the skin to treat cutaneous disorders of local skin infections like fungal infection, acne, psoriasis, etc.[8,9]. BRB in topical delivery dosage form has been reported to have antimicrobial and anti-inflammatory activity as per the result of *in vitro* and *in vivo* experiment[10–12]. Also, eugenol has been demonstrated as a promising antimicrobial and inflammatory agent by *in vitro* and *in vivo* experiment[13,14]. Based on the literature survey, it is hypothesized that the combination of berberine chloride and eugenol in formulated emulgel can be effective in controlled management of *S. aureus* and *Candida albicans* induces inflammation and skin infections. There are numerous ways for estimating BRB alone and in mixture with other phytochemicals such as rutin, quercetin, etc, via HPLC, HPTLC, and UV spectrometric approaches[15–25]. Also, various analytical methods for estimation of EGN alone, herbal formulation, and in combination with rosmarinic acid, piperine, and cinnamaldehyde like HPTLC, HPLC, and UV method have been reported[26–33]. As per the literature survey spectrophotometric method was not available for concurrent estimation of BRB and EGN in emulgel. So, an attempt was made for the development of the UV method because of its simplicity and huge application in analysis.

2. Materials and Method

2.1 Instrument and Materials

Uv Visible Double beam Spectrophotometer Shimadzu 1800 having spectral bandwidth of 2 nm and wavelength accuracy ± 1 nm with 1cm quartz cells was used. Berberine hydrochloride and Eugenol were purchased from Loba Chemie Pvt. Ltd. Distilled water was available in the lab by Millipore system and AR grade of methanol of Loba Chemie Pvt. Ltd. Mumbai, India were used.

2.2 Selection of Solvent

Phosphate buffer of pH 7 was selected as solvent, based on the literature and solubility studies[34,35].

2.3 Preparation of Standard Solution

Stock solutions of BRB and EGN were made by weighing 10 mg of each standard drug individually, then transferring them to a 50 ml volumetric flask with 5 ml phosphate buffer and sonicating them. It was then diluted with phosphate buffer until it reached 200 µg/ml. With phosphate buffer of pH 7, further dilutions were made to obtain the appropriate concentration.

2.4 Selection of Wavelength[36,37]

2.4.1 Simultaneous Equation Method

The BRB and EGN stock solutions were further diluted to achieve the necessary concentrations of 10 µg/ml and 5 µg/ml, respectively. The wavelengths of 263 nm and 280 nm were chosen for estimation of BRB and EGN by simultaneous equation approach based on the spectral pattern.

$$Cx = \frac{A_2 ay_1 - A_1 ay_2}{ax_2\, ay_1 - ax_1\, ay_2} \qquad Cy = \frac{A_2 ax_2 - A_2 ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

Where, Cx and Cy are the concentration of BRB and EGN, ax_1 and ax_2 are absorptivities of BRB at 263 and 280 nm, respectively. ay_1 and ay_2 are absorptivity of EGN at 263 and 280 nm respectively.

2.4.2 Absorbance Correction Method

It was observed from the spectrum, that at 345 nm only BRB has substantial absorbance, whereas, at 280 nm, both BRB and EGN showed substantial absorbance. Thus, the estimation of BRB was carried at 345 nm without any interference, as EGN has zero absorbance at 345 nm.

BRB's absorbance was subtracted from the total absorbance of the combination at 280 nm, yielding the corrected absorbance for EGN.

The concentrations of the solutions were calculated using the Beer-Lamberts Law:

$Cx = A_1/ax_1 * b$

$Cy = [A_2-(ax_2 * cx)] / ay_2$

where, A_1 and A_2 = Absorbance of the sample at 345 nm and 280 nm respectively,

ax_1 and ax_2 =Absorptivity of BRB at 345 nm,280 nm respectively,ay_2 = Absorptivity of EGN at 280 nm

2.4.3 Zero Crossing Derivative Spectrophotometric Method

The spectrum of standard BRB and EGN stock solutions containing 10 µg/ml and 5 µg/ml respectively, were scanned in the UV range (200-400 nm) and recorded. BRB and EGN spectra were captured and transformed into first, second, and third derivative spectra. The first derivative approach with Δ λ= 4 and scaling factor 4 was chosen for the study based on the spectral pattern and zero-crossing locations. It revealed normal zero crossing locations at 263.5 nm for BRB and 252.5 nm for EGN. So, 263.5 nm and 252.5 nm wavelengths were chosen from the overlain spectra (Figure 2a & 2b) and also, shown in Table 1.

2.5 Method Validation

ICH Q2 (R1) guideline was followed to validate the presented method[38]. The standard calibration curve was plotted for BRB and EGN at their selected wavelength and the correlation coefficient was calculated. The limit of detection (LOD = 3.3 σ/s, where σ is the standard deviation of the response and s is the slope) and limit of quantitation (LOQ = 10 σ/s) of BRB and EGN was computed. Intraday and interday precision was studied by analyzing three replicates of the standard solution at three concentration levels and six times the same amount of drug solution for repeatability study. Recovery studies of the proposed methods were carried out by the standard addition method, adding Known the amount of each drug to the reanalyzed emulgel solution at three levels 50%, 100%, and 150% of the label claim. % recoveries were calculated by applying the following formula

% Recovery = (Amount of drug found after addition of standard drug - Amount of drug found before the addition of standard drug) / (Amount of standard drug added) x 100

2.6 Analysis of Formulated Emulgel

0.1 gm emulgel (equal to 2 mg BRB and 1 mg EGN) was precisely weighed and transferred to a 25 ml volumetric flask containing 15 ml methanol. The volumetric flask was heated in the water bath at 60 ºC for 5 min and then the solution was centrifuged for 15 mins at 800 rpm and the volume was made up. The supernatant solution of 1 ml was diluted to 10 ml in a volumetric flask with phosphate buffer to obtain concentrations, of 8 µg/ml and 4 µg/ml of BRB and EGN, respectively. Using the developed simultaneous equation, absorption correction, and zero-crossing derivatives methods the concentrations of BRB and EGN present in formulated emulgel were calculated.

3. Result and Discussion

3.1 Method Development of UV Spectrophotometric Method

According to the pattern of spectra in the wavelength range of 200–400 nm, different methods, i.e., the simultaneous equation, absorbance correction, and zero-crossing first-order derivative approach were proposed for formulated emulgel containing BRB and EGN.

Table 1. Selection of zero-crossing points for BRB & EGN

Drugs	Zero crossing point (nm)	Detection wavelength (nm)
BRB	263.5	252.5
EGN	252.5	263.5

3.1.1 Simultaneous Equation Method

The absorption of the phytoconstituent BRB and EGN at wavelength maxima 263 nm and 280 nm was used in the simultaneous equation method.

3.1.2 Absorbance Correction Method

In absorbance correction method, the basic requirement that whole spectra should follow the Beers law for the developing an analysis method. which was satisfied by both these drugs. EGN show zero absorbance at 345 nm and at 280 nm both the analyte show absorbance, so this method can be adopted. The selected wavelengths were 345 nm (λ-max of BRB) and 280 nm (λ-max of EGN) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of BRB (345 nm) and EGN (280 nm) was shown in Figure 1.

3.1.3 Zero Crossing Derivative Spectrophotometric Method

The zero-crossing method allows more than one wavelength and precise identification and quantification of BRB and EGN in mixtures. The wavelength was selected in such a way that remains unaffected by the quantity of any other presence analyte and shows the absorbance near zero for one analyte and vice versa in the case of the estimation of another analyte. The spectra obtained in the zero-crossing first-order derivative was presented in Figure 2a and 2b. As a result, the simultaneous determination of BRB and EGN as a binary combination was carried out at 252.5 nm (zero-crossing wavelength of EGN) and 263.5 nm (zero-crossing wavelength of BRB). The optimum linear response to the analyte concentration was obtained by measuring the absolute value of the derivative spectrum at these wavelengths (Figure 2 a–b).

3.2 Validation of Proposed Method

For BRB and EGN in varied concentration ranges, the linear regression equation approach revealed linearity. The correlation coefficient of these drugs was found to be close to 1.000, i.e., 0.9995–0.9991 indicating good linearity as shown in Table 2. The linearity range for BRB and EGN was 4-20 µg/ml and 2-10 µg/ml respectively, in simultaneous equation, absorbance correction, zero crossing derivative method. Percentage RSD for repetability, intraday and interday was found to be less than 2 i.e. 0.961-1.883 obtained value were within the range (Table 3). The % recovery for both the drug was found within the range (98.39%-101.32%) as mention in Table 4, which indicates the validity of all three methods.

3.3 Assay of Formulated Emulgel

Quantitative assessment of BRB and EGN was effectively carried out by the proposed UV spectroscopic method in formulated emulgel (2% w/w BRB and 1% w/w EGN in 10g of emulgel). Average assay values for both the drug were within the range of 100.83-96.37 % of formulated emulgel (Table 5).

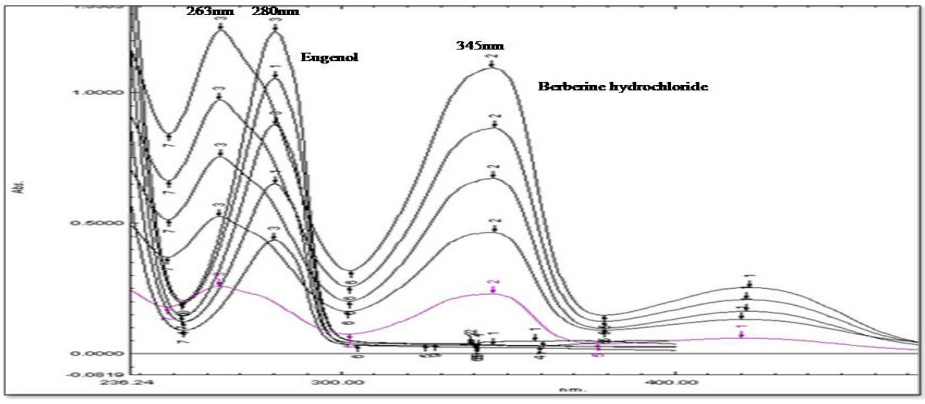
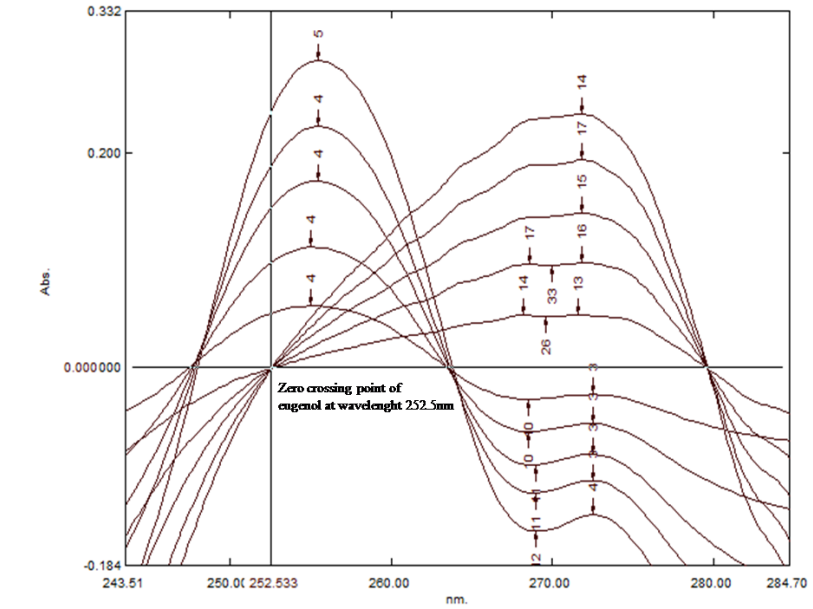
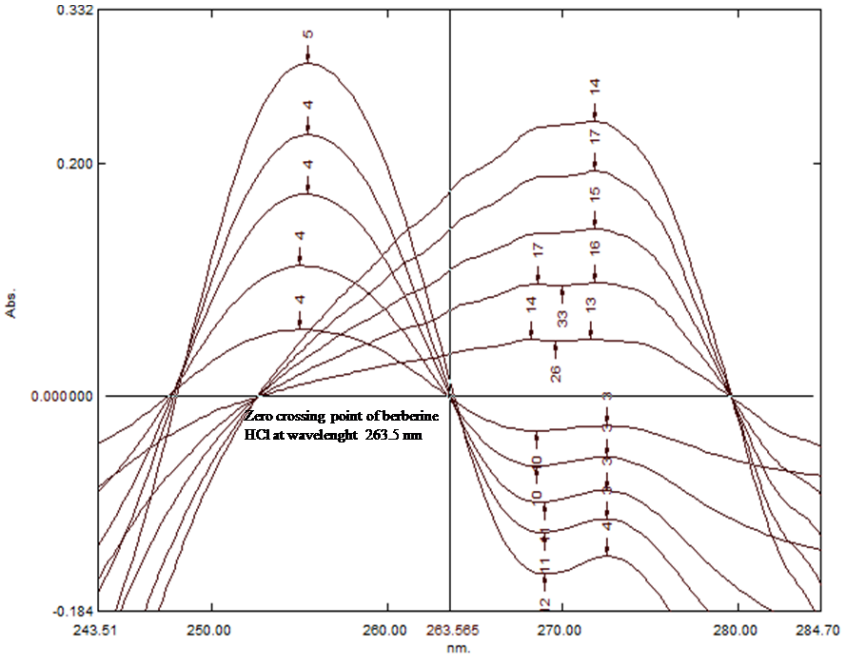


Figure 1. Overlain zero-order spectra of the standard solution 4-20 µg/ml of BRB and 2 - 10 µg/ml of EGN.



(a)



(b)

Figure 2. **(a)** First order derivative spectra for the estimation of BRB at 252.5 nm for linearity as EGN is showing zero crossing point. **(b)** First-order derivative spectra for the estimation of EGN at 263.5 nm for linearity as BRB is showing zero crossing point.

Table 2. Data of linear regression analysis of calibration curve

UV spectrophotometric methods	Drugs	Detection Wavelength (nm)	Linearity range (µg/ml)	Correlation coefficient	Regression equation*	LOD (µg/ml)	LOQ (µg/ml)
Simultaneous equation method	BRB	263	4-20	0.9995	y=0.060x + 0.0257	0.385	1.166
		280		0.9991	y=0.0447x+ 0.0251		
	EGN	263	2-10	0.9995	y=0.0199x+0.0128	0.412	1.257
		280		0.9994	y= 0.0617-0.0107		
Absorbance correction method	BRB	345	4-20	0.9992	y=0.0523x+0.0396	0.486	1.475
		280		0.9991	y=0.0447x+ 0.0251		
	EGN	280	2-10	0.9994	y= 0.0617-0.0107	0.605	1.835
Zero Crossing Derivative Method	BRB	252.5	4-20	0.9993	y=0.045x + 0.021	0.618	1.875
	EGN	263.5	2-10	0.9994	y=0.060x+0.023	0.385	1.167

*(n=5) average of five determinations

Table 3. Data of precision studies for the proposed method

UV-spectrophotometric methods	Drugs	Intraday studies (%RSD)**	Interday studies (%RSD)**	Repeatability studies (%RSD)*
Simultaneous equation method	BRB	1.578	1.672	1.342
	EGN	1.689	1.794	1.566
Absorbance correction method	BRB	1.356	1.560	1.147
	EGN	1.689	1.731	1.639
Zero-Crossing Derivative Method	BRB	1.178	1.767	0.961
	EGN	1.371	1.883	1.187

*(n=6) average of six determinations; ** (n=3) average of three determinations

Table 4. Data of accuracy studies for the proposed method

UV spectrophotometric methods	Drugs	% Recovery*		
		50 %	100 %	150 %
Simultaneous Equation	BRB	99.79 ± 1.56	100.09 ± 1.89	100.071 ± 1.78
	EGN	99.87 ± 1.78	99.23 ± 1.34	99.78 ± 1.67
Absorption correction method	BRB	99.01 ± 1.41	101.32 ± 1.10	100.54 ± 1.23
	EGN	100.16 ± 1.57	99.76 ± 1.67	99.90 ± 1.36
Zero crossing derivative method	BRB	98.83 ± 1.94	100.61 ± 1.72	100.21 ± 1.06
	EGN	98.39 ± 1.65	99.19 ± 1.94	98.93 ± 1.43

% Recovered mean ± SD* (n=3)

Table 5. Data of formulation analysis by the proposed method

Method	Drug	Labeled amount (w/w %)	Found amount (w/w %)*	% Drug found*	% RSD*
Simultaneous equation method	BRB	2	1.953 ± 0.03	97.59 ± 1.802	1.849
	EGN	1	0.963 ± 0.01	96.376±1.418	1.472
Absorbance correction method	BRB	2	1.946 ± 0.03	97.33 ± 1.627	1.669
	EGN	1	0.976 ± 0.01	97.67 ± 1.527	1.564
Zero crossing derivative method	BRB	2	2.016 ± 0.04	100.83 ± 1.631	1.614
	EGN	1	0.997 ± 0.01	99.74 ± 1.513	1.517

*(n=5) number of determinations

4. Conclusion

UV spectroscopy analysis methods which is cost effective and less time-consuming has been developed for quantifying BRB and EGN phytoconstituent present in formulated emulgel. Also, the obtained data of validation parameters were within the acceptable range of linearity, precision, and reproducibility for the simultaneous estimation of BRB and EGN. This proposed method can be an alternative quality control method for concurrent quantification of the cited drug in the formulation.

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6. Conflict of Interest

The authors declare no conflict of interest.

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