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VALIDATED ANALYTICAL APPROACH FOR SIMULTANEOUS ESTIMATION OF MICONAZOLE NITRATE AND EUGENOL IN SYNTHETIC FORMULATIONS

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ABSTRACT

The simultaneous estimation of Miconazole Nitrate and Eugenol in synthetic formulations is essential for ensuring quality, efficacy, and safety in pharmaceutical preparations. This study focuses on the development and validation of a robust analytical method for the precise quantification of these active ingredients. A high-performance liquid chromatography (HPLC) method was optimized using a suitable mobile phase, detection wavelength, and flow rate to achieve clear separation and accurate quantification. The method was validated as per ICH guidelines, evaluating parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ). The results demonstrated high accuracy and reproducibility, confirming the method's reliability for routine quality control analysis. The proposed method provides an efficient, sensitive, and reproducible approach for the simultaneous determination of Miconazole Nitrate and Eugenol, ensuring compliance with pharmaceutical standards.

Key words: ratio derivative approach, simultaneous equation, formulated emulgel, miconazole nitrate, first derivative (zero crossing) spectroscopic methods, and antifungal.

I. INTRODUCTION

Miconazole Nitrate and Eugenol are widely used in pharmaceutical formulations for their antifungal and antibacterial properties. Miconazole Nitrate, an imidazole antifungal agent, is commonly used for treating fungal infections by inhibiting ergosterol synthesis, thereby disrupting fungal cell membranes. Eugenol, a natural phenolic compound extracted from clove oil, exhibits antimicrobial, anti-inflammatory, and analgesic properties, making it a valuable component in various therapeutic applications.

The simultaneous estimation of these compounds in synthetic formulations is crucial for quality control and regulatory compliance. However, due to their structural differences and varying physicochemical properties, developing a validated analytical method poses significant challenges. Chromatographic techniques, particularly high-performance liquid chromatography (HPLC), have been widely adopted for the precise and accurate quantification of pharmaceutical compounds.

This study aims to develop and validate a simple, rapid, and sensitive HPLC method for the simultaneous estimation of Miconazole Nitrate and Eugenol in synthetic formulations. The method is validated following ICH guidelines, ensuring its reliability for routine quality control and pharmaceutical analysis. By optimizing chromatographic conditions, this study seeks to provide a robust analytical approach for efficient drug quantification, contributing to improved formulation standardization and regulatory compliance.

Infections caused by fungi continue to pose a serious risk to human health. Inappropriate and illogical usage of antifungal chemotherapeutics

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caused multidrug-resistant fungal infections, unwanted toxicity, and low therapeutic effectiveness.[1] Combination treatment is an option for treating infectious fungal illnesses, and new insights into the systems thought to have antifungal effects might inform the search for effective antifungal drugs.[2]

For fungal infections of the skin, such as vulvovaginitis, tinea pedis, or tinea cruris, the azole antifungal drug miconazole nitrate (MZL) is prescribed. Image 1a: MZL Antifungal mechanisms should be included. Inhibition of ergosterol manufacture and direct destruction of fungal cell membranes lead to changes in membrane fluidity and integrity, resulting in fungal cell lysis.3, 4

Eugenol, or 2-methoxy-4-prop-2-enylphenol as it is formally named in the chemical world, possesses a number of beneficial effects, including analgesic, anti-inflammatory, neuroprotective, antipyretic, antioxidant, and antifungal properties. The figure is labelled 1b. Clove oil has a unique family of microbiocidal phenylpropanoids, the principal component of which is EGL, which has a strong inhibitory effect on both bacteria and fungi. Both the destruction of the cell membrane and the leakage of lipids and proteins are possible outcomes.[5-7] The anti-fungal effects of miconazole nitrate and eugenol are enhanced when used together, as demonstrated in several studies. In addition, topical gels containing miconazole nitrate (such as nanoemulsion and microemulsion) are enhanced in their skin penetration and solubility by the addition of eugenol.Pages 8–10. Because of its aromatic nature, eugenol is having an additive impact on the MZL wavelength that was chosen. Therefore, it is crucial to find a way to estimate MZL and EGL concurrently that is easy to use, accurate, and reproducible. For both standalone and formulation-based MZL determination, several UV spectrophotometric, HPLC, and HPTLC approaches have been published.the years 2011–2014 Several analytical methods have been published for the analysis of MZL and other medicines, including mometasone furoate, nadifloxacin, lidocaine, econazole, metronidazole, hydrocortisone, and others.page(s) 15–26

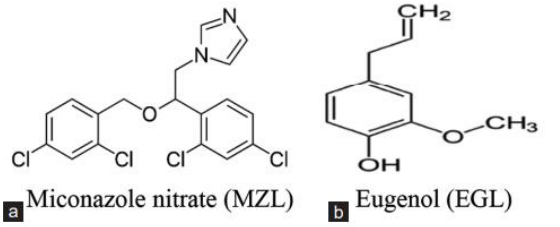

Figure 1: Chemical structures. (a) Miconazole nitrate; (b) Eugenol

Methods for estimating EGL alone and in combination with cinnamon oil, rosmarinic acid, piperine, and cinnamaldehyde have been developed and published by several authors using HPTLC, HPLC, and UV. pp. 27–38

For the purpose of determining MZL and EGL in emulgel formulation simultaneously, simpler, more sensitive, precise, accurate, and cost-effective UV spectroscopic techniques were attempted to be developed and validated [Figure 2].

II. MATERIALS AND METHOD

Chemical and reagent

A free sample of miconazole nitrate was sent by Novanta Health care LLP of Surat, Gujarat, India. Loba Chemie Pvt. Ltd. supplied the eugenol, while Samir Tech-Chem Pvt. Ltd. of Vadodara, Gujarat, India, supplied the methanol.

Apparatus

The entire experiment was conducted using a double beam UV visible spectrophotometer manufactured by Shimadzu (UV-1800, UV Probe, Kyoto, Japan) equipped with a matched quartz cell that had a path length of 1 cm.

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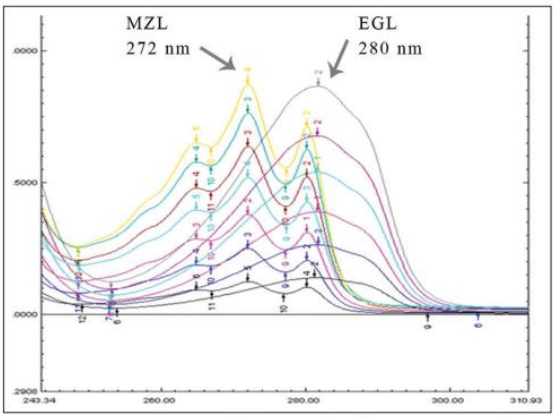

Figure 2: Overlain zero order spectra of the standard solution 100–600 µg/mL of MZL and 53–318 µg/mL of EGL

Preparation of standard solution

A 10 mL volumetric flask containing 1000 µg/mL of MZL was used to prepare a stock solution of the medication. Precisely 10 mg of the standard drug was weighed before transfer. Transferring precisely 0.05 mL of EGL (eugenol has a density of 1.067 g/mL) standard medication to a 10 mL volumetric flask allowed for the preparation of a stock solution of EGL. To get a concentration of 5300 µg/mL of EGL, it was further diluted with methanol until it reached the mark. To conduct linearity testing, further dilutions were prepared using methanol.

Selection of wavelength for simultaneous estimation of MZL and EGL[39]

Simultaneous equation method

The concentrations of 300 µg/mL and 159 µg/mL were achieved by further diluting the stock solution containing MZL and EGL, respectively, as mentioned above. Figure 2 shows that the spectral pattern informed the selection of the 272 nm and 280 nm wavelengths for the simultaneous equation approach of estimating MZL and EGL, respectively.

Zero-crossing derivative method

Spectra were collected after scanning standard stock solutions of MZL (300 µg/mL) and EGL (159 µg/mL) in the UV region (200-400 nm). First, second, and third derivative spectra were generated from the recorded spectra of MZL and EGL, respectively. For subsequent research, the approach with Δ lambda 4 and scaling factor 4 was chosen, considering its spectral pattern and zero crossing point. The absorbance for the measurement of MZL was shown in the first derivative spectra, which also displayed the usual zero-crossing point of EGL at 281 nm. The same holds true for the absorbance measurement of EGL; at 271 nm, the zero crossing point of the MZL is shown. The overlain spectra were used for MZL and EGL analysis, with 281 nm and 271 nm being the chosen wavelengths.

Ratio derivative method

Using reference spectra of MZL or EGL at varying concentrations, the ratio derivative technique divided the spectrum of the binary mixed solution of the two compounds to generate the ratio spectrum. Using 53 µg/mL of EGL as a divisor resulted in the optimised ratio spectra for MZL estimation. Similarly, when 600 µg/mL of MZL was utilised as a divisor, the ratio spectra of EGL were produced. After optimising the ratio spectrum, it was transformed into first, second, and third derivative spectra to create ratio derivative spectra. After converting the ratio spectra of MZL to the first derivative with a Δλ value of 2 nm and a scaling factor of 4, the optimised ratio derivative spectra were produced. The MZL analysis yielded 283 nm and 274 nm as analytical wavelengths, respectively. By transforming the EGL ratio spectra into the first derivative with a Δλ value of 2 nm and a scaling factor of 4, the ratio derivative spectra that were optimised for EGL estimation were obtained. For EGL analysis, the wavelengths that were obtained were 292 nm and 286 nm.

Formulation of emulgel

The necessary amount of Span 20 was dissolved in an oil phase consisting of liquid paraffin and isopropyl myristate to create an o/w emulsion. In contrast, the necessary amount of Tween 20 and preservative were dissolved in distilled water to create an external/aqueous phase. Separately,

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the watery and oily components were heated to around 60 °C. The oily phase was slowly added to the water phase while swirling constantly. The gel was made by mixing distilled water with Carbopol 934 P (1% w/w), which is 50% weight more than emulgel, and then shaking the mixture with a mechanical shaker for one hour. The produced gel was well mixed with the emulsion, and then, one drop at a time, triethanolamine was added to the dispersion system until it reached a semi-solid consistency.

Analysis of formulated emulgel

15 millilitres of methanol was added to 7.5 grammes of emulgel, which is equivalent to 15 milligrammes of MZL and 7.5 milligrammes of EGL. The mixture was then heated for 5 minutes in a water bath, centrifuged for 15 minutes at 600 rpm, and the volume was adjusted. The tube used for centrifugation was 50 millilitres. The concentrations of MZL and EGL were 300 µg/mL and 159 µg/mL, respectively, achieved by diluting the 10 mL supernant solution with methanol in a volumetric flask to 10 mL. The concentrations of MZL and EGL in the emulgel were determined using the proposed simultaneous equation, zero crossing derivatives, and ratio derivative techniques.

Parameters of analytical method

In accordance with ICH standards of Q2(R1), the established analytical methodologies have been verified. [40]

The correlation coefficient was computed for all the techniques indicated, and the standard calibration curves for MZL and EGL were displayed in the range of 100-600 µg/mL and 53-318 µg/mL, respectively, at the wavelengths they were chosen. The lowest detection and quantification concentrations were determined using the standard deviation of response and the mean slope of the calibration curve, respectively. On two separate days, researchers examined the discrepancy between the absorbance values of 200, 400, and 600 µg/mL of MZL and 106, 212, and 318 µg/mL of EGL. The amount of agreement in the obtained results was studied by analysing 300 µg/mL of MZL and 159 µg/mL of EGL six times, respectively. A matrix's accuracy can be defined as the degree to which its measured quantities agree with their true matrix contents. In order to carry out recovery experiments, three different concentrations (50, 100, and 150% of the reference drug solution) were spiked into the pre-analyzed emulgel solution (MZL: 300 µg/mL; EGL: 159 µg/mL). The percentage recovery was computed using the absorbance readings at certain wavelengths.

III. RESULTS AND DISCUSSION

Method development of UV spectrophotometric

Method

Quantification of formulations including emulgel, microemulsion, and nanoemulsion containing miconazole nitrate and eugenol/clove oil was done at 272 nm, which is the lambda max of miconazole nitrate alone. The spectra pattern of miconazole nitrate at 272 nm is interfered with by eugenol or clove oil because of its aromatic ring. Consequently, creating and testing a technique to estimate MZL and EGL simultaneously is crucial. references [9,10] We may estimate the mentioned pharmaceuticals in the formulated emulgel using three distinct methods: the simultaneous equation, the zero-crossing first-order derivative, and the ratio derivative technique, as shown by the UV spectra pattern in the 200-400 nm region of MZL and EGL.

Simultaneous equation method

Using the maximum absorption at 272 nm and 280 nm, respectively, the simultaneous equation approach was applied to the antifungal medication MZL and the phytoconstituent EGL. Absorbtivity values of 12.41 (ax1) and 10.31 (ax2) at 272 nm and 280 nm, respectively, were found for MZL and EGL. These numbers represent the mean of six separate estimates. Equations (1) and (2) were used to determine the

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drug concentrations by substituting the absorbance and absorption values (g/100 mL) at these wavelengths.

$$Cx = A2\ 16.85 – A1\ (24.76)/(-126.841) \quad (1)$$

$$Cy = A1\ (10.31) – A2\ (12.14)/(-126.841) \quad (2)$$

In this case, A1 is the absorbance at 272 nm and A2 is the absorbance at 280 nm of the sample solutions. The sample solution's MZL and EGL concentrations are denoted by Cx and Cy, respectively. Equations (1) and (2) may be solved to get Cx and Cy by replacing the values of A1 and A2.[39]

Zero-crossing derivative spectrophotometric method

By eliminating the possibility of drug interaction, the zero-crossing approach enables the accurate detection and quantification of MZL and EGL in combinations. We were able to quantify analytes with absorbances close to zero by carefully choosing their wavelengths, and we were able to estimate analytes with absorbances far from zero by doing the opposite. Figures 3a and 3b show the spectra that were derived from the zero crossing first order derivatives. Consequently, at 281 nm (the zero crossing wavelength of EGL) and 271 nm (the zero crossing wavelength of MZL), MZL and EGL were simultaneously determined in a binary combination. By analysing the derivative spectra at the specified wavelengths, we were able to determine the analyte amount's optimal linear response [Figure 3b]. With this information, we were able to utilise the linear regression equation to determine the unknown concentrations of MZL and EGL in the emulgel.[41]

Ratio derivative method

The standard spectra of MZL at various concentrations were used to split the stored spectra of binary mixes by wavelength in order to estimate EGL, and the inverse was also true. The ratio spectra of MZL were obtained by using 53 µg/mL of the standard solution of EGL as a divisor, after an examination of the effect of divisor concentration. The ratio spectra of EGL were obtained by dividing the binary mixture by the stored standard spectrum of MZL (600 µg/mL). A scaling factor of 4 and an interval of $\Delta\lambda = 2$ nm were used to trace the first derivative of these ratio spectra. In order to estimate MZL, we used the 283 nm and 274 nm wavelengths, and to determine EGL, we used the 286 nm and 292 nm wavelengths, as seen in Figure 4a and 4b. These wavelengths were readily used for the aforementioned drug estimations. At the aforementioned wavelengths, we examined the linear response to the analyte quantity and acquired ratio derivative spectra for various concentrations of MZL and EGL. The formula emulgel's unknown MZL and EGL concentrations were determined using the derived linear regression equation. [42]

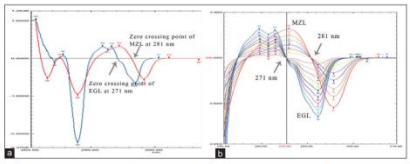

Figure 3: (a) First order derivative spectra for the estimation of MZL at 281 nm as EGN is showing zero crossing point and the estimation of EGL at 271 nm as MZL is showing zero crossing point. (b) First order derivative overlain spectra for the estimation of EGL at 271 nm for and MZL at 281 nm

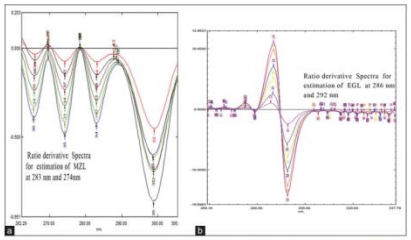

Figure 4: (a) Overlain first derivative ratio spectra of MZL (100–600 µg/mL) using standard spectrum of EGL (53 µg/mL) as divisor. (b) Overlain first derivative ratio spectra of EGL (53–318 µg/mL) using standard spectrum of MZL (600 µg/mL) as divisor

Validation of proposed method[40,43]

Several validation parameters were based on the "International Conference on Harmonisation" standard for analytical validation, which is detailed below. Range and linearity: The influence of different concentrations of MZL and EGL on absorbance at certain wavelengths was shown using a linear regression equation technique. There was a strong linear association between the reaction and concentration of MZL and EGL, as shown in Table 1, as the obtained correlation coefficient ranged from 0.9995 to 0.9999. We got the linearity range for MZL and

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EGL in the simultaneous equation, zero crossing derivative, and ratio derivative methods to be 100-600 µg/mL and 53-318 µg/mL, respectively. Reliability and Exactness: The % RSD of repeatability, intraday, and interday investigations was used to assess the dispersion variability among the measurement data. According to Table 2, the dispersion level was less than 2% of the RSD. The three approaches that were developed demonstrate precision based on these parameters. All three procedures were able to replicate the findings for MZL and EGL within the specified range (97.010%-101.53%), as shown in Table 3, demonstrating that the medicines were adequately recovered from the excipients.

Assay of formulated emulgel

A prepared emulgel containing 2% MZL and 1% EGL in 10 g of emulgel was successfully subjected to quantitative evaluation using the suggested UV spectroscopic approach. Table 4 shows that after 6 assessments of the prepared emulgel, the average test results for the two medications fell somewhere between 102.01% and 97-8.9%. Therefore, the created procedures may be used to analyse both medications in formed emulgel at the same time.

Table 1: Data of linear regression analysis of calibration curve of proposed methods

UV spectrophotometric method	Drugs	Detection wavelength (nm)	Linearity range (µg/mL)	Correlation coefficient	Regression equation*	LOD (µg/mL)	LOQ (µg/mL)
Simultaneous equation method	MZL	272	100–600	0.9995	Y=0.0031x–0.0153	1.908	5.782
		280		0.9996	Y=0.0031x–0.0184		
	EGL	272	53–318	0.9993	Y=0.0016x–0.0148	2.008	5.882
		280		0.9997	Y=0.016x+0.014		
Zero crossing derivative method	MZL	281	100–600	0.9995	Y=0.001x–0.0056	0.114	0.346
	EGL	271	53–318	0.9999	Y=0.006x–0.0021	0.804	2.437
Ratio derivative method	MZL	283	100–600	0.9998	Y=0.816x+0.3137	0.190	0.579
		274		0.9995	y=0.8994x+0.2233	0.280	0.898
	EGL	286	53–318	0.9994	Y=0.0387x–1.8116	0.012	0.036
		292		0.9995	Y=0.0398x–1.9113	0.023	0.052

*(*n*=5) average of five determination

Table 2: Data of precision studies for proposed methods

UV-spectrometric method	Drugs	Intraday studies (%RSD)**	Interday studies (%RSD)**	Repeatability studies (%RSD)*
Simultaneous equation method	MZL	1.356	1.985	0.606
	EGL	1.142	1.688	0.548
Zero crossing derivative method	MZL	1.356	1.560	1.147
	EGL	1.689	1.731	1.639
Ratio derivative method	MZL (283 nm)	1.683	1.549	0.908
	MZL (274 nm)	1.479	1.298	0.516
	EGL (286 nm)	0.959	1.671	1.193
	EGL (292 nm)	1.293	1.670	0.295

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

Table 3: Data of accuracy studies for proposed methods

Method	Drugs	% Recovery*		
		50%	100%	150%
Simultaneous equation	MZL	99.346±1.478	100.325±1.675	100.77±1.765
	EGL	97.622±1.651	98.472±1.582	98.591±1.562
Zero crossing derivative	MZL	98.399±1.653	99.195±1.948	98.932±1.438
	EGL	98.830±1.948	100.611±1.720	100.212±1.063
Ratio derivative	MZL (283 nm)	97.010±1.729	98.459±0.744	99.989±0.965
	MZL (274 nm)	97.333±1.116	98.048±1.196	99.508±0.713
	EGL (286 nm)	101.535±1.799	100.112± 1.274	100.458±1.092
	EGL (292 nm)	99.350±1.918	101.087±1.382	100.421±0.855

% recovered value mean±SD* (*n*=3)

Table 4: Data of formulation analysis by proposed method

Method	Drug	Labeled amount (w/w %)	Found amount (w/w %)*	% Drug found*	%RSD*
Simultaneous equation method	MZL	2	1.929±0.032	96.466±1.615	1.674
	EGL	1	0.973±0.019	97.266±1.682	1.737
Zero crossing derivative method	MZL	2	1.948±0.034	97.456±1.711	1.756
	EGL	1	0.958±0.018	95.833±1.144	1.896
Ratio derivative method	MZL	2	1.931±0.022	96.566±1.144	1.185
	EGl	1	0.952±0.0178	95.234±1.789	1.879

*(*n*=6) number of determinations

IV. CONCLUSION

The validated HPLC method developed in this study provides a simple, accurate, and reproducible approach for the simultaneous estimation of Miconazole Nitrate and Eugenol in synthetic formulations. The method was successfully validated as per ICH guidelines, ensuring its reliability in terms of linearity, precision, accuracy, specificity, LOD, and LOQ. The results confirm that this analytical technique is efficient and suitable for routine quality control and pharmaceutical analysis.

By offering a robust and sensitive quantification method, this study contributes to ensuring the quality, safety, and efficacy of pharmaceutical formulations containing Miconazole Nitrate and Eugenol. The proposed method can be further utilized for formulation development, regulatory submissions, and stability studies, enhancing standardization in the pharmaceutical industry.

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