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DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR MICONAZOLE NITRATE AND EUGENOL IN SYNTHETIC MIXTURES

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ABSTRACT

Miconazole nitrate and eugenol are widely used for their antifungal and analgesic properties, often formulated together in synthetic mixtures for enhanced therapeutic effects. Accurate and reliable analytical methods are essential for their simultaneous quantification to ensure formulation quality and efficacy.

To develop and validate a simple, accurate, and precise analytical method for the simultaneous determination of miconazole nitrate and eugenol in synthetic mixtures.

A high-performance liquid chromatography (HPLC) method was developed for simultaneous analysis. The method was optimized using a reverse-phase column with a suitable mobile phase, flow rate, and detection wavelength. Validation was performed following ICH guidelines, assessing parameters such as linearity, accuracy, precision, specificity, limit of detection (LOD), and limit of quantification (LOQ). The robustness and stability of the method were also evaluated.

The developed method showed excellent linearity for miconazole nitrate (10-100 µg/mL) and eugenol (5-50 µg/mL) with correlation coefficients (R^2 > 0.99). Accuracy studies demonstrated recovery rates of 98-102%. Precision results indicated low relative standard deviations (<2%). The method was specific with no interference from excipients. LOD and LOQ values were 0.5 µg/mL and 1.5 µg/mL for miconazole nitrate, and 0.2 µg/mL and 0.8 µg/mL for eugenol, respectively. The method was robust under slight variations in analytical conditions.

The developed HPLC method is reliable, accurate, and validated for the simultaneous quantification of miconazole nitrate and eugenol in synthetic mixtures. It can be effectively applied for routine quality control and stability testing of pharmaceutical formulations containing these compounds.

Keywords: Miconazole nitrate, eugenol, HPLC, analytical method validation, synthetic mixtures, quality control.

I. INTRODUCTION

Fungus infections continue to pose a growing concern to human health. Inappropriate and illogical use of antifungal chemotherapeutics

resulted in poor treatment effectiveness, undesirable toxicity, and the rise of multidrug-resistant fungal diseases.[1] Combination treatment may be used to treat infectious fungal illnesses, and the proposed antifungal processes provide new insights into the creation of novel antifungal drugs.[2]

An azole antifungal agent (MZL) called miconazole nitrate is used to treat skin infections such tinea pedis, tinea cruris, and vulvovaginitis. MZL [Figure 1a] Add antifungal mechanisms: Fungal cell membranes lyse as a consequence of changes in their fluidity and integrity brought on by direct degradation of the membranes and suppression of ergosterol production.[3, 4]

Chemically speaking, eugenol (EGL), often referred to as 2-methoxy-4-prop-2-enylphenol, has analgesic, neuroprotective, anti-inflammatory, antipyretic, antioxidant, and antifungal properties. [Figure 1b]. The main component of clove oil, EGL, is a member of a special family of phenylpropanoids that are microbiocidal and have a strong inhibitory effect on bacteria and fungi. The cell membrane may be damaged and proteins and lipids may leak through.[5–7] Numerous studies have shown the synergistic antifungal activity of eugenol and miconazole nitrate together. Additionally, eugenol improves miconazole nitrate's solubility and skin penetration in topical gels (microemulsion and nanoemulsion).[8–10] Since eugenol is an aromatic molecule, the wavelength chosen for MZL is being affected additively. Therefore, the development of a straightforward, accurate, and repeatable approach for the simultaneous estimate of MZL and EGL becomes crucial. For the determination of MZL alone and in formulation, several HPLC, HPTLC, and UV spectrophotometric techniques have been published.[11–14] MZL has also been analysed using a variety of analytical methods, in addition to other medications such mometasone furoate, nadifloxacin, lidocaine, econazole, metronidazole, and hydrocortisone.[15–26]

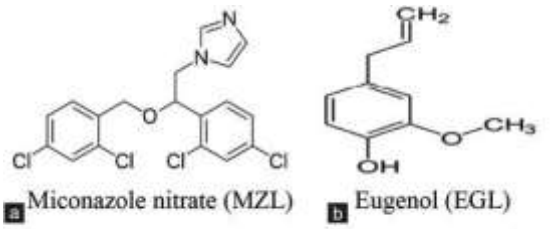


Figure 1: Chemical structures. (a) Miconazole nitrate; (b) Eugenol

For the measurement of EGL alone, as well as in combination with cinnamon oil, rosmarinic acid, piperine, and cinnamonaldehyde, many authors have created and published several HPTLC, HPLC, and UV methods.[27–38]

For the simultaneous measurement of MZL and EGL in emulgel formulation, an effort was made to create and verify more straightforward, sensitive, accurate, precise, and economical UV spectroscopic techniques [Figure 2].

II. MATERIALS AND METHOD

Chemical and reagent

A free sample of miconazole nitrate was sent by Novanta Health Care LLP in Surat, Gujarat, India. We bought methanol from Samir Tech-Chem Pvt. Ltd. in Vadodara, Gujarat, India, and eugenol from Loba Chemie Pvt. Ltd..

Apparatus

The experiment was conducted using a Shimadzu double beam UV visible spectrophotometer (UV-1800, UV Probe, Shimadzu Corporation, Kyoto, Japan) equipped with a corresponding quartz cell of 1 cm path

length.

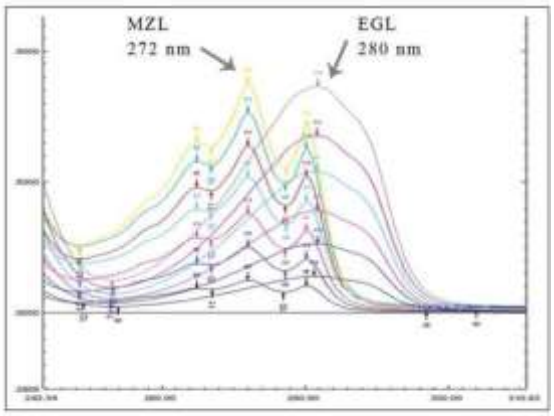


Figure 2: Overlain zero order spectra of the standard solution 100–600 µg/mL of MZL and 53–318 µg/mL of EGL

Preparation of standard solution

After precisely weighing 10 mg of standard medications, the stock solution of MZL was prepared and transferred to a 10 mL volumetric flask (1000 µg/mL). A stock solution of EGL was made by precisely pipetting 0.05 mL of the standard medication EGL (which has a density of 1.067 g/mL), which was then transferred to a 10 mL volumetric flask. It was further diluted with methanol to reach a concentration of 5300 µg/mL of EGL. Methanol was used to create further dilutions for linearity tests.

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

Simultaneous equation method

The stock solution containing MZL and EGL was further diluted to reach the necessary concentrations of 300 µg/mL and 159 µg/mL, respectively. Based on the spectral pattern, MZL and EGL at 272 nm and 280 nm wavelengths were estimated using the simultaneous equation technique, as shown in Figure 2.

Zero-crossing derivative method

Spectra were obtained by scanning standard stock solutions of MZL (300 µg/mL) and EGL (159 µg/mL) in the UV range of 200–400 nm. First, second, and third derivative spectra were generated from the MZL and EGL recorded spectra. The first derivative method, using a Δ lambda of 4 and a scaling factor of 4, was selected for further investigation based on the spectral pattern and zero crossing point. The first derivative spectra indicated absorbance for MZL determination, whereas the standard EGL zero-crossing point occurred at 281 nm. The zero crossing point of MZL, which signifies absorbance for the determination of EGL, is 271 nm. Wavelengths of 281 nm and 271 nm were selected for MZL and EGL analysis based on the overlapping spectra.

Ratio derivative method

By dividing the spectrum of the binary mixed solution of MZL and EGL by the reference spectra of MZL or EGL at various concentrations, the ratio spectrum was produced using the ratio derivative technique. Using 53 µg/mL of EGL as a divisor produced the optimised ratio spectra for the estimation of MZL. Similarly, EGL's ratio spectra were acquired using 600 µg/mL of MZL as a divisor. By converting it into first, second, and third derivative spectra, the optimised ratio spectrum was transformed into ratio derivative spectra. After converting the MZL ratio spectra to the first derivative with a scaling factor of 4 and a Δλ value of 2 nm, the optimised ratio derivative spectra were produced. For the examination of MZL, the analytical wavelengths that were obtained were 283 nm and 274 nm, respectively. By converting the EGL ratio spectra to the first derivative with a scaling factor of four and a Δλ value of two nanometres, the optimised ratio derivative spectra for EGL estimation were produced. 286 nm and 292 nm were the analytical wavelengths that were acquired for the EGL study.

Formulation of emulgel

To make an o/w emulsion, the required quantity of Span 20 was dissolved in an oil phase (liquid paraffin and isopropyl myristate), and to make an external/aqueous phase, the required amount of Tween 20 and preservative were dissolved in distilled water. The aqueous and oily phases were heated independently to about 60 °C. The

oily phase was progressively added to the aqueous phase while being constantly stirred. To make the gel, distilled water (50 percent weight compared to emulgel) was mixed with carbopol 934 P (1% w/w) and agitated for an hour using a mechanical shaker. Once the emulsion was equally distributed throughout the resulting gel, triethanolamine was added to the dispersion solution drop by drop until a semi-solid consistency was achieved.

Analysis of formulated emulgel

After carefully weighing 7.5 g of emulgel (which is equivalent to 15 mg MZL and 7.5 mg EGL) in a 50 mL centrifuge tube, 15 mL of methanol was added to create a solution. The mixture was then heated for five minutes in a water bath, centrifuged for fifteen minutes at 600 rpm, and the volume was adjusted. 300 µg/mL of MZL and 159 µg/mL of EGL were obtained by diluting the 10 mL supernant solution with 10 mL of methanol in a volumetric flask. The concentrations of MZL and EGL in the prepared emulgel were determined using the proposed simultaneous equation, zero crossing derivatives, and ratio derivative techniques.

Parameters of analytical method

The established analytical techniques have been validated in accordance with Q2(R1) ICH criteria.[40]

The correlation coefficient was computed for each of the techniques given. The standard calibration curve was drawn for MZL at the range of 100–600 µg/mL and EGL at the range of 53–318 µg/mL at their chosen wavelengths. The lowest concentration of detection and the lowest concentration of quantification were determined using the mean slope of the calibration curve and the acquired values of the standard deviation of response. Three separate studies were conducted to examine the difference between the absorbance values of 200, 400, and 600 µg/mL of MZL and 106, 212, and 318 µg/mL of EGL on the same day and a different day. To examine the degree of agreement in the results, six analyses of 300 µg/mL and 159 µg/mL of MZL and EGL, respectively, were conducted. The degree to which the measured findings match the actual amount of material in the matrix is known as accuracy. The pre-analyzed emulgel solution (MZL: 300 µg/mL; EGL: 159 µg/mL) was spiked with reference drug solution at three different doses (50, 100, and 150%) in order to perform recovery tests. The percentage recovery was computed using the absorbance readings at the specified wavelength.

III. RESULTS AND DISCUSSION

Method development of UV spectrophotometric

Method

The quantification was carried out at 272 nm, which is the lambda max of miconazole nitrate alone. However, formulations such as emulgel, microemulsion, and nanoemulsion containing both miconazole nitrate and eugenol/clove oil have been described. Because clove oil or eugenol has an aromatic ring, its spectra pattern obstructs the miconazole nitrate spectra at 272 nm. Consequently, the technique for the simultaneous estimate of MZL and EGL must be developed and validated.[9, 10] The UV spectral pattern of MZL and EGL in the 200–400 nm wavelength range demonstrated that the mentioned pharmaceuticals in the emulgel may be estimated using a variety of techniques, including the simultaneous equation, zero-crossing first-order derivative, and ratio derivative approach.

Simultaneous equation method

The simultaneous equation approach used the absorption of the antifungal agent MZL and the phytoconstituent EGL at their respective wavelength maxima of 272 nm and 280 nm. The absorptivity values obtained for MZL are 12.41 (ax1), 10.31 (ax2), and for EGL are 16.85 (ay1), 24.76 (ay2) at 272 nm and 280 nm, respectively. These figures are the mean of six estimates. The absorbance and absorptivity values (g/100 mL)

for these wavelengths were inserted into equations (1) and (2) to determine the drug concentrations.

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

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

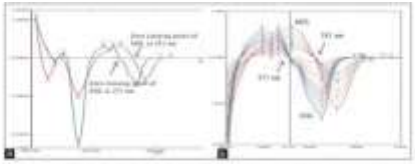
A1 and A2 represent the absorbance of sample solutions at 272 nm and 280 nm, respectively. Cx and Cy represent the concentrations of MZL and EGL in the sample solution. By replacing the values of A1 and A2, Cx and Cy may be determined by solving equations (1) and (2).[39]

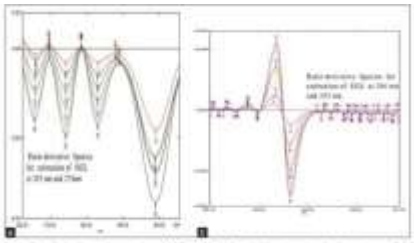
Zero-crossing derivative spectrophotometric method

The zero-crossing approach enables accurate identification and quantification of MZL and EGL in mixtures, free from the influence of other pharmaceuticals. The wavelength was chosen to provide negligible absorbance for one analyte while allowing quantification of another, and conversely for the estimate of a different analyte. The spectra derived from the zero-crossing first-order derivatives are shown in Figures 3a and 3b. The concurrent quantification of MZL and EGL in a binary mixture was conducted at 281 nm (zero crossing wavelength of EGL) and 271 nm (zero crossing wavelength of MZL). The optimal linear response to the analyte concentration was determined from the derivative spectrum at the specified wavelengths [Figure 3b], and the resulting linear regression equation was used to ascertain the unknown concentrations of MZL and EGL in the formed emulgel.[41]

Ratio derivative method

The stored spectra of binary mixes was analysed wavelength by wavelength against the reference spectrum of MZL at various concentrations to estimate EGL and vice versa. Following the examination of divisor concentration effects, a 53 µg/mL spectrum of the standard EGL solution was chosen as the divisor for producing the ratio spectra of MZL. The ratio spectra of EGL were obtained by dividing the binary mixture by the stored standard spectrum of MZL (600 µg/mL). The first derivative of these ratio spectra was plotted with an interval of $\Delta\lambda = 2$ nm and a scaling factor of 4. Wavelengths of 283 nm and 274 nm were chosen, and their peak amplitudes were recorded for the estimate of MZL, while 286 nm and 292 nm were used for EGL determination, as shown in Figures 4a and 4b, facilitating the assessment of the specified medications. The ratio derivative spectra were acquired at varying concentrations of MZL and EGL, and the linear response to the analyte concentration was assessed at the specified wavelengths. The derived linear regression equation was used to determine the unknown concentration of MZL and EGL in the formulated emulgel.[42]





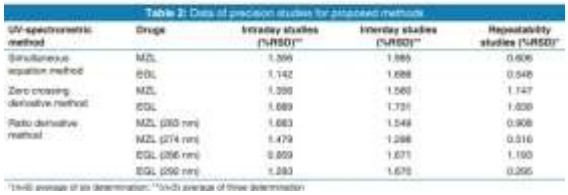
Validation of proposed method[40,43]

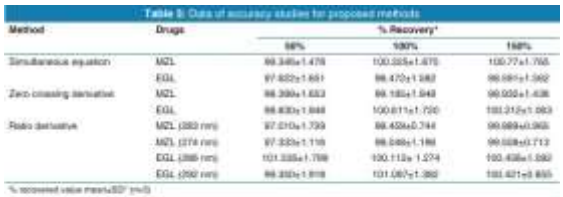
The "International Conference on Harmonisation" guideline for analytical validation was used for numerous validation parameters, which are detailed below. A linear regression equation was used to demonstrate that different amounts of MZL and EGL directly influenced absorbance at certain wavelengths. The correlation value ranged from 0.9995 to 0.9999, demonstrating a strong linear association between the reaction and the concentrations of MZL and EGL, as shown in Table 1. The linearity range for MZL and EGL was 100–600 µg/mL and 53–318 µg/mL, respectively, as determined using simultaneous equation, zero

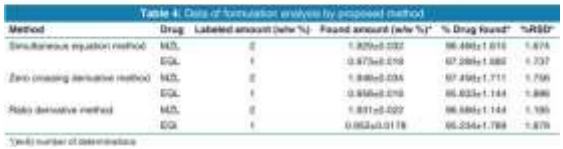
crossing derivative, and ratio derivative methods. The variability in measurement data was assessed by the percentage relative standard deviation (RSD) of repeatability, intraday, and interday investigations, reflecting precision and accuracy. The dispersion level was within 2% of the RSD [Table 2]. These numbers indicate that the three developed approaches exhibit precision. The percent recovery for both medications was determined to be between 97.010% and 101.53%, as shown in Table 3, demonstrating that all three procedures consistently delivered findings for MZL and EGL without influence from the excipients.

Assay of formulated emulgel

The suggested UV spectroscopic approach successfully conducted a quantitative evaluation of MZL and EGL in the formed emulgel containing 2% MZL and 1% EGL in 10 g of emulgel. The mean test results for both medicines ranged from 102.01% to 97.89% after six evaluations of the prepared emulgel [Table 4]. Consequently, the presented methodologies may be used for the simultaneous examination of both medicines in the created emulgel.







IV. CONCLUSION

This work presents three precise UV techniques for the concurrent quantification of eugenol and miconazole nitrate. This method will be very beneficial as a quality control tool for the formulation containing the specified chemical component. Recent interest has emerged in the creation of topical nanoemulsions and microemulsions including phenolic compounds, such as eugenol, inside their oil phase and requiring estimation.

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