RES MILITARIS Social Science Journal

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR MICONAZOLE NITRATE AND EUGENOL IN SYNTHETIC MIXTURE

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ABSTRACT

First of all, The risk of fungal infection to human health is still present and becoming worse. For the treatment of invasive fungal infections, combination therapy may be an optional strategy. The possible antifungal mechanisms provide fresh perspectives on the creation of innovative antifungal drugs. Numerous studies have shown the synergistic antifungal effects of combining eugenol and miconazole nitrate. Eugenol was thus added to a formulation in topical gels to improve skin penetration and miconazole nitrate availability. Consequently, a technique for measuring the aforementioned medications simultaneously in the emulgel formulation has been developed and verified by the use of UV spectrophotometric analysis. Supplies and Procedures: For the purpose of estimating both medications, absorbance was measured at 272 and 280 nm using the simultaneous equation approach. Using the first derivative (zero crossing) approach, the calculated values for miconazole nitrate and eugenol were 281 and 271 nm, respectively. Peak amplitudes at 286 and 292 nm for eugenol and 283 and 274 nm for miconazole nitrate were used in the ratio derivative approach. The developed techniques, which included criteria like specificity, linearity, range, precision, accuracy, limit of detection, and limit of quantification, were verified in accordance with the ICH recommendations. Findings and Discussion: For miconazole nitrate, all three test techniques demonstrated a clear correlation between response and concentration in the concentration range of 100–600 µg/mL, and for eugenol, 53–318 µg/mL. Within 2% of the RSD was the dispersion level. The results of recovery testing for both medications varied from 97 to 102%, suggesting that the techniques are successful.

In conclusion, it was discovered that every approach that was suggested was rapid, accurate, and affordable. Therefore, when calculating miconazole nitrate and eugenol in emulgel formulations or formulations including the aforementioned drugs, they may now be used for regular quality control analysis.

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

I. INTRODUCTION

The threat of fungus infection to human health is ongoing and getting worse. Antifungal chemotherapeutics were used inappropriately and irrationally, which led to the emergence of multidrug-resistant fungal infections, undesired toxicity, and poor therapeutic efficacy.[1] Infectious fungal diseases may be treated with combination therapy, and the putative antifungal mechanisms offer fresh perspectives on the development for new antifungal medications.[2]

The miconazole nitrate is an azole antifungal agent (MZL) used to treat skin infections such vulvovaginitis, tinea pedis, or tinea cruris. MZL [Figure 1a] Include antifungal mechanisms: Direct fungal cell membrane destruction and inhibition of ergosterol biosynthesis, which results in lysis of fungal cell membranes due to alterations in both membrane integrity and fluidity.[3,4]

Eugenol (EGL), also known as 2-methoxy-4-prop-2- enylphenol in chemical terms, has

analgesic, anti-inflammatory, neuroprotective, antipyretic, antioxidant, and antifungal activities [Figure 1b]. EGL, the primary constituent of clove oil, belongs to a unique class of microbiocidal phenylpropanoids and has a potent inhibitory impact on fungus and bacteria. Proteins and lipids may seep over the membrane and cell membrane may be destroyed.[5-7] Several papers have proven that combination of miconazole nitrate and eugenol has synergistic anti-fungal effect. Furthermore, eugenol increases the skin penetration and solubility of miconazole nitrate in topical gels (nanoemulsion and microemulsion).[8-10] Eugenol is an aromatic compound, so it is having the additive effect on the wavelength selected for MZL. Hence, it becomes essential to develop a simple, precise, and reproducible method for the estimation of MZL and EGL simultaneously. There are many reported HPLC, HPTLC, and UV spectrophotometric methods for the estimation of MZL alone and in formulation.[11-14] Analysis of MZL, along with other drugs such as mometasone furoate, nadifloxacin, lidocaine, econazole, metronidazole, hydrocortisone, and using several analytical techniques has also been reported.[15-26]

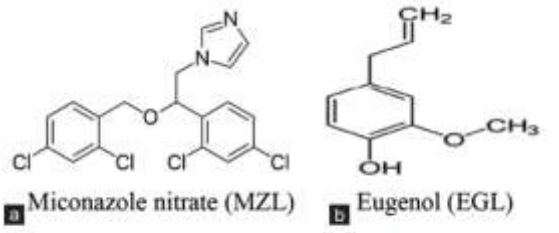


Figure 1: Chemical structures. (a) Miconazole nitrate; (b) Eugenol

Various authors have developed and reported different HPTLC, HPLC, and UV method, for the estimation of EGL alone, as well as combinations with cinnamon oil, rosmarinic acid, piperine, and cinnamaldehyde.[27-38]

Attempt was made to develop and validate simpler, sensitive, precise, accurate, and cost-effective UV spectroscopic methods for the simultaneous determination of MZL and EGL in emulgel formulation [Figure 2].

II. MATERIALS AND METHOD

Chemical and reagent

Miconazole nitrate was provided as gift sample from Novanta Health care LLP, Surat, Gujarat, India. Eugenol was purchased from Loba Chemie Pvt. Ltd. and methanol from Samir Tech-Chem Pvt. Ltd,Vadodara, Gujarat, India.

Apparatus

Shimadzu double beam UV visible spectrophotometer (UV-1800, UV Probe, Shimadzu Corporation, Kyoto, Japan) with matched quartz cell of 1 cm path length was used throughout the experiment.

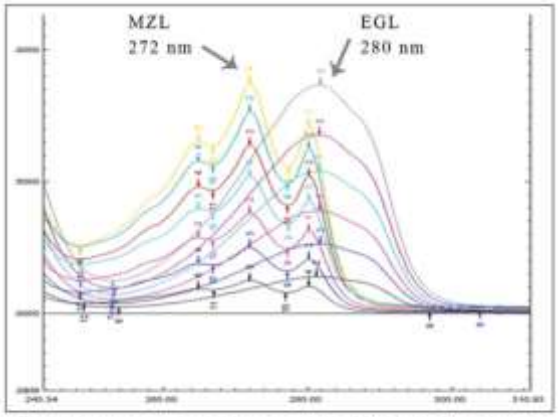


Figure 2: Overlain zero order spectra of the standard solution 100–600 µg/mL of MZL and 53–318 µg/mL of EGL

Preparation of standard solution

Stock solution of MZL was set ready by weighing accurately 10 mg of standard drugs and transferred to a 10 mL volumetric flask (1000 µg/mL). Stock solution of EGL was prepared by pipetting accurately 0.05 mL of EGL (the density of eugenol is 1.067 g/mL) standard drug which was then transferred to a 10 mL volumetric flask. It was further diluted up to mark with methanol to get 5300 µg/mL concentration of EGL. Further dilutions were made with methanol for linearity studies.

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

Simultaneous equation method

The above stock solution containing MZL and EGL was further diluted to get the desired

concentrations of 300 µg/mL and 159 µg/mL, respectively. Based on the spectral pattern, 272 nm and 280 nm wavelengths were selected for estimation of MZL and EGL by the simultaneous equation method as shown in Figure 2.

Zero-crossing derivative method

Standard stock solutions of MZL (300 µg/mL) and EGL (159 µg/mL) were scanned in the UV region (200–400 nm) and spectra were recorded. The recorded spectra of MZL and EGL were converted into first, second, and third derivative spectra. Based on the spectral pattern and zero crossing point of the first derivative method with Δ lambda 4 and scaling factor 4, was selected for further studies. The first derivative spectra showed the typical zero-crossing point of EGL at 281 nm but show absorbance for the determination of MZL. Similarly 271 nm is zero crossing point of MZL which show absorbance for the determination of EGL. From the overlain spectra, 281 nm and 271 nm were selected for MZL and EGL analysis.

Ratio derivative method

In the ratio derivative method, the ratio spectrum was obtained by dividing the spectrum of the binary mixture solution of MZL and EGL with the standard spectra of MZL or EGL of different concentrations. The optimized ratio spectra for the estimation of MZL were obtained when 53 µg/mL of EGL was used as a divisor. In the same way, the ratio spectra of EGL were obtained when 600 µg/mL of MZL was used as a divisor. The optimized ratio spectrum was converted into ratio derivative spectra by transforming it into first, second, and third derivative spectra. The optimized ratio derivative spectra were obtained when the ratio spectra of MZL were converted to the first derivative having Δλ value 2 nm and a scaling factor of 4. The obtained analytical wavelengths for the analysis of MZL were 283 nm and 274 nm, respectively. The optimized ratio derivative spectra for EGL estimation were obtained by converting the ratio spectra of EGL to the 1st derivative having Δλ value 2 nm and a scaling factor of 4. The analytical wavelengths obtained for the analysis of EGL were 286 nm and 292 nm.

Formulation of emulgel

An o/w emulsion was prepared by dissolving the required amount of Span 20 in an oil phase (liquid paraffin and isopropyl myristate), while an external/aqueous phase was prepared by dissolving the required quantity of Tween 20 and preservative in distilled water. Both the aqueous and oily phases were separately heated to around 60 °C. Gradually, the oily phase was added to the aqueous phase with continuous stirring. Gel was prepared by dispersing Carbopol 934 P (1% w/w) in distilled water (50% weight compared to emulgel) and shaking for 1 h using a mechanical shaker. Emulsion was mixed uniformly in the prepared gel, and at last, triethanolamine was added to the dispersion system drop by drop until semi-solid consistency was achieved.

Analysis of formulated emulgel

7.5 g of emulgel (equal to 15 mg MZL and 7.5 mg EGL) was precisely weighed in a 50 mL centrifuge tube, and a solution was prepared by adding 15 mL of methanol, heating it for 5 minutes in a water bath, centrifuged for 15 minutes at 600 rpm, and the volume was adjusted. The supernant solution of 10 mL was diluted to 10 mL in a volumetric flask with methanol to obtain concentrations of 300 µg/mL of MZL and 159 µg/mL of EGL, respectively. Using the developed simultaneous equation, zero crossing derivatives, and ratio derivative methods, the concentrations of MZL and EGL present in the formulated emulgel were calculated.

Parameters of analytical method

Validation of the developed analytical methods has been validated in pursuance of ICH guidelines of Q2(R1).[40]

The standard calibration curve was plotted for MZL in the range 100–600 µg/mL and EGL 53–318 µg/mL at their selected wavelengths, and the correlation coefficient was calculated for all the described methods. The obtained values of the standard deviation of response and the mean slope of the calibration curve were used to find the lowest concentration of detection and the lowest concentration of quantification. The deviation between the absorbance value 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 was studied 3 times. Six times the analysis of 300 µg/mL and 159 µg/mL of MZL and EGL, respectively, was done to study the level of agreement in the obtained values. Accuracy measures how close the measured results are to the actual quantity of material in the matrix. Hence, recovery tests were conducted by spiking reference drug solution to the pre-analyzed emulgel solution (MZL: 300 µg/mL; EGL: 159 µg/mL) at three different levels: 50, 100, and 150%. The absorbance values at designated wavelength were used to calculate the % recovery.

III. RESULTS AND DISCUSSION

Method development of UV spectrophotometric

Method

The formulation such as emulgel, microemulsion, and nanoemulsion containing both miconazole nitrate and eugenol/clove oil has been reported but the quantification was done at 272 nm which is the lambda max of miconazole nitrate alone. As eugenol or clove oil is having aromatic ring, and the spectra pattern of eugenol interferes with the miconazole nitrate spectra at 272 nm. Therefore, it is very important to develop and validate the method for the simultaneous estimation of MZL and EGL.[9,10] The UV spectrum pattern in the wavelength range 200–400 nm of MZL and EGL showed that different methods, that is, the simultaneous equation, zero-crossing first-order derivative, and ratio derivative approach, can be utilized for the estimation of the cited drugs in formulated emulgel.

Simultaneous equation method

The absorption of the antifungal drug MZL and phytoconstituent EGL at wavelength maxima of 272 nm and 280 nm was used in the simultaneous equation method. The absorptivity values determined are 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 values are average of six estimations. The absorbance's and absorptivity values (g/100 mL) at these wavelengths were substituted in equations (1) and (2) to obtain the concentration of drugs.

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

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

Where A1 and A2 are the absorbance of sample solutions at 272 nm and 280 nm, respectively. Cx and Cy are concentrations of MZL and EGL in sample solution. By substituting the values of A1 and A2, the Cx and Cy can be calculated by solving equations (1) and (2).[39]

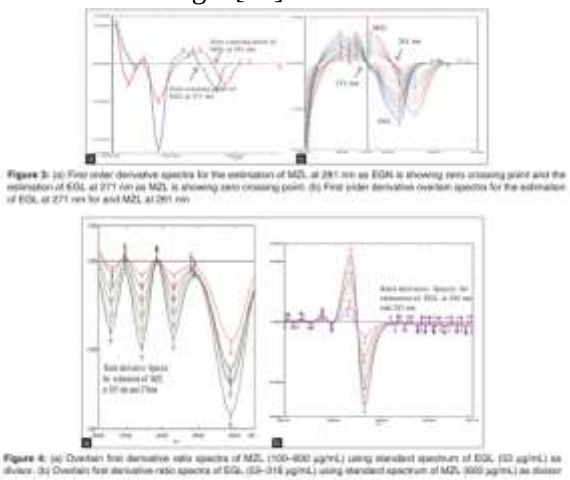
Zero-crossing derivative spectrophotometric method

The zero-crossing method allows precise identification and quantification of MZL and EGL in mixtures without the interference of other drugs. The wavelength was selected in such a way that absorbance near zero for one analyte and another analyte can be quantified, and vice versa for the estimation of another analyte. The spectra obtained in the zero crossing first order derivatives are presented in Figure 3a and 3b. As a result, the simultaneous determination of MZL and EGL in a binary combination was carried out at 281 nm (zero crossing wavelength of EGL) and 271 nm (zero crossing wavelength of MZL). The most favorable linear response to the analyte amount was measured from the derivative spectrum at the mentioned wavelengths [Figure 3b] and the

obtained linear regression equation was used to find the unknown conc. of the MZL and EGL in formulated emulgel.[41]

Ratio derivative method

The stored spectrum of binary mixtures was divided wavelength by wavelength by the standard spectrum of MZL of different concentrations for the estimation of EGL and vice versa. After studying the influence of divisor concentration, for obtaining the ratio spectra of MZL, 53 µg/mL spectra of standard solution of EGL was selected as a divisor. For obtaining the ratio spectra of EGL, the binary mixture was divided by the stored standard spectrum of MZL (600 µg/mL). The first derivative of these ratio spectra was traced with the interval of $\Delta\lambda = 2$ nm and a scaling factor of 4. Wavelengths 283 nm and 274 nm were selected, and their peak amplitudes were measured for the estimation of MZL and 286 nm and 292 nm for the EGL determination as shown in Figure 4a and 4b and easily adopted for the estimation of mentioned drugs. The ratio derivative spectra were obtained for different concentrations of MZL and EGL, and the linear response to the analyte amount was measured at the mentioned wavelengths. The obtained linear regression equation was used to find the unknown conc. of the MZL and EGL in the formulated emulgel.[42]

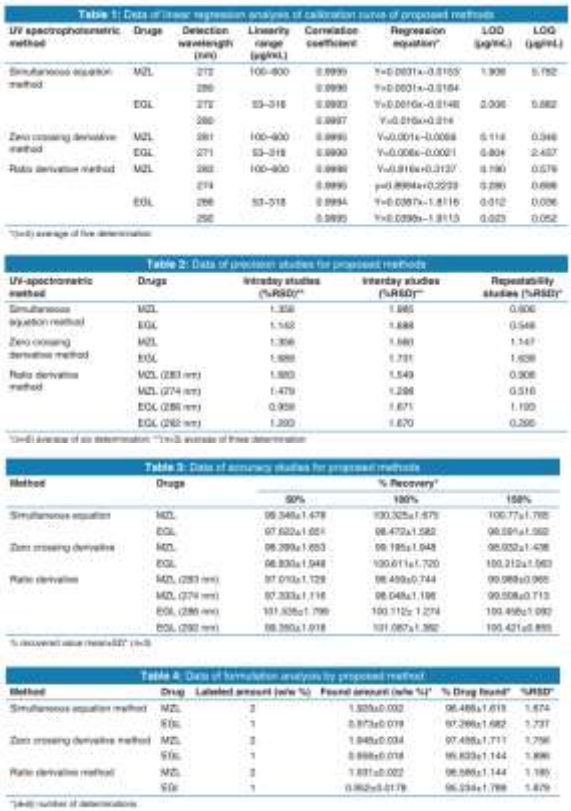


Validation of proposed method[40,43]

"International Conference on Harmonization" guideline of analytical validation was used for various validation parameters and is discussed below. Linearity and range: A linear regression equation approach was used to prove that varied concentrations of MZL and EGL were having a direct effect on the absorbance at selected wavelengths. The obtained correlation coefficient was between 0.9995 and 0.9999 indicating good linear relationship between response and concentration 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, in simultaneous equation, zero crossing derivative, and ratio derivative method was obtained. Precision and Accuracy: The dispersion variability among the measurement values was determined by the percentage RSD of, repeatability, intraday, and interday studies. The level of dispersion was within 2% of the RSD [Table 2]. these values show that the three developed methods are precise. The percent recovery for both drugs was found within the range (97.010%–101.53%) as mentioned in Table 3, which indicates that all three methods reproduced their results within the range for MZL and EGL without the interference of the excipients.

Assay of formulated emulgel

Quantitative assessment of MZL and EGL was effectively carried out by the proposed UV spectroscopic method in formulated emulgel (2% MZL and 1% EGL in 10 g of emulgel). The average assay values for both drugs were within the range of 102.01–97.89% after 6 times of assessment of the formulated emulgel [Table 4]. Hence, the developed methods can be a used for the analysis of both the drugs simultaneously in formulated emulgel.



IV. CONCLUSION

In this study, three straightforward, exact UV methods have been developed for the simultaneous measurement of eugenol and miconazole nitrate. As a quality control technique for the formulation including the mentioned chemical ingredient, this approach will be very helpful. Growing interest has been shown recently in the development of topical nanoemulsion and microemulsion formulations that include phenol chemicals, such as eugenol, in their oil phase and need estimate.

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