Ind. J. Pharm. Edu. Res., 2025; 59(3s):s1134-s1140.
https://www.ijper.org

Original Article

Quantification of Miconazole Nitrate and Eugenol in Formulated Emulgel by Planar Chromatography along its Stability Studies

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ABSTRACT

Introduction: Fungal infections are rapidly increasing and combination therapy using Miconazole Nitrate (MCZ) and Eugenol (EGN), may offer a promising approach for developing new antifungal medications. **Objectives:** The purpose of the demonstrated work was to develop and validate a stability-indicating HPTLC method for concurrent measurement of MCZ and EGN in formulated emulgel. **Materials and Methods:** The separation technique of the HPTLC method was aided by pre-coated silica gel $60F_{254}$ on aluminium and toluene: ethyl acetate: methyl alcohol (18:1:1 v/v/v) as mobile phase. The quantification of MCZ and EGN in formulated emulgels along with their stability studies was conducted. **Results:** The optimised conditions were used to develop the spotted drugs and showed a linear response from 100-600 ng/band (r^2=0.9995) for MCZ at R_f value of 0.33 and 53-318 ng/band (r^2=0.9996) for EGN at R_f value of 0.70. **Conclusion:** The proposed validated work is suitable for the quantification of the above-cited drugs under various stress conditions and quality control purpose.

Keywords: Eugenol, Miconazole Nitrate, HPTLC, Emulgel, Topical formulation.

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Received: 28-01-2025;
Revised: 07-03-2025;
Accepted: 13-05-2025.

INTRODUCTION

The continuous and worsening danger of fungal infections to the human population suggests that exploring combination therapy could provide novel approaches for developing antifungal drugs. The FDA authorized miconazole, an antifungal medication of the imidazole class, in 1974.[1] Chemically, Miconazole Nitrate (MCZ)is known as 1-[(2RS). [(2,4-dichlorobenzyl) oxy]-2-(2,4-dichlorophenyl) ethyl] nitrate of -1H-imidazole (Figure 1).[1] It prevents the formation of ergosterol, hinders the function of the membrane and interferes with enzymes attached to the membrane for its medication effect. It is used as a remedial agent for ringworm, jock itch, intestinal and oropharyngeal fungal infection. In the treatment of cutaneous, oral and vaginal mycoses, MCZ has been frequently employed. The most common application forms include semisolid dosage forms at 2.0% concentration level, either by MCZ alone or in combination with other antimicrobials or topical corticosteroids for the treatment of dermatitis.[2-4]

Eugenol (EGN), which is used as a pain reliever, a biocide and an antiseptic, is chemically known as 4-alkyl-2-methoxyphenol (Figure 1). Eugenol is an essential phytochemical with antimicrobial, analgesic, anti-inflammatory and anaesthetic effects. Aspergillus, Candida and dermatophytes are fungal species against which EGN demonstrates potent antifungal action. This is mainly because EGN damages the cell membrane, numerous virulence factors and its biofilm.[8] The WHO designated EUG as a substance that is nonmutagenic and Generally Recognized as Safe (GRAS). This naturally produced chemical is frequently used in the food and fragrance industries.[5,6]

Therefore, combining MCZ with EGN could lead to several advantages, including a reduced dosage of medications required, increased effectiveness and reduced toxicity, which ultimately aid in suppressing or eliminating biofilm and overcoming fungal infections brought on by drug-resistant Candida strains.[7] A literature review reveals different analytical methods for the quantification and validation of MCZ and EGN, single or in a mixture with other drugs in the commercial formulation as well as in serums. For the determination of MCZ both alone and in formulation, numerous HPLC, HPTLC and

DOI: 10.5530/ijper.20255177

UV spectrophotometric techniques have been reported.[8-11] It has also been claimed that MCZ has been analysed using a variety of analytical techniques along with other medications like mometasone furoate, nadifloxacin, lidocaine, econazole, metronidazole and hydrocortisone.[12-23]

The measurement of EGN in its formulation and with phytochemical like rosmarinic acid, cinnamon oil, cinnamaldehyde and piperine, have devised and published chromatographic methods like HPTLC, HPLC and UV methods.[24-37] Scientists have proved that MCZ and EGN have a synergistic effect and have formulated microemulsion and nanoemulsion of this combination.[38,39] Therefore, quantification of both aforementioned medications, for simultaneous estimation was important for quality control.

To ascertain the stability characteristics of the pharmaceutical compound, the ICH guideline mandates the necessity of conducting stress testing. HPTLC is becoming a common analytical technique. In contrast to HPLC, the main benefit of high-performance planar chromatography is that multiple test compounds can be run concurrently with a modest amount of mobile phase, reducing the amount of time and money needed for each analysis. The purpose of the demonstrated work was to develop and validate a stability-indicating HPTLC method for concurrent measurement of MCZ and EGN in formulated emulgel (2% w/w MCZ and 1% w/w EGN).

MATERIALS AND METHODS

The analytical grade gift samples MCZ and EGN were procured from Novanta Health Care LLP, Surat, Gujarat, India and Loba Chemie. Pvt. Ltd., respectively. All reagents and chemicals were purchased from Suvidhinath laboratories, Vadodara, India of pharmaceutical grade.

HPTLC method

The activation of pre-coated silica gel $60F_{254}$ is essential, which was done by prewashing it with methyl alcohol and then keeping it in the oven at 60°C for 5 min. The MCZ and EGN standard and sample solution were spotted with a Camag 100 µL hamilton syringe on an activated stationary phase in the band-shaped of 6 mm. Also, the mobile phase saturation was done for 30 min at the atmospheric condition 25±2°C with a relative humidity of 60±5%. Then, the above stationary phase was kept in a solvent chamber containing the mobile phase, toluene: ethyl acetate and methyl alcohol (18:1:1 v/v/v). After the mobile phase had moved 80% of the way through capillary action, TLC plates were dried using an air dryer with a stream of air. On a Camag TLC scanner III with a 272 nm detection wavelength and the WinCats software (version 3.15, Camag), densitometric scanning was carried out. The intensity of the diffusely reflected light was used to calculate the concentrations of MCZ and EGN.

Preparation of standard solutions

Precisely measured amounts of 10 mg of MCZ and 0.05 mL EGN (the density of eugenol is 1.067 g/mL) were separately placed into 2 distinct 10 mL volumetric flasks. These compounds were then dissolved and diluted, resulting in standard solutions with concentrations of 100 µg/mL for miconazole and 53 µg/mL for eugenol, respectively.

Analysis of formulated emulgel

A capped centrifuge tube (25 mL) contained accurately 7.5 g of formulated emulgel (equivalent to 15 mg MCZ and 7.5 mg EGN) was utilized for sample preparation. Then 15 mL of methanol was added to the above sample and warmed for 5-10 min and then at 600 rpm centrifuged for 15 min. Then the solvent was added up to mark. To acquire concentrations of 300 µg/mL of MCZ and 159 µg/mL of EGN, the supernatant solution was further diluted. Then the quantification of MCZ and EGN was done by the developed HPTLC method.

Method Validation

Parameters of Analytical Method

Confirmation of HPTLC methods was done following the guidelines outlined in ICH Q2 (R1) guideline.[40-42]

Specificity

The specificity of the developed method was determined by matching the R_f and obtained spectra for peak purity with those of the standard drugs and samples.

Linearity and range

Aliquots of standard solution of MCZ (100 µg/mL)and EGN (53 µg/mL) in the range of 1-6 µL were spotted on the TLC plate and the chromatogram was generated and assessed using the HPTLC method described above. By tracing the relationship between the peak area and the concentration (ng/band) that corresponds to each spot, the calibration curves for MCZ and EGN were created.

LOD and LOQ

The absolute variability of the peak area and the mean slope of the standard curve were important factors used in the formula given in the ICH guideline to find the concentration that can be detected at the lowest level and one that can be measured.

Precision and accuracy

The closeness between the peak areas in terms of RSD of 100, 300 and 500 ng/band of MCZ and 53, 159 and 265 ng/band of EGN on a similar day and an altered day was revealed statistically. The peak areas of 300 ng/band and 212 ng/band of MCZ and EGN, respectively, were studied six times. Recovery tests were done by adding reference drug solution to the pre-analysed emulgel solution (MCZ: 300 ng/band; EGN: 159 ng/band) at three

different levels. The obtained peak area were used to calculate the percent recovery.

Robustness of the method

Using a specific parameter, it was determined whether an analytical method could yield accurate results even when the experimental conditions changed a little bit**.** The amount of methyl alcohol, volume of mobile phase, chamber equilibration time and development distance were altered. The % RSD of the peak area and R_f were calculated for MCZ (600 ng/band) and EGN (318 ng/band).

Stability Studies

Acid Degradation

The 3 mL of MCZ (1000 µg/mL) and EGL (530 µg/mL) solutions were added to a flask (10 mL) containing 2 mL of HCl of 1N and kept aside for 1 hr. After that, the solution was neutralized by 2 mL of 1N NaOH, followed by addition of methanol and used for further analysis.

Alkaline Degradation

The 3 mL of MCZ (1000 µg/mL) and EGL (530 µg/mL) solutions were added to a flask (10 mL), containing 2 mL NaOH of 1N and kept aside for 1 hr. After that, the solution was neutralised by adding 2 mL of 1N HCl, followed by addition of methanol and used for further analysis.

Oxidative Degradation

The 3 mL of MCZ (1000 µg/mL) and EGL (530 µg/mL) solutions were added to a flask(10 mL) containing 2 mL of 1% H_2O_2 and kept aside for 1 hr. Then methanol was added and utilised for further analysis.

Thermal Degradation

For the thermal degradation study, the mixture of standard powder drugs MCZ and EGN (100 mg and 0.05 mL) was placed in a petri plate that was airtightly sealed in a preheated oven at 60°C for 24 hr. Appropriate dilutions were prepared in methanol and analysed under optimised chromatographic conditions.

Photolytic Degradation

The photolytic degradation was carried out by placing a mixture of the standard drugs MCZ and EGN (100 mg and 0.05 mL) in a petri plate that was airtightly sealed and exposed to sunlight for 1 hr. Appropriate dilutions were prepared in methanol and analysed under the optimised chromatographic conditions.

RESULTS AND DISCUSSION

Mobile phase optimization

A stability-indicating method was developed by optimising the HPTLC process. On TLC plates, MCZ and EGN were both detected and tested using various mobile phase ratios. The resolution and peak shape using toluene, ethyl acetate and methyl alcohol in a ratio 18:1:1 v/v/v (Figure 2) were found to be superior to those of the other solvent mixture. Therefore, the MCZ and EGN was separated at R_f value of 0.33 and 0.70 respectively.

Validation of the proposed method

Specificity

The selected mobile phase was capable enough to resolved the two analytes of MCZ and EUG successfully in the formulation and in stability study as shown in Figures 2 and 3. By correlating the UV spectra at the peak start, apex and end of the band of MCZ and EGN were determined with peak purity index 0.9999-0.9996 showing specificity of method.

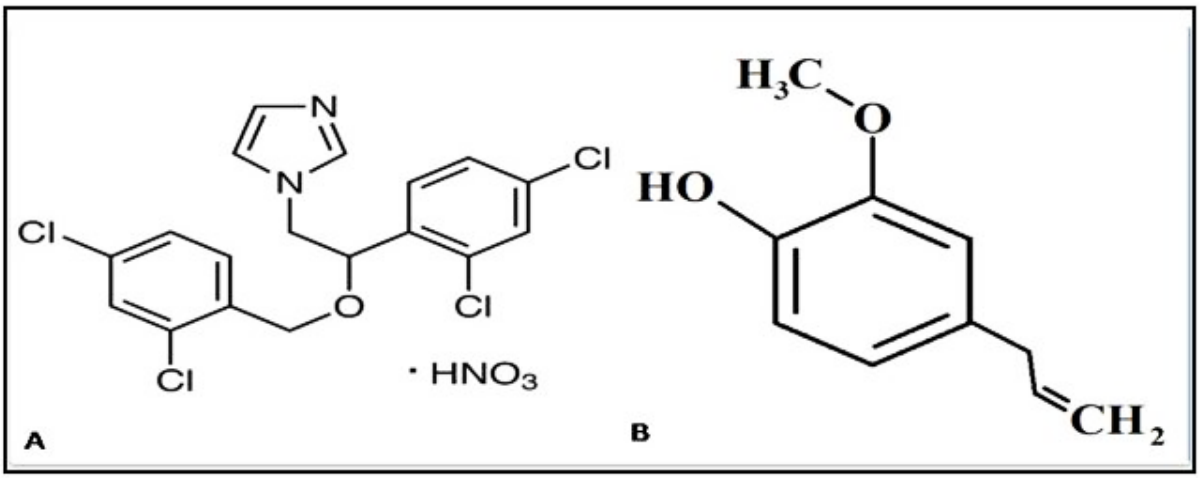

Figure 1: Structure of Miconazole nitrate (A) and Eugenol (B).

Table 1: Data of calibration curve MCZ and EGN obtained by HPTLC analysis.

Sl.No.	MCZ (ng/band)	MCZ Peak area (±SD) *	%RSD	EGN (ng/band)	MCZ Peak area (±SD) *	% RSD
1	100	716.9±4.533	1.633	53	1670±30.594	1.831
2	200	1133.1±5.527	1.477	106	3188.4±82.965	1.602
3	300	1516.2±17.31	1.117	159	4664±40.373	1.865
4	400	1940.52±2.780	1.432	212	6171±51.254	1.830
5	500	2357.6±5.029	1.133	265	7575.8±28.604	1.775
6	600	2711.4±5.594	1.206	318	8999.4±30.278	1.336

*(*n*=5) number of determination.

Table 2: Data of Intraday and Interday precision studies of MCZ and EGN.

Conc. ng/band	Intra-day Precision		Inter-day Precision		Conc. ng/band	Intra-day Precision		Inter-day Precision	
	Mean±SD*	%RSD	Mean±SD*	%RSD		Mean±SD*	%RSD	Mean±SD*	%RSD
100	729±8.7607	1.200	720 ± 10.004	1.389	53	1684.33±14.011	1.831	1673.66±8.020	1.472
300	1556±25.166	1.616	1527 ± 6.082	1.398	159	4660±52.915	1.135	4684.66±78.487	1.675
500	2377±20.420	1.859	2357 ± 15.394	0.653	265	7586.66±15.275	1.201	7605±44.440	0.584

*(*n*=3) number of determination.

Linearity

The proportional responses were seen in the concentration ranges of MCZ (100-600 ng/band) and EGN (53-318 ng/band) (Table 1). A 3D chromatogram of standard MCZ and EGN was shown in Figure 2. The correlation coefficients for the calibration curves of MCZ and EGN were found to be 0.9995 and 0.9998, respectively with regression equations representing the linearity of the method are y=4.0201x+322.25 for MCZ and y=27.664x+246.48 for EGN.

Precision

The dispersed values obtained for MCZ and EGN was calculated in term of % RSD as 0.951 and 1.312, respectively, for the repeatability test. Also, for intra-day and inter-day precision, values indicate that the closeness among the obtained values was within the acceptable range i.e., less than 2 in term of % RSD as shown in Table 2.

Accuracy

The % recovery of MCZ and EGN was found to be in the range of 98.399-99.199 and 98.830-100.611, respectively. Based on the obtained data, the developed method was accurate (Table 3).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD for MCZ and EGN was calculated as 1.603 and 0.620 ng/band respectively. The LOQ for MCZ and EGN were calculated as 4.857 and 1.879 ng/band respectively.

Robustness

Robustness data shows that at small but deliberate changes the % RSD of peak area was less than 2. The analysed data is mentioned in Table 4.

Stability study outcomes

Acid hydrolysis

The chromatographic analysis of the cited drug has revealed additional peaks at R_f values of 0.24, which are susceptible to acidic-induced degradation. The obtained chromatograph was accountable for 57.57% and 72.25% recovery of MCZ and EGN, respectively as shown in Figure 3 (A) and Table 5.

Alkaline hydrolysis

The chromatographic analysis of the cited drug in alkaline conditions has revealed additional peaks at R_f values of 0.09 and 0.29. They were accountable for 54.42% and 89.15% recovery, MCZ and EGN respectively and susceptible to alkaline-induced degradation as shown in Figure 3(B) and Table 5.

Oxidative degradation

The chromatographic analysis of the cited drug has revealed additional peak at R_f values of 0.09 and 0.25. This indicates that MCZ and EGN are susceptible to H_2O_2 induced degradation and were accountable for 64.53% and 94% recovery, respectively as shown in Figure 3 (C) and Table 5.

Photodegradation

The chromatographic analysis of the cited drug revealed additional peaks at R_f values of 0.09 and 0.58. This indicates that MCZ and EGN was susceptible to sunlight-induced degradation and was accountable for 56.71% and 99.81% recovery of MCZ and EGN, respectively as shown in Figure 3 (D) and Table 5.

Thermal degradation

The chromatographic analysis of the cited drug revealed additional peaks at R_f values of 0.09 and 0.13 suggesting that MCZ and EGN was susceptible. Preheated oven at 60°C induced degradation and was accountable for 70.81% and 98.15% recovery of MCZ and EGN, respectively shown in Figure 3 (E) and Table 5.

Table 3: Recovery study of MCZ and EGN by the HPTLC method.

Recovery Level (%)	Amount of MCZ in emulgel (ng/band)	Standard MCZ (ng/band)	Mean±SD*	Amount of EGN in emulgel(ng/band)	Standard EGN (ng/band)	Mean±SD*
50	300	150	98.399±1.655	159	79.5	98.830±1.948
100		300	99.199±1.948		159	100.611±1.720
150		450	98.932±1.438		238.5	100.212±1.063

*(*n*=3)number of determinations.

Table 4: Robustness study data of MCZ and EGN.

Sl. No.	Modification	MCZ 600 (ng/band)	EGN 318 (ng/band)
		% RSD*	% RSD*
1	Organic modifier (1±0.1 mL)	0.727	0.983
2	M/P Volume (20±5% v/v)	0.813	0.671
3	Chamber saturation time (15±5 min)	1.364	0.865
4	Development distance (80±5 mm)	0.715	0.803

*(*n*=3) number of determination.

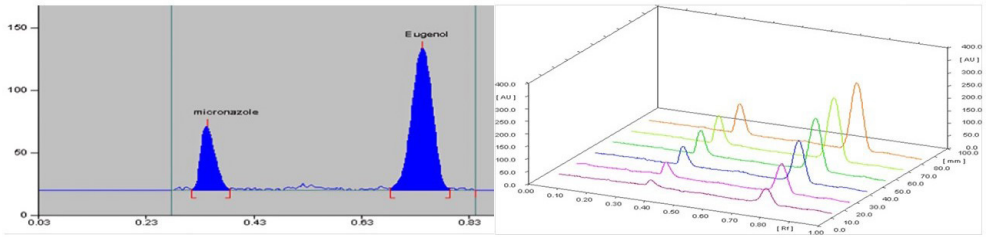

Figure 2: HPTLC chromatogram of MCZ (Miconazole nitrate) and EUG (Eugenol) in optimized mobile phase and 3D overlay Chromatogram of MCZ and EUG.

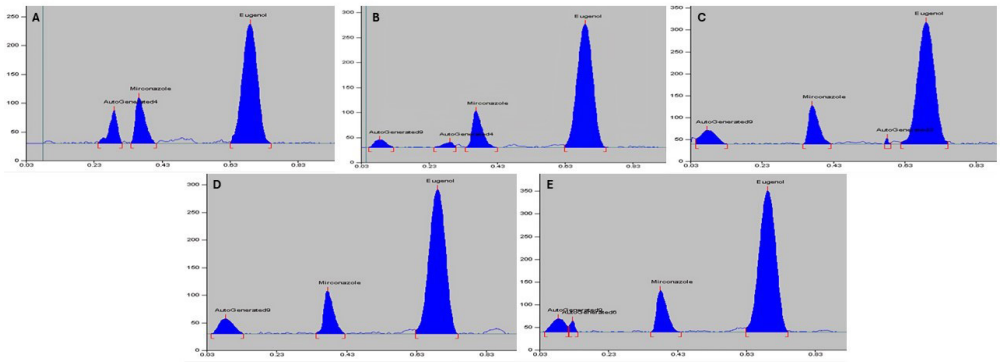

Figure 3: Densitometric chromatograms of MCZ and EGN(600-318 ng/band) derived under different condition for forced degradation study. (A) - Acid degradation (1N HCl) study, (B) - Alkaline degradation (1N NaOH), (C) - Oxidative degradation (1% H_2O_2), (D) - Photo degradation, (E) - Thermal degradation.

Table 5: Summary of forced degradation study of MCZ and EGN in different stress condition.

Sl. No.	Stress Condition	% Recovery of MCZ	% Recovery of EGN
1.	Acid hydrolysis (1N)	57.57	72.25
2.	Alkali hydrolysis (1N)	54.42	89.15
3.	Oxidation (1% H_2O_2)	64.53	94
4.	Photolytic degradation	56.71	99.81
5.	Thermal degradation	70.81	98.15

Table 6: Results of the assay of MCZ and EGN in formulated emulgel.

Sl. No.	Drugs	Amount (%w/w)		Drug Content (%)*	%RSD
		Labelled	Found*		
1	MCZ	2	1.969±0.0261	98.450±1.303	1.323
2	EGN	1	0.975±0.013	97.467±1.250	1.283

*(*n*=6) values of 6 determination.

Analysis of emulgel Fomulation

The total drug contained in the optimised batch of emulgel was 15 and 7.5 mg of MCZ and EGN, respectively. The quantified drug content was determined to be 98.450±1.303 for MCZ and 97.467±1.250 for EGN, as shown in Table 6.

CONCLUSION

The outcomes of the analysis performed using the stated methodology exhibit a linear response for MCZ (100-600 ng/band) and EGN (53-318 ng/band). The recently produced procedure was discovered to be straightforward, particular, accurate, speedy and reproducible and can be employed to quantify miconazole nitrate and eugenol simultaneously. Drug compounds were well separated from degradation products created under varied stress settings using the developed method. The stress condition such as acid, alkaline hydrolysis, oxidation and photolytic conditions can affect MCZ and EGN stability.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Piparia, Waghodia, Vadodara, Gujarat, India for providing all the facilities throughout the work. Also, thankful to Novanta Health care LLP in Surat, Gujarat, India for providing miconazole nitrate as gift sample.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

EGN: Eugenol; **FDA:** Food and drug administration; **HPLC:** High performance thin layer chromatography; **HPTLC:** High Performance thin layer chromatography; **LOD:** Limit of detection; **LOQ:** Limit of Quantification; **MCZ:** Miconazole Nitrate; **R_f:** Retardation factor; **RSD:** Relative standard deviation; **TLC:** Thin layer Chromatography; **WHO:** World health organization.

SUMMARY

Fungal infections are increasing rapidly and combination therapy, including eugenol and miconazole nitrate, may provide a new approach to developing antifungal medications. Therefore, the purpose of the demonstrated work was to develop and validate a stability-indicating HPTLC method for concurrent measurement of MCZ and EGN in formulated emulgels. The outcomes of the analysis performed using planar chromatography exhibit a linear response for MCZ (100-600 ng/band) and EGN (53-318 ng/band). The recently produced procedure was discovered to be straightforward, particular, accurate, speedy and reproducible. Also, MCZ and EGN were well separated from degradation products created under varied stress settings and can be employed for their quantification.

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Cite this article: Zanwar AS, Sen DB, Vyas PH, Burle SS, Ruikar DB, Sen AK. Quantification of Miconazole Nitrate and Eugenol in Formulated Emulgel by Planar Chromatography along its Stability Studies. Indian J of Pharmaceutical Education and Research. 2025;59(3s):s1134-s1140.