ORIGINAL ARTICLE

Analytical HPLC Method for Determination of Miconazole Nitrate and Eugenol in Formulated Emulgel

Aarti S. Zanwar[1], Dhanya B. Sen[1], Pruthvi H. Vyas[1], Sachin B. Zanwar[2], Dillip Kumar Dash, Ashim Kumar Sen[1]

[1]Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, India, [2]Faculty of Pharmacy, Kalabhavan Campus, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

Abstract

Aim: Conventional antifungal medications face challenges, such as recurrence of infection, medication resistance, and drug-related toxicity. Biofilm-associated cells exhibit resistance due to drug-efflux pumps and metabolic conditions. A new formulation containing miconazole nitrate (MIZ) (antifungal drug) and eugenol (EUL) (anti-biofilm agent) increases the effectiveness of antifungal activity. This necessitates a new high-pressure liquid chromatography (HPLC) method to develop for quality control analysis. **Materials and Methods:** The proportion of solvent (acetonitrile: methanol:ammonium acetate buffer, pH 6.5) in the ratio of 25:55:20 v/v/v resolved the cited drugs on the C_8 column at 238 nm by the HPLC method. The developed method was validated as per the International Council for Harmonisation Q2 guideline for different parameters. **Result and Discussion:** The linearity and range for MIZ (50–250 µg/mL) and EUL (26.5–132.5 µg/mL) solution were performed by the mentioned HPLC method. The interferences of the excipients, MIZ and EUL, were negligible, as the recovery percentage ranged from 98.867% to 101.482%. The quantified drug content was determined to be 98.450 ± 1.303 for MIZ and 97.467 ± 1.250 for EUL by the developed and validated HPLC method. **Conclusion:** The proposed research was appropriate for the examination of MIZ and EUL content in the formulated emulgel by the developed HPLC method.

Key words: Antifungal, eugenol, high-pressure liquid chromatography, method development, miconazole nitrate, validation

INTRODUCTION

Fungal infections are increasing globally due to the use of antibiotics, radiation therapy, certain immunosuppressive agents, and intensive care units. The main clinical issues with conventional antifungal drugs include the recurrence of fungus infections and the development of medication resistance, mainly due to fungal biofilm development.[1] Biofilm-associated cells exhibit inherent resistance to antifungal medications due to their continuous activation of drug-efflux pumps and some modified metabolic conditions. In addition, they offer physical protection from antifungal drugs, partly through the production of an extracellular matrix.[2,3] A new antifungal formulation is required to destroy the biofilm produced by the fungi to increase the efficiency of the current antifungal drug. Therefore, the addition of an antibiofilm agent may increase the effectiveness of antifungal medications,such as miconazole nitrate (MIZ) and eugenol (EUL) work together to inhibit Candida and the biofilm community, as per the evidence reported. Furthermore, EUL increases skin penetration and increases the availability of MIZ in topical gels.[4,5] Therefore, for quality control analysis of these two drugs in the formulated emulgel, the high-pressure liquid chromatography (HPLC) method needs to be developed and validated.

Address for correspondence:
Aarti S. Zanwar, Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat, India. Phone: 91-9724628289.
E-mail: aarti.zanwar@gmail.com

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The FDA approved MIZ [Figure 1], an antifungal medication belonging to the imidazole class, in 1974. Chemically, MIZ is known as 1-[(2RS)-2-[(2,4-dichlorobenzyl) oxy]2,4-dichlorophenyl-2-ethyl]imidazole nitrate (-1H). It displays its antifungal activity by blocking the production of ergosterol, impairing the barrier function of the membrane, and damaging enzymes attached to the membrane. Inhibiting 14-alpha-lanosterol demethylase, a crucial enzyme in the manufacture of ergosterol, is the commonly acknowledged mechanism of action of azole antifungals. MIZ is soluble in DMSO and very slightly soluble in water and methanol. It is now used in several medicinal formulations, including vaginal suppositories, oral gels, lotions, ointments, and injections. The common dosage forms of MIZ are semisolids at a concentration level of 2.0% w/v, alone or in combination with other antimicrobials or anti-inflammatory steroids.[6,7] EUL, 4-allyl-2-methoxyphenol, a phytochemical [Figure 1], interferes with the function of cell membranes in fungi, suppresses components that contribute to virulence, and stops the production of fungal biofilms. Its solubility in water is less and more in organic solvents and fixed oil. The extracellular polymeric substances of microbial biofilms may be broken down by EUL. The existing literature on the pharmacological effects of EUL indicates noteworthy characteristics such as anti-inflammatory, antioxidant, pain reliever, antibacterial, and antifungal actions which have a noteworthy impact on human health. *Aspergillus*, *Candida*, and *Dermatophytes* are some of the fungal species against which EUL has reported strong antifungal action.[8,9]

The published paper reveals a variety of techniques, including potentiometry[10] and spectrophotometry for the estimation of MIZ and EUL alone[11-15] or in combination of metronidazole,[16] econazole,[17] nystatin,[18] scopoletin,[19] and hydrocortisone acetate.[20] Different HPLC methods for quantification of MIZ and EUL alone has been reported.[21-23] Also,quantification and separation of cited drug in combination with other drugs, such as mometasone furoate,[24,25] hydrocortisone,[25] lidocaine hydrochloride,[26] clotrimazole, tinidazole,[27] clobetasol[28] etc. by chromatographic method has been mentioned in research paper. Planar chromatography was also utilized for the estimation of both citated drugs[29] and other drugs such as nadifloxacin,[30] cinnamon oil,[31] rosmarinic acid,[32] cinnamaldehyde, and piperine.[33-36] Today, HPLC is a vital qualitative and quantitative method frequently used for quality control. It is the most adaptable, secure, trustworthy, and quick chromatographic method for determining the quality of medicinal ingredients. In reported formulations of MIZ such as nanoemulsion and microemulsion, only MIZ was estimated though it also contains EUL or clove oil. EUL is an aromatic compound, so its analysis is important to avoid misleading responses.[37,38]

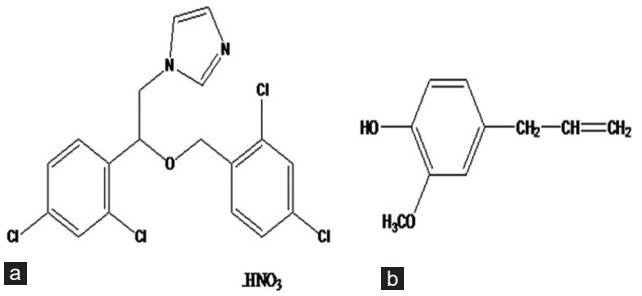

Figure 1: Structure of miconazole nitrate (a) and eugenol (b)

The primary goal of the work is to create and validate a novel HPLC technique capable of concurrently identifying MIZ and EUL within a formulated emulgel.

MATERIALS AND METHODS

Pharmaceutical-grade EUL was acquired from Lobachemi. Pvt. Ltd. and MIZ from Novanta Health Care LLP. All chemicals and reagents of analytical quality were purchased from Suvidhinath Laboratories, Vadodara, India.

Preparation of stock and standard solution

A separate volumetric flask (10 mL) was placed and precisely weighed MIZ (10 mg) and EUL (0.05 mL; the density of EUL is 1.067 g/mL) were added and diluted with methanol. A standard solution that contains 1000 µg/mL of MIZ and 530 µg/mL of EUL was obtained and diluted to investigate various parameters.

Instrumentation

A highly sensitive Adventurer-Pro, AVG264C electronic balance, HPLC Younglin (S.K) Gradient system equipped with a UV 730 detector and Autochro-3000 software. For the separation, a Thermo C_8 column with the dimension of 250 mm × 4.6 mm, particle size 5 µm was used.

Analysis of formulated emulgel

A capped centrifuge tube (50 mL) was used for sample preparation. An accurately weighed 7.5 g of formulated emulgel (equivalent to 15 mg MIZ and 7.5 mg EUL) was placed and 15 mL of methanol was added and melted in a water bath at 45°C. The volume was made up and then centrifuged for 15 min at 600 rpm. The obtained supernatant solution was further diluted to get 200 µg/mL of MIZ and 106 µg/mL of EUL and quantified by the developed HPLC method.

Validation of chromatographic method[39-41]

Specificity

The standard drug solution, a placebo solution (containing only excipients), and a sample solution were analyzed and

their chromatograms were compared to see the interference among excipients, MIZ, and EUL.

Linearity

Working solutions of MIZ and EUL were evaluated for linearity and range. The approach was investigated by measuring the peak area at the corresponding concentration. The calibration curve for the MIZ (50–250 µg/mL) and EUL (26.5–132.5 µg/mL) was computed at selected wavelengths using an optimized mobile phase. Regression analysis was carried out using the least square method.

Accuracy

The value of closeness to the true value, i.e., the concentration of spiking standard solution at three levels (50%, 100%, and 150%) was calculated. The pre-analysed sample solution (MIZ: 50 µg/mL; EUL: 26.5 µg/mL) was used for recovery studies.

Precision

For the precision study, we measured the level of dispersion for repeatability, intraday, and interday values. The closeness between the response values of MIZ (150 µg/mL) and EUL (79.5 µg/mL) was evaluated 6 times, and variation was calculated as % RSD. To determine variation within and across days, the responses of three replicate injections of MIZ at concentrations of 50, 150, and 250 µg/mL and EUL at concentrations of 26.5, 79.5, and 132.5 µg/mL were recorded and expressed as % RSD.

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 International Council for Harmonisation (ICH) guideline to find the concentration that can be detected and quantified at its lowest level.

Robustness

A small change in the buffer, pH (6.5 ± 0.2), flow rate (1 ± 0.1 mL/min), and methanol volume (55 ± 2 mL) was used to test the robustness of the method. Peak area and retention time values were obtained and statistically analyzed.

System suitability

Variations in response to the six standard solutions (100 µg/mL and 53 µg/mL) of MIZ and EUL were noted to evaluate the HPLC system suitability by keeping the same chromatographic conditions.

RESULTS AND DISCUSSION

The formulated emulgel consists of a mixture of MIZ and EUL, along with the excipients. Therefore, for the quantification of the above-active compound, HPLC is the most effective separation-related analytical technique. The chemical properties of both the drugs differ from each other; therefore, different solvents and stationary phases were examined to get a well-resolved peak for the cited drugs. Trials were initially conducted using both C_8 and C_{18} columns. However, the peaks obtained with the C_{18} column exhibited more tailing and poor peak shapes. In contrast, using the C_8 column resulted in better symmetrical and resolved peaks. Furthermore, this was achieved with a mobile phase of acetonitrile, methanol, and ammonium acetate buffer (pH 6.5) in a ratio of 25:55:20 v/v/v. In this case, an above-mobile phase with a pH of 6.5 contributes to the sharp and symmetrical peaks observed with the C_8 column.

The recorded chromatogram is revealed in Figure 2. The MIZ and EUL were retained on the columns at 2.10 and 4.31 min and were detected at 238 nm wavelength with a flow rate of 1 mL/min.

Specificity

During the allotted run time, no interfering peaks were seen, and the predicted method was determined to be specific.

Linearity

The linearity and range for MIZ (50–250 µg/mL) and EUL (26.5–132.5 µg/mL) solution were performed by the mentioned HPLC method. The obtained data of peak area obtained at different concentrations were utilized for plotting calibration graphs and mentioned in Table 1. The obtained correlation coefficient was 0.9998 for MIZ and 0.9996 for EUL, which indicates a direct relationship between the amount of drug and the obtained peak area. The obtained regression equation for MIZ was y = 4620.7x + 39045 and EUL was y = 20767x + 49926.

Accuracy

The interferences of the excipients, MIZ, and EUL were negligible according to recovery studies. The recovery percentage range was found to be from 98.867% to 99.462% and 100.889% to 101.482%, respectively, for MIZ and EUL [Table 2].

Precision

The values in terms of relative standard deviations obtained for MIZ and EUL were 1.157 and 1.125, respectively, for the repeatability test. Furthermore, for intra-day and inter-day precision, the % RSD range was between 1.115 and 1.648 for MIZ and 1.155 and 1.898 for EUL [Table 3].

Table 1: Data for calibration curve of MIZ and EUL by the developed HPLC method

S. No.	MIZ			EUL		
	Conc. (µg/mL)	Peak area (±SD)*	% RSD	Conc. (µg/mL)	Peak area (±SD)*	% RSD
1	50	635879±15811.39	1.190	26.5	1070327±171475.1	1.091
2	100	895317.1±53665.63	1.851	53	1573375±1419317	1.611
3	150	1150394±96510.1	1.746	79.5	2143942±1945859	1.789
4	200	1405379±130690.5	1.714	106	2709942±1460222	1.808
5	250	1651013±158113.9	1.742	132.5	3253687±1955194	1.706

MIZ: Miconazole nitrate, EUL: Eugenol, RSD: Relative standard deviation, SD: Standard deviation

Table 2: Results of recovery studies

Drugs	Recovery level	Pre-analyzed emulgel (µg/mL)	Standard added (µg/mL)	% recovery Mean±SD*
Miconazole nitrate	50	100	50	99.053±1.813
	100		100	98.867±1.704
	150		150	99.426±1.683
Eugenol	50	53	26.5	100.925±0.756
	100		53	101.482±0.696
	150		79.5	100.889±0.985

*Three number of estimation

Table 3: Results of precision measurement parameter

Precision parameter	MIZ		EUL	
	Conc. (µg/mL)	%RSD	Conc. (µg/mL)	%RSD
Repeatability (*n*=6)[a]	150	1.157	79.5	1.125
Intraday (*n*=3)[a]	50	1.115	26.5	1.327
	150	1.592	53	1.289
	250	1.217	79.5	1.289
Interday (*n*=3)[a]	50	1.160	26.5	1.155
	150	1.241	53	1.689
	250	1.648	79.5	1.898

[a]=number of determinations, RSD: Relative standard deviation, MIZ: Miconazole nitrate, EUL: Eugenol

The obtained values indicate that the closeness is within an acceptable range.

LOD and LOQ

The sensitivity of the method was evaluated by calculating the LOD value, i.e., 8.58 and 0.89 µg/ml and LOQ values of 26.00 and 2.69 µg/mL for MIZ and EUL, respectively.

Robustness

The proposed method underwent examination of all the parameters described under robustness studies, but no notable changes were observed in retention time or peak area, as given in Table 4. The outcome indicates the robustness of the method.

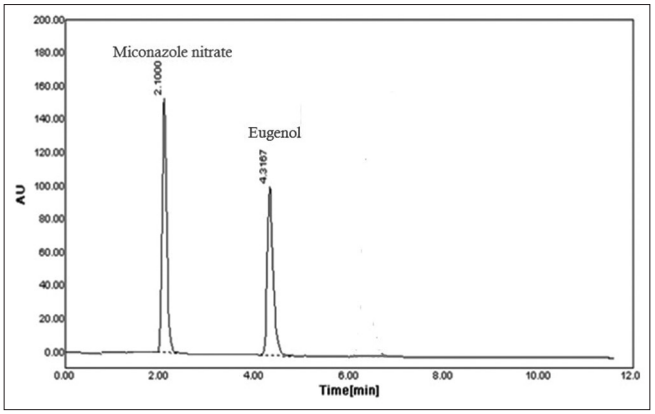

Figure 2: High pressure liquid chromatography chromatogram of miconazole nitrate and eugenol at 238 nm

System suitability test

System appropriateness tests were conducted, and the findings showed that the parameters examined were within the ICH standards' tolerable limit. Hence, the method was suitable for the intended analysis [Table 5].

Drug content uniformity

The total drug used during the preparation of the optimized batch of emulgel was 15 and 7.5 mg of MIZ and EUL, respectively. Hence, the quantified drug content of MIZ and EUL was found to be 99.722 ± 1.804 and 100.064 ± 1.192, as given in Table 6.

Table 4: Results of robustness study by developed high-pressure liquid chromatography method

S. No.	Modification in the mobile phase	RSD			
		MIZ		EUL	
		R_t	Peak area	R_t	Peak area
1	Methanol (55±2 mL)	0.342	1.521	1.201	1.647
2	pH (6.5±0.2 unit)	0.527	1.695	0.207	1.713
3	Flow rate (1±0.1 mL/min)	0.794	1.156	1.239	0.931

(*n*=3) Number of determinations, RSD: Relative standard deviation, MIZ: Miconazole nitrate, EUL: Eugenol

Table 5: Results of system suitability studies

Parameters	Values	
	MIZ	EUL
Peak area (%RSD ≤2)*	0.827	0.592
Retention time (%RSD ≤2)*	0.157	0.347
No. of theoretical plates (Mean±SD) * >2000	7347.98±635.727	9606.980±669.204
Tailing factor (Mean±SD, ≤ 2)*	1.278±0.144	1.072±0.112

*(*n*=6) number of determination, MIZ: Miconazole nitrate, EUL: Eugenol, RSD: Relative standard deviation, SD: Standard deviation

Table 6: Drug content uniformity

Sr. No.	Drugs	Drug Content (%)*	% RSD
1	MIZ	99.722±1.804	1.810
2	EUL	100.064±1.192	1.192

*Mean±Standard deviation (*n*=6) values of six determination

CONCLUSION

The mentioned HPLC analytical method can distinguish the analyte from excipients, which are present in formulated emulgel. The method is quicker since it requires less time for analysis. Consequently, it is practical to utilize it for regular quality examination of MIZ and EUL in the formulated emulgel.

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