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Research Article

Development and Validation of a Stability-Indicating RP-HPLC Method for Estimation of Miconazole Nitrate in an In-House Formulated Nanoemulsion

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Abstract: Miconazole nitrate is a broad-spectrum imidazole antifungal agent widely used for the treatment of fungal infections such as candidiasis, dermatophytosis, and other superficial fungal disorders. However, its therapeutic effectiveness is limited due to poor aqueous solubility, inadequate permeability, and reduced bioavailability. Nanoemulsion drug delivery systems have emerged as promising carriers for enhancing the solubility, penetration, and therapeutic efficacy of lipophilic drugs. The present investigation aimed to develop and validate a simple, precise, accurate, and robust reverse phase high performance liquid chromatographic (RP-HPLC) method for quantitative estimation of Miconazole nitrate in an in-house formulated nanoemulsion system.
Nanoemulsion formulations were prepared using oleic acid as oil phase, Tween 80 as surfactant, and propylene glycol as co-surfactant by high shear homogenization technique. Various formulation parameters were optimized to obtain stable nanoemulsions with satisfactory physicochemical characteristics. Chromatographic separation was achieved using RP-C18 column with optimized mobile phase under isocratic conditions and UV detection at 232 nm.
The developed method was validated according to ICH Q2(R1) guidelines for parameters including specificity, linearity, precision, accuracy, robustness, limit of detection, and limit of quantification. The developed method exhibited excellent linearity with correlation coefficient greater than 0.999. Precision studies demonstrated %RSD values less than 2%, while recovery studies confirmed acceptable accuracy within 98–102%. The developed method was successfully applied for assay of Miconazole nitrate in the prepared nanoemulsion formulation. The study concluded that the developed RP-HPLC method was reliable, reproducible, and suitable for routine quality control analysis and stability studies of Miconazole nitrate nanoemulsion formulations.
Keywords: Miconazole nitrate, Nanoemulsion, RP-HPLC, Method validation, Antifungal, ICH guidelines

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1. Introduction

Fungal infections, particularly dermatophytic and candidal infections, represent a significant global health burden, often necessitating topical antifungal therapy. Miconazole nitrate, an imidazole derivative, is a potent broad-spectrum antifungal agent effective against Candida species and dermatophytes. However, its poor aqueous solubility and limited permeability pose challenges in achieving adequate therapeutic concentrations at infection sites, resulting in inconsistent clinical efficacy [1].

Miconazole nitrate is a synthetic imidazole derivative extensively employed as a broad-spectrum antifungal agent for the treatment of superficial fungal infections including candidiasis, pityriasis versicolor, dermatophytosis, and other opportunistic fungal infections [2]. The antifungal activity of Miconazole nitrate is attributed to inhibition of ergosterol biosynthesis, an essential component of fungal cell membranes. Disruption of ergosterol synthesis results in increased membrane permeability, leakage of intracellular constituents, and eventual fungal cell death [3].

Despite its broad therapeutic utility, Miconazole nitrate suffers from several formulation-related challenges. The drug exhibits poor aqueous solubility and low permeability, leading to inadequate dissolution and reduced bioavailability [3]. Conventional dosage forms such as creams and ointments often show poor penetration through biological membranes and require repeated application to maintain therapeutic drug levels. These limitations have necessitated the development of advanced drug delivery systems capable of improving solubility, permeation, and sustained release of the drug.

Nanoemulsion-based drug delivery systems have emerged as promising carriers for enhancing the therapeutic performance of poorly soluble drugs. Nanoemulsions are isotropic, kinetically stable colloidal dispersions composed of oil phase, aqueous phase, surfactant, and co-surfactant with droplet size generally ranging between 10–500 nm [4]. Due to their nanosized droplets and high surface area, nanoemulsions improve drug dissolution, absorption, permeation, and bioavailability [5]. They also provide advantages such as improved physical stability, uniform drug distribution, controlled drug release, and enhanced penetration through skin and mucosal membranes [6].

The successful development of nanoemulsion formulations requires appropriate analytical methods for quantitative estimation of drug content during formulation development, stability testing, and quality control analysis. Among various analytical techniques, reverse phase high performance liquid chromatography (RP-HPLC) is considered one of the most reliable analytical tools because of its specificity, sensitivity, reproducibility, and accuracy [7]. RP-HPLC methods are widely used in pharmaceutical industries for quantitative estimation of active pharmaceutical ingredients and impurities.

Several analytical methods have been reported for estimation of Miconazole nitrate in bulk drugs and conventional dosage forms [8–10]. However, very limited validated analytical methods are available for estimation of Miconazole nitrate specifically in nanoemulsion formulations. The presence of surfactants, oils, and co-surfactants in nanoemulsions can interfere with chromatographic analysis and therefore necessitates development of selective and robust analytical methods.Hence, the present study was designed to develop and validate a simple, accurate, precise, and reproducible RP-HPLC method for estimation of Miconazole nitrate in an in-house formulated nanoemulsion. The developed method was validated according to ICH Q2(R1) guidelines and applied successfully for quantitative estimation of Miconazolenitrate in the prepared nanoemulsion formulation.

The present research therefore aimed to develop an accurate, sensitive, and validated RP-HPLC method for estimation of Miconazole nitrate in an in-house formulated nanoemulsion. The study also focuses on formulation optimization and evaluation of the nanoemulsion for physicochemical stability and drug entrapment, ensuring its potential as an improved antifungal delivery system

2. Materials And Methods

Miconazole nitrate was obtained as a gift sample from Yarrow Chem Industry, Mumbai. Oleic acid was used as oil phase. Tween 80 served as surfactant while propylene glycol was used as co-surfactant. Methyl cellulose was used as thickening agent and methyl paraben as preservative. Methanol, acetonitrile, potassium dihydrogen phosphate, sodium hydroxide, and distilled water of analytical grade were used throughout the study.

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The study employed UV-visible double beam spectrophotometer, digital weighing balance, magnetic stirrer, Brookfield viscometer, pH meter, cooling centrifuge, high shear homogenizer, particle size analyzer, FTIR spectrophotometer, and HPLC systemequipped with UV detector.

2.1 Formulation Development of Nanoemulsion

The nanoemulsion formulations were prepared using high shear homogenization method. Oleic acid was selected as oil phase due to its excellent drug solubilization capacity. Tween 80 was selected as surfactant because of its high hydrophilic-lipophilic balance (HLB) value suitable for oil-in-water nanoemulsions. Propylene glycol was used as co-surfactant to reduce interfacial tension and improve formulation stability.Initially, accurately weighed quantity of Miconazole nitrate was dissolved in oleic acid with continuous stirring to prepare oil phase. Tween 80 and propylene glycol were mixed separately to prepare surfactant mixture. Distilled water was mildly heated to approximately 40–45°C and added slowly to the oil phase under continuous stirring. The obtained coarse emulsion was subjected to high shear homogenization for reduction of droplet size and formation of stable nanoemulsion. (Table 1)

Table 1: Composition of Nanoemulsion Formulations

Sr.no	Formulation	Miconazole Nitrate (mg)	Tween-80 (ml)	Oleic Acid(ml)	Propylene glycol (ml)	Distilled water (ml)
1	NE-1	100	18±1	80	6±1	100
2	NE-2	100	15±0	80	5±0	100
3	NE-3	1000	18±1	80	5±0	100
4	NE-4	1000	15±0	80	6±1	100
5	NE-5	100	21±2	80	6±1	100

2.2 Evaluation of Nanoemulsion

The prepared nanoemulsion formulations were evaluated for physical appearance, pH, viscosity, particle size, centrifugation stability, and drug content. Formulations were visually examined for homogeneity, transparency, and phase separation. The pH was measured using digital pH meter. Viscosity was measured using Brookfield viscometer at room temperature. Particle size analysis was performed using Malvern particle size analyzer.

2.3 HPLC Method Development

Different chromatographic conditions were investigated to achieve optimum peak symmetry, retention time, and resolution. Several combinations of methanol, acetonitrile, and phosphate buffer were evaluated during preliminary trials. Detection wavelength was selected after scanning standard solution of Miconazole nitrate in UV region between 200–400 nm. Maximum absorbance was observed at 232 nm and selected for further analysis.Optimized chromatographic separation was achieved using RP-C18 column with mobile phase consisting of acetonitrile and phosphate buffer under isocratic elution conditions. Flow rate was maintained at 1.0 mL/min and injection volume was fixed at 20 µL (Table2) and (Figure2).

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Table 2: Optimized Chromatographic Conditions

Column	C18(ThermoHypersilgold)/4.6x250 mm
Flow Rate	1 ml/min
Wavelength	280 nm
Injectionvolume	20µl
ColumnovenTemperature	Ambient
Run Time	10 minutes
MobilePhase	Mixtureofwater(0.1%OPA):ACNin ratio 30:70 % v/v

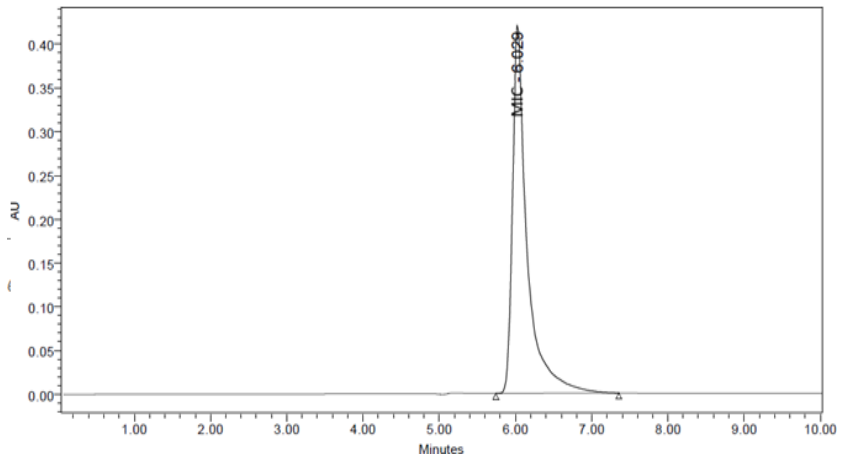

Figure 2: Optimized Chromatogram of Miconazole Nitrate

2.4 Preparation of Standard and Sample Solutions

Accurately weighed 10 mg of Miconazole nitrate was transferred into 10 mL volumetric flask and dissolved in methanol to obtain stock solution of 1000 µg/mL. Appropriate dilutions were prepared using mobile phase.Nanoemulsion formulation equivalent to 10 mg of Miconazole nitrate was extracted with methanol, sonicated, filtered through 0.45 µ membrane filter, and diluted appropriately before chromatographic analysis.

2.5 Validation of Developed RP-HPLC Method

The developed RP-HPLC method was validated according to ICH Q2(R1) guidelines.

2.5.1 System Suitability

System suitability parameters including retention time, theoretical plates, tailing factor, and %RSD of peak area were evaluated prior to analysis (Figure 3).

Table 3: System Suitability Parameters

Sr.No	**Peakarea**	**Retention Time**	**Symmetry**	**No.oftheoretical Plates**
	MIC	**MIC**	**MIC**	**MIC**
1	160500	6.03	1.60	8530
2	161600	6.05	1.50	8525
3	160900	6.15	1.65	8622
4	160700	6.10	1.60	8667
5	160900	6.12	1.60	8545
Mean	160920	6.09	1.59	8578
S.D	414	0.05	0.05	63.36
%R.S.D.	0.25	0.81	2.4	0.67

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2.5.2 Linearity

Linearity was evaluated over concentration range of 50–150 µg/mL. Serial dilutions were prepared and injected under developed chromatographic conditions (Table 4) and (Figure 3).

Table 4: Linearity Data

Sr.No.	% Level	MIC	
		Conc. (µg/ml)	Meanpeakarea
1	50	50	91520
2	80	80	145800
3	100	100	180520
4	120	120	216650
5	150	150	272500

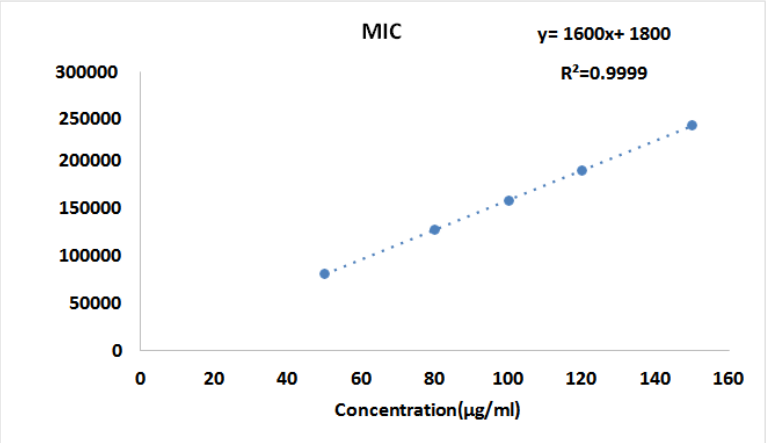

Figure 3: Calibration Curve

2.5.3 Precision

Repeatability and intermediate precision studies were performed by multiple injections of standard solutions. The %RSD values were found below 2%, indicating good precision (Table 5).

Table 5: Precision Study

Sr.no.	% Assay of LC	
	MIC	
	Set– I	Set-II
1	99.95	99.8
2	99.90	99.2
3	100	99.9
Average	99.8	
SD	0.82	
% RSD	0.80	

2.5.4 Accuracy

Accuracy studies were carried out by recovery method at 80%, 100%, and 120% levels. Standards were spiked in sample solution at three levels and recovery studies were calculated (Table6)

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Table 6: Accuracy Study

	MIC		
	Levels		
	80%	100%	120%
Amtadded (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amttaken (µg/ml)	80	100	120
	80	100	120
	80	100	120
Amtrecovered (µg/ml)	79.95	99.80	119.85
	79.90	99.95	119.90
	79.90	99.95	119.85
% Recovery	99.87	99.80	99.75
	99.75	99.95	99.83
	99.75	99.95	99.75
Mean%recovery	98.79	99.92	99.78
% RSD	0.06	0.05	0.04

2.6. Application of HPLC method for analysis of MIC nano emulsion

The prepared nano emulsion was analysed by developed HPLC method. The recovery was calculated. A chromatogram representing analysis of MIC in nanoemulsion is presented in figure 3.

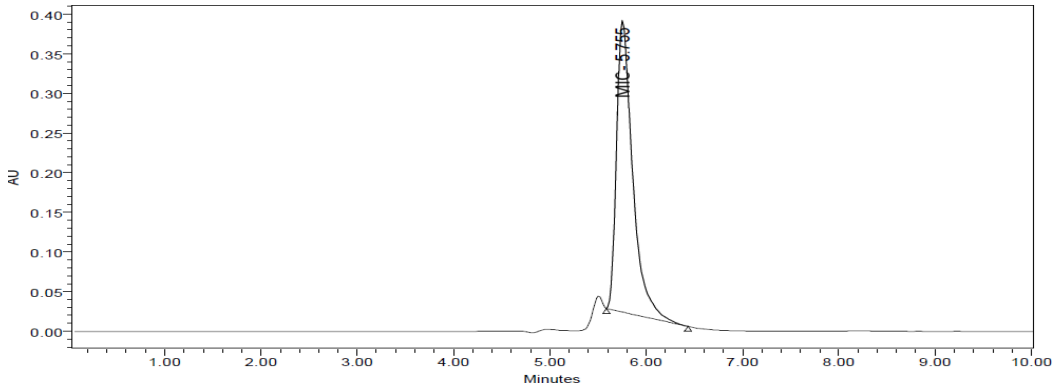

Figure 3: HPLC analysis of MIC nano emulsion.

3. Results And Discussion

The developed RP-HPLC method provided sharp, symmetrical, and well-resolved peaks for Miconazole nitrate without interference from excipients present in nanoemulsion formulation. The optimized chromatographic conditions resulted in satisfactory retention time and acceptable system suitability parameters.

The calibration curve demonstrated excellent linearity over selected concentration range with correlation coefficient greater than 0.999, confirming proportional relationship between concentration and peak area. Precision studies indicated high reproducibility with %RSD values below 2%. Recovery studies demonstrated excellent accuracy within acceptable range of 98–102%.

The robustness study confirmed that small deliberate changes in chromatographic parameters did not significantly affect the performance of the method. The developed method was found to be specific, accurate, precise, and suitable for routine quality control analysis.

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The prepared nanoemulsion formulations appeared homogeneous and stable without evidence of phase separation or creaming. The optimized formulation exhibited satisfactory pH, viscosity, and particle size suitable for pharmaceutical application. The small droplet size of nanoemulsion may contribute toward enhanced dissolution and penetration of Miconazole nitrate.

4. Conclusion

The present investigation successfully developed and validated a simple, rapid, accurate, precise, and robust RP-HPLC method for estimation of Miconazole nitrate in an in-house formulated nanoemulsion. The developed method complied with ICH Q2(R1) validation requirements for specificity, linearity, precision, accuracy, robustness, LOD, and LOQ. The chromatographic method demonstrated satisfactory reproducibility and selectivity for quantitative estimation of Miconazole nitrate in nanoemulsion formulation. The developed nanoemulsion formulation exhibited acceptable physicochemical properties and stability characteristics. Incorporation of Miconazole nitrate into nanoemulsion system may improve solubility, permeability, and therapeutic efficacy of the drug. The validated RP-HPLC method can be effectively utilized for routine quality control analysis, formulation development, and stability studies of Miconazole nitrate nanoemulsion formulations.

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