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Original Article

Validated high-performance thin-layer chromatographic (HPTLC) method for simultaneous determination of nadifloxacin, mometasone furoate, and miconazole nitrate cream using fractional factorial design

Kalpana G. Patel[*], *Pratik M. Shah*, *Purvi A. Shah*, *Tejal R. Gandhi*

Department of Quality Assurance, Anand Pharmacy College, Anand, India

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ABSTRACT

A high-performance thin-layer chromatographic method for simultaneous determination of nadifloxacin, mometasone furoate, and miconazole nitrate was developed and validated as per International Conference on Harmonization guidelines. High-performance thin-layer chromatographic separation was performed on aluminum plates precoated with silica gel $60F_{254}$ and methanol:ethyl acetate:toluene: acetonitrile:3M ammonium formate in water (1:2.5:6.0:0.3:0.2, % v/v) as optimized mobile phase at detection wavelength of 224 nm. The retardation factor (R_f) values for nadifloxacin, mometasone furoate, and miconazole nitrate were 0.23, 0.70, and 0.59, respectively. Percent recoveries in terms of accuracy for the marketed formulation were found to be 98.35–99.76%, 99.36–99.65%, and 99.16–100.25% for nadifloxacin, mometasone furoate, and miconazole nitrate, respectively. The pooled percent relative standard deviation for repeatability and intermediate precision studies was found to be < 2% for three target analytes. The effect of four independent variables, methanol content in total mobile phase, wavelength, chamber saturation time, and solvent front, was evaluated by fractional factorial design for robustness testing. Amongst all four factors, volume of methanol in mobile phase appeared to have a possibly significant effect on retention factor of miconazole nitrate compared with the other two drugs nadifloxacin and mometasone furoate, and therefore it was important to be carefully controlled. In summary, a novel, simple, accurate, reproducible, and robust high-performance thin-layer chromatographic method was developed, which would be of use in quality control of these cream formulations.

* *Corresponding author*. Department of Quality Assurance, Anand Pharmacy College, Anand, Gujarat, 388001, India.
E-mail address: kalpanapatel.pharma@gmail.com (K.G. Patel).

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1. Introduction

Nadifloxacin (ND), chemically (RS)-9-fluoro-8-(4-hydroxy-piperidin-1-yl)-5-methyl-1-oxo-6,7-dihydro-1H,5H-pyrido [3,2,1-ij] quinoline-2-carboxylic acid (Figure 1A), is a potent antibacterial drug. ND has not yet been officially described in any pharmacopoeia. Mometasone furoate (MF), a glucocorticoid, chemically 9α,21-dichloro-llβ-hydroxy-16α-methyl 3,20-dioxopregna-l,4-dien-17-yl furan-2-carboxylate (Figure 1B), is used for antiinflammatory and antipruritic properties [1,2]. Miconazole nitrate (MN), is an antifungal drug, chemically known as (RS)-1-[2-(2,4dichlorophenylmethoxy)-2-(2,4-dichlorophenyl)ethyl] lH-imidazole nitrate (Figure 1C). It is used to exhibit a broad spectrum of antimicrobial activity for systemic and local treatment of vaginal and topical fungal infections [3]. A combination of all these three drugs available as a cream has been used for the treatment of dermatoses topically.

A literature survey revealed various stabilities, indicating reverse-phase high-performance liquid chromatography (HPLC) and spectrophotometric methods for MF and MN, and HPLC and high-performance thin-layer chromatographic (HPTLC) methods for all three drugs individually and in combination with other drugs [4–23]. Spectrophotometric and HPTLC methods for all three drugs individually and in combination for simultaneous estimation have also been reported [24–29]. However, development of a HPTLC method for simultaneous estimation of ND, MF, and MN in combined dosage form has not been reported.

Recently, HPTLC is widely employed for the quantification of drugs because of low maintenance cost, lower analysis time, low mobile phase consumption per sample, and need for minimal sample clean-up. It facilitates automated application of samples and scanning of plates and, moreover, HPTLC as method recently has been proposed to be included in various pharmacopoeia [30–33].

Analytical quality by design (AQbD) is a systematic approach of method development that begins with predefined objectives and emphasizes method understanding and its performance, based on sound science and quality risk management [34]. The main objective of AQbD is to reduce variations in the measurements by controlling various factors that affect method performance thereby resulting in less variation in interlaboratory studies and assuring reproducibility. Design of experiment (DoE) is an integral part of AQbD that includes use of experimental design, mathematical model generation by ANOVA analysis, and graphical representations, showing correlation between factors and response [35–38]. Therefore, design of experimentation is required to study the effect of previously identified factors affecting the method and defining a robust AQbD design space where the method can be operated anywhere in that region. Method transfer and reproducibility in interlaboratory studies are the potential benefits of AQbD [39–40].

This research article focuses on the determination of robustness of HPTLC analytical method by fractional factorial design (FFD). Among the various experimental designs, FFD as a response surface was preferred for prediction of nonlinear response and also due to its flexibility, in terms of experimental runs and information related to the factor's main and interaction effects. Therefore a novel, simple, accurate, reproducible HPTLC method was developed for simultaneous estimation of ND, MF, and MN in pharmaceutical dosage form, using FFD design for robustness testing. Therefore, this research paper describes the development of HPTLC method for simultaneous estimation of ND, MF, and MN using the DoE approach for method validation.

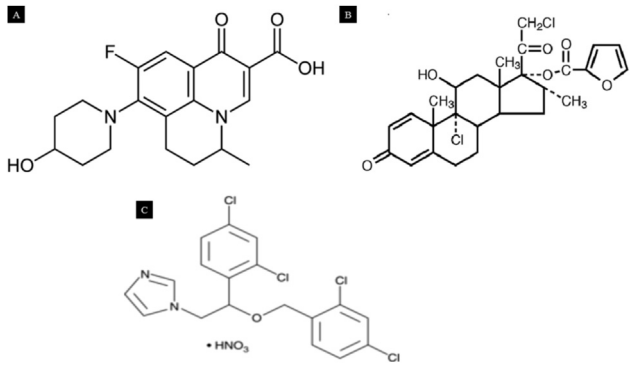

Figure 1 – (A) Chemical structures of nadifloxacin; (B) mometasone furoate; (C) miconazole nitrate.

2. Materials and methods

2.1. Materials

Working standards of ND and MN were kindly provided as a gratis sample from Hetero Drugs Limited, Hyderabad, India and MF from Cipla Ltd., Mumbai, India. All solvents and chemicals used were purchased from Merck Specialities Pvt. Ltd., India. Marketed cream formulation; Bactimax cream (Ajanta Pharma Ltd., Mumbai) used in this study was procured from the local market.

2.2. Instrumentation

Linomat 5 applicator (Camag, Switzerland), twin trough chamber (20 × 10 cm; Camag, Switzerland), TLC scanner IV (Camag, Switzerland), win CATS version 1.4.6 software (Camag, Switzerland), Microsyringe (Linomat syringe 659.0014, Hamilton–Bonaduz Schweiz, Camag, Switzerland), UV chamber (Camag, Switzerland), precoated silica gel $60F_{254}$ aluminium plates (20 × 10 cm, 100 μm thickness; Merck, Darmstadt, Germany) were used in the study.

2.3. Preparation of standard solutions

A stock solution of ND, MF, and MN was prepared separately by weighing accurately 10 mg of drug followed by dissolution in methanol in a 100-mL volumetric flask and dilution up to the mark with methanol, to obtain a concentration of 100 μg/mL. This stock solution was appropriately diluted with methanol to obtain a working standard solution for ND, MF, and MN.