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Simultaneous estimation of Nadifloxacin and Mometasone Furoate in topical cream by HPTLC method

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Abstract

The present work describes HPTLC method for analysis of Nadifloxacin and Mometasone furoate in topical cream. The separation was carried out on Merck precoated silica gel aluminium plate 60 F_{254} using Dichloroethane: Diethyl ether: Ammonia: Methanol: Ethyl acetate (6: 3: 0.2: 1.75: 3.5 v/v) as mobile phase. The densitometric scanning was carried out at 254 nm. Response was found to be linear in the concentration range of 1000–3000 ng /band with correlation coefficient (r^2 = 0.997) for Nadifloxacin and 100-300 ng/band with correlation coefficient (r^2 = 0.995) for Mometasone furoate. The method was validated as per ICH guidelines. The method was successfully applied for the analysis of drugs in pharmaceutical formulation.

Key words: Nadifloxacin, Mometasone furoate, HPTLC.

INTRODUCTION

Nadifloxacin (NAD), chemically known as (9-Fluoro-6, 7-dihydro-8-(4-hydroxy piperidino)-5-methyl-1-oxo-1*H*, 5*H*-benzo [*ij*] quinolizine-2-carboxylic acid). It is a fluoroquinolone antibiotic used for the treatment of skin infections with susceptible bacteria, Acne vulgaris (Inflamed lesions) [1–3]. Where as Mometasone Furoate (MF), (9α, 21-dichloro-11β, 17-dihydroxy-16α-methylpregna-1, 4-diene-3, 20-dione 17-(2-furoate)) is used for mixed infection of skin [4-6].

Literature survey reveals HPLC determination of Nadifloxacin in rat plasma [7] & UV–visible spectroscopic studies, which is interactions between polyamidoamine (PAMAM) dendrimers and quinolones (nadifloxacin and prulifloxacin) [8]. For simultaneous determination of MF with other drugs, RP-HPLC [9, 10, and 11], UV [4] and HPTLC [12] methods are reported. No HPTLC method is reported so far for the estimation of NAD and MF in combined pharmaceutical formulations; hence we have developed a HPTLC method for the simultaneous

estimation of Nadifloxacin and Mometasone Furoate in bulk and pharmaceutical formulation. Structure of Nadifloxacin and Mometasone Furoate is shown in **Figure 1.**

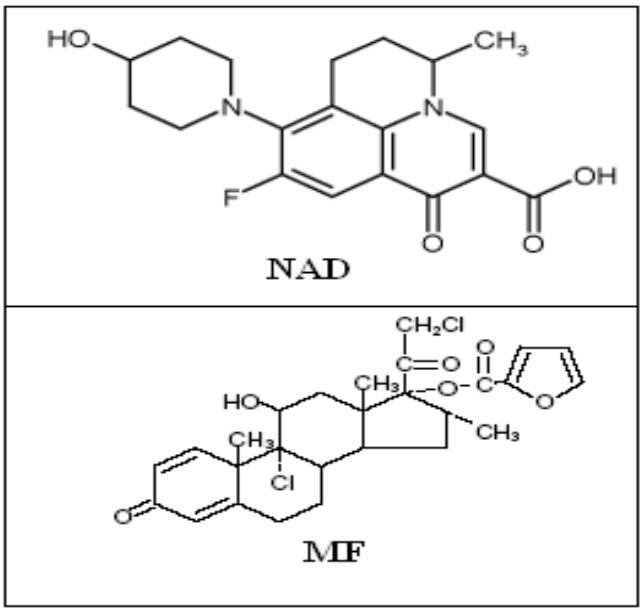

Figure 1. Structures of NAD and MF.

MATERIALS AND METHODS

2.1. Materials

Nadifloxacin and Mometasone Furoate were supplied as a gift samples by Cipla Ltd (Goa, India). All chemicals and reagents used were of AR grade.

2.2. HPTLC method and chromatographic conditions:

The standard solutions ranging from 1000-3000 ng/band of NAD and 100-300 ng/band of MF were applied on precoated silica gel 60 GF_{254} plate in the form of bands with 100 µl sample syringe using automatic sample applicator LINOMAT V. The linear ascending development was carried out in 10 cm ×10 cm twin trough glass chamber which was already saturated for 10 mins with the mobile phase. The mobile phase consists of Dichloroethane: Diethyl ether: Ammonia: Methanol: Ethyl acetate (6: 3: 0.2: 1.75: 3.5 v/v). After development plate was immediately dried with the help of dryer and was observed under UV chamber. The well resolved band of drug was scanned at 254 nm with Camag TLC scanner III densitometer controlled by WINCAT's software. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

2.2.1. Standard Solutions and Calibration Graphs

Stock solution was prepared by dissolving 100 mg of Nadifloxacin and 10 mg of Mometasone Furoate in 100 ml methanol, from which 1 ml was further diluted to 10 ml with methanol. Final concentration prepared was 100 ng/µl of NAD and 10 ng/ µl of MF. The standard solutions were applied in a concentration range of 1000–3000 ng/band for Nadifloxacin and 100-300 ng/band for MF. The plate was developed on previously described mobile phase. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

2.2.2. Analysis of Formulation

A quantity of cream equivalent to 10 mg of NAD (1 g of cream) was weighed and transferred to a 100 ml volumetric flask and added 30 ml of methanol, the solution was warmed for 5-10 mins,

ultrasonicated for 20 mins and volume was made up to the mark with methanol. The solution was filtered using Whatman paper No. 41. 10 µl Filtrate of sample solution was applied on HPTLC plate to obtain final concentration, 1000 ng/band of NAD and 100 ng/band of MF. Six sample solutions were applied and after chromatographic development peak areas of the spots were measured at 254 nm and concentrations in the samples were determined using multilevel calibration curves.

2.2.3. Method validation

The proposed method was optimized and validated as per the International Conference on Harmonization (ICH) guidelines [13].

RESULTS AND DISCUSSION

3.1. Optimization of Procedures

While selection of mobile phase different proportions of methanol, chloroform, ammonia, Dichloroethane, ethyl acetate, diethyl ether were tried. Ultimately mobile phase containing Dichloroethane: Diethyl ether: Ammonia: Methanol: Ethyl acetate (6: 3: 0.2: 1.75: 3.5 v/v) was finalized as optimal for obtaining well defined and resolved peaks. The spots developed were dense, compact and typical peaks of drugs were obtained. Peaks were symmetrical in nature and no tailing was observed when plates were scanned at 254 nm as shown in **Figure 2**.

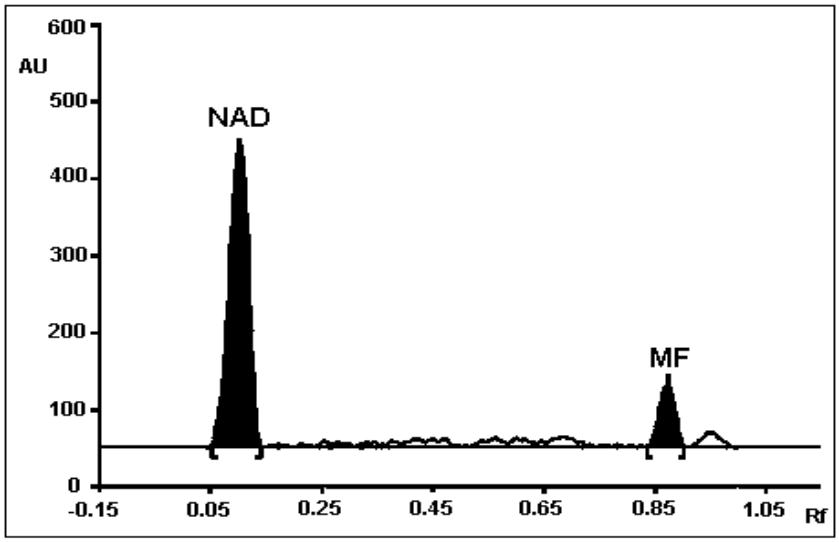

Figure 2. Standard densitogram of NAD and MF mixed standard

3.2. Method Validation

NAD and MF showed good correlation coefficient in concentration range of 1000–3000 ng/band and 100-300 ng/band respectively as shown in **Table 1**.

Table 1: Linear regression data for calibration curves for NAD (n = 3)

Parameter	NAD	MF
Detection Wavelength (nm)	254	
Linearity range (ng/band)	1000-3000	100-300
Correlation Coefficient (r)	0.997	0.995
Linear Regression Equation (y = mx + c)		
Intercept (c)	771.9	5.415
Slope (m)	5.274	10.032

The spots were observed at Rf 0.12 and 0.85 in the densitogram of the drugs extracted from cream. There was no interference from the excipients commonly present in the creams. The % assay for Nadifloxacin and Mometasone Furoate were found to be 100.22 ± 0.73 % RSD and 100.30 ± 1.20 % RSD respectively as shown in **Table 2**.

Table 2: Results of marketed formulation analysis

DRUG	LABEL CLAIM (mg/g of cream)	% LABEL CLAIM*	SD	% RSD
NAD	10	100.22	0.74	0.73
MF	1	100.30	1.21	1.20

*Average of six determinations

The low % RSD value indicated the suitability of this method for routine analysis. Good correlation was obtained between standard and sample spectra of Nadifloxacin and Mometasone Furoate. % RSD was found to be less than 2 % for day to day variations, which proves that method was precise. Results are shown in **Table 3**.

Table 3: Statistical evaluation of precision of developed method

PRECISION	% LABEL CLAIM		SD		% RSD	
	NAD	MF	NAD	MF	NAD	MF
Intraday (n= 6)	100.58	100.73	0.92	1.60	0.92	1.59
Interday (n= 3×3)	100.68	101.38	1.14	1.31	1.13	1.29

Results of recovery studies are shown in **Table 4**.

Table 4: Result from recovery studies (n = 3)

DRUG	AMOUNT TAKEN (ng per band)	AMOUNT ADDED (ng/band)	AMOUNT RECOVERED (ng/band)	% RECOVERY	SD	% RSD
NAD	1000	800	797.28	99.66	0.55	0.55
	1000	1000	996.98	99.70	0.46	0.46
	1000	1200	1194.17	99.51	0.50	0.50
MF	100	80	79.91	99.89	0.77	0.77
	100	100	99.51	99.51	0.42	0.42
	100	120	119.73	99.77	0.41	0.41

Table 5: Robustness of method (n = 3)

SR. NO.	PARAMETERS	VARIATION	MEAN* ± SD	
			NAD	MF
1.	Mobile phase composition	± 2 % (Dichloroethane)	0.12 ± 0.02	0.86 ± 0.02
2.	Chamber saturation period	± 10 %	0.12 ± 0.01	0.86 ± 0.02
3.	Development distance	± 10 %	0.12 ± 0.01	0.86 ± 0.02
4.	Time from application to development	0, 10, 20, 30 min	0.12 ± 0.01	0.87 ± 0.01
5.	Time from development to scanning	0, 30, 60, 90 min	0.12 ± 0.00	0.86 ± 0.00

Method was found robust and results are given in **Table 5**.

CONCLUSION

The proposed HPTLC method was validated as per ICH guidelines. The %RSD and standard error calculated for the method were low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Hence, it can be concluded that the developed HPTLC method was accurate, precise and selective and can be employed successfully for the estimation of Nadifloxacin and Mometasone furoate in topical cream.

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