Densitometric development and validation of mupirocin in ointment dosage form

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ABSTRACT

This paper presents a simple, rapid, precise, accurate and robust High Performance Thin Layer Chromatographic (HPTLC) method for estimation of mupirocin (MUP) in ointment dosage form. The mobile phase used in the study comprised of dichloromethane: ethyl acetate: methanol (8:1:2, v/v/v). Chromatographic separation of drug was performed on aluminium plates precoated with silica gel 60 F_{254} as the stationary phase. The densitometric evaluation of separated zones was carried out at 226 nm. A retardation factor of MUP was found to be 0.57 ± 0.02. Linearity of MUP was found to be in the concentration range of 200-3000 ng/band. The Method was validated for linearity, accuracy, precision, specificity and robustness in accordance with International Conference on Harmonisation [ICH] guidelines. The proposed HPTLC method has been successfully applied for the analysis of drug in ointment dosage formulation.

Keywords: Mupirocin, HPTLC, Validation, ICH.
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INTRODUCTION

Mupirocin (MUP) is an antibacterial agent produced by fermentation using the organism *Pseudomonas fluorescens* [1]. Chemically it is (*E*)-(2*S*, 3R, 4R, 5S)-5-[(2*S*, 3S, 4S, 5S)-2, 3-epoxy-5-hydroxy-4-methylhexyl] tetrahydro-3, 4-dihydroxy-β-methyl-2*H*-pyran-2-crotonic acid, ester with 9-hydroxynonanoic acid [2-4]. Literature survey revealed UV [5], HPLC [6, 7] methods for analysis of MUP. Nowadays HPTLC is a routine analytical technique. It has been well reported that a number of samples can be run concurrently with the use of a smaller amount of mobile phase than in HPLC [8 - 11].

No research data has been found for estimation of mupirocin by HPTLC in ointment dosage form. Hence the present study was undertaken to develop and validate HPTLC method for mupirocin in ointment dosage form.

MATERIALS AND METHODS

A reference standard of mupirocin was gifted by Nulife Pvt. Ltd., Pune, India. Brand of Bactroban ointment (mupirocin USP 2.0 % w/w) was purchased from local market. All chemicals and reagents of analytical grade were purchased form Merck specialities Pvt. Ltd. (Mumbai, India).

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Instrumentation
HPTLC plates were prewashed with methanol and activated at 120°C for 15 min prior to chromatographic analysis. The sample solution was applied on precoated silica gel aluminium plates 60F$_{254}$ (20 × 10 cm with 250 μm thickness; E. Merck, Darmstadt, Germany) in the form of bands of 6 mm width, located 8 mm from bottom and 15 mm apart with a camag syringe (100 μL) using a Camag Linomat V (Switzerland) applicator with constant application rate of 150 nLs^{-1}. The slit dimension was kept at 5 × 0.45 mm with densitometric scanning speed of 10 mm/s. HPTLC plate was then developed, with 20 mL solvent system consisting of dichloromethane: ethyl acetate: methanol (8:1:2, v/v/v). Linear ascending development was carried out in a 20 x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with solvent system. The chamber saturation time for mobile phase was 20 min at room temperature (25 ± 2°C) with relative humidity of 60 ± 5%. The length of chromatographic run was 8 cm. After development the plate was removed from the chamber and air-dried followed by densitometric scanning at 226 nm using a Camag TLC Scanner-III with winCATs software version 1.4.4 in the reflectance-absorbance mode. Deuterium lamp was used as a source of radiation in the range of 200 - 400 nm.

Preparation of standard stock solution
Standard stock solution with a concentration of 1000 μg/mL of MUP was prepared in methanol.

Selection of detection wavelength
After HPTLC development, bands were scanned over the range of 200-400 nm. The compound showed maximum absorbance at 226 nm and hence was selected for densitometric analysis (Figure 1).
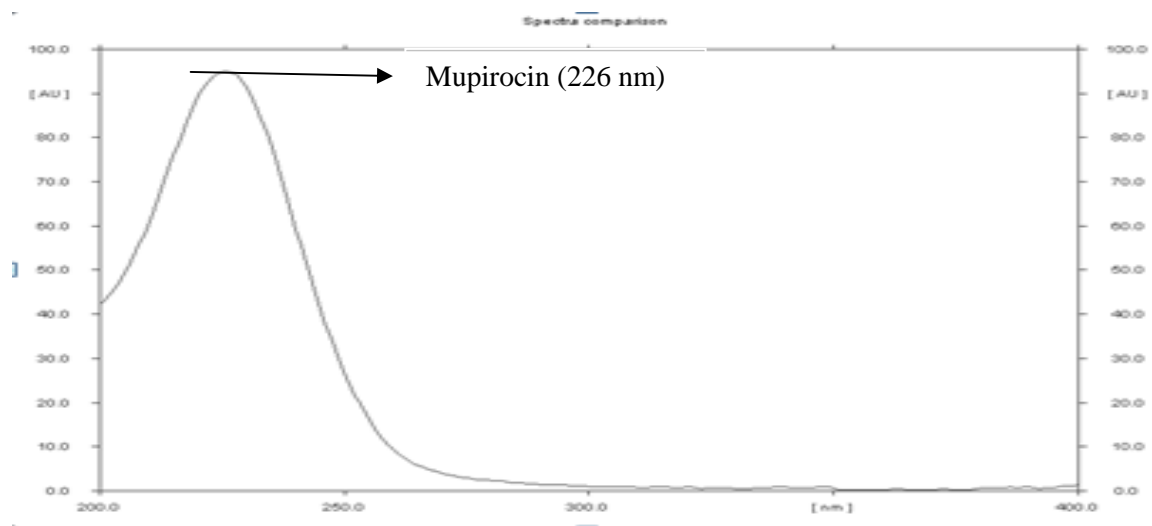

Figure 1: UV Spectrum of mupirocin standard

Optimization of the HPTLC method
The TLC procedure was optimized with a view to develop assay method for mupirocin. The method was optimized using various solvents such as methanol, toluene, ethyl acetate, ethanol, water, n-hexane, dichloromethane, glacial acetic acid, formic acid. After several trials, the mobile phase consisting of dichloromethane: ethyl acetate: methanol (8: 1: 2, v/v/v) gave good results and hence selected for analysis.

Method validation
The proposed HPTLC method was optimized and validated as per ICH guidelines Q2 (R1) [12].

Linearity and Range
Linearity was determined by applying stock solution of mupirocin on the HPTLC plate in the concentration range of 200 – 3000 ng/band. Study was performed six times. The plate was developed using above mentioned optimized mobile phase. Peak area versus concentration was subjected to least square linear regression analysis and the intercept, slope and correlation coefficient for the calibration were determined. Correlation coefficient alone is not sufficient to prove linearity; hence residual analysis was also done.

Precision
The precision of the method was verified by intraday and interday precision studies. Intraday studies were performed in the same laboratory by the same analyst, using single equipment, same reagents, sample, HPTLC plate using three different concentrations (500, 1000, 2000 ng/band) of the MUP in triplicate within one working

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day. The interday precision of the method was performed by repeating studies within same laboratory by different analysts, using different equipments, reagents and HPTLC plates on three successive days. The peak areas were recorded and relative standard deviation (RSD) was calculated.

Sensitivity
Sensitivity was determined by establishing the limit of detection (LOD) and the limit of quantitation (LOQ). LOD and LOQ were found to be 3.3 σ/S and 10σ/S respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration graph.

Robustness studies
The effect of small, deliberate variation of the analytical conditions on the retention factor, the peak areas of the mupirocin was determined. Factors varied were mobile phase composition (± 0.1 mL), amount of mobile phase (± 0.5 %), time from application to development (+ 10 min) and from development to scanning (+ 10 min). One factor at a time was changed to study the variation.

Specificity
Specificity of the method was determined by analyzing standard drug and test samples. The densitograms obtained from mupirocin were compared with the densitograms obtained from sample solution. The peak purity of MUP was determined by studying the spectrum at three different regions at peak start (S), peak apex (M) and peak end (E) positions of the band i.e. r (start, middle) and r (middle, end).

Recovery study
Recovery studies of the drug were carried out for the accuracy parameter at three levels i.e. multiple level recovery studies. Sample stock solution from ointment formulation was prepared. To the above prepared solution, 80 100 and 120 % of the standard drug solutions were added. Dilutions were made and recovery studies were performed.

Solution stability
The solution stability of MUP was determined at 0, 6, 12, 18, 24, 48 h. of storage at room temperature. The stability of the solutions was determined by comparing peak areas at each time point against freshly prepared solutions.

Analysis of marketed formulation
To determine the content of MUP in ointment (Bactroban®, label claim: 2% w/w), 2.296 gm of mupirocin ointment equivalent to 0.04592 gm of MUP was weighed and added in 100 ml volumetric flask. Then it was mixed with 70 ml of methanol and solution was sonicated for 10 min. The volume of resulting solution was made up to 100 ml with methanol. The % assay was determined.

RESULTS AND DISCUSSION

Linearity
The drug response was linear over the concentration range between 200-3000 ng/band for Mupirocin (Table 1 and Figure 2).
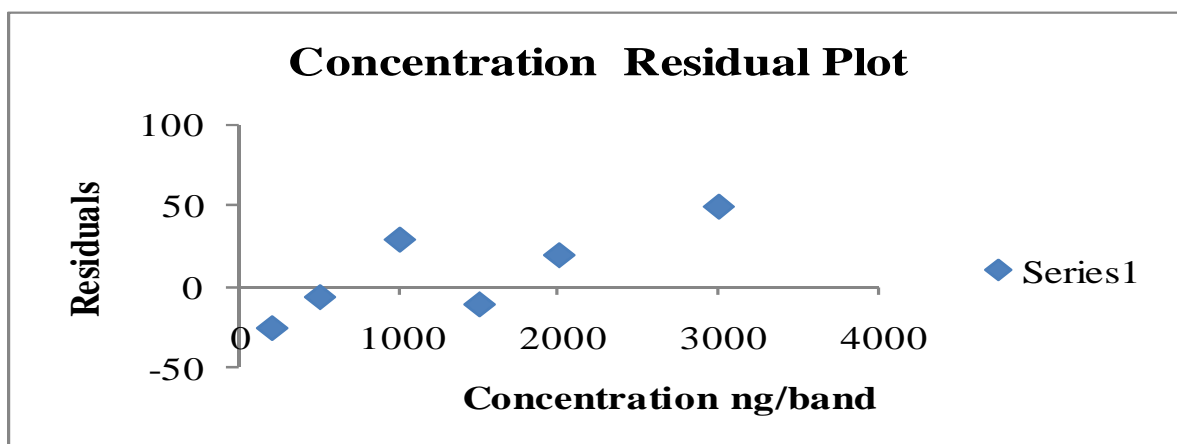

Figure 2: Concentration residual plot of mupirocin

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Table 1: Linear regression data for the calibration curve (n = 6) of MUP

Parameters	
Linearity range (ng/band)	200 – 3000
r^2	0.999
Slope	2.633
Intercept	758.400

n - Number of determinations.

Precision

The developed method was found to be precise as the RSD values for repeatability and intermediate studies were < 2% as recommended by ICH guidelines (Table 2).

Table 2: Precision studies

Concentration (ng/band)	Intraday Precision (n=3)		Interday precision (n=3)	
	Mean peak area $\pm$ SD	% RSD	Mean peak area $\pm$ SD	% RSD
500	2050.750 $\pm$ 12.915	0.629	2037.001 $\pm$ 14.776	0.725
1000	3657.500 $\pm$ 21.001	0.574	3642.433 + 15.250	0.418
2000	6428.060 $\pm$ 38.641	0.601	6357.033 + 34.754	0.546

n - Number of determinations.

Sensitivity

The LOD and LOQ for mupirocin were found to be 44.520 and 134.912 ng/band, respectively.

Robustness of the method

The standard deviation of peak areas were calculated for each parameter and % RSD was found to be < 2, indicating robustness of the method (Table 3).

Table 3: Robustness testing

Robustness testing (n = 3)		
Parameter	SD of Peak Area for Mupirocin	% RSD
Mobile phase composition [Dichloromethane] ($\pm$ 0.1 ml)	13.186	0.639
Amount of mobile phase ($\pm$ 5%)	15.147	0.734
Time from application to development (+ 10 min)	12.113	0.586
Time from development to scanning (+ 10 min)	12.851	0.622

n - Number of determinations.

Specificity

The peak purity of Mupirocin was found by observing the spectra at the start, apex, peak end positions of the band i.e., r (S, M) = 0.999 and r (M, E) = 0.999. A good correlation (r = 0.999) was also obtained between standard and sample spectra of mupirocin.

Recovery studies

As seen from the data, a recovery of the Mupirocin was found to be 99.172-100.575 % which indicates that the proposed densitometric method is reliable for estimation of mupirocin in ointment dosage form (Table 4).

Table 4: Recovery studies

Recovery studies (n = 3)					
Amount taken (ng/band)	Amount added (ng/band)	Total amount (ng/band)	Amount recovered (ng/band)	% Recovery $\pm$ SD	% RSD
1000	800	1800	1785.100	99.172 $\pm$ 12.264	0.687
1000	1000	2000	2009.267	100.463 $\pm$ 10.231	0.509
1000	1200	2200	2212.667	100.575 $\pm$ 11.872	0.536

n - Number of determinations.

Analysis of marketed formulation

Validity of the proposed HPTLC method was applied for estimation of mupirocin in ointment dosage form (Figure 3). The percent content of mupirocin in marketed formulation was found to be 99.62%.
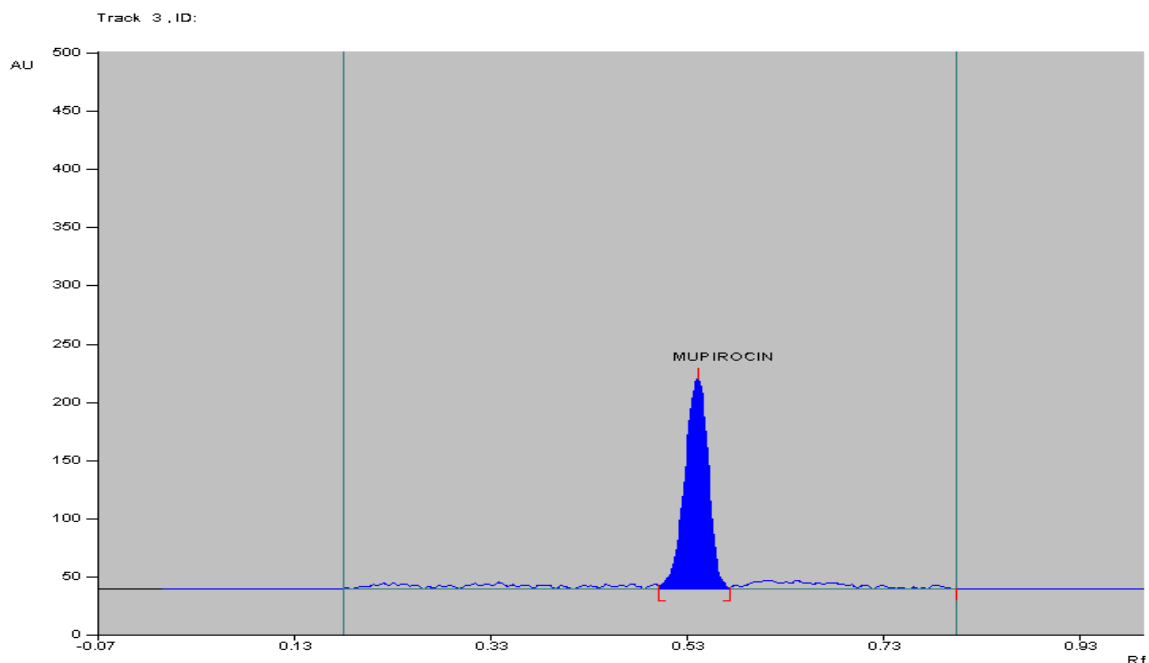
Figure 3: Representative densitogram of mupirocin

CONCLUSION

It is shown that the method was linear, accurate, precise, reproducible, repeatable, and selective, proving the reliability of the method [13 - 16]. Hence, the method is recommended for routine quality control analysis of the formulation used in the study [17].

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