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DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF MUPIROCIN IN BULK AND OINTMENT DOSAGE FORM

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ABSTRACT

A simple, gradient RP- HPLC method has been developed and validated for Mupirocin in bulk and ointment formulation. The successful estimation was carried out of the drug product is developed on C(18) column reversed-phase using Methanol: Phosphate buffer (20:80 v/v) as mobile phase composition. The flow rate was adjusted to 1.0 mL/minute and the absorption maxima were observed at 270 nm utilizing Shimadzu SPD-20A Prominence UV-Vis detector. Mupirocin showed a good and precise linearity in the range 20-100 µg/mL. The HPLC, assay shows the purity ranging 98.95 to 103.07% for ointment formulation. The mean percentage purity is 101.47%. The chromatographic retention time of Mupirocin was found to be 7.3 minutes. The statistical analysis shows the method accuracy. Various forced degradation studies was conducted on Mupirocin ointment to examine the stability of the drug. The developed method validated according to the ICH guidelines.

KEYWORDS: Mupirocin, RP-HPLC, UV, Validation and forced degradiation.

INTRODUCTION

Mupirocin comes under the drug class of antibiotic.[1] Mupirocin (MUP) is chemically 9-[(E)-4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-[[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl]methyl] oxan-2-yl]-3-methylbut-2-enoyl]oxynonanoic acid.[2] Mupirocin is used in the treatment of topical bacterial skin infections, which is effective against Gram-positive bacteria topically; for example, furuncle, impetigo, open wounds.[3] Mupirocin is widely used for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA).[4] Mupirocin is inactive for most anaerobic bacteria, mycobacteria, mycoplasma, chlamydia, yeast and fungi.[5] The mechanism of action of Mupirocin is by inhibiting the bacterial isoleucyl-tRNA synthetase and by blocking protein synthesis.[5] The structural analogy with isoleucyn and it interacts reversible with aminoacid specifically at the active site of the enzyme. The depletion of cellular levels of isoleucine-charged transfer RNA leads to the arrest of protein synthesis.[6] According to the literature review several methods has been developed for Mupirocin, like UV spectroscopy, capillary electrophoresis, HPTLC and HPLC method.[7-12] The thorough literature survey revealed that a few stability-indicating normal and RP-HPLC methods for Mupirocin in respective dosage forms are available but all of these methods are specific to the bulk drugs. The proposed aim of the study was to develop simple, accurate, specific and precise RP-HPLC method for the estimation of Mupirocin in the bulk and pharmaceutical formulation.

MATERIALS AND METHODS

Chemicals: The Mupirocin powder reference standard (RS) was purchased from Sigma, Germany. The Avoban 2% w/w marketed drug of Mupirocin, manufactured and marketed by Avelon Pharma**,** purchased from Jazan local Pharmacy, Saudi Arabia. The HPLC grade Methanol was purchased from Merck.

Instrumentation

RP-HPLC instrumentation: Shimadzu LC-20 AT HPLC system, using SPD-10 detector (SPD- M20A, Japan). A Zorbax Eclipse Plus, Agilent Technology column (150mm x 4.6mm, 5µm) with Pore size 95Å. The column temoerature was maintained at a 27ºC and the flow rate 1.0 ml/min. The injection volume is 20µl, 270nm was set as a wavelength and the HPLC run time was seted for 15 minutes. Phosphate buffer was prepared

RP-HPLC Standard solutions

Preparation of Mobile phase: Accreatly weighed 1.35g of KH_2PO_4 transfered in to 1 liter volumaetric flask and dissolved by 500 ml of HPLC grade water and the pH was adjusted to 6 by gradual adding of phosphoric acid, the resulting solution was filtered with 0.45µ membrane filter. The final mobile phase was prepared by adding the ratio of (20:80 v/v) Methanol and phosphate buffer.

Preparation of Mupirocin Stock solution: Accurately 2 mg Mupirocin was taken in 100 ml volumetric flasks and mixed with 100 ml of mobile phase solution. For 5 minutes the resulting solution is kept in the sonicator. The concentration of 20-100 µg/ml was achived by diluting the standard stock solution with mobile phase. Mupirocin powder freely soluble in methanol.

Preparation of sample solution: 1 gm of marketed sample of Avoban 2% w/w ointment weighed accurately and equivalent of 20 mg of Mupirocin transferred into 25ml volumetric flasks and dissolved with 25 ml mobile phase and filtered through Whatman 1 filter paper. Further dilutions were made based on the required concentrations.

Solution stability: The prepared drug solution stability was analysed during the time of analysis and also repeated the same analysis method on same day with different time intervels. The same analysis is repeated after 24 hrs by keeping the drug solution under laboratory temperature (35 ± 1°C) and in refrigeration (6 ± 1°C).

Forced degradation study: Mupirocin ointment was put into different stress conditions to perform degradation studies. The degradation study was conducted to examine the proposed assay technique. Mupirocin is freely soluble in Methanol and also methonol is the portion of mobile phase, so methol was used as solvent. In all the experiments Mupirocin ointment contents equivalent to 20mg Mupirocin was weighed. Accordig to the stock solution procedure the solutions was prepared. 60 µg/ml of Mupirocin is taken for every analysis. The drug solution was treated with acid, base, oxidative, dry and wet heat and direct sun light (photolytic stress). According to the proposed method the resulting drug samples were examined.

Acid degradation study: The stability of Mupirocin ointment in acidic state was examined by treating with different strength of Hydrochloric acid 0.1N to 4N HCL. Solution of 60 µg/ml Mupirocin is taken for this study was treated with 4N hydrochloric acid in presence of methanol. The treated drug solution was kept in dark chamber at 35°C for 12 hours.

Alkali degradation study: The stability of the Mupirocin ointment in alkaline condition was examined by treating with different strength of sodium hydroxide 0.1N to 4N NaOH. Solution of 60 µg/ml Mupirocin is taken for this study was treated with 4N sodium hydroxide in presence of methanol. The treated drug solution was kept in dark chamber at 35°C for 12 hours.

Oxidation study: The stability of Mupirocin ointment under oxidative condition using hydrogen peroxide was examined. Solution of 60 µg/ml Mupirocin is taken for this study was treated using 20 % H_2O_2 in methanol. The treated drug solution was incubated at 35ºC for 12 hours.

Wet heat study: Solution of 60 µg/ml Mupirocin is taken for this study treated with HPLC grade water and the resulting solution was kept in dark chamber at 35°C for 12 hours.

Dry heat study: To conduct a dry heat analysis the drug solution was prepared by 2gm of Mupirocin ointment approximately in a clean aluminium foil and kept in an oven at 35°C for 12. The resulting Mupirocin was weighed and solutions were prepared same as the preparation of stock solution procedure, 60 µg/ml of Mupirocin is taken for analysis.

Photo stability study (Sun light): 2gm of Mupirocin ointment on a glass dish and exposed to direct sunlight. Exposing Mupirocin ointment over a period of 4 hours carried the testing. The resulting Mupirocin ointment was weighed and solutions were prepared same as the preparation of stock solution procedure, 60 µg/ml of Mupirocin is taken for analysis.

RESULTS AND DISCUSSION

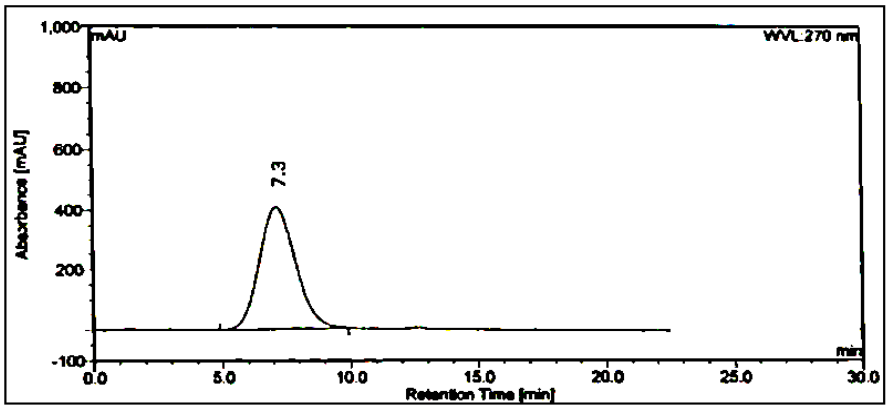

Fig. 1: A Typical Chromatogram of Mupirocin Standard

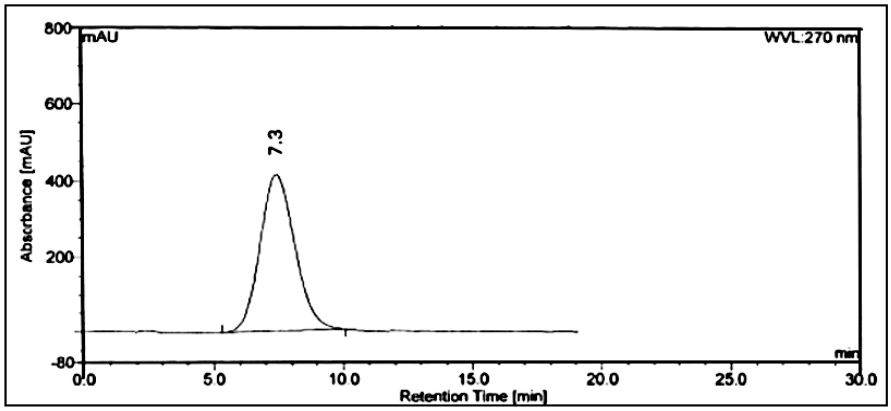

Fig. 2: A Typical Chromatogram of Mupirocin ointment

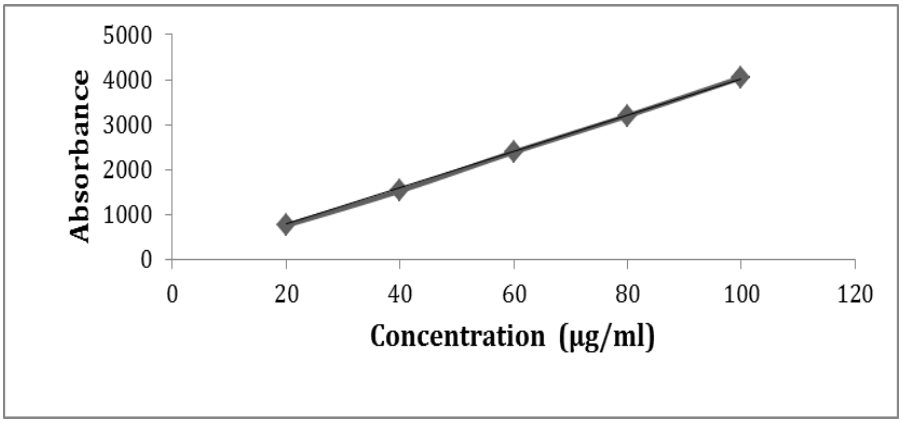

Fig. 3: Calibration graph of Mupirocin 20-100µg/ml precision

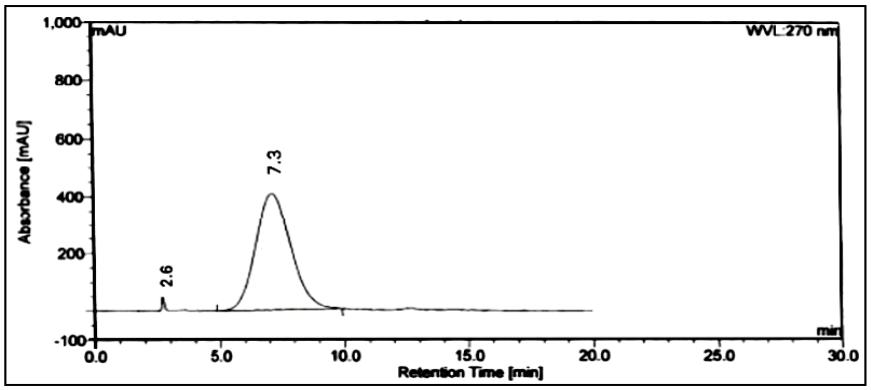

Fig. 4: Chromatogram of Mupirocin under oxidation condition by RP-HPLC method

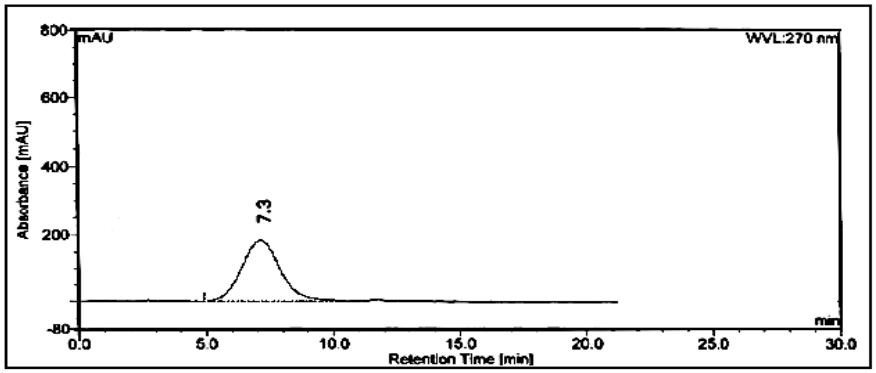

Fig. 5: Chromatogram of Mupirocin under wet heat condition by RP-HPLC method

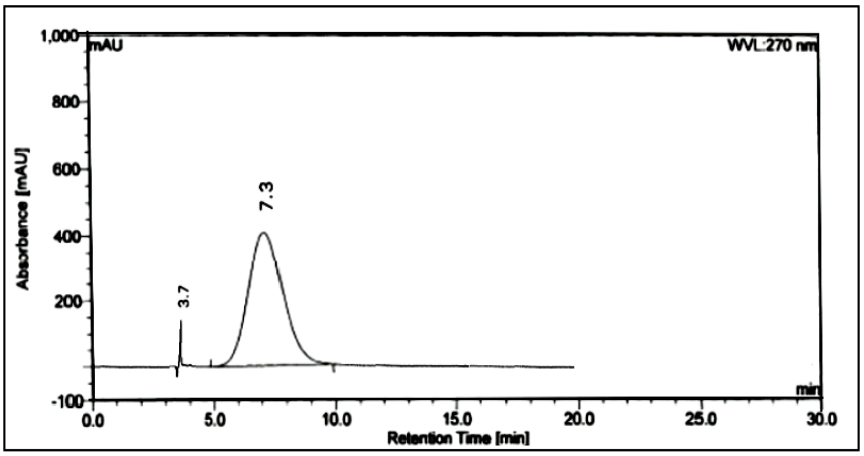

Fig. 6: Chromatogram of Mupirocin under dry heat condition by RP-HPLC method

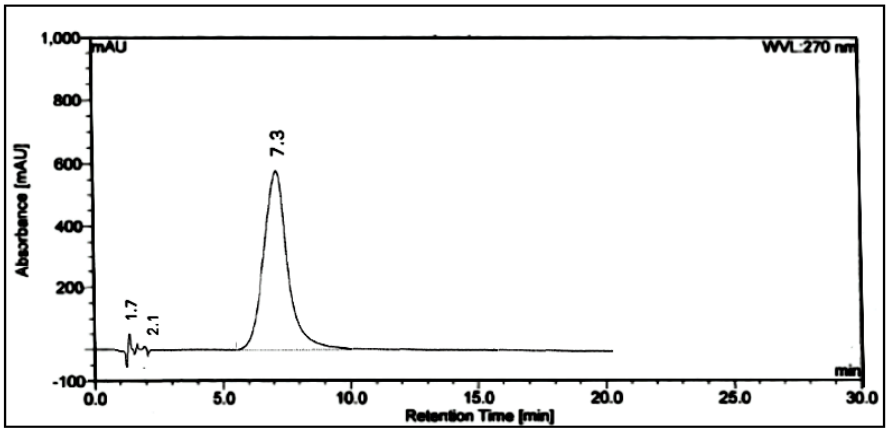

Fig. 7: Chromatogram of Mupirocin under Photo stability condition by RP-HPLC method

Table. I: HPLC conditions for estimation of Mupirocin

Parameters	Description
Column	Agilent Technology column C_{18} (150mm x 4.6mm, 5µm)
Column temperature	25 ± 1°C
Mobile phase	Methanol: Phosphate buffer (20:80 v/v)
Detection	Photodiode array detection at 270 nm
Injection volume	20 µl
Flow rate	1.0 ml min^{-1}

Table. 2: HPLC linearity data for Mupirocin

Concentration (µg/ml)	Peak area
20	764.25
40	1528.95
60	2293.27
80	3197.45
100	4049.69

Table.III : Recovery studies of Mupirocin ointment formulation

S No	Drug	Amount of Drug present in preanalyzed Sample (µg/ml)	Amount of Standard drug (RS) added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery	Mean recovery in Percentage
1.	Mupirocin	20	40.00 60.00 100.00	60.73 80.76 100.62	101.21 100.95 100.62	100.92

Table. IV: Method precision data of Mupirocin by RP-HPLC method

Mupirocin 60 µg/ml (n=4)	Retention time	Area
1	7.321	2273.67
2	7.291	2143.27
3	7.223	2203.49
4	7.341	2253.22
Mean	7.3	2218.21
S.D [a]	0.0321	123.432
% CV[b]	0.453	0.543

4 observations

Table. V: Intermediate precision data of Mupirocin by RP-HPLC method

Mupirocin µg/ml	Inter-day measured mean area ± S.D.[a]	%CV[b] (n^c=4)	Intra-day measured mean area ± S.D.[a]	%CV[b] (n^c=4)
20	757.85± 4.45	0.9842	697.54± 4.75	1.065
40	1599.75±3.05	0.9964	1679.75±2.05	0.987
60	2273.22±5.56	1.0432	2376.22±4.16	0.996

n^c = 4 observations

Table. VI: Results of Limit of detection & limit of quantification

Parameters	Mupirocin
LOD (µg/ml)	0.20
LOQ (µg/ml)	0.30

Table. VII: Results of system suitability parameters

SNo	Parameters	Mupirocin
1.	Theoretical plates	4557
2.	Tailing factor	0.977
3.	Resolution factor	2.74
4.	Retention time	7.3± 0.2
5.	Calibration range or Linear dynamic range	20-100 µg/ml

Table.VIII: Quantitative estimation (Assay) data of Mupirocin

S No	Drug	Label claim (mg/Oin)	Amount found (mg/Oin)	Mean amount found (mg/ Oin)	Percentage purity (% w/w)	Mean percentage purity (% w/w)	% Deviation
1.	Mupirocin	20	20.77	20.32	103.07	101.47	+ 0.6
			20.40		102.10		+1.1
			20.21		101.05		+0.5
			19.79		98.95		-1.0
			20.44		102.20		+0.4

Table. IX : Results of statistical parameters Statistical parameters

SNo	Parameters	Mupirocin
1.	Standard deviation (SD)	3.03
2.	Relative standard deviation (RSD)	0.0776
3.	% RSD	0.716
4.	Standard error (SE)	0.02286
5.	Correlation Coefficient (r)	0.9987
6.	Slope (a)	40.711
7.	Intercept (b)	17.176
8.	Regression equationY = (aX+b)	Y = 40.711 X + 17.176

n= 4 observations

Table. X: Summary of Force degradation of Mupirocin by RP-HPLC method

SNO	Stress condition/ state	Time	% Assay ± S.D. [a](n^b=5)
1	Acidic 4N HCL(35 ^oC)/ solution	12 hrs	97.774 ± 1.732
2	Alkali 4N NaOH (35 ^oC)/ solution	12 hrs	99.223± 0.7243
3	**Wet** Wet heat (35 ^oC)/ solution	12 hrs	97.077±1.023

4	Dry heat (35 °C)/ solid	12 hrs	94.551±1.343
5	Oxidative 20 % H_2O_2 (35 °C)/ solution	12 hrs	98.567±1.232
6	Photo stability/ solid	4 hrs	82.764±1.333

S.D.[a] is standard deviation for n[b] = 5 observations

Method optimization

Chromatogram with good shape peaks and good retention time shows good resolution for Mupirocin and forced degradation products. The proposed method was to identify the number of degradation products formed during the stressed conditions. The typical RP-HPLC conditions are presented in **Table 1**. The good separation of Mupirocin and the products degraded peak under stressed conditions shows the success of the method. The HPLC chromatogram of Mupirocin standard and Mupirocin ointment is presented in **figure 1 and 2.**

Method validation: The method proceeded to achive sencitive, easy and economical for degermination and estimation by HPLC from ointment formulation. Based on the ICH recommended guidelines the experimental was validated.

Linearity: The proposed method Linearity was examined for five concentrations. The concentration ranges from 20-100 µg/ml. The Mupirocin standard linearity was determined by the plotting graph concentration vs absorbance. By absorbance as a functional of analyte concentration linearity was evaluated for Mupirocin. The linearity graphs presented in **figure 3,** and data presented in **Table 2**. The system suitability is demonstrated by the linearity analysis.

Accuracy: The recovery experiment shows the accuracy of the method. The good recovery shows the method was accurate. The analysis for recovery was performed by known amount of Mupirocin working standard added to pre-analysed solution of formulation in the test concentration range of (40%, 60% and 100 %). For each recovery level three samples was prepared and repeated for 3 consecutive days. The statistical results for recovery study are well within the range (S.D. < 2.0). The Mupirocin drug recoveries results are presented in **Table 3.**

Precision: The proposed method precision (repeatability) experiment results of are shown in T**able 4.** In the proposed method intraday and interday precision was examined by analyzing the responeses of the sample on the same day for 4 repeatations and 3 alternate days for 20-80 µg/ml concentration range of Mupirocin. The obtained results are represented in % RSD. The % CV of the proposed method was precise as the values < 1.0 % for the repeatability study. The precision data are presented in **Table 5**.

Specificity: The standard reference and the drug formulation shows specificity of the method. The RP-HPLC chromatogram of Mupirocin both bulk and the ointment formaulation are presented in **figure 1, 2.** The bulk and ointment formaulation retention time was found to be 7.3. For the ointment formulation there was no excipients interference was detected, which shows the specificity of the method. The proposed method shows the ability to determine the analyte in presence of excipients.

Limit of detection and quantitation

The limit of detection and quantification for Mupirocin is presented in table 6.

System suitability: For the system suitability parameters five repeats of standards and two repeats of sample preparation are injected, the data is presented in **table 7.** The Assay data of Mupirocin presented in **table 8.**

Statistical Parameters: The obtained assay results are subjected to the coefficient of variation, statistical analysis, regression equation and standard deviation are presented in **table 9.**

Force degradation of Mupirocin in formulation

Mupirocin showed slight and moderate degradation in dry heat, oxidative and Photo stability (Sun light) condition for a short period of time. **Table 10** indicates the degradation of Mupirocin under different stress conditions. According to the ICH guidelines of the forced degradation study of Mupirocin was examined.

Alkali degradation

The stability of the Mupirocin ointment in alkaline condition was examined. There was no degraded product was separated from Mupirocin. The chromatogram of alkali-degraded result was compared with the formulation and standard chromatogram. The result shows around 1–2 % of the Mupirocin drug is degraded and in alkaline condition the drug was highly stable.

Acid degradation

The stability of the Mupirocin ointment in acid condition was examined. The chromatogram of acidic condition product was compared with the formulation and standard chromatogram. The result shows around 1–2 % of the Mupirocin drug is degraded. The result shows that drug in acidic condition was highly stable.

Oxidation

The stability of the Mupirocin ointment in oxidation condition was examined. It was observed around 2-4% degradation was taken place on exposure to 20 % H_2O_2 for 12 hrs. The chromatogram of oxidation product was compared with the formulation, standard chromatogram and blank H_2O_2. No degradation product peaks was obseved. The 20 % H_2O_2 peak time observed at RT 2.6,

and in the peak height and area no significant decrease with time presented in **figure 4.** The result shows that the drug was highly stable to oxidative conditions.

Wet heat (Hydrolysis)

The stability of the Mupirocin ointment in neutral condition, 1–3 % drug degradation was observed after 12 hrs of incubation at 35ºC. The chromatogram of wet heat degraded product was compared with the formulation and standard chromatogram presented in **figure 5**. In the wet degraded chromatogram Mupirocin peak area and height was decreased and no degradation peak was observed.

Dry heat

The chromatogram for Mupirocin in dry heat shows the drug is slightly unstable as compare to acid, base and wet degradation. Around 3-7% drug degradation was observed. The chromatogram of dry heat degraded product was compared with the formulation and standard chromatogram. In the dry degraded chromatogram the drug decomposed into minor degradation product. The result shows no significant decrease in the peak height and peak area with time presented in **figure 6.**

Photo stability (Sun light)

The chromatogram for Mupirocin under photo stablilty study was found to be unstable after drug exposure to direct sunlight for 4 hours. Almost 10-20% of the drug degraded in 4 hours. Two minor drug degradation peaks is observed between 1-2.5 minute and also there was a significant increase in the drug peak height and decrease in peak area was observed presented in **figure 7.**

CONCLUSION

The force degradation study was performed according to the guidelines of International Conference on Harmonization (ICH), The developed RP-HPLC method shows the accuracey, sensitive and stability indicating. The developed method is rapid, reproducible. The developed method can be used for the routine analysis for Mupirocin formulations.

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