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**Research Article**

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# Development and Validation of Analytical Method by RP-HPLC Technique for Determination of Mupirocin Lithium in Pharmaceutical Dosage Form

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## ABSTRACT

*A novel, sensitive and robust reverse phase liquid chromatographic method was developed for assay of mupirocin lithium in ointment or cream as its dosage form. For chromatographic study, water symmetry C8 (150 mm × 4.6 mm, 5 µm) column was used in on isocratic system at ambient temperature. The selected mobile phase made up of buffer and acetonitrile in proportion 74:26% (v/v). The flow rate of 1 ml / min. was used in study. The effluent study was done at wavelength 221 nm. The retention time was observed at 5.3 min. The linearity was observed in concentration range as 50 to 150 µg/ml. The correlation coefficient is less than 1. The ICH guidelines were used for system suitability, linearity, accuracy and precision studies. The accuracy and precision were found to be well within the acceptable limit. The method was successfully applied for assay of mupirocin lithium in cream or ointment with good recoveries.*

**Keywords**: Mupirocin lithium; Triethyl amine; Phosphoric acid; Acetonitrile; HPLC

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## INTRODUCTION

The research article gives important insight into validation and application of RP-HPLC method for the assay of mupirocin lithium in bulk drug and dosage form i.e., ointment or cream.

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.

It is used in the treatment for topical bacterial skin infection, methicillin-resistant Staphylococcus aureus. It is effective against Gram-positive bacteria topically. Mupirocin is not effective for most anaerobic bacteria such as mycobacterium, mycoplasma, chlamydia, yeast and fungi. It inhibits the bacterial isoleucyl-tRNA synthetase and blocked the protein synthesis. Due to structural similarity with isoleucy, it interacts reversible with amino-acid specifically at the active site of the enzyme. It gives depletion of cellular levels of iso-leucine-charged transfer RNA which leads to the prevent of protein synthesis. According to the literature review several methods has been developed for Mupirocin, like HPLC [1-3] and UV spectroscopy, methods [4-6]. 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 (Figure 1).

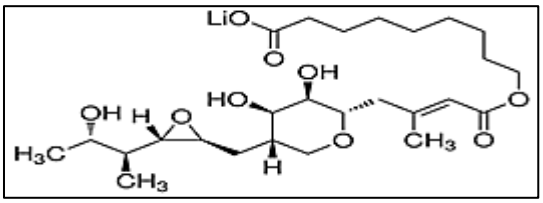


**Figure 1: Structure of mupirocin lithium**

## MATERIALS AND METHODS

### Instrumentation
The Merck-Hitachi La-Chrome HPLC with isocratic pump and UV detector equipped was used. The EZChrom Elite software was used for quantification of recorded chromatograms.

### Materials and Reagents
Mupirocin lithium, reference standard was obtained as gift sample with certificate of analysis. HPLC grade acetonitrile and AR grade of tri-ethyl amine and phosphoric acid were used from Merck. The Millipore water system was used HPLC grade water.

### Preparation of Mobile Phase
The buffer and acetonitrile in ratio as 74:26% (v/v) as mobile phase. They were sonicated for degassed.

### Preparation of Buffer Solution
A 1 ml of AR grade tri-ethyl amine was dissolved in 1000 ml of HPLC grade water and pH of solution was adjusted to 5.1 with the help of dilute phosphoric acid.

### Conditions of Chromatographic Separation
The column was used as Water symmetry C8 (150 mm × 4.6 mm, 5 µm) and flow rate was 1. 0 ml/min. The UV detector was adjusted at 221 nm and injection volume was 15 µl for each injection.

### Standard Solution
Weigh about 10 mg of standard mupirocin lithium accurately in 10 ml of standard flask. Add about 5 ml of diluent (buffer: acetonitrile (74:26% v/v)) It was sonicated for 5 minutes for complete dissolved the mupirocin lithium. The solution was further diluted to mark to give concentration as 1000 µg/ ml.

### Sample Preparation
A 5 gm of marketed sample of 2% w/w ointment was weighed accurately. It is equivalent of 10 mg of mupirocin lithium. It was transferred into 100 ml volumetric flasks and sonicated with dissolved with 25 ml mobile phase and filtered through whatmann filter paper. Further dilutions were made based on the required concentrations. A 15 µl was injected for analysis.

### Method Development
A UV spectrum of the drug is given in Figure 2 and chromatogram of the drug assayed is in Figure 3.

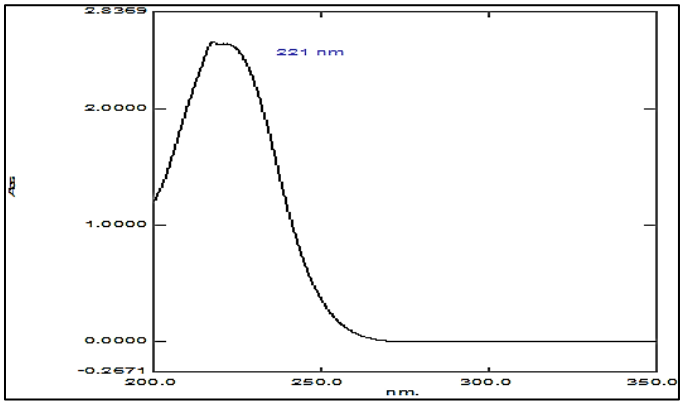


**Figure 2: UV spectrum of mupirocin lithium**

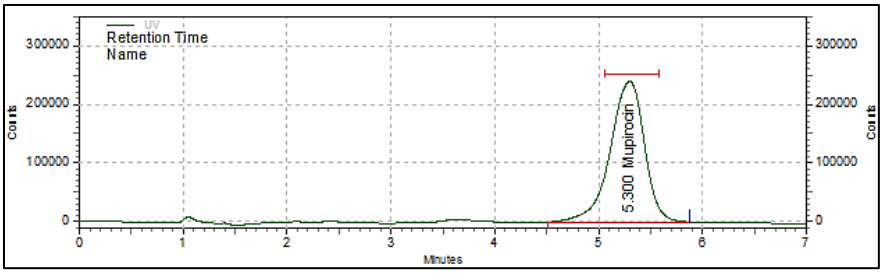


**Figure 3: Chromatogram of standard mupirocin lithium**

## Validation of Method

For mupirocin lithium, the validation of proposed method was carried out by using given parameters. For validation the standard addition was used. The good performance of the system was studied with different Parameters such retention time, area, % area and asymmetry. Such parameters were shown in Table 1.

**Table 1: System performance parameters for mupirocin lithium (n=6)**

| Retention time | symmetry factor | Area | % Area |
|---|---|---|---|
| 5.300 | 0.86012 | 5510700 | 100.00 |

## System Suitability

A standard solution of mupirocin lithium was prepared. The working standard was injected in six replicate in HPLC. The system suitability parameters were calculated from standard chromatograms. The % RSD was calculated from six replicates for peak areas and retention time. The data is given in Table 2.

**Table 2: Values of system precision**

| No. | Retention Time | Observed peak Area |
|---|---|---|
| 1 | 5.3 | 5566767 |
| 2 | 5.3 | 5559311 |
| 3 | 5.31 | 5505788 |
| 4 | 5.31 | 5510700 |
| 5 | 5.307 | 5454550 |
| 6 | 5.307 | 5458039 |
| mean | 5.31 | 5509192.5 |
| SD | 0 | 47839.38 |
| RSD | 0.09 | 0.87 |

## Linearity

The linearity study was carried for mupirocin lithium at concentrations level 50%, 80%, 100%, 120% and 150% in ranging from 50 to 150 µg/ml. The linearity curve was plotted by plotting area against concentration of the drugs. The regression equation was given as y = 54276 x - 98883. The correlation coefficient ($r^2$) was 0.9998 and concentration range indicated above. The linearity graph is given in Figure 4.

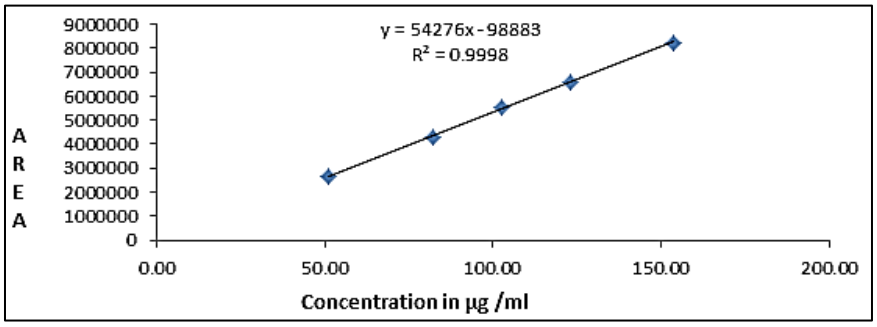


**Figure 4: Linearity curve**

The results are summarized in the Table 3.

**Table 3: Parameters of linearity study**

| Parameter | Mupirocin lithium |
|---|---|
| Range of linearity in µg /ml | 50-150 |
| Slope | 54276 |
| Intercept | -98883 |
| Coefficient of correlation | 0.9998 |

## Accuracy

The accuracy study of the method was carried by recovery experiments in the concentration range as 80%, 100% and 120% in 3 replicates with test concentration. The % recovery studies and RSD were calculated and presented in Table 4.

**Table 4: Accuracy - % Recovery**

| Standard in % | Replicate no | standard in mg | Recovered area | Amount added in µg / ml | Amount recovered in µg / ml | Recovery In % | Recovery ( mean) |
|---|---|---|---|---|---|---|---|
| 80 | 1 | 10.31 | 4309237 | 82 | 80.17 | 97.77 | 97.83 |
| | 2 | 10.36 | 4311131 | 82 | 80.21 | 97.82 | |
| | 3 | 10.32 | 4314432 | 82 | 80.27 | 97.89 | |
| 100 | 1 | 10.25 | 5509010 | 102.5 | 102.5 | 100 | 100.05 |
| | 2 | 10.21 | 5511448 | 102.5 | 102.54 | 100.04 | |
| | 3 | 10.13 | 5516162 | 102.5 | 102.63 | 100.13 | |
| 150 | 1 | 10.22 | 6622831 | 123 | 123.22 | 100.18 | 100.33 |
| | 2 | 10.29 | 6633858 | 123 | 123.42 | 100.35 | |
| | 3 | 10.36 | 6641660 | 123 | 123.57 | 100.46 | |
| | | | | | Mean of recovery | | 99.4 |

*Average of triplicate analysis.

## Method Precision

For precision study six replicates were injected. It was observed that the value of relative standard deviation present with the limits. The results of precision were summarized in the Table 5.

**Table 5: Precision – method precision**

| Replicate no | Sample in mg | Recovered Peak Area | % Assay |
|---|---|---|---|
| 1 | 10.21 | 5521714 | 99.84 |
| 2 | 10.22 | 5517122 | 99.85 |
| 3 | 10.19 | 5491742 | 99.1 |
| 4 | 10.27 | 5495604 | 99.95 |
| 5 | 10.2 | 5479764 | 98.98 |
| 6 | 10.29 | 5482458 | 99.9 |
| | Mean Assay | | 99.6 |
| | SD | | 0.439 |
| | RSD | | 0.441 |

## Stability of Solution

The study of stability of solution was carried out up to 24 hrs at room temperature. No significant changes were observed in results. It gave that the drug solution was stable in the given solvent system. The slight changes were made in the chromatographic conditions for robustness study. The observed chromatogram does no showed marked changes hence the proposed method was robust (Table 6).

**Table 6: Stability of solution**

| Parameter | Variation |
|---|---|
| flow rate | ± 0.2 ml |
| mobile phase composition | ± 0.2 units |
| wavelength | ± 5 nm |

## Application of Method to Dosage

The validated high performance liquid chromatographic method was applied for determination of mupirocin lithium its formulation. A portion of ointment equivalent to 10 mg of mupirocin lithium was weighed accurately. It was dissolved in 10 ml of diluents and sonicated for 15 minutes and filtered through whatmann filter paper. It gave the concentration 1000 µg/ml. The working sample solution was prepared by diluting 10 ml of 1000 µg/ml solution to

100 ml with diluent to give 100 µg/ml. Under specified conditions 15 µl of this solution was injected at each time. The analyte peaks were compared with respective standard and chromatogram. The analyte peak was given in Figure 5.

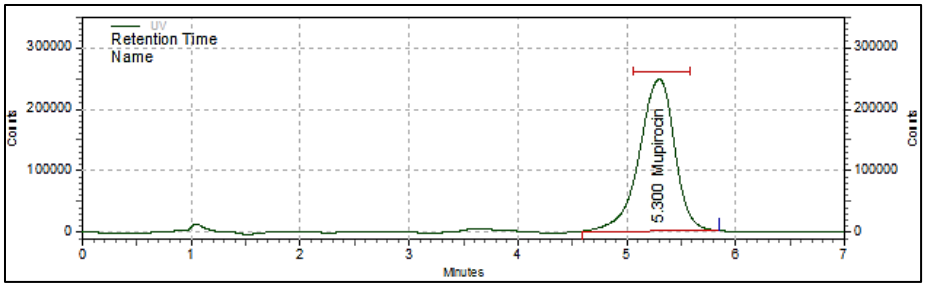


**Figure 5: Chromatogram of mupirocin lithium (sample)**

## RESULTS

In the proposed method, the retention time of mupirocin lithium was 5.3 min. The study of linearity was in the range of 50-150 µg/ml. The regression equation was given as y = 54276 x - 98883 where X represents amount of mupirocin lithium in µg /ml. and y is peak area of respective concentration. From the linearity curve the coefficient of co-relation was obtained as 0.9998. It exhibits excellent correlation between area of peak and given concentration of mupirocin lithium. The method precision reports the relative standard deviation as 0.441which is less than 2.0%. The total mean recovery was 99.66. Hence method is found to be highly accurate. The selected mobile phase gave good resolution of peaks and during the run time, no interfering peaks were found in the chromatogram. It indicates there is no interference of excipents used in the formulation during analysis.

## DISCUSSION

The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory due to the low values of standard deviation such as 0.439 and % RSD as 0.441. The literature reveals, previous methods has large retention time for resolution of mupirocin lithium hence literature methods were time consuming. Due to longer retention time methods they were not economical. But the proposed method has less retention time hence it is found to be more economical. The proposed method is also useful for stability study of drug as per ICH guidelines.

## CONCLUSION

Thus the proposed RP-HPLC method has relatively short retention time as 5.3 as compared to methods suggested in literature. The values of standard deviation and % RSD indicate more precision, accuracy, linearity, robustness of method; hence it is used for assay of mupirocin lithium from ointment due to its simplicity and non-interference of other peaks. Hence such RP-HPLC method is also useful for the assay of mupirocin lithium in various stages of quality control as per ICH guidelines.

## ACKNOELEDGEMENT

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