RP-HPLC Method for Estimation of Mupirocin in Bulk and Pharmaceutical Formulation

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ABSTRACT
Simple, sensitive, accurate, precise RP-HPLC method is developed for estimation of mupirocin in ointment formulation. Separation is achieved on Phenomenex C-18 column 150x4.6 mm, 5µ using mobile phase methanol: phosphate buffer pH 3.0 (70:30) at the flow rate 1.0 ml/min and effluent was monitored at 220 nm. The retention time of mupirocin is 4.5 min. The detector response is linear for the concentration range of 10-50 µg/ml. The recoveries are found in the range of 96-98 %. This method is validated as per ICH guidelines. The validated method can be successfully applied to the estimation of mupirocin in bulk drug and dosage form.

Key-words: Mupirocin, RP-HPLC method.

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INTRODUCTION
Mupirocin or pseudomonic acid chemically (9-[(2E)-4-[(2S,3R,4R,5S)-5-(2S,3S,4S,5S)-2,3-epoxy-5-hydroxy-4-methylhexyl]–3,4-dyhydroxy-3,4,5,6tetrahydro-2H-pyran-2-yl]-3-methylbut-2-enoyl]oxy)nonanoic acid) is official in USP. It is antibiotic derived from Pseudomonas fluorescens; discovered in 1971 and has in vitro activity against a range of Gram-positive and some Gram-negative bacteria. Mupirocin acts as protein synthesis inhibitor by targeting the isoleucyl-tRNA synthetase, it mimics isoleucyl-adenylate. Mupirocin reversibly binds to bacterial isoleucyl-tRNA synthetase, an enzyme which promotes the conversion of isoleucine and tRNA to isoleucyl-tRNA. Prevention of functioning of these enzymes results in the inhibition of bacterial protein and RNA synthesis. Mupirocin binds to the plasma proteins to the extent of 75% and has a half-life of 20-40 min. Mupirocin is used for the treatment of topical bacterial infections in furuncle, impetigo, open wounds etc. It is also useful in the treatment of methicillin-resistant Staphylococcus aureus (MRSA), which is a significant cause of death in hospitalized patients. Mupirocin is available in market in the form of ointment and cream. It is slightly soluble in water; freely soluble in dehydrated alcohol, in acetone, and in dichloromethane [1-4]. Literature survey reveals few spectroscopic, HPLC methods for the estimation of Mupirocin are reported. UV spectrophotometric method for the estimation of mupirocin calcium has been reported [5]. HPLC method for the measurement of mupirocin concentrations in
both skin layers and percutaneous samples has been reported [6]. Another HPLC method for estimation of Mupirocin in PEG bases is also reported [7]. Stability-indicating RP-HPLC [8], Liquid Chromatography-Tandem Mass Spectrometry [9] are reported for the estimation of mupirocin. There is no reported method for simple RP-HPLC for pure mupirocin so, the present research work was to develop and validate chromatographic method for the estimation of mupirocin in bulk and ointment formulation.

Structure of Mupirocin

![Structure of Mupirocin](image)

Experimental:

Pure bulk drug mupirocin was obtained from Glenmark Pharmaceutical Ltd. Nasik, India. Bactroban (Marketed by GSK) the marketed brand of mupirocin was bought from local market which contains Mupirocin 2%w/w. All HPLC grade solvents and reagents, water and Grade I Whatman filter paper, 0.45µm were used throughout the procedure.

Preparation of Standard Stock Solution

10 mg of standard mupirocin was weighed and transferred to a 10 ml volumetric flask then dissolved in the methanol. The volume was made up to the mark with same solvent to obtain conc. of 1000 µg/ml of mupirocin. From the resulting solution 1 ml was diluted to 10 ml with same solvent to obtain conc. of 100 µg/ml of mupirocin and labeled as ‘Std Stock Mupirocin’.

Selection of Analytical Wavelength

1ml of Std Stock solution was diluted to 10 ml with mobile phase and scanned in the range of 200-300 nm.

Selection of Mobile Phase and its Strength

Solution of mupirocin was prepared in methanol and injected into the HPLC system. The solution was analyzed using different combinations of methanol: phosphate buffer pH3 (60:40, 70:30, 80:20), at flow rate of 1ml/min for 10 minutes run time.

Chromatographic Conditions

- **Analytical Column**: Phenomenex C18 column (150 mm × 4.6 mm, 5 µm)
- **Mobile Phase**: Methanol : Phosphate Buffer pH 3 (70:30)
- **Flow Rate**: 1 ml/min
- **Injection Volume**: 20 µl
- **Detection Wavelength**: 220 nm

Preparation of Mobile Phase

- Methanol and phosphate buffer of pH3 in the ratio (70:30) was selected in RP-HPLC method development for estimation of mupirocin.

Preparation of phosphate buffer pH3:

Accurately weighed 0.34gm of potassium dihydrogen phosphate transferred in the 250 ml volumetric flask and dissolved in HPLC water, then volume was made up to the mark with HPLC water and, then adjusted the pH to 3.0 by using o-phospheric acid.

Mobile phase was prepared by mixing 350 ml of methanol, 150 ml of phosphate buffer
(pH3) and filtered through 0.45 µm nylon filter using Vacuum Pump and ultrasonicated for 30 min for degassing.

Spectra of Mupirocin between 200-300nm in Mobile Phase

Chromatogram of Blank in Optimized Chromatographic Conditions
Validation of RP-HPLC Method:

1. Linearity
From the ‘Std Stock’ (100 µg/ml) solution, 1, 2, 3, 4 and 5 ml were transferred in a series of 10ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain the conc. of 10, 20, 30, 40 and 50 µg/ml.

The solutions were filtered through syringe filter and 20 µl injected into the HPLC system and their chromatogram were recorded under the chromatographic conditions as described above after getting a stable baseline. Peak areas were recorded for all the peaks. Calibration curve of mupirocin was constructed by plotting the peak area of mupirocin v/s conc. of mupirocin.

2. Range
The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the curve.

3. Accuracy
Recovery study was carried out by standard addition method by adding the known amount of mupirocin (reference standard) to the preanalyzed sample at three different concentration levels i.e. 80 %, 100 %, and 120 % of assay concentration and percent recoveries were calculated.

From the 100 µg/ml Sample Stock solution 1.5ml was transferred to four different 10ml volumetric flasks separately along with 0, 1.2, 1.5, 1.8 ml from the 100 µg/ml Std Stock solution. The volume was made up to the mark with mobile phase. All the solutions were filtered through syringe filter and injected into the HPLC system and their chromatograms were recorded under the same chromatographic conditions after getting a stable baseline. Peak areas were recorded for all the peaks. From the above data percent recoveries were calculated.
Overlain Chromatogram of Mupirocin (10-50 µg/ml)

Calibration Curve of Mupirocin of RP-HPLC Method

Results of Accuracy for RP-HPLC Method

\[ y = 26.005x \]

\[ R^2 = 0.9983 \]
4. Precision
The precision of an analytical method was studied by performing Repeatability and intermediate precision.

a) Repeatability: From the 100 µg/ml Std Stock solution, 3 ml was transferred in 10ml volumetric flasks. The volume was made up to the mark with mobile phase to obtain the conc. of 30 µg/ml. The solution was filtered through syringe filter and 20 µl injected into the HPLC system and its chromatogram was recorded under the same chromatographic conditions after getting a stable baseline. Peak area was recorded. The procedure was repeated thrice.

b) Intermediate Precision
Intra-day Precision: Intra-day precision was determined by analyzing the standard solution of mupirocin (30 µg/ml) at 10.00am and 3.00pm on same day following the procedure of repeatability.

Variation by different analyst: Combined standard solutions of mupirocin (30 µg/ml) was prepared and analyzed by Analyst 1 and Analyst 2, separately.

5. Limit of Detection
Detection limit was determined based on the standard deviation of peak areas of same concentrations i.e. standard solution of mupirocin (30 µg/ml) prepared six times.

6. Limit of Quantitation
Quantitation limit was determined based on the standard deviation of peak areas of same concentrations i.e. standard solution of mupirocin (30 µg/ml) prepared six times.

7. Robustness
Standard solution of mupirocin (30 µg/ml) was prepared and analyzed at different flow rates (0.9, 1.0, 1.1 ml/min) separately.

8. System Suitability
Sample solution of mupirocin (30 µg/ml) was prepared and analyzed six times. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they comply with the recommended limit or not.

Results of System Suitability Parameters

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Theoretical Plates (N)</th>
<th>Retention Time (min)</th>
<th>Tailing Factor (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>3552</td>
<td>4.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Analysis of Mupirocin in Ointment Formulation
An accurately weighed quantity of ointment equivalent to about 10 mg of mupirocin was dissolved in 10 ml of methanol, sonicated for 10 min and filtered through whatman.
filter paper No. 41. 1 ml of this solution was diluted to 10 ml with methanol. 3 ml of resulting solution diluted to 10 ml with mobile phase. This solution was filtered through syringe filter and injected into HPLC system. The chromatogram was recorded under the same chromatographic conditions as described above after getting a stable baseline and Peak area was recorded. The amount of mupirocin present in the ointment was calculated using calibration curve equation, y = 26.00x.

**Assay Results of Ointment Formulation by RP-HPLC Method**

<table>
<thead>
<tr>
<th>Concentration of formulation (µg/ml)</th>
<th>Amount estimated (µg/ml)</th>
<th>% Label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 µg/ml</td>
<td>30.9 µg/ml</td>
<td>103 %</td>
</tr>
</tbody>
</table>

**Results and Discussion:**

The above method has been optimized after several permutations and combinations of mobile solvents with stationary phase C\textsubscript{18}, i.e. methanol: phosphate buffer pH3 in the ratio of 70:30. Using C\textsubscript{18} column we got good results. Mupirocin show the maximum absorbance at 220nm. Hence, HPLC analysis was carried out at 220nm.

**Validation of HPLC Method**

The peak response is proportional to concentration and linear in the range of 10-50 µg/ml. The correlation coefficient is 0.998 which is well within the acceptance criteria. The range is from 50-150% of the test concentration. The percentage recoveries of the results indicate that the recoveries are well within the acceptance range, therefore, method is accurate. The percentage RSD (<2) values obtained shows that the method developed is précised at repeatability and intermediate precision level. Detection limit is calculated based on standard deviation of response and slope. Quantification limit is calculated based on standard deviation of response and slope. Due to change in the flow rate, no significant changes were found in the chromatogram, the method developed is robust. Study of resolution, retention, tailing factor and capacity factor shows system is suitable for this method.

Amount of drugs present in the marketed formulation (Bactroban) was calculated using equation. Amount of mupirocin was found to be 103% of label claim. This method can be employed for routine analysis of mupirocin.

**Conclusion:**

Attempts were made to develop RP-HPLC method for Mupirocin from bulk drug and Bactroban ointment. RP-HPLC method was developed and validated as per ICH guidelines using Methanol: Phosphate buffer (70:30), pH 3.0 as mobile phase. Retention time of Mupirocin was found to be 4.5 min at the wavelength, 220 nm and flow rate, 1 ml/min.

The method was found to be simple yet accurate, precise and reproducible. So this method can be used for the routine quality control analysis of Mupirocin in bulk drug as well as ointment formulation.
Summary of RP-HPLC Method

**Acknowledgements:**
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10. ICH Harmonized Tripartite Guideline, Text on Validation of Analytical Procedure. Q2 (R1).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Mupirocin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Linearity Range (µg/ml)</td>
<td>10-50</td>
</tr>
<tr>
<td>2.</td>
<td>Regression Equation (y = mx+c)</td>
<td>26.00x+0.00</td>
</tr>
<tr>
<td>3.</td>
<td>Correlation Coefficient (r²)</td>
<td>0.998</td>
</tr>
<tr>
<td>4.</td>
<td>LOD (µg/ml)</td>
<td>0.15</td>
</tr>
<tr>
<td>5.</td>
<td>LOQ (µg/ml)</td>
<td>0.47</td>
</tr>
<tr>
<td>6.</td>
<td>Analysis of Tablets (% Assay)</td>
<td>104%</td>
</tr>
<tr>
<td>7.</td>
<td>% Recovery</td>
<td>96-98</td>
</tr>
<tr>
<td>8.</td>
<td>Intra Day Precision (%RSD)</td>
<td>1.29</td>
</tr>
<tr>
<td>9.</td>
<td>Repeatability (%RSD)</td>
<td>1.23</td>
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