The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
21(1) S (1) 842-859, 2026 www.thebioscan.com

MUPIRICONE OINTMENT ANALYTICAL METHOD DEVELOPMENT AND VALIDATION USING RP-HPLC TECHNIQUES

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DOI: https://doi.org/10.63001/tbs.2026.v21.i01.S.I(1).pp842-859

Keywords:

ICH Guidelines, RP-HPLC, Mupirocin, Assay, Chromatography.

Received on: 25-02-2026

Accepted on: 10-03-2026

Published on:20-03-2026

ABSTRACT

For the assay investigation of Mupirocin Ointment in the pharmaceutical dosage form, a straightforward, affordable, specific, accurate, exact, and verified reverse-phase high-performance liquid chromatography (RP-HPLC) method has been devised. Using the mobile phase Phosphate Buffer: Acetonitrile (ACN) (75:25% v/v) at a flow rate of 1.0 ml/min, the chromatographic separation was accomplished on a C-18 column (300 mm x 3.9 mm, 10 µ particle size) at 25°C. A UV detector operating at 229 nm was used to obtain quantification. Mupirocin USP was found to have a retention time of 6.696 ± 0.05 minutes. The suggested approach was verified in compliance with ICH recommendations for Mupirocin ointment test investigations. Mupirocin in its medicinal dosage form was effectively determined using the described method with satisfactory separation.

INTRODUCTION:

Analytical chemistry is the science and practice of identifying the elements or compounds that make up a substance. It is essential to the advancement of science and entails detecting, separating, and calculating the number of related components in a sample of matter. The product's quality may differ from the necessary standards, but when doing an analysis, one must also ensure that the analysis's quality meets the necessary standards. Pharmaceutical product testing includes chemical, physical, and occasionally microbiological tests. It is estimated that the UK spends £10 million per year on analysis alone, and such analytical procedures may be found in a wide range of industries, including food, drinks, cosmetics, detergents, metals, paints, water, agrochemicals, biotechnological products, and medicines. [1,2,3].

Analytical chemistry is classified into the following types based on the necessity for analysis:
Qualitative Analysis identifies the chemical species present in a sample, whether organic or inorganic.

Quantitative Analysis is used to determine the amount of organic or inorganic constituent species present in a sample.

Analytical methods are primarily characterized as instrumental, classical, or wet-chemical procedures based on the property detected in the final measurement process.

Chemical Analysis involves measuring the mass or volume of reacting chemicals using methods such as gravimetric and volumetric.

Instrumental Methods that rely on the measurement of electrical, optical, thermal, and other properties, as well as those based on determining the extent to which radiation is absorbed or the intensity of emitted radiation, necessitate the use of a suitable instrument, such as a spectrophotometer, HPLC, oven, and NMR, among others. [4] Instrumental analysis is widely used in research and applications today.

Instrumental Analysis Techniques [5]

Many instrumental methods are employed in pharmaceutical analysis, among which important approaches are:

Separation techniques include chromatographic procedures, i.e. TLC, GC, or HPLC may be relevant for an analysis depending on different parameters such as sample solubility or volatility, separation efficiency, analyte concentration, detection limit, analysis cost, and so on.

Spectrometric techniques, including as UV, IR, NMR, and MASS, are commonly used to analyze the drug of interest alone in a matrix containing excipients, degradation products, contaminants, additives, and so on. It also comprises plasma, atomic, X-ray flame, absorption or emission spectrometry, and so on.

Electro-analytical techniques: Electro-analytical methods of analysis deal with electrical signals applied to the sample and/or monitor the sample's electrical properties, such as amperometry, polarography, electrogravimetry, conductometry, potentiometry, and so on.

Thermo-analytical techniques: This analytical approach involves the interaction of heat with the substance. Examples are DSC, DTA, and the Thermo Gravimetric Technique.

Modern hyphenated techniques: These techniques involve advances in drug analysis by combining one instrumental technique with another to refine the analysis of drug, impurity, degradation product, and so on.

For example, GC-MS, LC-MS, and GC-IR. Thus, analytical science entails the application of a variety of techniques to determine the analyte present in sample matrices. These approaches offer advantages in analytical sciences for determining a specific analyte [6]

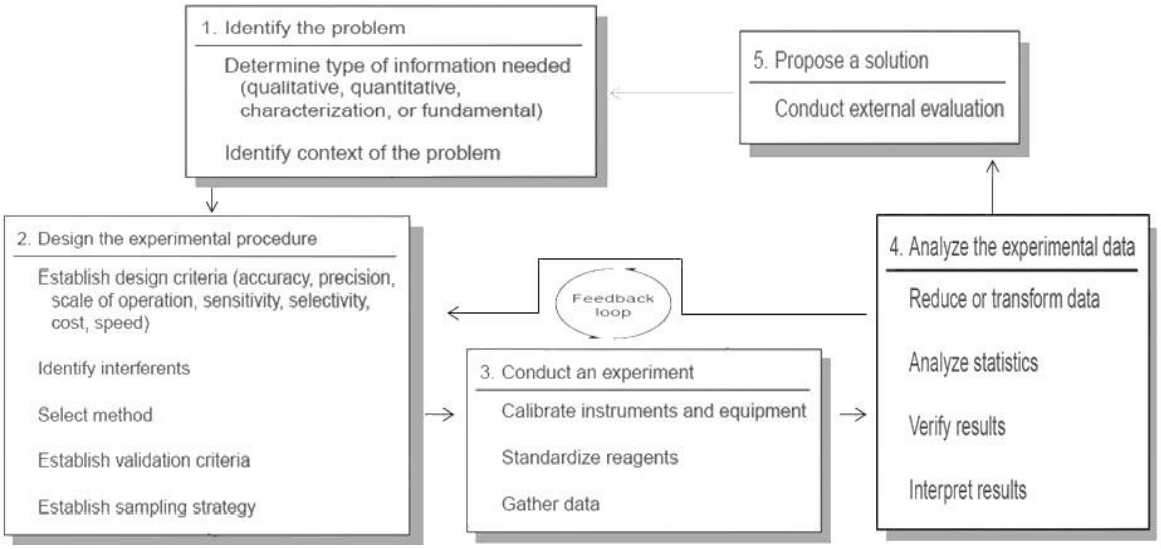

Fig.1.1: Flow diagram for analytical approach [7]

CHROMATOGRAPHY :

The main purpose of chromatography is to separate the components in a sample by distributing them between two phases, one of which is stationary and the other is movable. The stationary phase can be a liquid or solid supported on a solid or gel, and it can be packed in a column, spread as a layer, or distributed as a film. The mobile phase, which travels on the stationary phase, can be either liquid or gaseous[8].Chromatography was first created by Russian botanist Michael Tswett in 1903 to separate colored plant pigment by passing a petroleum ether extract through a glass column filled with calcium carbonate powder. It has evolved into various related but distinct forms that allow the components of complicated mixtures of organic or inorganic components to be separated and quantified, making it, generally speaking, the widely used separation technique in analytical chemistry. The image illustrates the categorization of chromatographic methods.

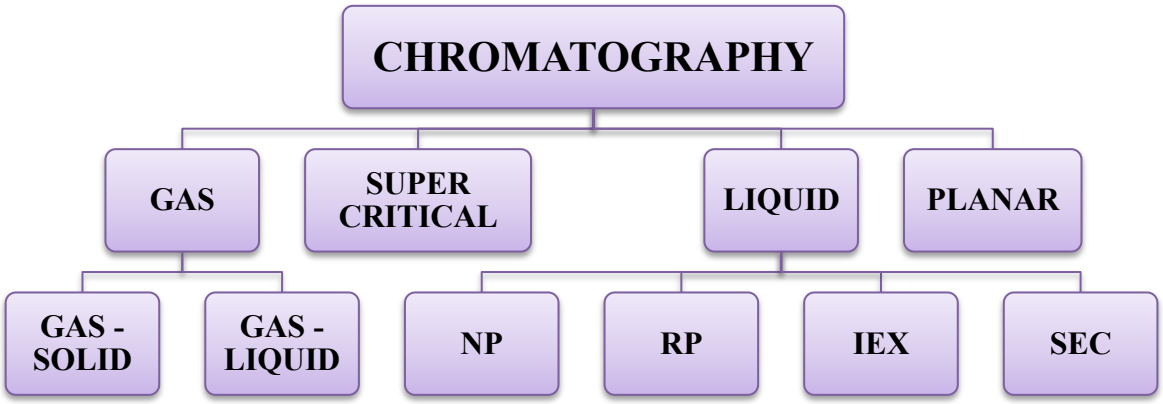

Fig.1.2: Classification of chromatography

HIGH PERFORMANCE LIQUID CHROMATO

GRAPHY

Because it performs better than ambient pressure or low-pressure liquid chromatography and has a faster separation speed, high pressure liquid chromatography is also known as high performance liquid chromatography. The pharmaceutical sector uses the HPLC technology for a wide range of samples. It is the preferred technique for verifying the purity of novel drug candidates found in pharmaceutical dosage forms, tracking modifications or the expansion of drug synthesis processes, conducting in-process testing to create new formulations or dosage forms, and ensuring the quality of finished drug products. [11]

Liquid chromatography in which the liquid mobile phase is mechanically pushed over a column holding the stationary phase is referred to as high performance liquid chromatography (HPLC). HPLC is an analytical method that separates, quantifies, and analyzes chemical mixture components using specialized instrument design. The detection mechanism is used in conjunction with a data recording system to gather component data after the sample of interest is introduced to a solvent flow route and passed through a column filled with specialized material for component separation. The pharmaceutical research and development department now uses HPLC for analyte analysis.

HPLC separation methods:

1. Analysis of tiny (<2000 Da) organic molecules in the normal phase or reverse phase
2. Ion chromatography, or ion analysis
3. Size exclusion chromatography: a method for separating polymers Enantiomeric purity is determined using chiral HPLC. [12]

HPLC components: [13]

The block diagram in Fig. 1.3 below illustrates the key components of contemporary chromatography, or HPLC.

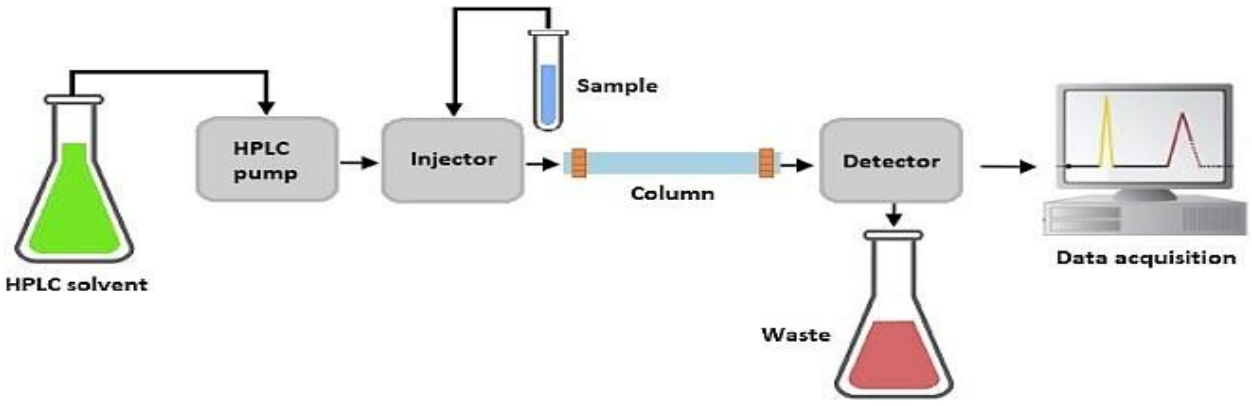

Fig.1.3: Block diagram showing the components of HPLC Method Development in HPLC [14]

The development of high performance liquid chromatography methods appears complicated due to the large range of equipment, columns, eluent, and operational parameters involved. The analyte's characteristics have an impact on the process. Developing a high-performance liquid chromatography (HPLC) method for analyte separation is a daily challenge for many chromatographers. HPLC method development frequently adheres to a set of procedures. Our approach to method development is founded on a number of factors. There is currently a solid practical understanding of chromatographic separation, which changes depending on the sample and experimental conditions. This understanding of the chromatographic process should serve as the foundation for any methodical approach to HPLC technique development. Most of the time, it just takes a few experiments to accomplish the separation. In other situations, a significant amount of experimentation is necessary.

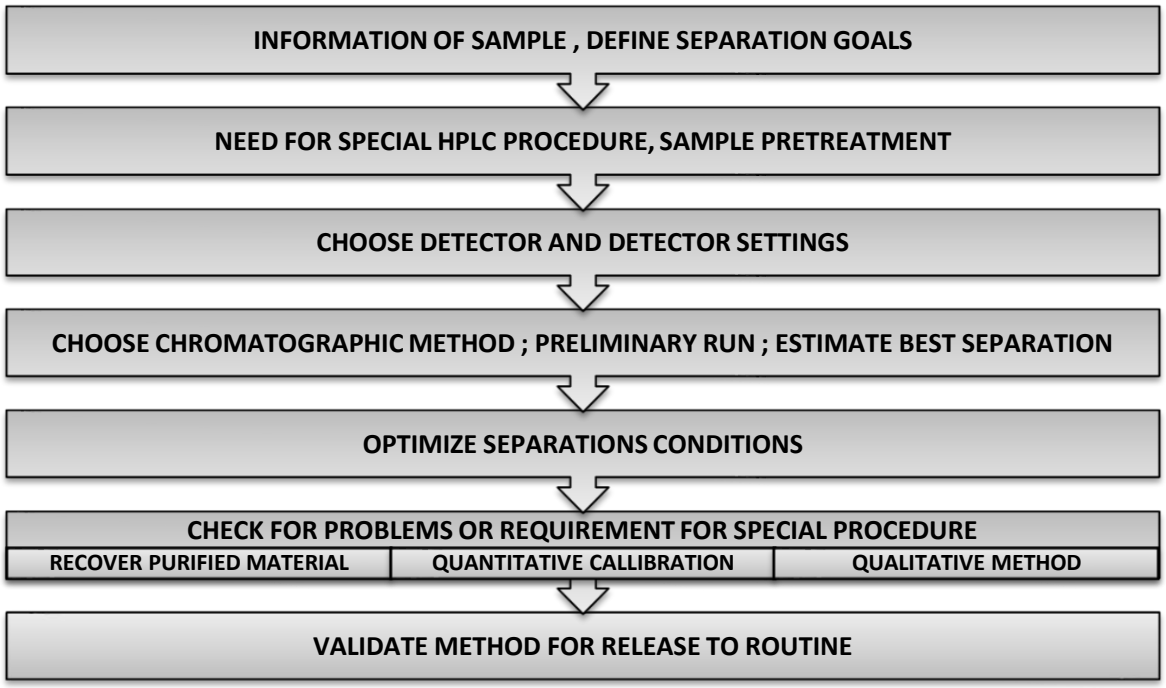

Fig.1.4: Steps in HPLC method development

Nature of Sample:

Before starting to create a method, we must review what is known about the sample. The sample's chemical makeup should provide useful hints. Some chromatographers attempt to match the sample's "chemistry" to the ideal beginning HPLC settings. The following is a summary of the different sample-related information that may be significant:

- ✓ The quantity of chemicals in the sample

- ✓ The compound's chemical structure (functionality)
- ✓ The chemicals' molecular weights and pKa values
- ✓ The chemicals' UV spectra and their concentration range in the relevant samples

Sample preparation and detection

Samples come in a variety of forms:

a. Injection-ready solutions.
b. Solutions requiring volumetric manipulation, such as dilution, buffering, or adding internal standards.
c. Solids must be dissolved or removed.
d. Samples need pretreatment to avoid interference and safeguard the column or equipment from harm.

Some samples require partial separation (pretreatment) before to HPLC in order to remove interferences, concentrate the sample analyte, or eliminate "column killers".

Separation Improvement :

Separation / resolution is a critical prerequisite in quantitative HPLC analytical techniques. Typically, for samples with five or less components, baseline resolution more than 1.5 can be easily reached for the desired bands. Resolution often declines over the column's life and can vary day to day due to tiny differences in separation circumstances. As a result, while developing methods for simple mixes, resolution (Rs) values of 2 or more should be the target.

TABLE 1: Separation goals in HPLC method development.

Goal	Comments
Resolution	For precise and robust quantitative analysis, Rs must be greater than 1.5.
Separation time	A separation time of less than 5-10 minutes is ideal for common procedures.
Quantitation	≤2% (RSD) for assays, ≤5% for less demanding analyses, and ≤15% for trace studies.
Pressure	<150 bar is preferred, <200 bar is normally required (new column assumed).
Peak height	A narrow peak is ideal for high signal-to-noise ratios.
Solvent consumption	To minimize solvent consumption, it is recommended to utilize a minimum amount of mobile phase per run.

SOLVENTS USED IN HPLC:

The choice of mobile phase solvents can have a significant impact on the ease and sensitivity of HPLC detection. The lowest usable cut-off wavelength is significant for ultraviolet detectors; solvent refractive index (RI) has an effect on the sensitivity of RI detection for samples; and solvent volatility (boiling point) is important for evaporating light-scattering detectors.

Ideally, solvents employed as mobile phases in HPLC should have the following

characteristics:
1. High solubility of the sample components.
2. Not corrosive to HPLC system components.
3. Low cost, UV transparency, and high purity.

Table 3: Various Solvents for HPLC analysis [16]

Solvent Name	UV cut off (nm)	Refractive Index20^{o}C	Polarity (P)	Boiling Point (^{o}C)	Viscosity (cP)
Acetone	330	1.3587	5.1	56.29	0.36
Acetonitrile	190	1.3441	5.8	81.60	0.38
Chloroform	245	1.4458	4.1	61.15	0.57
Cyclohexane	200	1.4242	0.2	80.72	1.0
Tetrahydrofuran	212	1.4072	4.0	66.0	0.55
1-Butanol	215	1.3993	3.9	117.5	2.98
Methanol	205	1.3284	5.1	64.7	0.55
Hexane	195	1.3749	0.1	68.7	0.31
Water	190	1.3330	10.2	100	0.81
DMF	268	1.4384	6.5	166.1	0.84

1.5.6. SELECTION OF DETECTOR

To choose a good detector, essential characteristics include sensitivity at low concentrations, linearity across a wide range, and tolerance to temperature or solvent composition changes. Most tiny compounds in the pharmaceutical sector possess chromospheres that absorb well in the ultraviolet range[17].
There are two main types of detectors: bulk property detectors and solute property detectors. Bulk property detectors are incompatible with gradient elution because they rely on some bulk property of the eluent, such as refractive index. The solute property detectors can be spectrophotometric, fluorescence, or electrochemical detectors that measure some form of physical or chemical detector.
They react to a physical or chemical feature of the solute and, ideally, are independent of the mobile phase. As a result, solute property detectors can be applied to gradient elution. UV detectors are the primary choice since they are convenient and applicable to the majority of samples.
Alternate detectors may be used for

- Samples with low UV absorption.
- Analyte concentrations are insufficient for UV detection.
- Matrix interferences.

Qualitative structural information can be required. [18]

Selection of Mobile Phase [20]:
Compatibility with solvents, sample solubility in the eluent, polarity, light transmission, viscosity, stability, and pH are all important considerations when selecting mobile-phase solvents. When mobile phase solvents are combined together, they should be miscible and do not precipitate. Dichloromethane and water, for example, are immiscible in most formulations and should not be utilized as mobile phase components.

Selection of pH [21]

pH selection has a significant impact on separation selectivity, with a 10-fold difference in ionic sample retention [21]. Most silica-based columns have a pH range of 2 to 8, while pH-stabilized columns have a pH range of 1 to 14.

$HA \longleftrightarrow A^+ H^+$

$B^+H^+ \longleftrightarrow B\ H^+$.

Hydrophobic (More retained in RPC) Hydrophilic (Less retained in RPC)

Hydrophobic and hydrophilic materials have different retention rates in RPC (more or less).

MATERIALS AND METHODS:

ACTIVE PHARMACEUTICAL INGREDIENTS

Table no.4. API and Supplier

API	Supplier
Mupirocin USP	RU, Ahemadabad

MARKETED FORMULATION

Table no.5. Details of Marketed Formulation

Company Name	Brand Name	Dose	Dosage form
Glaxo SmithKline Pharmaceuticals Ltd	T - BACT	Mupirocin 2%	Ointment

CHEMICALS:

Table no. 6. List of chemicals

Sr.no.	Chemicals	Grade	Manufacturer
I.	Acetonitrile	HPLC	Vizag Chemicals
II.	Monobasic sodium Phosphate	AR	Vizag Chemicals
III.	Sodium hydroxide	AR	Vizag Chemicals
IV.	Hydrochloric acid	AR	Vizag Chemicals

INSTRUMENTS/ EQUIPMENT

Table no.7. List of Instruments / Equipment

Sr.no.	Instrument name	Make
I.	HPLC	Shimadzu
II.	Water bath	Lab India
III.	pH meter	Mettler Toledo
IV.	Ultrasonicator	Ultrasonics
V.	Digital analytical balance	Labindia

RESULT:

RP-High Performance Liquid Chromatography Method:

INSTRUMENTATION

The chromatographic apparatus utilized to design and validate this method was an Elite LaChrome RP-HPLC Instrument with a quaternary pump. A sample injector with a 20µL loop, C18 column (300mm × 3.9µm particle size), and DAD detector were employed. The HPLC equipment is supplied with open lab software for peak acquisition and measurement.

Table no.8. Instrument specification

Parameter	Specification
Instrument Name	High Performance Liquid Chromatography (HPLC)
Manufacturer	Shimadzu Corporation, Japan
Model	LC-20AD / Prominence Series
Pump Type	Binary / Isocratic / Gradient Pump
Flow Rate Range	0.001 – 10.0 mL/min
Flow Rate Accuracy	±1%
Maximum Operating Pressure	40 MPa (400 bar)
Injector Type	Manual / Auto sampler
Injection Volume	0.1 – 100 µL
Column Oven Temperature	Ambient to 85 °C
Detector Type	PDA Detector
Wavelength Range	190 – 700 nm
Wavelength Accuracy	±1 nm
Data Processor	Shimadzu LCsolution Software

Mobile Phase	Aqueous & Organic solvents
Column Used	C18 (ODS), 150 × 4.6 mm (typical)
Power Supply	230 V AC, 50 Hz
Application	Qualitative & Quantitative analysis

CHROMATOGRAPHIC CONDITION

The chromatographic analysis was done on an Agilent C18 column (300mm ×3.9mm, 10 µm particle size). The mobile phase was composed of ACN and Phosphate Buffer (pH adjusted to 6.3 with sodium hydroxide) in a ratio of 25:75 v/v. The flow rate was 2.0 mL/min, injection volume was 20 µL, and detection was done at 229 nm with a photodiode array detector (PDA).

MOBILE PHASE PREPARATION

Selection of Solvent System:

The solvent was chosen after determining the solubility of the medication in several solvents such as water and acetonitrile.Mupirocin is soluble in both water and ACN.ACN was used as a solvent system for the development of this RP-HPLC technique due to its solubility and low cost.

Prepretion of Solution :

Preparation of 5N Sodium Hydroxide Solution System :

To make a 5 N Sodium hydroxide solution, accurately weigh around 20.0gm of sodium hydroxide and transfer it to a 100.0 ml volumetric flask. Dilute with distilled water to 100.0 ml.Close properly with the stopper and shake vigorously to dissolve entirely.

Preparation of 6 N hydrochloric acid :

Take cautiously 25.5 mL. Pour concentrated hydrochloric acid into a 50.0 mL volumetric flask containing 25.0 mL of pure water. Then, dilute with pure water until the volume reaches 50.0 ml. Close properly with the stopper and shake thoroughly.

Prepation of Standard :

Transfer 10.0 mg of Mupirocin USP Working Standard to a 100.0 mL volumetric flask. In a volumetric flask, add approximately 25.0 mL of acetonitrile and sonicate for 10.0 minutes using a sonicator. Then dilute with pH 6.3 phosphate buffer until the volume reaches 100.0 ml. Close properly with the stopper and shake vigorously to dissolve entirely. The final concentration of Mupirocin USP is 100.0 mcg/mL.

Prepation of Resolution Solution :

To prepare the resolution solution, pipette out 10.0 ml of the standard solution and adjust the pH to 2.0 using 6N hydrochloric acid. Allow the solution to stand for 2.0 hour before adjusting the pH to 6.3 ± 0.2 with 5 N sodium hydroxide.

Preparation of Test :

Weigh accurately about 0.5 gm (equal to 10.0 mg) of Nupirocin ointment into a 100.0 ml volumetric flask, then add about 25.0 ml of acetonitrile and sonicate on the sonicator for 10 minutes. Then dilute with pH 6.3 phosphate buffer until the volume reaches 100.0 ml. Close properly with the stopper and shake vigorously to dissolve entirely. The final concentration of Mupirocin USP is 100.0 mcg/mL. Filter resolution, standard,

and test solutions using a 0.45µm syringe filter.

Preparation for Mobile Phase:
Prepare pH 6.3 phosphate buffer.
Prepare 0.05M monobasic sodium phosphate.

Weigh 6.9gm of monobasic sodium phosphate accurately and transfer it to a 1000.0 ml volumetric flask. Dilute with distilled water to the capacity of 1000.0 ml. Close properly with the stopper and shake vigorously to dissolve entirely. Adjust pH to 6.3 ± 0.2 with 10N sodium hydroxide. To prepare the mobile phase, mix a volume of acetonitrile with prepared buffer solution in the ratio of 250:750. After mixing, pass through 0.45µm or 0.2µm Nylon membrane filter paper and degas.

TRIAL - I

FIRST TRIAL
CHROMATOGRAPHIC CONDITIONS: As per monograph • Column: 25cm,4.6mm C18 5µm • Temprature: 25°C • Flow rate: 2.0ml/min • Injection volume: 20µl • Wavelength: 229 nm ❖ MP: (0.05M) PH 6.3 phosphate buffer : ACN 750 : 250
Solution Preparation: In resolution solution, RRT & Resolution not in limit.
LIMITS: ASSAY: 90-110% **RRT: 0.9 FOR mupirocin acid hydrolysis product, 1.0 for mupirocin Resolution: Between mupirocin acid hydrolysis product and mupirocin is NLT 2.0**

SECOND TRIAL
CHROMATOGRAPHIC CONDITIONS: • Column: 30cm,3.9mm C18 10µm • Temprature : 25°C • Flow rate: 2.0ml/min • Injection volume: 20µl • Wavelength: 229 nm ❖ MP: (0.1M) PH 6.3 phosphate buffer : ACN 72 : 28
✓ % Assay: Batch no. MUPO13 = 75.88% MUP014 = 77.56% MUPO15 = 75.99% MUPO10 = 76.91% ✓ In resolution solution, RRT for hydrolysis product and mupirocin was 0.7806, 1.2810 respectively & Resolution was 3.4229.

LIMITS: ASSAY: 90-110% **RRT: 0.9 FOR mupirocin acid hydrolysis product, 1.0 for mupirocin Resolution: Between mupirocin acid hydrolysis product and mupirocin is NLT 2.0**

THIRD TRIAL **(MP, Dilutions, chromatographic conditions as per USP) CHROMATOGRAPHIC CONDITIONS:** • Column: 30cm,3.9mm C18 10µm • Temprature : 25°C • Flow rate: 2.0ml/min • Injection volume: 20µl • Wavelength: 229 nm ❖ MP: (0.05M) PH 6.3 phosphate buffer : ACN 750 : 250
✓ % Assay: Batch no. MUPO13 = 77.85% MUP014 = 75.89% MUPO15 = 85.65% MUPO10 = 86.14% ✓ In resolution solution, RRT for hydrolysis product and mupirocin was 0.6488, 1.5412 respectively & Resolution was 4.2737
LIMITS: ASSAY: 90-110% **RRT: 0.9 FOR mupirocin acid hydrolysis product, 1.0 for mupirocin Resolution: Between mupirocin acid hydrolysis product and mupirocin is NLT 2.0**

System Appropriateness As A Resolution Solution:

The relative retention periods for the Mupirocin acid hydrolysis product and Mupirocin are around 0.9 and 1.0, respectively. The resolution of the Mupirocin acid hydrolysis product to Mupirocin is not less than 2.0.

System Suitability For Standard Solution:

The tailing factor is not greater than 2.0. Column efficiency is not less than 1500 theoretical plates. The Relative standard deviation for replicate injections should not exceed 2.0%.

Calculation:

Calculate the Assay for Mupirocin USP by comparing the Standard and Test chromatograms, taking into account the Standard Area, the Test Area, the Working Standard Weight and its dilution, the Weight of the test sample and its dilution, and the Working Standard Potency.

% Assay = (As / Cs) * (Wt_std / Vol_std) * (Vol_samp / Wt_samp) * (1 / Ps) * 100

RP-HPLC Method Validation :

1.Linearity

To ensure linearity, accurately weigh approximately 0.020gm (20mg) of Mupirocin USP. In a clean 100.0ml volumetric flask, add about 50.0ml of mobile phase and shake thoroughly to dissolve the sample entirely. To get a final concentration of 200µg/ml, add 100.0ml of mobile phase to the remaining volume.

% Conc. of Sample	Conc. (PPM)	Mean Response (Area)	Statistical analysis	
50	80	4711685	Correlation	0.999
80	90	5158791		
100	100	5604013		
120	110	6311451	Intercept	16066
150	120	6474953	Slope	13588

Sr. No.	Drug	LOD
I.	Mupirocin USP	0.3694

Acceptance Criteria :

The coefficient of determination (r2) should not be less than 0.99.The residuals plot should show no curvature.The y intercept should not deviate significantly from zero (for example, its area response should be less than 5.0% of that of the midrange concentration value).

Detection Limit :
To do regression analysis, repeat the assay three times with 80 µg/ml Mupirocin USP injection solutions.To determine the LOD, compute the SD and then use the slope.
• Where σ represents the standard deviation of the blank.
• S represents the slope of the calibration curve.
LOD = 3.3 * σ / S.

Acceptance Criteria :
The limit of detection is the initial concentration at which the analyte's signal-to-noise ratio equals 3:1.

Quantitation Limit :
Repeat the assay three times and inject solutions containing 80 µg/ml Mupirocin USP.Perform a regression analysis. Calculate SD & take slope to calculate the LOQ.

Sr. No.	Drug	LOQ
I.	Mupirocin USP	1.1197

Acceptance Criteria:
The limitation of quantitation is achieved when the signal-to-noise ratio is 10:1 and the variability between injections is < 10%.

Accuracy (recovery):-
Accuracy expresses the degree of agreement between the value discovered and the value regarded as a conventional true value or an acceptable reference value. It is frequently expressed as the recovery achieved by the assay of known, additional concentrations of analytes. Samples (spiked placebos) are often created to cover 80%, 100%, and 120% of the nominal sample preparation

concentration. These samples are analyzed, and the recoveries for each are calculated.

$$\% \text{ Recovery} = \frac{\text{Recovered Am}}{\text{ActualAmount of I}}$$

Solution Preparation (Sample Procedure:

Spike the placebo with correctly weighed APIs of Mupirocin USP, then mix in the dry aliquot. Inject each sample three times and analyze using the analytical procedure, which is suitably bracketed by the standard. Inject samples from lowest to highest concentrations. Calculate the percentage RSD for each weight at each level.

Table no 11 : Preparation of placebo

Recovery Level %	Amount of placebo added (mg)	Amount of API (mg)	Area
80-1	0.010	0.08	4729094
80-2	0.010	0.08	455874
80-3	0.010	0.08	4858730
100-1	0.010	0.010	5485749
100-2	0.010	0.010	5560496
100-3	0.010	0.010	5541854
120-1	0.010	0.012	6575401
120-2	0.010	0.012	6688329
120-3	0.010	0.012	6614991

Table 12: Data sheet for Accuracy

% Conc. Sample	Theoretical Concentration (µg/mL)	Concentration Recovered (µg/mL)	% Recovery	Mean % Recovery
80%-1	49.861	49.860	100.0	100.2
50%-2	51.107	50.401	101.4	
50%-3	49.762	50.212	99.1	
100%-1	100.047	100.147	100.1	
100%-2	102.927	103.750	100.8	

• Acceptance Criteria:

The average percent recovery of boosted placebos should be 100±2.0% across three weight sets. Individual sample recoveries should range from 90.0% to 110.0%.

Specificity: - Weigh about 0.010g (10mg) of Mupirocin USP. Working standard: In a clean 100.0ml volumetric flask, add about 50.0ml of mobile phase and shake well to fully dissolve the sample. To get a final concentration of 100µg/ml, add 100.0ml of mobile phase to the remaining volume.

• Prepation of Test Solution :

To prepare the test solution, weigh 0.5 gm (equal to 10.0 mg) of Nupirocin ointment into a 100.0ml volumetric flask. Add 25.0ml of acetonitrile and sonicate for 10 minutes.

Then, dilute with mobile phase until the volume reaches 100.0ml. Close properly with the stopper and shake vigorously to dissolve entirely. The final concentration of Mupirocin USP is 100 µg/mL.

• Preparing the placebo:
Weigh 0.5gm placebo in a clean. To get a final concentration of 5000µg/ml, add 50.0ml of mobile phase to a volumetric flask, shake well, sonicate for 10 minutes, then dilute to 100.0ml with mobile phase.

- **Determination:**

Inject blank, standard, or placebo and test for high performance interference of contaminants and results. Inject 20µl of standard and test on High Performance Liquid Chromatography at 229 nm. Compare the findings.

- **Precision :**

Precision refers to the repeatability of a method. Weigh accurately around 0.010gm (10mg) of Mupirocin USP. Working standard: In a clean 100.0ml volumetric flask, add about 50.0ml of mobile phase and shake well to fully dissolve the sample. To get a final concentration of 100µg/ml, add 100.0ml of mobile phase to the remaining volume.

- **Determination:**

Six injections of a known concentration of the standard preparation were repeated under prescribed chromatography conditions. Calculate the mean and relative standard deviations (% RSD) for the six sample preparations.

Injection	Area
1	5662937
2	5715275
3	5689798
4	5666490
5	5702929
6	5702929
Mean	5690059
SD	20317.2140
% RSD	0.357

Acceptance criteria:
RSD of test or recovery results should not exceed 2.0%.

Intermediate Precision: -
Description of Intermediate Precision:

Intermediate accuracy relates to fluctuations within a laboratory, such as between various days, instruments, and analysts. Ruggedness was the formal name for intermediate accuracy.

Determination :
To determine repeatability, perform six repetitions on different days and time intervals (e.g., morning, afternoon, evening).

Acceptance criteria:
% RSD of test or recovery results should not exceed 2.0%.

Acceptance Criteria:
The coefficient of determination (r2) should not exceed 0.99.The residuals plot should show no curvature. The y intercept should not deviate significantly from zero (for example, its area response should be less than 5% of that of the nominal 100% concentration value).

CONCLUSION:
A HPLC technique for Mupirocin in ointment dosage form was developed and validated in accordance with ICH criteria.The findings of this validation are summarized in the report.The results are found to meet the acceptance requirements

for each of the parameters. The peak of Mupirocin USP was found to be well separated at 6.6 minutes. The devised technique was validated for ICH-compliant metrics such as system appropriateness, accuracy, precision, linearity, specificity, ruggedness, robustness, and solution stability.

REFERENCES :

1. Sudha Tantry and Ajay Kumar,Green chemistry aspects in Analytical Chemistry applications,Research Journal of Chemical Sciences,ISSN 2231-606X Vol. 13(2), 29-39, June (2023).
2. Anastas, P. T., & Warner, J. C. (1998). Principles of green chemistry. Green chemistry: Theory and practice, 29, 14821-42.
3. Williamson, T. C., & Anastas, P. T. (Eds.). (1998). Green chemistry: Frontiers in benign chemical syntheses and processes. Oxford University Press.
4. Mut Günzler, I. I., & Williams, A. (2001). Handbook of analytical techniques. Evolution, 1, 1-2.
5. Maryam Abdoljavadi, Mahboubeh Masrournia,D etermination of dopamine using carbon paste electrode modified with multi-wall carbon nanotube by differential pulse stripping voltammetry.Clinical Biochemistry,Volume 44, Issue 13, Supplement, September 2011, Page S319.
6. Ilgaz Akseli, Girindra N. Mani, Cetin Cetinkaya,Non-destructive acoustic defect detection in drug tablets,International Journal of Pharmaceutics Volume 360, Issues 1–2, 6 August 2008, Pages 65-76.
7. Harshita Joshi* , Dr. Anju Goyal,Method Development And Validation For UvVisible Spectroscopy And High-Performance Liquid Chromatography: A Review,© 2025 IJCRT | Volume 13, Issue 1 January 2025 | ISSN: 2320 - 2882.
8. Rajmane, K. P. (n.d.). a review method develpoment and validation. Retrieved from Asian Journal of Pharmaceutical Analysis (AJPA) is an international, peer-reviewed journal, devoted to pharmaceutical analysis. https://ajpaonline.com/AbstractView.aspx?PID= 2023-13-2-10
9. Cuatrecasas P, W. M. (n.d.). Separation techniques: Chromatography. Retrieved from https://pmc.ncbi.nlm.nih.gov/articles/PMC52064 69/#abstract1
10. Beaux j, j. k. (n.d.). understanding and implementing efficient analytical method development and validation.
11. Manasa Padma Meduri*; Pooja Agarwal; G. Vimala; Nafiza Banu. The development and validation studies of RP-HPLC method - A review. World J Pharm Sci. 2016; 4(1): 85-92.
12. Rachit Shukla*1, Prashant Kumar Singh2, Savita Upadhyay3,A

Comprehensive Review of High-Performance Thin Layer Chromatography (HPTLC),April 2023 Vol.:27, Issue:1.

13. McPolin O. An introduction to HPLC for pharmaceutical analysis. Lulu. Com ; 2009 Mar 1.
14. Virus ED, Sobolevsky TG, Rodchenkov GM. Introduction of HPLC/orbitrap mass spectrometry as a screening method for doping control. Journal of mass spectrometry. 2008 Jul;43(7):949-57.
15. Kirbiš A, Marinšek J, Flajs VC. Introduction of the HPLC method for the determination of quinolone residues in various muscle tissues. Biomedical Chromatography. 2005 May;19(4):259-65.
16. Ahuja S, Dong M, editors. Handbook of pharmaceutical analysis by HPLC. Elsevier; 2005 Feb 9.
17. Rashed Ahmed,High-Performance Liquid Chromatography (HPLC): Principles, Applications, Versatality, Efficiency, Innovation and Comparative Analysis in Modern Analytical Chemistry and In Pharmaceutical Sciences,Clinical Investigation (2024) Volume 14, Issue 9.
18. Abdu Hussen Ali*,High-Performance Liquid Chromatography (HPLC): A review,June 2022,Annals of Advances in Chemistry 6(1):010-02,DOI:10.29328/journal.aac.1001026.
19. Gerber F, Krummen M, Potgeter H, Roth A, Siff rin C, Spoendlin C. Practical aspects of fast reversed-phase high-performance liquid chromatography using 3 microm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. J Chromatogr A. 2004 May 21;1036(2):127-33. doi: 10.1016/j.chroma.2004.02.056. PMID: 15146913.
20. Xiang Y, Liu Y, Lee ML. Ultrahigh pressure liquid chromatography using elevated temperature. J Chromatogr A. 2006 Feb 3;1104(1-2):198-202. doi:,10.1016/j.chroma.2005.11.118. PMID: 16376355.
21. Bergh JJ, Breytenbach JC. Stability-indicating high-performance liquid chromatographic analysis of trimethoprim in pharmaceuticals. J Chromatogr. 1987 Jan 30;387:528-31. doi: 10.1016/s0021-9673(01)94565-0. PMID: 3558640.
22. Rushikesh Bachhav*, Piyush Bachhav, Mayur Bhamare, Ruchita Bachhav, Ganesh Sonawane, Kajal Pansare, Dhananjay Patil,Review of High Performance Liquid Chromatography and Its Applications,Research & Reviews: Journal of Pharmacy and Pharmaceutical Sciences,e-ISSN: 2320-1215.

23. Krishna M. Vaishnav1* , Amol V. Sawale2 , Shreyash S. Padmawar3 , Shiwani A Gulhane,Review On: Recent Advancement In Hyphenated Techniques,© 2023 Ijrar August 2023, Volume 10, Issue 3.
24. Wilson ID, Brinkman UA. Hyphenation and hypernation: The Practice and Prospects of Multiple Hyphenation. J Chromatogr A, 2003; 1000: 325-56.
25. Szpunar J, Lobinski R, Prange A. Hyphenated Techniques for Elemental speciation in Biological Systems. Appl spect, 2003; 57(3): 102A-12A.
26. Joshi RR, Gupta KR, Patil SS. Hyphenated Technique- A Boon To Analytical World. IJPSR, 2012; 3(11): 4184-91.
27. Patel KN, Patel JK, Patel MP, Rajput GC, Patel HA. Introduction to Hyphenated Techniques and Their Applications in Pharmacy. Pharm Method. 2010, 1(1): 1-13.
28. Gunasekar Manoharan,Development And Validation Of A Stability-Indicating Rp-Hplc Method For The Estimation Of Mupirocin In Bulk And Ointment Dosage Form,European Journal Of Pharmaceutical And Medical Research.
29. Hughes J And Mellows G. Interaction Of Pseudomonic Acid A With Escherichia Coli B Of Isoleucyl-Trna Synthetase. J. Biochem, 1980; 191(1): 209-219.
30. Alexandra Tucaliuc 1, Alexandra Cristina Blaga 2, Anca Irina Galaction 3, Dan Cascava,Mupirocin: Applications And Production,2019 May;41(4-5):495-502.Doi: 10.1007/S10529-019-02670-W. Epub 2019 Mar 29.
31. Arti P. Parmar1 , Dr.Dilip G.Maheshwari*2,Development And Validation Of Analytical Method For Simultaneous Estimation Of Mupirocin And Mometasone Furoate In Topical Formulation By Rp-Hplc,Arti P. Parmar Et Al. / International Journal Of Pharma Sciences And Research