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Research Article

**ANALYTICAL METHOD DEVELOPMENT AND VALIDATION
OF MUPIRICON OINMENTS BY RP-HPLC METHODS**Samadahan M.Kambale¹, Vishal S. More², M.J.Chavan³^{1,2,3}Department of Chemistry, Amrutvahini College Sangamner S.K. Dist Ahmednagar-422608(MH)**Abstract:**

A simple, economical, specific, accurate, precise and validated reverse-phase high-performance liquid chromatography (RP-HPLC) method has been developed for the assay study of Mupirocin Ointment in the pharmaceutical dosage form. The chromatographic separation was achieved on C-18 column (300 mm x 3.9 mm, 10 μ particle size) at 25°C temperature using mobile phase Phosphate Buffer : Acetonitrile (ACN) (75:25 % v/v) at flow rate 1.0 ml/min. Quantification was achieved with a UV detector at 229 nm. The retention time of Mupirocin USP was found to be 6.696 ± 0.05 min. The proposed method was validated according to ICH guidelines concerning assay studies for Mupirocin ointment. The developed method with good separation successfully applied for the determination of Mupirocin in its pharmaceutical dosage form.

Keywords : RP-HPLC, Mupirocin, Assay, Chromatography, ICH Guidelines.**Corresponding author:****Samadahan M.Kambale,**

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INTRODUCTION:

The science and art of determining the composition of materials in terms of elements or compounds containing in them is the analytical chemistry. It plays pivotal role in the development of science and involves separating, identifying and determining the related number of components in a sample of matter. The quality of product may deviate from standard required but in carrying out an analysis one also must certain that the quality of the analysis itself is of the standard required. The chemical, physical and sometimes microbiological analyses involve in the testing of Pharmaceutical product. It has been estimated that in the UK 10 million is spent each year on analysis alone and such analytical processes can be found in industries as diverse as those producing food, beverages, cosmetics, detergents, metals, paints, water, agrochemicals, biotechnological products and pharmaceuticals. [1,2,3].

According to need of analysis analytical chemistry can be classified as following types:

- **Qualitative analysis** which reveals determine the chemical identification of the constituent species which may be of organic or inorganic in a sample.
- **Quantitative analysis** use to determine or establish how much of a constituent species may be organic or inorganic is present in a sample.

Analytical methods are mainly classified into instrumental method & classical or wet- chemical methods based on the property that is observed in the final measurement process.

Chemical methods of analysis involve measurements of the mass or the volume of the substances of reacting solution,

E.g. Gravimetric method, volumetric method, etc.

Instrumental methods which depend upon the measurement of electrical, optical thermal and other properties and those based upon determination of the extent to which radiation is absorbed or upon assessment of the intensity of emitted radiation, It requires the use of a suitable instrument e.g. Spectrophotometer, HPLC, Oven and NMR etc. [4]
Now a day many research and application are to be

done by using instrumental analysis.

Techniques of Instrumental Analysis [5]

Many instrumental methods are used in pharmaceutical analysis, of which important methods are –

Separation techniques: These are the chromatographic methods i.e.

TLC, GC or HPLC which may applicable for an analysis depends on various parameter like solubility or volatility of the sample, separation efficiency, concentration of analyte, limit of Detection, analysis cost etc.

Spectrometric techniques: It include UV, IR, NMR, MASS etc. spectrometric techniques are usually used in the analysis of the drug of interest alone in the matrix of excipient, degradation product, impurities, additives, etc. It also includes Plasma, Atomic, X-Ray Flame, Absorption or Emission Spectrometry, etc.

Electro-analytical techniques: Electro-analytical method of analysis deals with electrical signal to the sample and/or monitor the electrical property of the sample e.g. Amperometry, Polarography, Electrogravimetry, Conductometry, Potentiometry, etc.

Thermo-analytical techniques: This analytical technique includes interaction of heat with the material. e.g. DSC, DTA, Thermo Gravimetric Technique, etc.

Modern hyphenated techniques: These techniques involve advancement in drug analysis by the combination of one instrumental technique with other in order to refine the analysis of drug, impurity, degradation product etc.

e.g. GC-MS (Gas Chromatography-Mass spectrometry), LC-MS (Liquid Chromatography-Mass spectrometry), GC-IR (Gas Chromatography-infrared spectroscopy).

Thus, analytical science involves use of various techniques for determination of analyte present in the sample matrices. These techniques have advantage in analytical sciences in order to determination of specific analyte [6].

Analytical approaches

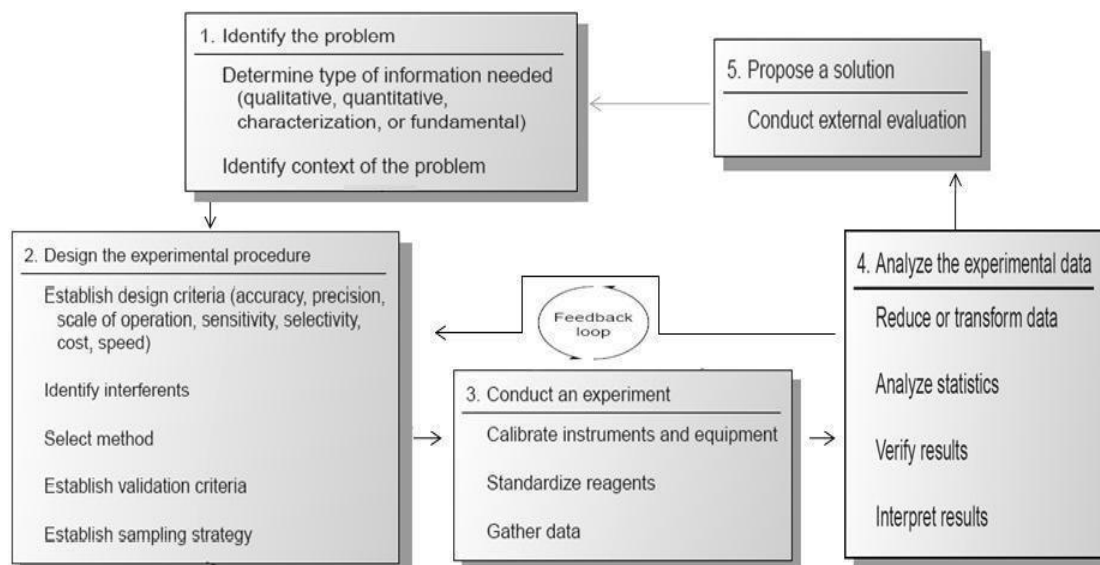


Fig.1.1: Flow diagram for analytical approach ^[7]

Chromatography

Chromatography is the method used primarily for the separation of components present in the sample, in which the component are distributed between two phases one of which is stationary phase and other phase is movable, the stationary phase may be a solid or liquid supported on a solid or a gel, and it may be packed in the column, spread as layer or distributed as film. The mobile phase may be gaseous or liquid which moves on the stationary phase. ^[8] Chromatography was originally developed

by Russian botanist Michael Tswett in 1903 for the separation of colored plant pigment by producing a petroleum ether extract through a glass column packed with powdered calcium carbonate. It is now, in general, the widely used separation technique in analytical chemistry having developed into several related but quite different form that enable the components of complex mixture of organic or inorganic components to be separated and quantified. The classification of chromatographic techniques shown in figure .

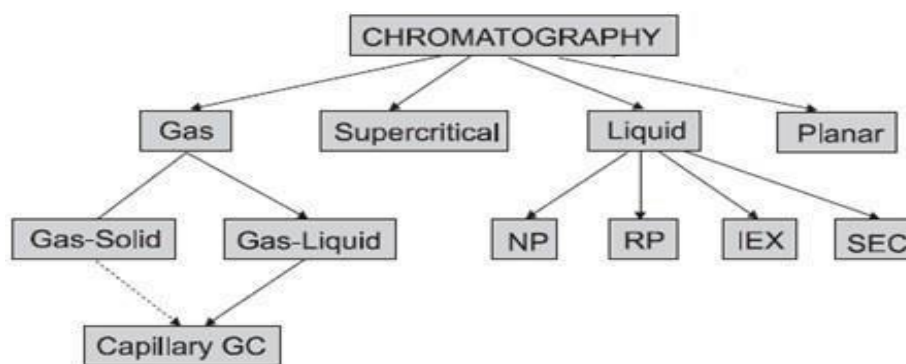


Fig.1.2: Classification of chromatography

High Performance Liquid Chromatography

High pressure liquid chromatography is also called high performance liquid chromatography because it offers high performance over ambient pressure or low- pressure liquid chromatography has higher separation speed. HPLC technique is used in the pharmaceutical industry for a wide variety of samples used. It is the method of choice for checking the purity of new drug candidates present in pharmaceutical dosage form, monitoring changes or the scale up of synthetic procedures of drugs, in process testing for developing new formulations or dosage form, and quality control or quality assurance of final drug products. [11]

High performance liquid chromatography (HPLC) is the term used to describe liquid chromatography in which the liquid mobile phase is mechanically pumped over a column containing the stationary phase. HPLC is an analytical process utilizing special instrument design to separate, quantify and analyze component of chemical mixture. Sample of interest

are introduced to a solvent flow path; carried through a column packed with specialized material for component separation; and component data is obtained through the combination of the detection mechanism coupled with a data recording system. Analysis of analyte by HPLC is presently used in pharmaceutical research and development department.

Modes of separation of HPLC:

1. Normal phase or reverse phase -- analysis of small (<2000 Da) organic molecule
2. Ion chromatography -- analysis of ions
3. Size exclusion chromatography -- for separation of polymers Chiral HPLC -- determination of Enantiomeric purity. [12]

Components of HPLC: [13]

The essential features of modern chromatography i.e. HPLC are illustrated in the block diagram shown below in Fig.1.3.

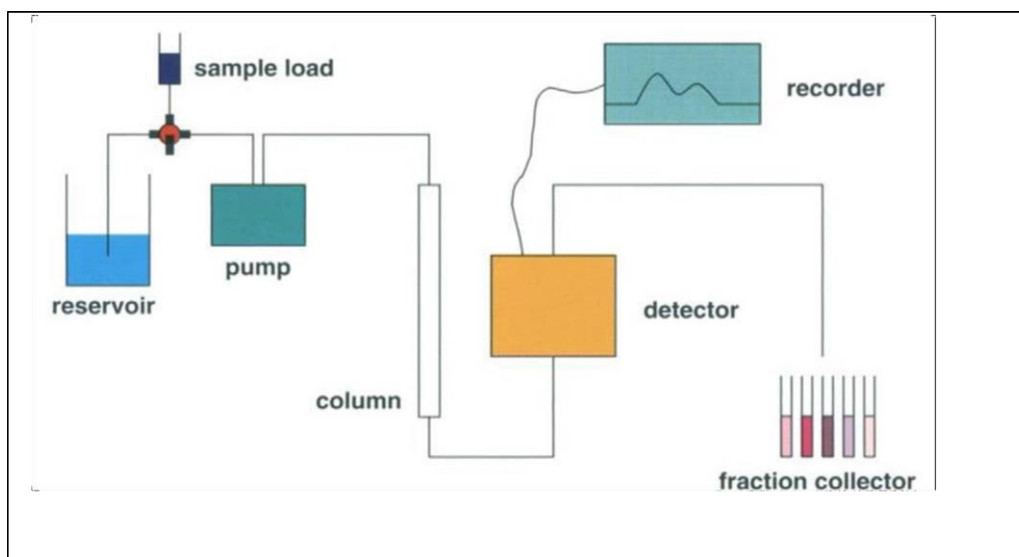


Fig.1.3: Block diagram showing the components of HPLC Method Development in HPLC [14]

The wide variety of equipment, columns, eluent and operational parameters involved make high performance liquid chromatography method development seem complex. The process is influenced by the nature of the analyte. Every day many chromatographers face the need to develop a high- performance liquid chromatography (HPLC) method for the separation of analyte. Metho development of HPLC often follows the series of steps. Our Method development philosophy is based on several considerations. Today exists a good practical understanding of chromatographic separation and it varies with the sample and with its experimental conditions. Any systematic approach to HPLC method development should be based on this knowledge of chromatographic process. In most cases, the separation can be achieved easily with only a few experiments. In other cases, a considerable amount of experimentation requires.

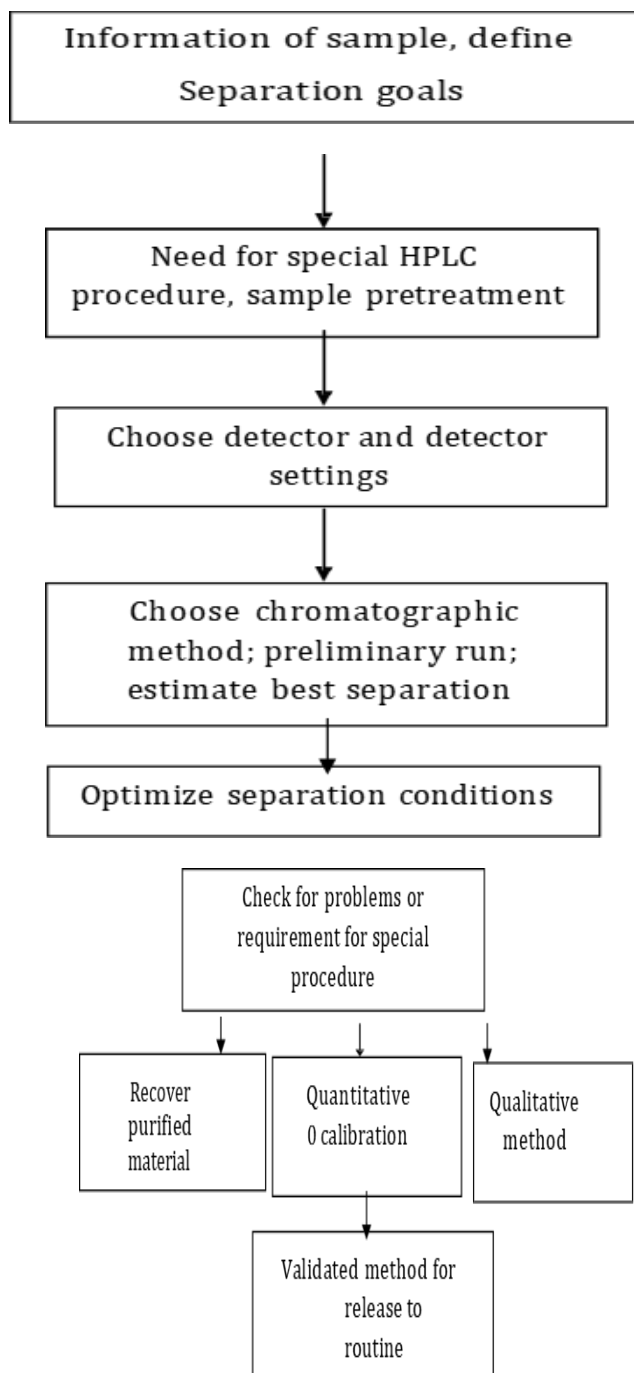


Fig.1.4: Steps in HPLC method development

Nature of sample

We need to review what is known about the sample before beginning method to be developed. The valuable clues should be provided by chemical composition of the sample .

Some of the chromatographers try to match the "chemistry" of the sample to a best choice of initial HPLC conditions. The various sample related information that can be important are summarized as:

- Number of compounds present in sample
- Chemical structure (Functionality) of the compound

- Molecular weights of the compounds
- pKa values of the compounds
- U.V spectra of the compounds
- Concentration range of the compounds in samples of interest

Sample pretreatment and detection

Samples come in various forms:

- Solutions that is ready for injection.
- Solutions that require dilution, buffering, addition of an internal standard or other volumetric manipulation.
- Solids that must first be dissolved or extracted.
- Samples that require sample pretreatment to remove interference and or protect the column or equipment from damage.

Some samples are requiring a partial separation (pretreatment) prior to HPLC, because of a need to remove the interferences, sample analyte concentrate, or eliminate "column killers".

Separation Improvement

Separation or resolution is a primary requirement in quantitative HPLC analysis technique. Usually for samples containing five or fewer components, baseline resolution greater than 1.5 can be obtained easily for the bands of interest. Resolution usually degrades during the life of the column and can vary from day to day with minor fluctuations in separation conditions. Therefore, values of resolution (Rs) equal to 2 or greater should be the goal during method development for simple mixtures.

Table 1: Separation goals in HPLC method development

Goal	Comments
Resolution	precise and rugged quantitative analysis requires that Rs be greater than 1.5
Separation time	<5-10 minute is desirable for routine procedures
Quantitation	≤2% (RSD) for assay; ≤5% for less-demanding analyses; ≤15% for trace analyses
Pressure	<150 bar is desire, <200 bar is usually essential (new column assumed)
Peak height	narrow peak is desirable for large signal to noise ratios
Solvent consumption	minimum mobile-phase use per run is desirable

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Table 2: Separation goals in HPLC method development

Goal	Comments
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Pressure	<150 bar is desire, <200 bar is usually essential (new column assumed)
Peak height	narrow peak is desirable for large signal to noise ratios
Solvent consumption	minimum mobile-phase use per run is desirable

Solvents used in HPLC

The choice of mobile phase solvents can have a sonorous effect on ease and sensitivity of HPLC detection. The lowest usable cut-off wavelength is important for Ultraviolet detectors, solvents refractive index (RI) show effect on the sensitivity of RI detection for sample, and solvent volatility (boiling point) is important for evaporating light-scattering detectors.

Ideally, solvents which used as HPLC mobile phases should have following characteristic:

High solubility of the sample components

Noncorrosive to HPLC system components

Low cost, UV transparency, High purity.

Table 3: Various Solvents for HPLC analysis^[16]

Solvent Name	UV cut off (nm)	Refractive Index _{20°C}	Boiling Point (°C)	Polarity (P)	Viscosity (cP)
Acetone	330	1.3587	56.29	5.1	0.36
Acetonitrile	190	1.3441	81.60	5.8	0.38
1-Butanol	215	1.3993	117.5	3.9	2.98
Chloroform	245	1.4458	61.15	4.1	0.57
Cyclohexane	200	1.4242	80.72	0.2	1.0
DMF	268	1.4384	166.1	6.5	0.84
Water	190	1.3330	100	10.2	0.81
Tetrahydrofuran	212	1.4072	66.0	4.0	0.55
Hexane	195	1.3749	68.7	0.1	0.31
Methanol	205	1.3284	64.7	5.1	0.55

1.5.6. Selection of Detector

To select a good detector, the basic criteria include sensitivity at low concentration, linearity over a wide range, and tolerance to temperature or solvent composition changes. In the pharmaceutical industry, most small molecules contain chromospheres that display good absorbance in UV region.^[17]

There are basically two types of detectors- the bulk property detectors and solute property detectors. The bulk property detectors function on some bulk property of the eluent, such as refractive index and are not suitable for gradient elution. The solute property detectors may be spectrophotometric, fluorescence and electrochemical detectors may be by measuring some type of physical or chemical detectors. They respond to a physical or chemical property of the solute, and ideally, they are independent of the mobile phase. As a result, the solute property detectors can be used with gradient elution. UV detector is the first choice because of its convenience and applicability in case of most of the samples. Alternate detectors may be required when:

- Samples have little or no UV absorption.
- Analyte concentrations are too low for UV-detection.
- Matrix interferences.

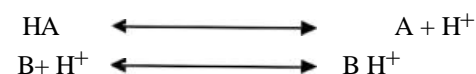
Qualitative structural information may be required.^[18]

Selection of Mobile Phase^[20]

For choice of mobile-phase solvents include compatibility between solvents, solubility of the sample in the eluent, polarity, light transmission, viscosity, stability, and pH. The mobile-phase solvents should be miscible and should not precipitate when they are mixed together. Dichloromethane and water are the example which is immiscible at most compositions and should not be used as mobile-phase components.

Selection of pH^[21]

pH is a factor affect the selectivity of the separation; change in pH can result in 10-fold change in retention of ionic samples. Organic molecule that contains one or more functional groups capable of acidic or basic behavior in the usual pH range: $2 < \text{pH} < 8$ for most silica-based columns and $1 < \text{pH} < 14$ for pH-stable columns.



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

MATERIALS AND METHODS :**Active Pharmaceutical Ingredients****Table no.4. API and Supplier**

API	Supplier
Mupirocin USP	AVCOP, Sangamner

Marketed Formulation**Table no.5. Details of Marketed Formulation**

Company Name	Brand Name	Dose	Dosage form
Nulife Pharmaceutical, Pvt. Ltd	Nupirocin	Mupirocin 2%	Ointment

Chemicals:**Table no. 6. List of chemicals**

Sr.no.	Chemicals	Grade	Manufacturer
1.	Acetonitrile	HPLC	Merck
4.	Hydrochloric acid	AR	Merck
5.	Sodium hydroxide	AR	Merck
6.	Monobasic sodium Phosphate	AR	Merck

Instruments/ Equipment**Table no.7. List of Instruments / Equipment**

Sr.no.	Instrument name	Make
1.	Water bath	Shimadzu
2.	HPLC	Agilent technology
3.	Digital analytical balance	Shimadzu
4.	Ultrasonicator	Ultrasonic cleaner
5.	pH meter	Systonics

Result:**RP-High Performance Liquid Chromatography Method:****Instrumentation**

The chromatographic system used to perform development and validation of this method consisted of an Elite LaChrom RP-HPLC Instrument equipped with quaternary pump. Sample injector with a 20 μ L loop, C18 column (300mm \times 3.9 μ m particle size) and DAD detector used. The HPLC system equipped with open lab software for data acquisition and quantification of peaks.

Table no.8. Instrument specification

HPLC Parameter	Specification
Manufacture	Agilent
Model	1260
Software	Elite LaChrom
Mode of Operation	Quaternary system
Flow rate	0-5ml
Composition range	0-100% programmable in 1% increments for each 4 components A B C and D total composition sum to 100%
Automatic solvent sparging	Yes
Pressure maxima	400bar
Programmed press limit	400bar
Detector	DAD detector

Chromatographic condition

Chromatographic analysis was performed Agilent C18 (300mm \times 3.9mm 10 μ m particle size) column. The mobile phase consisted of ACN and Phosphate Buffer (pH adjusted to pH 6.3 with the help of sodium hydroxide) in the ratio of (25:75 v/v). The flow rate was 2.0 mL/min, the injection volume was 20 μ L, and detection was performed at 229 nm using a photodiode arraydetector (PDA).

Mobile Phase Preparation**Selection of Solvent System:**

The selection of solvent was made after assessing the solubility of the drug in different solvents like water, and Acetonitrile. Also Mupirocin is soluble in water and ACN. According to their solubility and economical point of view ACN was selected as a solvent system for this RP-HPLC method development.

Preparation of Solution:**Preparation of 5 N Sodium hydroxide solution:**

Weigh accurately about 20.0 gm Sodium hydroxide and transfer to 100.0 ml volumetric flask and dilute up to 100.0 ml with distilled water. Close properly with stopper and shake well to dissolve completely.

Preparation of 6 N Hydrochloric acid:

Take carefully 25.5 ml Concentrated Hydrochloric acid into a 50.0 ml volumetric flask having 25.0 ml distilled water. Then dilute up to the volume 50.0 ml with distilled water. Close properly with stopper and shake well.

Preparation of Standard:

Weigh accurately 10.0 mg of Mupirocin USP Working Standard and transfer carefully into a 100.0 ml volumetric flask. Add about 25.0 ml of acetonitrile in volumetric flask and sonicate on sonicator for 10.0 minutes. Then dilute up to the volume 100.0 ml with pH 6.3 phosphate buffer. Close properly with stopper and shake well to dissolve completely. Final concentration of Mupirocin USP is 100.0 mcg/ml.

Preparation of Resolution solution:

Pipette out 10.0 ml from standard solution adjust the pH 2.0 using 6N hydrochloric acid, allow to stand for 2.0 hour, and again adjust the pH of same solution to 6.3 ± 0.2 using 5 N sodium hydroxide.

Preparation of Test: -

Weigh accurately about 0.5 gm (equivalent to 10.0 mg) of Nupirocin ointment in to 100.0 ml of volumetric flask to that add about 25.0 ml of acetonitrile in volumetric flask and sonicate on sonicator for 10.0 minutes. Then dilute up to the volume 100.0 ml with pH 6.3 phosphate buffer. Close properly with stopper and shake well to dissolve completely. Final concentration of Mupirocin USP is 100.0 mcg/ml. Resolution solution, standard and test solution filter through syringe filter 0.45 μ m.

Preparation of Mobile Phase:**Preparation of pH 6.3 phosphate buffer
Preparation of 0.05M monobasic sodium phosphate**

Weigh accurately about 6.9 gm of Monobasic sodium phosphate and transfer into a 1000.0 ml volumetric flask and dilute up to the volume 1000.0 ml with Distilled water. Close properly with stopper and shake well to dissolve completely. Then adjust pH 6.3 ± 0.2 with 10N Sodium hydroxide.

Preparation of Mobile phase: -

A Mixture of volume of Acetonitrile and above prepared Buffer solution in the proportion or ratio of Acetonitrile 250: Buffer 750. After mixing filter through 0.45 μ m or 0.2 μ m Nylon membrane filter paper and degas.

TRIAL – 1**FIRST TRIAL****CHROMATOGRAPHIC CONDITIONS:** As per monograph

- Column: 25cm, 4.6mm C18 5 μ m
- Flow rate: 2.0 ml/min
- Temp: 25°C
- Wavelength: 229 nm
- Injection volume: 20 μ l
- ❖ 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:	
<ul style="list-style-type: none"> Column: 30cm,3.9mm C18 10μm Flow rate: 2.0ml/min Temp: 25°C Wavelength: 229 nm Injection volume: 20μl ❖ 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	

<ul style="list-style-type: none"> Flow rate: 2.0ml/min 	
3 TRIAL	
(MP, Dilutions, chromatographic conditions as per USP) CHROMATOGRAPHIC CONDITIONS:	
<ul style="list-style-type: none"> Column: 30cm,3.9mm C18 10μm Temp: 25°C Wavelength: 229 nm Injection volume: 20μl ❖ 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 suitability for resolution solution:

Relative retention times are about 0.9 for the Mupirocin acid hydrolysis product and 1.0 for Mupirocin.

Resolution between the Mupirocin acid hydrolysis product and Mupirocin is not less than 2.0.

System suitability for standard solution:

Tailing factor is not more than 2.0.

Column efficiency is not less than 1500 theoretical plates.

The Relative standard deviation for replicate standard

deviation for replicate injections is not more than 2.0%.

Calculation:

Calculate the Assay for Mupirocin USP by comparison of Standard chromatogram, Test chromatogram i.e. Consideration of Standard Area, Test Area, Working Standard Weight and their dilution, Weight of test sample and their dilution & working Standard Potency.

$$\% \text{ Assay} = \left(\frac{A_s}{C_s} \right) * \left(\frac{Wt_std}{Vol_std} \right) * \left(\frac{Vol_samp}{Wt_samp} \right) * \left(\frac{1}{Ps} \right) * 100$$

RP-HPLC Method Validation**1.Lineariry**

For linearity, accurately weigh about 0.020gm (20mg) of Mupirocin USP. Working standard in clean 100.0ml volumetric flask add about 50.0ml of

mobile phase shake well to dissolve the sample completely. Make up the remaining volume to 100.0ml with mobile phase to get a final concentration of 200µg/ml.

Table no 9 :Linearity in RP-HPLC validation .

% Conc. of Sample	Conc. (PPM)	Mean Response (Area)	Statistical analysis	
50	80	4711686	Correlation	0.999
80	90	5158792		
100	100	5604014	Intercept	16067
120	110	6311452		
150	120	6474954		
			Slope	13589

Sr. No.	Drug	LOD
1.	Mupirocin USP	0.3695

Acceptance Criteria:

Coefficient of determination (r^2) should not less than 0.99. There should be no curvature in the residuals plot. The y intercept should not significantly depart from zero (e.g., area response of y intercept should be less than 5.0% of the response of the midrange concentration value).

Detection Limit: -

Perform three repetition of assay concentration and inject solutions of concentrations 80 µg/ml Mupirocin USP Perform the regression analysis. Calculate SD & take slope to calculate the LOD.

$$\text{LOD} = 3.3 * \sigma / S.$$

- Where σ is the standard deviation of the blank.
- And S is the slope of the calibration curve.

Acceptance Criteria:

The limit of detection is the first concentration at which the analyte has a signal- to- noise ratio of 3:1.

Quantitation Limit: -

Perform three repetition of assay concentration and inject solutions of concentrations 80 µg/ml Mupirocin USP respectively. Perform the regression analysis. Calculate SD & take slope to calculate the LOQ.

Sr. No.	Drug	LOQ
1.	Mupirocin USP	1.1198

Acceptance Criteria:

The limit of quantitation is the level at which a signal-to-noise ratio of 10:1 is obtained and the difference between injections is less than 10%.

Accuracy (Recovery): -

Accuracy expresses the closeness of agreement between the value found and the value that is accepted as either a conventional true value or an accepted reference value. It may often be expressed as the recovery by the assay of known, added amounts of analyte. Samples (spiked placebos) are prepared normally covering 80%, 100% &120% of the nominal sample preparation concentration. These samples are analyzed and the recoveries of each are calculated.

$$\% \text{ Recovery} = \frac{\text{Recovered Amount}}{\text{Actual Amount of Pure Drug}} \times 100$$

Preparation of solution (Sample) Procedure:

Spike the placebo with accurately weighed API's of Mupirocin USP and mix the dry aliquot into the placebo mixture. Inject each sample three times and analyze according to the analytical method, adequately bracketed by standard. Inject samples from the lowest concentration to the highest concentration. Calculate the % RSD for each individual 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	4729095
80-2	0.010	0.08	455875
80-3	0.010	0.08	4858731
100-1	0.010	0.010	5485750
100-2	0.010	0.010	5560497
100-3	0.010	0.010	5541855
120-1	0.010	0.012	6575402
120-2	0.010	0.012	6688330
120-3	0.010	0.012	6614992

Table 12: Data sheet for Accuracy

% Conc. Sample	Theoretical Concentration (µg/mL)	Concentration Recovered (µg/mL)	% Recovery	Mean % Recovery
80%-1	49.862	49.861	100.0	100.2
50%-2	51.108	50.402	101.4	
50%-3	49.762	50.213	99.1	
100%-1	100.048	100.148	100.1	
100%-2	102.928	103.751	100.8	

- Acceptance Criteria:**

The percent recovery of the spiked placebos should be within 100±2.0% for the average of each set of three weights. Each individual sample recovery should lie within the range of 90.0% to 110.0%.

- Specificity: -**

Accurately weigh about 0.010gm (10mg) of Mupirocin USP Working standard in clean 100.0ml volumetric flask add about 50.0ml of mobile phase shake well to dissolve the sample completely. Make up the remaining volume to 100.0ml with mobile phase to get a final concentration of 100µg/ml.

- Preparation of test solution:**

Weigh accurately about 0.5 gm (equivalent to 10.0 mg) of Nupirocin ointment in to 100.0ml of volumetric flask to that add about 25.0ml of acetonitrile in volumetric flask and sonicate on sonicator for 10.0 minutes. Then dilute up to the volume 100.0ml with mobile phase.

Close properly with stopper and shake well to dissolve completely. Final concentration of Mupirocin USP is 100µg/ml.

- Preparation of placebo:**

Weigh a quantity of 0.5gm placebo in a clean 100.0ml of volumetric flask, disperse in 50.0ml of mobile phase, shake well, sonicate for 10minutes and dilute to 100.0ml with mobile phase to get a final concentration of 5000µg/ml.

- Determination:**

Inject individually of Blank, standard, placebo and test on High Performance interference of impurities

and results. Inject 20µl individually of standard and test on High Performance Liquid Chromatography at 229 nm and compare the results.

- Precision:**

- Repeatability (Method Precision)**

Accurately weigh about 0.010gm (10mg) of Mupirocin USP Working standard in clean 100.0ml volumetric flask add about 50.0ml of mobile phase shake well to dissolve the sample completely. Make up the remaining volume to 100.0ml with mobile phase to get a final concentration of 100µg/ml.

- Determination:**

Replicate six injections of the standard preparation of known concentration were injected as per chromatographic conditions specified. Calculate the mean and relative standard deviations (% RSD) of the six sample preparations.

Table no 13 : Sample preparation

Injection	Area
1	5662938
2	5715275
3	5689799
4	5666491
5	5702930
6	5702930
Mean	5687872
SD	20317.2141
% RSD	0.357

- Acceptance criteria:**

The % RSD of the assay or recovery values should not be greater than 2.0%.

Intermediate Precision: -**Description of Intermediate Precision:**

Intermediate precision refers to variations within a laboratory as with different days, with different instruments, by different analysts, and so forth. Intermediate precision was formally known as ruggedness.

Determination:

Perform the repeatability analysis with six repetitions on different days and on same day with some time interval (Morning, afternoon, evening).

Acceptance criteria:

The % RSD of the assay or recovery values should not be greater than 2.0%

Acceptance Criteria:

Coefficient of determination (r^2) should not greater than 0.99. There should be no curvature in the residuals plot. The y intercept should not significantly depart from zero (e.g., area response of y intercept should be less than 5% of the response of the nominal 100% concentration value.

CONCLUSION:

A HPLC method for Mupirocin was developed and validated in Ointment dosage form as per ICH guidelines. The results of this validation are as summarized in the report. The results are found to be complying with the acceptance criteria for each of the parameter.

The peak of Mupirocin USP was found well separated at 6.6 min. The developed method was validated for various parameters as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, ruggedness, robustness and solution stability.

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