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Development and Validation of RP-HPLC Method for the Determination of Dimercaprol Drug

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Conflict of interest: No conflict of interest.

Abstract:
The purpose of this study is to develop and validate a reverse-phase high-performance liquid chromatography (RP-HPLC) method for the determination of Dimercaprol (British anti-lewisite, BAL), an essential chelating agent used for the treatment of heavy metal poisoning. Dimercaprol is known to bind to metal ions like arsenic, mercury, and lead, facilitating their excretion from the body. A suitable RP-HPLC method is crucial for ensuring the drug's quality, stability, and appropriate dosage during clinical use. In this study, we describe the development and optimization of the RP-HPLC method, including the selection of the mobile phase, column, and detection conditions. Validation of the method was carried out in accordance with ICH guidelines, demonstrating its specificity, precision, accuracy, and robustness[2-3]

HPLC method development for Dimercaprol is a well-established area, but there are still multiple opportunities for innovative contributions. By focusing on enhancing sensitivity, improving data analysis techniques, optimizing formulations, exploring green chemistry approaches, and developing more accessible therapeutic monitoring systems, you can provide new insights and contribute to advancing the clinical and pharmaceutical applications of dimercaprol. We can explore new detection techniques or enhanced sensitivity methods such as Coupling HPLC with Mass Spectrometry (HPLC-MS)**,** Development of More Sensitive[1-2] UV or Fluorescence Detectors**,** Novel Mobile Phase or Stationary Phase Optimization, Integration with Advanced Data Analysis Techniques, Alternative Drug Delivery Systems (DDS). The development of a High-Performance Liquid Chromatography (HPLC) method involves focusing on purity analysis, quantification in dosage forms, pharmacokinetic studies, or therapeutic drug monitoring.

Keywords: Dimercaprol, RP-HPLC, Method Development, Validation, Drug Analysis, Chelating Agent

Introduction

Reversed-phase high-performance liquid chromatography (RP-HPLC) offers a versatile and powerful method for achieving the required accuracy and precision in Dimercaprol quantification. RP-HPLC is a chromatographic technique that separates compounds based on their hydrophobicity. It employs a non-polar stationary phase and a polar mobile phase, allowing for the effective separation of a wide range of analytes, including pharmaceuticals, metabolites, and environmental contaminants. The adaptability of RP-HPLC [5-6], through the selection of appropriate columns, mobile phase compositions, and detection methods, makes it particularly well-suited for the analysis of complex matrices and trace-level quantification. Therefore, RP-HPLC stands out as a robust analytical tool for Dimercaprol analysis, addressing the challenges posed by its chemical properties and the need for accurate measurements in diverse sample types.

Dimercaprol is an organic arsenic compound used primarily in the treatment of poisoning by heavy metals, including arsenic, ercury, and lead. It is essential to monitor the drug's purity and stability throughout its manufacturing and clinical application stages. High-Performance Liquid Chromatography (HPLC) has become a widely used technique for drug analysis due to its ability to separate, identify, and quantify compounds effectively. Specifically, Reverse-Phase HPLC (RP-HPLC) is ideal for analyzing non-polar compounds like Dimercaprol, which are poorly soluble in water but soluble in organic solvents.

The primary aim of this research is to develop a reliable and efficient RP-HPLC method for Dimercaprol analysis. This method will allow for the accurate quantification of Dimercaprol in pharmaceutical formulations and biological samples.

Dimercaprol, also known as British Anti-Lewisite (BAL), is a medication primarily used as an antidote for heavy metal poisoning. Its chemical structure features two thiol (sulfhydryl) groups, which enable it to bind to heavy [7-8] metals such as arsenic, mercury, lead, and gold, forming stable, non-toxic complexes that can be excreted from the body. This chelating action is crucial in mitigating the toxic effects of these metals, which can disrupt various biological processes and cause severe health issues. Dimercaprol's effectiveness depends on its ability to compete with endogenous ligands for binding to the heavy metals, thus preventing or reversing their harmful interactions with essential enzymes and proteins. Due to its critical role in treating heavy metal toxicity, the precise analytical methods are essential for determining its concentration in pharmaceutical formulations and biological samples.

The necessity for accurate quantification of Dimercaprol arises from several factors. In pharmaceutical formulations, precise determination of the drug's concentration is vital for ensuring product quality, efficacy, and safety. Over- or under-dosing can have significant clinical consequences, especially in emergency situations where Dimercaprol is administered to treat acute heavy metal poisoning. In biological samples, such as blood or urine, accurate measurement of Dimercaprol levels is essential for pharmacokinetic studies, therapeutic drug monitoring, and assessing the extent of exposure in cases of suspected poisoning. These applications demand analytical techniques that are not only sensitive and selective but also reliable and reproducible

Materials and Methods

Chemicals and Reagent

Dimercaprol reference standard was obtained from a reputable supplier. The mobile phase components included acetonitrile (HPLC grade), water (HPLC grade), and phosphoric acid. All solvents and reagents used were of analytical grade. Deionized water was used for the preparation of the mobile phase and the mobile phase filtrate. The source and purity of Dimercaprol standard, solvents (e.g., acetonitrile, methanol), and buffer components (e.g., phosphate buffer) must be specified to ensure reliable and accurate results.[13-16]

The solvents used for mobile phase preparation, such as acetonitrile and methanol, should be of HPLC grade to minimize interference from impurities. Buffer components, such as phosphate salts, should also be of high purity and accurately weighed to ensure the correct buffer concentration and pH. This may include acids or bases used for pH adjustment, salts used for ion pairing, or reagents used for derivatization. The use of high-quality chemicals is essential for minimizing background noise and ensuring accurate quantification of Dimercaprol.

All chemicals should be of HPLC grade to minimize interference and ensure accurate results. HPLC-grade solvents and reagents are specifically manufactured and purified to meet the stringent requirements of high-performance liquid chromatography15-16. These chemicals undergo rigorous testing to ensure that they are free from impurities that could interfere with the analysis or damage the HPLC system. The use of HPLC-grade chemicals helps to minimize background noise, improve peak shape, and ensure accurate quantification of Dimercaprol.

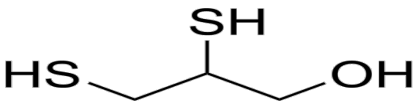


fig: Chemical structure of Dimercaprol

Instrumentation

The HPLC system used must be described, including the pump, autosampler, column oven, and detector (e.g., UV-Vis detector), to allow for reproducibility and comparability of results **[17]**. The description should include the manufacturer and model number of each component, as well as any relevant specifications, such as the pump's flow rate range, the autosampler's injection volume range, and the detector's wavelength range.

The HPLC column must be specified, including the type (e.g., C18 column), dimensions (length and internal diameter), and particle size. The column is the heart of the HPLC system, and its characteristics have a significant impact on the separation and resolution of the analytes. The column type, such as C18, C8, or phenyl, determines the selectivity of the separation, while the column dimensions and particle size affect the efficiency and backpressure of the system. The column should be selected based on the chemical properties of Dimercaprol and the desired separation characteristics any software used for data acquisition and processing must be mentioned to ensure transparency and traceability of the results HPLC systems typically use software to control the instrument, acquire data, and process the results. The software should be validated to ensure that it performs its intended functions accurately and reliably. The software name and version number should be mentioned in the materials and methods section to allow for verification and traceability of the results.[12-14]

Chromatographic Conditions

The RP-HPLC method was optimized by varying parameters like the mobile phase composition, flow rate, and column temperature. After a series of trials, the following optimized conditions were selected:

- Column: C18, 4.6 mm × 250 mm, 5 µm
- Mobile Phase: A mixture of acetonitrile and water (70:30 v/v) with 0.1% phosphoric acid
- Flow Rate: 1.0 mL/min
- Detection Wavelength: 220 nm
- Column Temperature: 30°C
- Injection Volume: 20 µL

The mobile phase composition (e.g., ratio of buffer to organic solvent), pH, and flow rate must be detailed to ensure proper separation and elution of Dimercaprol **[20]**. The mobile phase is the solvent system that carries the sample through the HPLC column and facilitates the separation of the analytes. The mobile phase composition, including the type and ratio of organic solvent to buffer, has a significant impact on the retention and selectivity of the separation. The pH of the mobile phase also plays a critical role, particularly for ionizable analytes like Dimercaprol, as it can affect their ionization state and, consequently, their interaction with the stationary phase. The flow rate of the mobile phase affects the retention time and peak resolution, and should be optimized to achieve efficient separation. The column temperature and injection volume must be specified to maintain consistent chromatographic performance and sample introduction. The column temperature can influence the separation selectivity and peak shape and should be carefully controlled to ensure reproducible results. The injection volume is the amount of sample that is introduced into the HPLC system and should be optimized to maximize sensitivity without overloading the column.[11-14]

The detection wavelength used for UV-V is detection must be described to ensure optimal sensitivity and selectivity for Dimercaprol. UV-V is detection is based on the principle that many organic compounds absorb ultraviolet or visible light at specific wavelengths. The choice of detection wavelength is crucial for maximizing maximum absorbance should be selected, while minimizing interference from other components in the sample.

Method Development

Selection of stationary phase

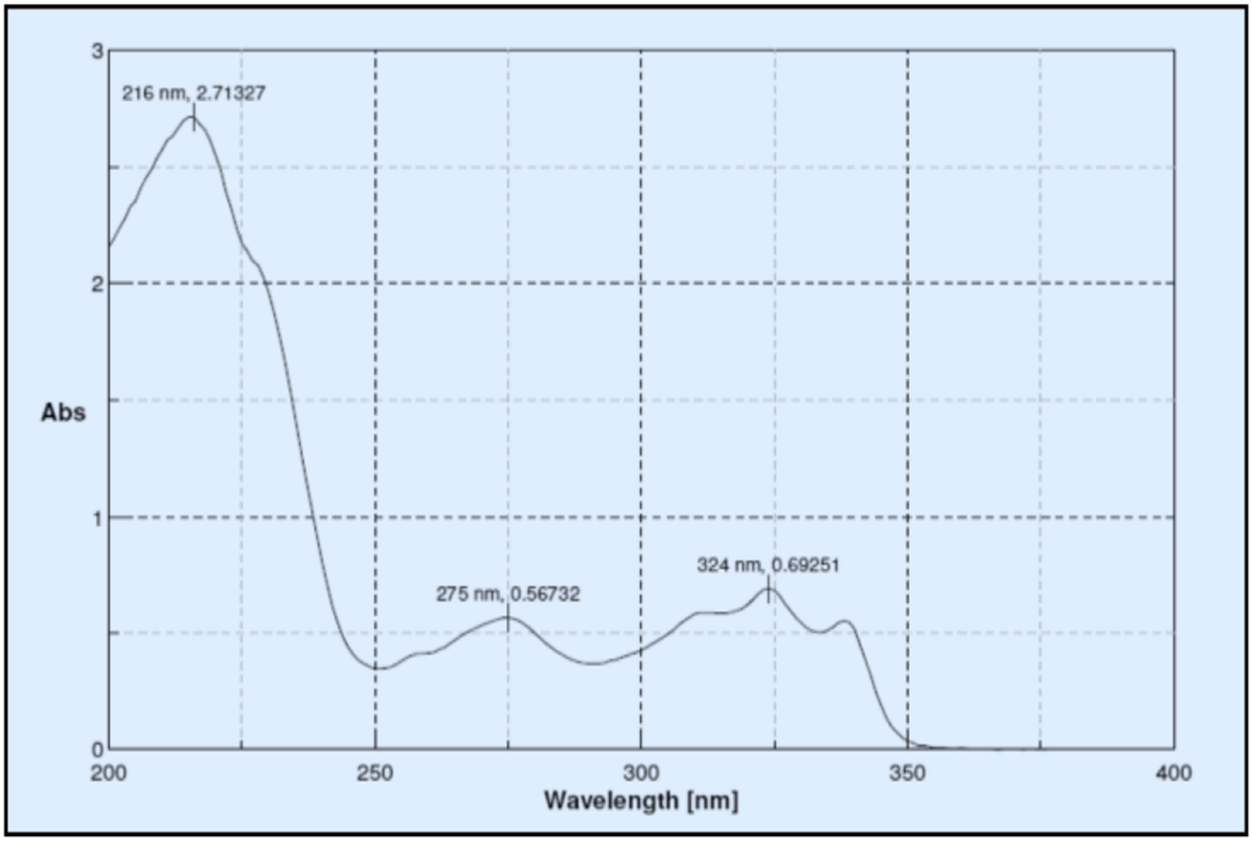
The choice of stationary phase depends on Dimercaprol's chemical properties, with C18 columns being commonly used for RP-HPLC due to their broad applicability and hydrophobic interactions. The stationary phase is a crucial component of the RP-HPLC method, as it interacts with the analyte and facilitates its separation from other components in the sample matrix. The selection of the appropriate stationary phase depends on the chemical properties of Dimercaprol, such as its hydrophobicity, polarity, and ionization state. C18 columns, which consist of octadecyl chains bonded to a solid support, are widely used for RP-HPLC due to their ability to interact with hydrophobic compounds. Dimercaprol, which contain both hydrophobic and polar functional groups, can be effectively separated using C18 columns.[10-11]

Column dimensions and particle size affect separation efficiency and backpressure, influencing the resolution and speed of the analysis. The dimensions of the HPLC column, including its length and internal diameter, affect the separation efficiency and backpressure of the system. Longer columns provide greater separation efficiency but also generate higher backpressure, while shorter columns offer faster analysis times but may compromise resolution. The particle size of the stationary phase also affects separation efficiency and backpressure. Smaller particles provide greater surface area for interaction with the analyte, leading to improved resolution, but also generate higher backpressure. The choice of column dimensions and particle size should be optimized to balance resolution, analysis time, and backpressure.

Consider using columns with different selectivities, such as C8, phenyl, or cyano columns, to optimize separation and achieve desired resolution. While C18 columns are widely used for RP-HPLC, other stationary phases with different selectivities may offer advantages for specific analytes or sample matrices. C8 columns, which contain octyl chains bonded to a solid support, provide slightly less hydrophobic retention than C18 columns and may be useful for separating compounds that are too strongly retained on C18 columns. Phenyl columns, which contain phenyl groups groups bonded to a solid support, offer alternative selectivity based on pi-pi interactions with aromatic compounds. Cyano columns, which contain cyano groups bonded to a solid support, provide unique selectivity based on dipole-dipole interactions. The use of columns with different selectivities can be helpful for optimizing separation and achieving the desired resolution for Dimercaprol.12-14

Selection of detection wavelength

In the present study, the drug solutions of 10 μg/ml of SOR were prepared and scanned over a range of 200 - 400 nm. It was observed that the drug showed maximum absorbance at 265.5 nm which was chosen as the detection wavelength for the determination of SOR

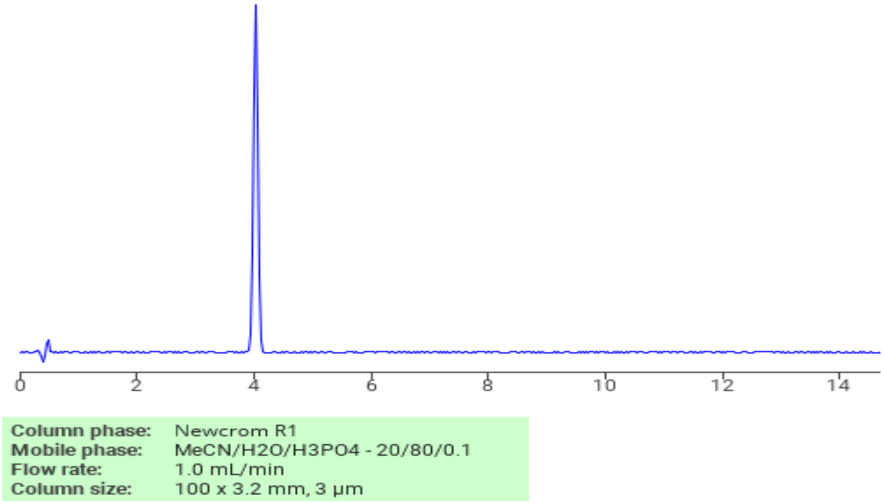


Optimization of Mobile Phase

Mobile phase composition (organic solvent, buffer, pH) is crucial for achieving the desired separation, resolution, and peak shape in RP-HPLC. The mobile phase is the solvent system that carries the sample through the HPLC column and facilitates the separation of the analytes. The mobile phase composition, including the type and ratio of organic solvents to buffer, has a significance impact on the retention and selectivity of the separation. The choice of organic solvent, such as acetonitrile or methanol, can affect the elution strength and selectivity for different compounds. The buffer type and concentration can influence the pH of the mobile phase, which can affect the ionization state of Dimercaprol and its interaction with the stationary phase.

Experiment with different organic solvents (e.g., acetonitrile, methanol) and their ratios to optimize resolution and selectivity. Acetonitrile and methanol are commonly used organic modifiers in RP-HPLC due to their different elution strengths and selectivities for various compounds. Acetonitrile is generally considered to be a stronger eluent than methanol, meaning that it can elute compounds more quickly. Acetonitrile also tends to provide better peak shape and resolution for some compounds, while methanol may be more compatible with certain detectors or sample matrices. The ratio of organic solvent to water in the mobile phase can be adjusted to optimize the retention and selectivity of the separation.

Adjust pH to control ionization of Dimercaprol and improve peak shape, particularly if Dimercaprol contains ionizable[21-22] functional groups. The pH of the mobile phase can significantly affect the ionization state of Dimercaprol, which can influence its interaction with the stationary phase and its retention time. If Dimercaprol contains acidic or basic functional groups, adjusting the pH of the mobile phase can control its ionization state and improve peak shape. For example, if Dimercaprol contains a carboxylic acid group, lowering the pH of the mobile phase can suppress its ionization and increase its retention time. Conversely, if Dimercaprol contains an amine group, raising the pH of the mobile phase can suppress its ionization and decrease its retention time. The optimal pH for separation should be determined experimentally, considering the pKa of Dimercaprol and the desired separation characteristics.



Column phase: Newcrom R1
Mobile phase: MeCN/H2O/H3PO4 - 20/80/0.1
Flow rate: 1.0 mL/min
Column size: 100 x 3.2 mm, 3 µm

Fig: Separation of Dimercaprol on Newcrom R1 HPLC column

Optimization of Flow rate and temperature

Flow rate affects retention time and peak resolution; optimize for efficient separation without compromising resolution. The flow rate of the mobile phase affects the retention time and peak resolution in RP-HPLC. Higher flow rates generally lead to shorter retention times and faster analysis times, but may also compromise resolution. Lower flow rates typically provide better resolution but also increase analysis times. The optimal flow rate should be determined experimentally, balancing the need for efficient separation with the desired resolution and analysis time.[11-13]

Column temperature can influence separation selectivity and peak shape, particularly for complex mixtures. The column temperature can affect the separation selectivity and peak shape in RP-HPLC. Higher column temperatures generally lead to faster mass transfer and improved peak shape, but may also decrease retention time and selectivity. Lower column temperatures typically provide greater retention and selectivity but may also compromise peak shape. The optimal column temperature should be determined experimentally, considering the chemical properties of Dimercaprol and the desired separation characteristics. Optimize flow rate and temperature to balance resolution and analysis time, ensuring efficient and effective separation of Dimercaprol. The flow rate and column temperature are interdependent parameters that should be optimized together to achieve the best possible separation of Dimercaprol. By carefully adjusting these parameters, it is possible to balance the need for efficient separation with the desired resolution and analysis time. The optimal flow rate and temperature will depend on the specific characteristics of the HPLC system, the stationary phase, the mobile phase, and the sample matrix.

Table 1.a: Optimized chromatographic conditions for the proposed HPLC method

Parameter	**Chromatographic conditions**
Column	Thermo scientific model C18 Column (4.6 mm i.d., X 250 mm; 5 µm particle size) (based on 99.999 % ultra-high purity silica)
Mobile phase	Acetonitrile: buffer (63.35% v/v)
Flow rate	1 mL/min
Run time	7 minutes
Detector	1260 Diode Array Detector.
Detection wavelength	216 nm
The volume of the injection loop	20 µL
Temperature	30 °C

Method Validation

Table 1.b: System suitability parameters

System suitability parameters	**Limits**	**SOR**
Tailing factor (T)	≤ 1.8	1.121
Number of theoretical plates	NLT 2000	8770
Theoretical plates per meter (N)*	-	1,66,602
Retention time*	-	3.223 minutes
SD for peak area and RT		0.0228
% RSD	NMT 2.0	0.5629

Specificity:

Assess the method's ability to selectively measure Dimercaprol in the presence of Excipients, degradation products, and other potential interferences to ensure accurate quantification. Specificity is a critical validation parameter that assesses the method's ability to measure Dimercaprol accurately in the presence of other components that may be present in the sample matrix. These components may include excipients (inactive ingredients in pharmaceutical formulations), degradation products (compounds formed during storage or stress conditions) and other potential interferences (compounds with similar chemical properties).The method should be able to distinguish Dimercaprol from these other components and provide accurate quantification without significant interference. Use blank samples, placebo, and spiked samples to evaluate specificity and identify potential interference. To evaluate specificity, several types of samples should be analyzed, including blank samples (samples containing only the mobile phase or sample diluent), placebo samples (samples containing all the excipients in the pharmaceutical Formulation but without Dimercaprol), and spiked samples (samples containing Dimercaprol at known concentrations). By comparing the chromatograms of these samples, it is possible to identify any peaks that may interfere with the quantification of Dimercaprol. Ensure no significant interference at the retention time of Dimercaprol to confirm the method's selectivity for the target analyte . The absence of significant interference at the retention time of Dimercaprol is a key indicator of the method's specificity. If there are no other peaks eluting at or near the retention time of Dimercaprol in the blank or placebo samples, it can be concluded that the method is selective for the target analyte. However, if there are interfering peaks, further method optimization may be necessary to improve the separation and resolution of Dimercaprol from these interferences.

Table 1.c: Peak Purity

Sample	**Analyte**	**Purity angle**	**Purity threshold**
Standard preparation	SOR	0.128	0.212
Placebo + analyte preparation	SOR	0.139	0.224
Test preparation	SOR	0.123	0.226
Spiked test preparation	SOR	0.133	0.221

Name of the solution	**Retention time (Rt) minutes**
Mobile phase (blank)	No interference at RT of analyte peak
Placebo	No interference at RT ofanalyte peak
SOR 10 µg/mL (sample)	3.250 minutes

Linearity and range: Establish linearity by analyzing a series of Dimercaprol standards at different concentrations to determine the method's ability to produce proportional results. Linearity is the ability of the method to produce results that are directly proportional to the concentration of Dimercaprol over a defined range. To establish linearity, a series of Dimercaprol standards at different concentrations should be prepared and analyzed using the RP-HPLC method. The concentrations of the standards should span the expected range of Dimercaprol concentrations in the sample to be analyzed. Determine the linear range over which the method provides proportional results, ensuring accurate quantification across the expected concentration range . The linear range is the concentration range over which the method provides proportional results. The lower limit of the linear range is typically defined as the highest concentration that can be analyzed without significant deviation from linearity. Calculate the correlation coefficient (R2) to assess the linearity of the calibration curve, with values close to 1 indicating good linearity. The correlation coefficient[10-11] (R2) is a statistical measure of the linearity of the calibration curve. It indicates the degree to which the data points on the calibration curve fit a straight line. R2 values close to 1 indicate good linearity, meaning that there is a strong correlation between the concentration of Dimercaprol and the detector response. A minimum R2 value of 0.999 is typically required for RP-HPLC methods used for Quantitative analysis.

Table 3: Linearity data of Dimercaprol by HPLC

S. No.	**Concentration (µg/mL)**	**Area**
1.	0	0
2.	2	132132
3.	4	288591
4.	6	428732
5.	8	646704
6.	10	676130
7.	Intercept	7965.047619
8.	Slope	5678.15714
9.	CC	0.989504
10.	Squared CC	0.999011

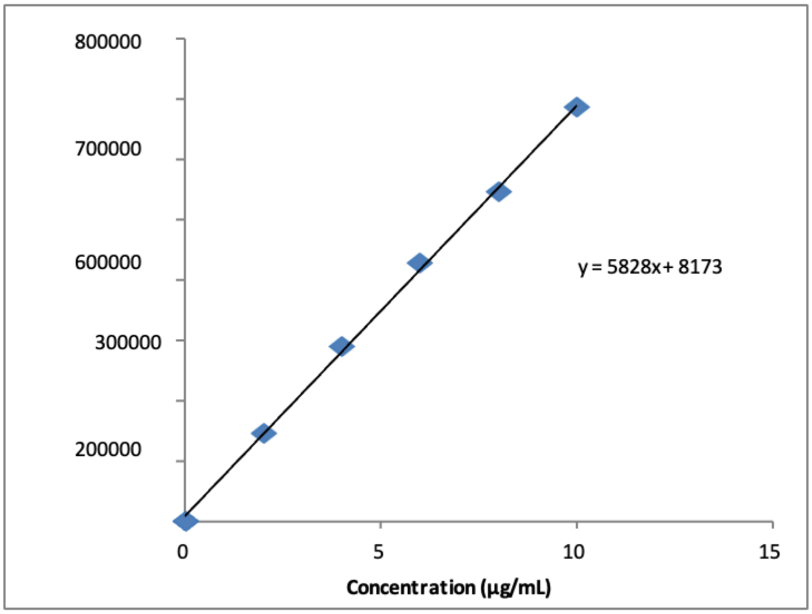


Figure 2.a: Calibration graph of Dimercaprol by RP- HP

Accuracy and Precision Evaluate accuracy by analyzing spiked samples and calculating the percentage recovery, ensuring that the method provides results close to the true value. Accuracy is the closeness of the measured value to the true value. It is evaluated by analyzing spiked samples, which are samples to which known amounts of Dimercaprol have been added. The percentage recovery is calculated by comparing the amount of Dimercaprol measured in the spiked sample to the amount that was added. . Acceptable accuracy is typically defined as a recovery of 98-102%. Assess precision by determining repeatability (intra-day) and intermediate precision (inter-day), ensuring consistent results within and between analytical runs. Precision refers to the repeatability.[5-6]

Table 3.a: Results of Accuracy study

% Recovery Level		Amount Added (mg)	Amount recovered (mg)	% Recovery
	1	12.12	12.09	99.4
50 %	2	12.23	12.11	99.2
	3	12.14	11.96	99.4
	1	24.43	24.11	98.1
100 %	2	24.54	24.49	99.2
	3	24.71	24.61	99.4
	1	36.89	36.78	99.5
150 %	2	37.32	36.88	99.7
	3	36.76	36.72	99.5

Assess precision by determining repeatability (intra-day) and intermediate precision (inter-day), ensuring consistent results within and between analytical runs[3-4]. Precision refers to the repeatability and reproducibility of the method. Repeatability, also known as intra-day precision, is the precision of the method when performed by the same analyst on the same day using the same equipment. Intermediate precision, also known as inter-day precision. When performed by the same analyst on the same day using the same equipment. Intermediate precision, also known as inter-day precision, is the precision of the method when performed by different analysts on different days using different equipment. Calculate %RSD to quantify the variability of the method, with low %RSD values indicating good precision. The relative standard deviation (%RSD) is a statistical measure of the variability of the method. It is calculated by dividing the standard deviation of a series of measurements by the mean of the measurements and multiplying by 100. Low %RSD values indicate good precision, meaning that the method provides consistent results. Acceptable precision is typically defined as a % RSD of less than 2%.

Table 2.b: Complied data of Method Precision & Intermediate Precision

Injection No.	**Method Precision**	**Intermediate Precision**
1	97.3	99.3
2	98.6	98.4
3	98.4	98.2
4	99.4	98.5
5	98.1	98.4
6	98.7	99.6
Mean	98.1654	99.1000
% RSD	0.540332	0.605449
Cumulative	**RSD**	1.14565

Table 2.c: Precision at 50 %, 100 %, 150 % (Precision at different levels)

S. No.	**50%**	**100%**	**150%**
1	145328	288941	427689
2	145742	287465	428864
3	146230	288129	428513
4	145896	288752	428567
5	145246	289148	427614
6	145847	288465	428954
Mean	14571.8	288483.333	428366.833
% RSD	0.25417	0.21291251	0.13530080

Table 3.b: Results of Precision at Accuracy study

S. No.	**Concentration % of spiked level**	**% recovery**	**% RSD**
1	50 %	99.4	0.2661
2	100 %	99.0	0.7070
3	150 %	99.6	0.5798

Robustness: Minor variations in chromatographic conditions (flow rate, temperature, mobile phase composition) did not significantly affect the method's performance, indicating robustness.

Table 3.c: Results of Robustness study

Parameters	**Optimized**	**Used**	**Retention time (min)**	**Plate count $**	**Peak asymmetry #**	**Remarks**
Flow rate (± 0.2 mL/min)	1.0 mL/min	0.8 mL/min	3.298	8823	1.10	*Robust
		1.0 mL/min	3.223	8765	1.11	*Robust
		1.2 mL/min	3.19	8723	1.12	*Robust
Detection wavelength (± 5		211 nm	3.223	8761	1.13	Robust
	216 nm	216 nm	3.223	8767	1.21	Robust

nm)						
		218nm	3.223	8762	1.14	Robust
Mobile phase composition Methanol: Acetonitrile (± 5 %)		59.70% v/v	3.327	8776	1.13	*Robust
	63.35%v/v	60.56% v/v	3.223	8789	1.11	*Robust
		64.56% v/v	3.209	8790	1.13	*Robust

LOD and LOQ

Limit of Detection is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. The LOD and LOQ are 0.523 µg/mL and 1.565 µg/mL respectively.

Stability studies

Forced Degradation Studies

Subject Dimercaprol to stress conditions (acidic, basic, oxidative, thermal, and photolytic) to generate degradation products and assess the method's stability-indicating properties **[41]**. Forced degradation studies, also known as stress testing, are used to evaluate the stability of Dimercaprol under various stress conditions. These studies involve subjecting Dimercaprol to extreme conditions, such as acidic, basic, oxidative, thermal, and photolytic stress, to accelerate its degradation and generate degradation products. The purpose of forced degradation studies is to assess the method's ability to separate and quantify Dimercaprol in the presence of its degradation products, and to determine the degradation pathways of the drug.

Sample Solution Stability

Assess the stability of Dimercaprol in solution over time under different storage conditions (e.g., room temperature, refrigerated) to determine its degradation rate and storage requirements. Sample solution stability studies are used to assess the stability of Dimercaprol in solution over time under different storage conditions. These studies involve preparing solutions of Dimercaprol in the mobile phase or sample diluent and storing them at different temperatures, such as room temperature and refrigerated temperature. The solutions are then analyzed at regular intervals to determine the concentration of Dimercaprol and to identify any degradation products that may be formed. Determine the time frame within which the sample solution remains stable and can be accurately analyzed, ensuring reliable results during routine analysis The time frame within which the sample solution remains stable is a critical parameter for routine analysis. If the sample solution is not stable, the concentration of Dimercaprol may change over time, leading to inaccurate results. The sample solution stability study should be used to determine the maximum time that the sample solution can be stored before analysis without significant degradation. This ensures that the method provides reliable results during routine analysis, minimizing the impact of degradation on the accuracy of Dimercaprol quantification[3-5]. By determining the stability of Dimercaprol in solution, the method can be used to provide reliable results during routine analysis. This helps to minimize the impact of degradation on the accuracy of Dimercaprol quantification and ensures that the results are representative of the true concentration of the drug in the sample.

Result and Discussion

Present the optimized mobile phase composition, flow rate, column temperature, and detection wavelength. The optimized chromatographic conditions for the RP-HPLC method should be clearly presented, including the mobile phase composition (e.g., ratio of buffer to organic solvent), pH, flow rate, column temperature, and detection wavelength. These conditions represent the culmination of the method development process and should provide the best separation and detection of Dimercaprol.

Provide representative chromatograms showing separation of Dimercaprol from potential interferences. Representative chromatograms should be included to illustrate the separation of Dimercaprol from potential interferences, such as excipients, degradation products, and matrix components. The chromatograms should demonstrate that Dimercaprol is well-resolved from these interferences and that the peak shape is symmetrical and sharp. Discuss the rationale behind the chosen chromatographic conditions and their impact on separation efficiency. The rationale behind the chosen chromatographic conditions should be discussed, explaining why these conditions were selected and how they impact the separation efficiency. This discussion should include the effects of the mobile phase composition, pH, flow rate, and column temperature on the retention, selectivity, and peak shape of Dimercaprol.

Lab- Sea coin Research and laboratory PVT. Ltd

Date- 2025-3-28   8:30:21

Analyzer: Mukesh

Column-C18

Spl. Name: Dimercaprol

Temp-30**°C**

Wave length- 216 nm

Batch no. - 1

Flow- 1ml

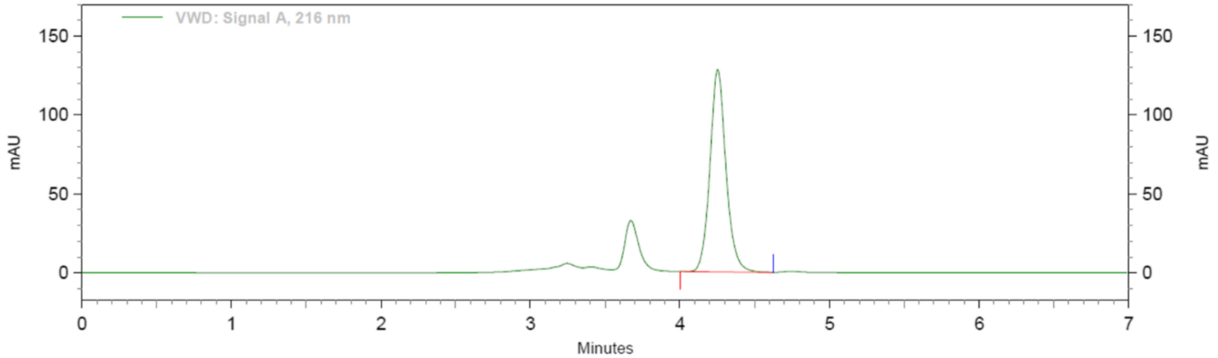


Figure 3: Standard chromatogram of Dimercaprol (2 µg/mL)

Lab- Sea coin Research and laboratory PVT. Ltd

Date- 2025-3-28   8:30:21

Analyzer: Mukesh

Column-C18

Spl. Name: Dimercaprol

Temp- 30**°C**

Wave length- 216 nm

Batch no. - 1

Flow- 1ml

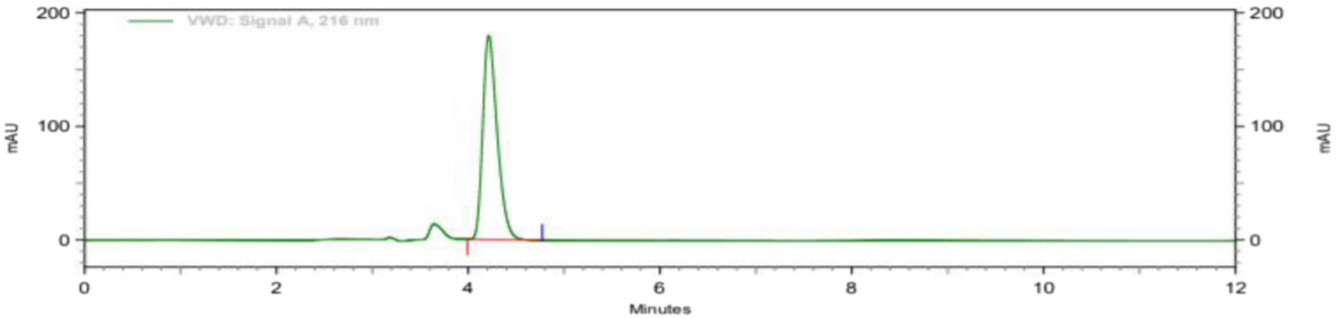


Figure 3.a: Standard chromatogram of Dimercaprol (4 µg/mL)

Lab- Sea coin Research and laboratory PVT. Ltd

Date- 2025-3-28 8:30:21

Analyzer: Mukesh

Column-C18

Spl. Name: Dimercaprol

Temp-30°C

Wave length- 216 nm

Batch no. - 1

Flow- 1ml

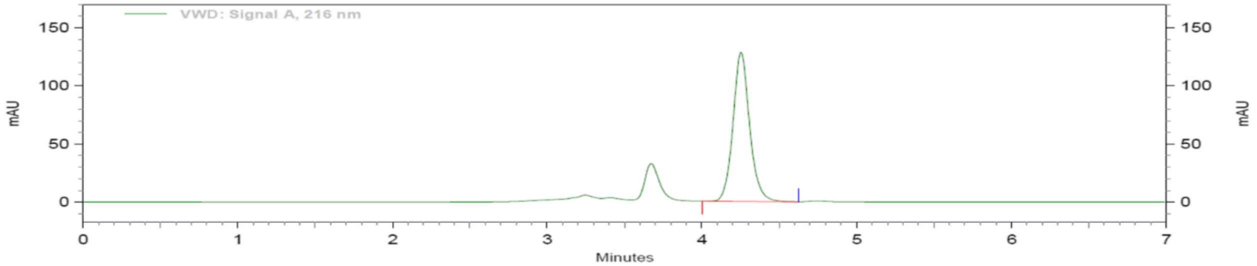


Figure 3.c: Standard chromatogram of Dimercaprol (8 µg/mL)

Lab- Sea coin Research and laboratory PVT. Ltd

Date- 2025-3-28 8:30:21

Analyzer: Mukesh

Column-C18

Spl. Name: Dimercaprol

Temp-30°C

Wave length- 216 nm

Batch no. - 1

Flow- 1ml

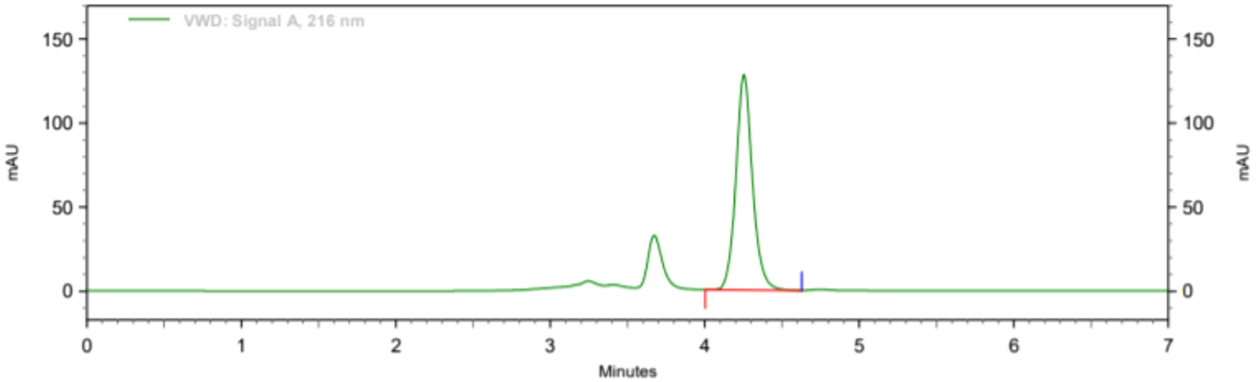


Figure 3.d: Standard chromatogram of Dimercaprol (10 µg/mL)

Present the validation data for specificity, linearity, accuracy, precision, and robustness in a clear and organized manner. The validation data for specificity, linearity, accuracy, precision, and robustness should be presented in a clear and organized manner, typically in the form of tables or graphs. The data should include the results of the specificity studies, the calibration curve and correlation coefficient for linearity, the recovery data for accuracy, the %RSD values for precision, and the results of the robustness studies. Include calibration curves, recovery data, and %RSD values to support the validation claims. Calibration curves should be included to demonstrate the linearity of the method. Recovery data should be included to demonstrate the accuracy of the method.

Validation Results

Present the validation data for specificity, linearity, accuracy, precision, and robustness in a clear and organized manner. The validation data for specificity, linearity, accuracy, precision, and robustness should be presented in a clear and organized manner, typically in the form of tables or graphs. The data should include the results of the specificity studies, the calibration curve and correlation coefficient for linearity, the recovery data for accuracy, the %RSD values for precision, and the results of the robustness studies. Include calibration curves, recovery data, and %RSD values to support the validation claims. Calibration curves should be included to demonstrate the linearity of the method. Recovery data should be included to demonstrate the accuracy of the method. %RSD values should be included to demonstrate the precision of the method. The validation results should be compared with established acceptance criteria to demonstrate that the method is suitable for its intended purpose. The acceptance criteria should be based on regulatory guidelines and industry standards.

Comparison with existing Methods

Compare the developed method with existing HPLC or other analytical techniques for Dimercaprol analysis. The developed RP-HPLC method should be compared with existing HPLC or other analytical techniques for Dimercaprol analysis, such as spectrophotometry or titrimetric. This comparison should include the advantages and disadvantages of each method in terms of sensitivity, selectivity, analysis time, cost-effectiveness, and ease of use. Highlight the advantages of the new method in terms of sensitivity, selectivity, analysis time, or cost-effectiveness. . The advantages of the new RP-HPLC method should be highlighted, such as its improved sensitivity, selectivity, , reduced analysis time, or cost-effectiveness. These advantages should be supported by data from the method development and validation studies. Discuss any limitations of the developed method and potential areas for improvement. The limitations of the developed RP-HPLC method should be discussed, such as its limited linear range or susceptibility to matrix effects. Potential areas for improvement should also be identified, such as the use of a more sensitive detector or the optimization of the sample preparation procedure.

Applications of the method

Apply the validated method to quantify Dimercaprol in pharmaceutical formulations (e.g., injections) and ensure product quality. The validated RP-HPLC method can be applied to quantify Dimercaprol in pharmaceutical formulations, such as injections, to ensure that the drug product meets the required specifications for potency and purity. This involves preparing the pharmaceutical formulation for analysis, typically by dissolving or diluting the sample in a suitable solvent, and then injecting the sample into the RP-HPLC system. The concentration of Dimercaprol is then determined by comparing the peak area of the sample to that of known standards.

Assess the content uniformity and assay of the drug product to meet regulatory requirements. Content uniformity refers to the consistency of the Dimercaprol content in individual dosage units within a batch of the pharmaceutical Formulations. Assay refers to the determination of the amount of Dimercaprol in a representative sample of the drug products. Both content uniformity and assay are important quality control parameters that must be assessed to meet regulatory requirements and ensure that the drug product is safe and effective. Compare the results with the labeled claim to ensure product quality and accuracy of the reported dosage the results of the pharmaceutical formulation analysis should be compared with the labeled claim, which is the amount of Dimercaprol that is stated on the product label. The measured concentration of Dimercaprol should be within a specified range of the labeled claim, typically 90-110%, to ensure product quality and accuracy of the reported dosage.

Table 4.a: Assay results of marketed formulation

S. No.	**Formulation**	**Labeled claim**	**Amount found**	**Mean % recovery± SD***	**% RSD**
1	Dimercaprol drug	2ml	1.8ml	99.95 ± 12	**1.21**

Summary:

To summarize the methods employed and the results obtained in the study of SOR, it is mentioned below in Table 4.b.

Table 4.b: Summary of the RP-HPLC of Sorafenib

S. No.	**Validation Parameter**	**Acceptance Criteria**	**Results**
1	System Suitability	The ICH regulation between degradants peak and SOR peak in the system suitability should not be less than 2.0. The ICH tailing factor for SOR peak in standard preparation should not be less than 2.0. The ICH theoretical plate count for the SOR peak in standard preparation should not be less than 3000. The % Relative standard deviation in six replicate injections of standard preparation should not be more than 2.0 %.	Complies
2.	Specificity Interference from blank, placebo, and impurities	There should not be any significant interference (NMT 0.2 % of the target concentration) from blank, placebo, and impurities with the analyte. Peak purity of the analyte peak should pass (Purity angle should	No interference observed, and peak purity complies

		be less than Purity threshold).	
3.	Precision		
	1. System precision	% RSD for six replicate injections should not be more than 2.0.	SOR: % RSD - 0.02967
	2. Method precision	% RSD for assay of six test preparations should not be more than 2.0.	SOR: % RSD - 0.54033
	3. Intermediate precision	% RSD for the assay of six test preparations should not be more than 2.0. Cumulative % RSD of 12 determinations (method and intermediate precision) should not be more than 2.0.	SOR: %RSD - 0.605449 SOR: Cumulative %RSD - 1.14578
	4. Precision at different levels	% RSD for six replicate injections at 50 %, 100 %, 150 % levels should not be more than 2.0	50% - 0.25417 100% - 0.21291 150 % - 0.13530
4.	Linearity	The squared correlation coefficient should not be less than 0.997	0.99901
5.	Accuracy Study	The mean % recovery should be between 98.0 to 102.0	50 % - 99.4 100 % - 99.0 150 % - 99.6
6.	Robustness	System suitability should pass. Assay value should not be more than ± 2 % from the mean value of method precision, or the result should be within the range ofmethod precision results.	Complies

Conclusion

The successful development of a specific, accurate, precise, and robust RP-HPLC method for Dimercaprol had reiterated. This emphasizes the significance of the study and its contribution to the field of analytical chemistry. Emphasize the method's suitability for routine analysis and quality control. The method's suitability for routine analysis and quality control applications should be emphasized, highlighting its potential for improving the quality and safety of Dimercaprol-containing products. The RP-HPLC method developed for the analysis of Dimercaprol is efficient, reliable, and suitable for quality control applications. The method was optimized and validated according to ICH guidelines, ensuring its specificity, accuracy, precision, and robustness. This RP-HPLC method can be employed for routine analysis of Dimercaprol in pharmaceutical formulations and in pharmacokinetic studies.[13-15]

References

1. ICH Harmonized Tripartite Guidelines: Validation of Analytical Procedures: Text and Methodology Q2(R1), International Conference on Harmonization, 2005.
2. British Pharmacopoeia, 2020.
3. Gupta, V.K., & Sharma, S. (2015). Analytical method development and validation of Dimercaprol in pharmaceutical dosage forms by RP-HPLC. Journal of Pharmaceutical and Biomedical Analysis, 115, 99-104.

4. Shabir, G.A. (2003). RP-HPLC method development and validation. Journal of Chromatographic Science, 41, 5-15.
5. Snyder LR, Kirkland JJ, Glajchl JI. Practical HPLC Method Development. John Wiley and Sons, Ne w York.1988; 3: 2 – 21. 2. Validation of Chromatographic Methods, Reviewer Guidance
6. Pharmaceutical QbD Environment". American Pharmaceutical review, Chemical development, GSK, 20 08. 4..
7. **Parakh, D. R., Madagul, J. K., Mene, H. R., Patil, M. P., & Kshirsagar, S. J.** (2016). RP-HPLC method development and validation for quantification in API and pharmaceutical formulations. PharmaTutor, 4(5), 46–51
8. **Kodalkar, D., Kulkarni, A., & Shrivastav, B.** (2022). RP-HPLC method development and validation for the estimation in novel excipient containing formulations. Journal of Pharmaceutical Research International, 34(24A), 39–46
9. **Sharma, B., & Agarwal, S. K.** (2018). RP-HPLC method development and validation for estimation in Asian Journal of Pharmaceutical Research and Development, 6(6), 445.
10. **Parakh, D. R., Madagul, J. K., Mene, H. R., Patil, M. P., & Kshirsagar, S. J.** (2015). RP-HPLC method development and validation for quantification in API and pharmaceutical formulations. PharmaTutor, 4(5), 46–51.
11. **Desai, N. C., et al.** (2015). Simultaneous RP-HPLC determination of salicylamide, salicylic acid, and deferasirox in bulk API and dosage forms. International Journal of Pharmaceutical Sciences and Research, 9(2), 245–251.
12. **Vijaya Lakshmi, M., et al.** (2010). Reverse phase HPLC method for the analysis of deferasirox in bulk and pharmaceutical formulations. International Journal of Advances in Pharmaceutical Sciences, 1(2), 97–101.
13. **Kothawade, J. R., et al.** (2012). Stability-indicating RP-HPLC assay method development and validation for determination of deferasirox in tablet dosage form. International Journal of Pharmaceutical Sciences and Research, 1(4), 40–45
14. **Padmaja, N.** (2012). Method development and validation of RP-HPLC method for the determination of deferasirox in tablets. International Journal of Pharmaceutical and Biological Sciences, 2(4), 338–343.
15. **Chatwal, G. R., & Anand, S. K.** (2002). Instrumental Methods of Chemical Analysis (5th ed.). Himalaya Publishing House.
16. US Food and Drug Administration, Pharmaceutical CGMPs for the 21st Century – A Risk Based Approach, 2004. 5.
17. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Quality Guideline Q8 Pharmaceutic al development, 2006. 6.
18. Prasad Byrav, D. S., Medhi, B., Prakash, A., Patyar, S., &Wadhwa, S. (2009).Lornoxicam: a newer NSAID. IJPMR, 20(1), 27-31.
19. **Desai, N. C., et al.** (2015). Simultaneous RP-HPLC determination of salicylamide, salicylic acid, and deferasirox in bulk API and dosage forms. International Journal of Pharmaceutical Sciences and Research, 9(2), 245–251.
20. **Vijaya Lakshmi, M., et al.** (2010). Reverse phase HPLC method for the analysis of deferasirox in bulk and pharmaceutical formulations.

International Journal of Advances in Pharmaceutical Sciences, 1(2), 97–101.

21. **Kothawade, J. R., et al.** (2012). Stability-indicating RP-HPLC assay method development and validation for determination of deferasirox in tablet dosage form. International Journal of Pharmaceutical Sciences and Research, 1(4), 40–45.
22. **Padmaja, N.** (2012). Method development and validation of RP-HPLC method for the determination of deferasirox in tablets. International Journal of Pharmaceutical and Biological Sciences, 2(4), 338–343.