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Analytical Method Development and Method Validation of Enoxaparin Sodium Using Uv-Visible Spectrophotometer

Shreesanth AN[1], Sowmya C[2], Srimathi R[3], Saniya Mehvish S[4], Sujatha K[5*]

[1]Department of pharmaceutical chemistry, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai-16
Mail: shreesanth868@gmail.com
[2]Department of Pharmaceutics, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai-16
Mail: drcsowmya@sriramachandra.edu.in
[3]Department of pharmaceutical chemistry, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai-16,
Mail: drsrimathi.r@sriramachandra.edu.in
[4]Department of pharmaceutical chemistry, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai-16
Mail: saniyamehvish2810@gmail.com
[5*]Department of pharmaceutical chemistry, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai-16,
Mail: sujatha.k@sriramachandra.edu.in

ABSTRACT: Enoxaparin Sodium is a well-known low molecular weight heparin that is used for the prevention and management of various thromboembolic disorders. This study was conducted with an objective of developing a rapid, accurate, and economical UV-Visible spectrophotometric method for quantification of Enoxaparin Sodium in formulation aligning with principles of affordable healthcare and sustainable manufacturing by minimizing solvent use and analytical costs. Spectral analysis was performed using 0.1 M Hydrochloric Acid as solvent, with λmax of 232 nm.

It was observed that the method had good linear relationship within the concentration range from 5 µg/mL to 25 µg/mL with a correlation coefficient value of 0.9980. Validation of the test was done in accordance with the guidelines of ICH Q2 (R1).The precision of the method was confirmed by low %RSD values for both intraday (0.4750%) and interday studies (<0.5%). The method demonstrated good repeatability with %RSD of 0.4663%. Accuracy studies showed satisfactory recovery in the range of 99–102%, indicating the reliability of the method thereby supporting quality assurance in pharmaceutical supply chains.

It was discovered that the limits of detection (LOD) and quantitation (LOQ) were 0.1542 µg/mL and 0.4642 µg/mL, respectively, indicating sufficient sensitivity. Robustness testing showed that small variations in wavelength (±2 nm) did not significantly affect the results, with % deviation less than 2%.his cost-effective and eco-friendly approach contributes to responsible consumption and supports innovation in analytical infrastructure for resource-limited settings.

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INTRODUCTION

Enoxaparin sodium, commonly known by the brand names Lovenox and Clexane, is a widely used anticoagulant (blood thinner) medication belonging to the low molecular weight heparin (LMWH) family. Treatment and prevention of dangerous blood clots are its main uses.[1-2]

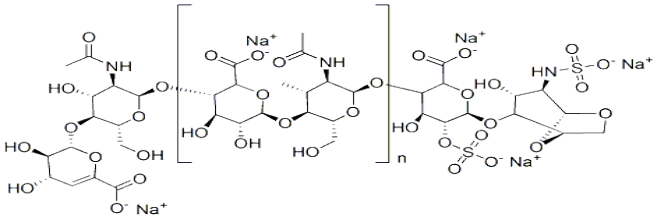
It is an important factor involved in the prevention and management of thromboembolic disorders, including disorders such as deep vein thrombosis (DVT) and pulmonary embolism (PE), as well as those related to cardiovascular diseases such as myocardial infarction. Due to its proven clinical effectiveness, predictable pharmacokinetic profile, and reduced risk of adverse effects compared with conventional unfractionated heparin (UFH), enoxaparin has become one of the most commonly prescribed anticoagulants worldwide.[3-5]

The development of enoxaparin sodium represents a major advancement in anticoagulant therapy. Traditionally, unfractionated heparin was widely used for the management of thrombosis; however, it had several limitations including variable patient response, the need for continuous laboratory monitoring, and a higher risk of bleeding and thrombocytopenia.[5-8]

Low molecular weight heparins such as enoxaparin were developed to overcome these limitations, thereby offering a safer and more convenient therapeutic alternative. Enoxaparin has been extensively studied in clinical trials and is now incorporated into international guidelines for the management of venous thromboembolism and acute coronary syndromes.[9-11]

Chemically, enoxaparin is a complex mixture of sulfated polysaccharide chains that exert anticoagulant activity by interacting with natural inhibitors in the blood coagulation system.

ENOXAPARIN SODIUM



MATERIALS AND METHODS

Materials and insturuments:

0.1M Hcl, Enoxaparin sodium working reference standard, the UV analysis was carried out using SHIMADZU UV-1780. The UV visible spectrophotometer employs a deuterium lamp and is paired with spectra treats software for analysis.[12-14]

UV spectrophotometric method development and validation

Preparation of standard solution:

Approximately 10mg of standard drug was weighed and transferred into 100ml standard flask. The drug is dissolved with 100ml of 0.1 M HCl, producing a first stock solution at a concentration of 100 µg/ml. From this pipette out 1ml of the above solution and dilute using 0.1 M HCl to produce a final concentration of 10µg/ml in a 10ml volumetric flask as a working.[15]

Method validation

Validation of the methods was done following the ICH guidelines for method validation found in document number Q2 RI from The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Linearity:

The ability of the method is determined by preparing the linear concentration of standard working dilutions (5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml and 25 µg/ml) and measuring the absorbance of each working solution in a particular wavelength. Limit of acceptance is calculated by measuring R2 by plotting the linearity graph and obtaining slope.

Limit of acceptance should be R2 ≤ 0.999

Precision:

Intraday precision, also referred to as repeatability, measures the ability of the method to be consistent on the same day. The intraday precision evaluates the difference in results that arise from analyzing several samples by same analyst in the same equipment. For inter day precision three consecutive days of standard solutions were analyzed using the suggested UV procedure of measuring the absorbance for the standard working solution (concentration-15µg/ml).

Limit of acceptance should be < 2%

Limit of detection and Limit of Quantification (LOD & LOQ):

Calculation of LOD and LOQ was made based on the signal-to-noise ratio. The smallest quantity of the analyte present in the sample is referred to as LOD while LOQ is the smallest quantity of the analyte that can be quantified. Both LOD and LOQ have been calculated using the slope and standard deviation of the response. The standard deviation has been calculated using the calibration curve.

Accuracy:

Accuracy of the test was established through recovery experiments that were carried out using three different concentrations (80%, 100% and 120%). Replicate analysis for each concentration was done thrice, and the percentage recovery together with %RSD were calculated, results found to be within acceptable limits (98%-102%).

Robustness:

Robustness was assessed by deliberately varying conditions during the analysis with respect to wavelengths (±2 nm) and different values of λmax. Robustness of the method is confirmed since %RSD was found to be below 2.0%.

Repatability:

The repeatability of the experiment was determined through measuring absorbance six times for the working concentration of a standard solution using the same experimental conditions. The value of %RSD for the absorbance measurements is less than 2.0%, which means that the experiment is repeatable.[16-23]

RESULTS AND DISSCUSSION

Method validation for UV method:

Determination of λ max:

The λ max of Enoxaparin sodium was found to be 232 nm (Abs 0.235) in 0.1M HCl solution as the solvent.

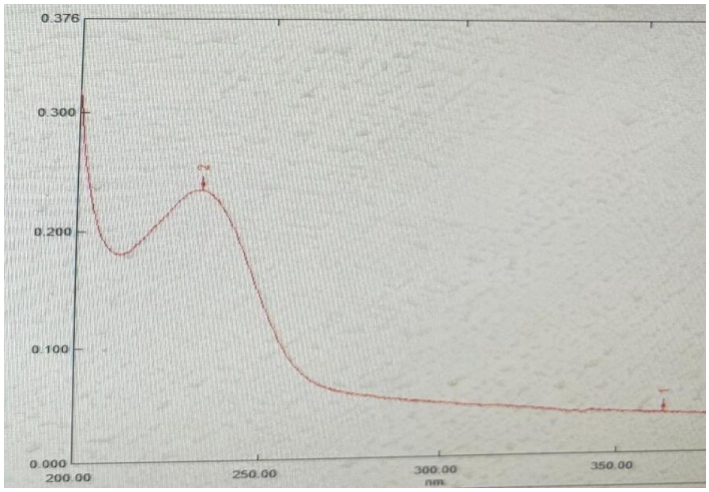


Figure 1: Lamada Max of enoxaparin sodium.

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Linearity:

Table 1: Linearity of Enoxaparin sodium by UV method.

S.NO	Concentration(µg/ml)	Absorbance
1	5	0.137
2	10	0.284
3	15	0.417
4	20	0.570
5	25	0.741
6	Correlation coefficient- r^2	0.9980

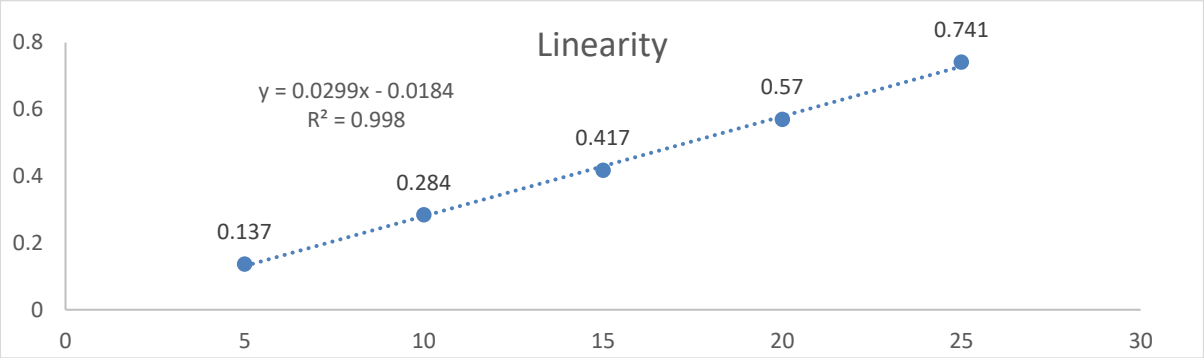

Figure: 2 Linearity graph of Enoxaparin sodium by UV method.

Enoxaparin sodium showed a linear relationship within the concentration range of 5 to 25 µg/ml. A very good linearity (r2 = 0.9980) was observed. The equation for linearity was y = 0.0299x+0.0184

Interday precision

The Interday precision results of Enoxaparin sodium by UV method as shown in table 2.

Table :2 Inter-day precision of Enoxaparin sodium by UV method.

Day	Absorbance	Mean	Standard deviation	% RSD
Day-1	0.421 0.419 0.421	0.42033	0.0011	0.2747
Day-2	0.426 0.423 0.425	0.42466	0.0015	0.3596
Day-3	0.419	0.42066	0.0020	0.4948

- The values of %RSD for all three days are considerably less than 1%, indicating high precision of the measurements.
- The mean absorbance values remain almost constant for all three days with slight variation.

- The standard deviations are also low, again highlighting the precision of UV absorbance measurements.

Intraday precision:

The Intraday precision results of Enoxaparin sodium by UV method as shown in table 3.

Table 3: Intraday precision of Enoxaparin sodium by UV method.

S.NO	Conc (µg/mL)	Absorbance	Mean	SD	%RSD
1	15	0.419	0.421	0.002	0.4750
2	15	0.423			
3	15	0.421			

The %RSD value of 0.4750 % suggests high precision in intra-day analysis. Any %RSD value that is below 1% is regarded as acceptable in most analytical methods since it suggests low variability and high repeatability. The mean absorbance value of 0.421 is comparable to the individual absorbance values with very little variance.

Repeatability:

The Repeatability results of Enoxaparin sodium by UV method as shown in table 4.

Table 4: Repeatability of Enoxaparin sodium by UV method:

S.NO	Conc (µg/mL)	Absorbance	Mean	SD	%RSD
1	15	0.423	0.4216	0.0019	0.4663
2		0.421			
3		0.423			
4		0.419			
5		0.424			
6		0.420			

The %RSD value of 0.4663 % indicates good Repeatability. In analytical techniques, a %RSD value of less than 1% is typically regarded as acceptable.

Limit of detection and Limit of quantification by UV method

The method demonstrates good sensitivity. The LOD was found to be (0.1542) indicating that the analyte can be detected at low concentration. LOQ was founded to be (0.4642), proving that the technique is able to detect the analyte at this concentration level with good accuracy and precision. Thus, these findings indicate that the UV spectrophotometric technique has proven itself suitable for the detection and quantitative determination of the analyte at low concentration levels.

Accuracy:

The Accuracy results of Enoxaparin sodium by UV method as shown in table 5.

Table 5: Accuracy of Enoxaparin sodium by UV method.

PERCENTAGE	Absorbance	% RECOVERY	AVERAGE
80%	0.219	99.12%	99.9%
	0.224	101.25%	

	0.220	99.62%	
100%	0.279	99.04%	100.33%
	0.283	100.08%	
	0.283	100.08%	
120%	0.349	102.33%	102.99%
	0.354	103.75%	
	0.351	102.91%	

The Accuracy of Enoxaparin sodium by the UV method were evaluated at three different concentration levels: 80%, 100%, and 120%.

The results show that the UV technique for the determination of Enoxaparin sodium concentration is highly accurate and precise for all concentration levels tested, since the mean values of the recovery percentages were approximately 100%. The uniformity of the recovery percentage among the various concentrations implies that the technique can be applied effectively in the analysis of the drug concentration.

Robustness:

The Robustness results of Enoxaparin sodium by UV method as shown in table 6.

Table 6: Robustness of Enoxaparin sodium by UV method:

The optimized wavelength **(**Λmax) was 232nm-0.235 abs

	Wavelength	Absorbance	% Difference
Λ-2nm	230	0.231	1.7021
Λ+2nm	234	0.238	1.2765

The absorbance values showed only slight variation with deliberate changes in wavelength ±2nm. All % differences were found to be below2% indicating that small wavelength variation does not affect significantly. The method is robust with respect to wavelength variation.

CONCLUSION

The UV-Visible spectrophotometry method developed is new, simple, accurate and economical for the analysis of Enoxaparin Sodium in its pharmaceutical formulation. It is found to be linear, precise, accurate and robust in nature.

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