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Development And Validation of UV-Visible Spectrophotometer Method for Estimation of Enoxaparin Sodium Spiked in Human Plasma

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Abstract
A simple and rapid UV method was developed for the estimation of Enoxaparin sodium in human plasma by extracting the Enoxaparin sodium from spiked human plasma using water as diluent. The absorption maximum for the drug was observed at 231nm in water. The calibration curve was linear in the range of 10 - 650 µg/ml. The % recovery of the proposed method was found to be 93.81 - 97.34 %. The method was successfully applied for estimation of Enoxaparin sodium in bulk and injectables.

Keywords
Enoxaparin sodium, UV spectrometry, method development, validation.

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1. INTRODUCTION:
Enoxaparin sodium belongs to the group of low molecular weight heparin [1] (figure 1). It is official in EP, IP [2], BP [3] and USP [4]. The IUPAC name is tetrasodium;(2R,3R,4S)-2-[(2R,3S,4R,5R,6S)-5-acetamido-6-[(1R,2R,3R,4R)-4-[(2R,3S,4R,5R,6R)-5-acetamido-6-[(4R,5R,6R)-2-carboxylato-4,5-dihydroxy-6 [[(1R,3R,4R,5R)-3-hydroxy-4-(sulfonatoamino)-6,8-dioxabicyclo[3.2.1]octan-2-yl]oxy]oxan-3-yl]oxy-2-(hydroxymethyl)-4-methyloxan-3-yl]oxy-6-carboxylato-2,3-dihydroxycyclohexyl]oxy-4-hydroxy-2-(sulfooxymethyl)oxan-3-yl]oxy-3,4-dihydroxy-3,4-dihydro-2H-pyran-6-carboxylate. The molecular formula is (C26H42N2O37S5) and its molecular weight is 1134.9g/mol. It is white amorphous powder, freely soluble in water and insoluble in organic solvents. The mechanism of action of Enoxaparin sodium is anti-thrombin dependent. Enoxaparin sodium is an anticoagulant that helps prevent the formation of blood clots. It is used to treat or prevent a type of blood clot called deep vein thrombosis (DVT)[5], which can lead to blood clots in the lungs. Enoxaparin sodium has greater bioavailability and longer half-life than unfractionated heparin, permitting less frequent subcutaneous administration. Enoxaparin sodium injections are available in market with brand name CLEXANE, TENOXA-0.6, SANKARIN, EVAPARIN and many other brands are available in the market. In the present study an attempt was made to develop a simple, precise, and accurate method for the estimation of drug in pharmaceutical dosage form and validate as per International Conference on Harmonization (ICH) guidelines [6].

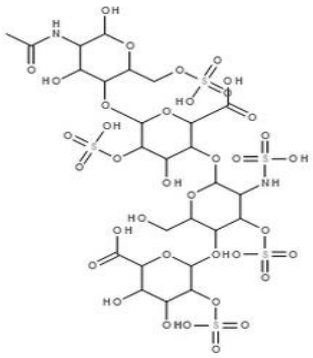

Figure 1: Enoxaparin sodium

2. MATERIALS AND METHODS:

2.1. Instrument:

A double beam UV-Visible spectrophotometer "ELICO SL 210" and double beam UV-Visible spectrophotometer "SYSTRONIC 2203".

2.2. Chemicals:

Enoxaparin sodium was obtained as gift sample from pharmaceutical industry, Hyderabad, Telangana, India. CLEXANE injection (20mg/0.2ml) was purchased from local market. Human plasma was procured from the healthy volunteers. Analytical reagents such as acetonitrile, water was used.

3. EXTRACTION OF ENOXAPARIN SODIUM FROM PLASMA:

One microlitres of plasma was transferred into a centrifuge tube and spiked with fixed aliquots of working standard solution of Enoxaparin sodium and vortexed, 5 ml of acetonitrile was added and sonicated for 10 min. The above solution was centrifuged for 15 min at 1500 rpm. The supernatant layer was removed, and the absorbance was measured at 231 nm.

4. METHOD DEVELOPMENT:

4.1. Preparation of standard solution:

Standard solution of Enoxaparin sodium was prepared by taking 10mg in 10ml volumetric flask containing distilled water and the volume was made up to the mark with distilled water (stock solution). From the above solution 1ml was pipetted out into 10ml volumetric flask and made up to the mark with water to get 100µg/ml.

4.2. Selection of wavelength:

Solutions of 100µg/ml of Enoxaparin sodium were prepared and the solution was scanned in the spectrum mode from 200nm to 400nm. The maximum absorbance of Enoxaparin sodium was observed at 231nm (figure 2).

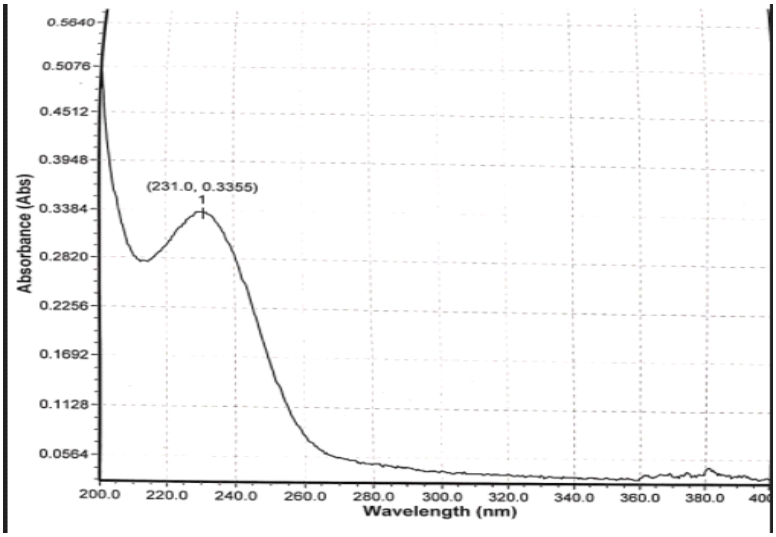

Figure 2: UV spectrum of standard solution (100µg/ml).

4.3. Optimization of parameters:

All the optimization parameters at room temperature. Enoxaparin sodium was found to yield clear colorless solution with distilled water, showing maximum absorbance at 231nm. Different concentrations and different volumes were tried for all the solvents. The optimum concentration and volume were selected on the basis of their ability to give maximum absorbance.

4.4. Preparation of calibration curve:

Standard stock solution of Enoxaparin sodium was further diluted to get concentration in the range of 10-650µg/ml. The resultant absorbance of the solution was measured at 231nm against distilled water as blank.

5. METHOD VALIDATION:

The developed method was validated according to ICH guidelines. The proposed method was validated in terms of specificity, linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ.

5.1. Specificity:

Definition: The ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components (Figure 3).

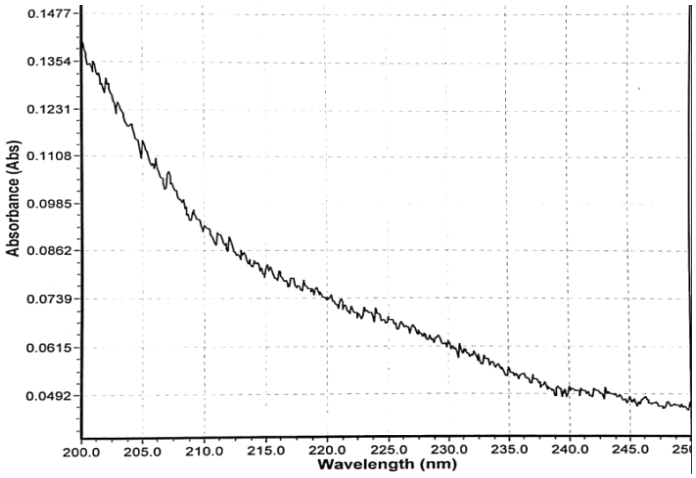

Figure 3: Blank spectrum

5.2. Linearity:

Definition: Ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

The proposed spectroscopic method was found to be linear in the range of 10-650µg/ml with correlation coefficient was 0.9993 (figure 4), slope 0.0031 and intercept 0.014 was shown in Table 1.

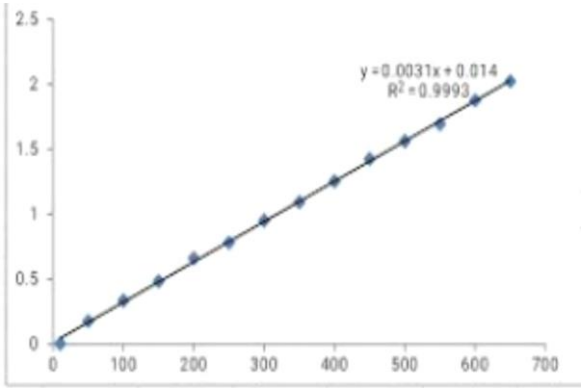

Figure 4: Linearity study

S. NO.	PARAMETERS	RESULTS
1.	Absorbance maximum(nm)	231
2.	Linearity and range(µg/ml)	10-650µg/ml
3.	Slope	0.0031
4.	Correlation coefficient	0.9993
5.	Y-intercept	0.014

Table 1: Results of quantitative determination of Enoxaparin sodium.

5.3. Precision:

Definition: Degree of agreement between a series of measurement obtained from multiple sampling of same homogenous sample.

The precision of the proposed method was estimated in terms of inter-day and intra-day precision wherein the method was repeated for 6 times respectively. The results shown in table 2 indicating %RSD of less than 2% each level clearly indicate that the proposed method was precise enough for the analysis of drug.

%RSD = (SD of measurement/mean value of measurement) x 100.

Concentration	Intraday precision (%RSD)	Inter-day precision (%RSD)	
300µg/ml	0.0885%	Day 1	Day 2
		0.1695%	0.3568%

Table 2: Results of precision studies.

5.4. Accuracy:

Definition: Closeness of test results obtained by that procedure to the true values [7].

The accuracy of the method was determined by performing recovery studies by spiking standard solution to that of the human plasma at three different levels i.e., 50%, 100%, 150%. Values of %recovery greater than 90-125% indicate that the proposed method was accurate for the analysis of drug and the results were reported in Table 3.

%Recovery = (Amount found/ Amount added) x 100

Level	Amount of standard added (µg/ml)	Pre-analysed sample (µg/ml)	% Recovery
50%	50	200	93.81%
100%	100	200	95.59%
150%	150	200	97.34%

Table 3: Results of accuracy studies.

5.5. Robustness:

Definition: Deliberate changes in the method are made such as wavelength. The ±nm from the fixed wavelength.

The robustness of the proposed method was evaluated by changing wavelength. The %RSD was calculated. The low values of %RSD obtained after small deliberate changes in method indicates that the method was robust, and the results were presented in Table 4.

S. NO.	Concentration	Wavelength	% RSD
1.	300µg/ml	229nm	0.2923%
2.		230nm	0.488%
3.		232nm	0.1053%
4.		233nm	0.1072%

Table 4: Results of robustness studies.

5.6. Ruggedness:

Definition: The degree of reproducibility of the results obtained by the analysis of the sample under a variety of conditions such as different analyst and different instrument.

The ruggedness of the proposed method was evaluated by varying conditions different analyst and different instrument ("ELICO SL 210" and "SYSTRONIC 2203"). The %RSD was calculated. The low values of %RSD obtained by changing the conditions indicates that the method was rugged, and the results were presented in Table 5.

S. NO	Concentration	Analyst	% RSD	Instrument	% RSD
1.	300µg/ml	Analyst 1	0.0553%	Instrument 1 (ELICO)	0.0537%
2.		Analyst 2	0.0277%	Instrument 2 (SYSTRONIC)	0.0401%

Table 5: Results of ruggedness studies.

5.7. LOD:

Definition: It is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated under the stated experimental condition.

LOD = 3.3 x SD/ slope.

The LOD of the proposed method was found to be 0.8952µg/ml.

5.8. LOQ:

Definition: It is the lowest amount of analyte in the sample which can be quantitatively determined with acceptable precision and accuracy under the stated experimental conditions.

LOQ = 10 x SD/ slope.

The LOQ of the proposed method was found to be 2.7129µg/ml

5.9. Analysis of injectable formulation studies (Assay):

The CLEXANE injection of Enoxaparin sodium was prepared by taking 10mg/0.1ml in 10ml volumetric flask and diluted with water to obtain 1000µg/ml. From the above solution pipette out 2ml in 10ml volumetric flask to obtain 200µg/ml. The absorbance of the dilution was observed at selected wavelength and the concentration was obtained from calibration curve method.

The % assay is calculated by using the following formula

% Assay = [(Absorbance of the sample/ Absorbance of the standard) *(Concentration of the standard/ Concentration of the sample)] *100.

= 103%.

6. CONCLUSION:

A simple and selective Spectrophotometric method was developed for the estimation of Enoxaparin sodium in bulk and injectables formulation. The developed method was validated as per ICH guidelines.

7. ACKNOWLEDGEMENT:

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