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Development and Validation of RP-HPLC Method for the Determination of Enoxaparin in Bulk Drug and Pharmaceutical Dosage Form.

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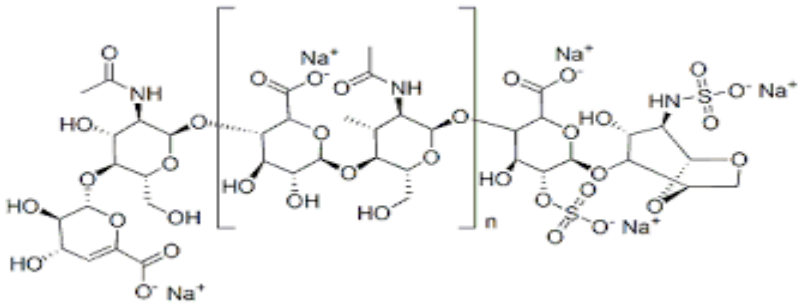
ABSTRACT

The reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed to quantify Enoxaparin in raw material and pharmaceutical formulations using C18 analytical reverse-phase column. Enoxaparin API was used as an internal standard. Mobile phase consisted of methanol-water (10:91 v/v), pumped at a flow rate of 0.9 ml/min at ambient temperature and the retention time was about 6.28 min with symmetrical peaks. (enoxaparin) was detected by ultraviolet absorbance at 232 nm with no interference of commonly used excipients. The method was linear over the concentration range 0.5–25 µg/mL (R2 = 0.9995). The limit of detection of enoxaparin was 0.1804 µg/mL and the limit of quantitation was 0.546 µg/ml. The results obtained showed a good agreement with the declared contents in case of pharmaceutical formulations. The proposed method is rapid, accurate, economical and selective and it may be used for the quantitative analysis of metformin in xomparin injection because of its sensitivity and reproducibility.

Keywords: RP-HPLC; metformin; xomparin injection.

INTODUCTION



STRUCTURE OF ENOXAPARIN

Enoxaparin sodium belongs to the group of low molecular weight heparins chemically it is 6-[5-acetamido-4,6-dihydroxy-2-(sulfooxymethyl)oxan-3-yl]oxy-3-[5-(6-carboxy-4,5-dihydroxy-3 sulfooxyoxan-2-

yl)oxy-6-(hydroxylmethyl)-3-(sulfoamino)-4-sulfooxyoxan-2-yl]oxy-4-hydroxy-5-sulfooxyoxane-2-carboxylic acid1.The empirical formula of Enoxaparin sodium is C42H59N3Na4O35S2 and its molecular weight is 1322.000 g/mol, Brands: Lovenox Injection (150mg), structural formula (Fig. 1). Enoxaparin sodium is a white crystalline powder. It is practically soluble in water and freely soluble in 0.1 N HCl. It also dissolves freely in Ethanol, methanol, dissolves sparingly in Phosphate Buffer pH 6.8. Enoxaparin sodium has a greater bioavailability and longer half-life than unfractionated heparin, permitting less frequent subcutaneous administration. In well controlled trials in surgical patients at high risk of deep venous thrombosis (DVT)2. In clinical studies, Enoxaparin sodium has also prevented coagulation of extracorporeal circulation, maintaining the patency of the circuit in patients undergoing haemodialysis. Thus, Enoxaparin sodium represents an effective alternative in the prophylaxis and treatment of thrombosis, with the convenience of less frequent administration than unfractionated heparin and the possible advantage of a lesser propensity for bleeding complications. Enoxaparin sodium injections are available in market with brand name Lovenox (150 mg), Critnox (60 mg), Venoxtaj (80 mg) and many other brands are available in the market. Literature survey reveals that very few methods are available for the estimation of Enoxaparin sodium alone or in combination with other drugs and in its dosage form. 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].

EXPERIMENTAL

Table 1: Drug and Drug Supplier

Name of Drug	Drug Supplier
Enoxaparin	SWAPNROOP DRUGS AND PHARMACEUTICALS

List of reagents & chemicals used

Table 2: List of Reagents and Chemicals used

Sr. No.	Name of chemicals	Manufacturer.
1.	Acetonitrile (HPLC grade)	Merck Ltd., India
2.	Methanol (HPLC grade)	Merck Ltd., India
3.	0.05% OPA (HPLC grade)	Merck Ltd., India
4.	water (HPLC grade)	Merck Ltd., India

2 Selection of formulation: From the literature survey and market survey we selected Maxide formulation for work.

Marketed Preparation**:**

Table No.3: List of brand names of combined formulations of Enoxaparin

Sr. No	Brand name	Formulation	Available strength	Address of manufacturer
1.	Xomparin	Injection	Enoxaparin sodium I.P 60mg	Care Formulation Labs

The marketed preparation was obtained from local market and is referred here after in this thesis by the name as such.

- **Instruments:**

The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector, (DAD) &Gradient Detector. Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 250mm; 5µm), and UV730D Absorbance detector and running chemstation 10.1 software.

Method Development Standard stock solution

Accurately weighed Enoxaparin sodium (5 mg) was transferred to a 10 mL volumetric flask, dissolved in 100 ml diluent = Methanol+0.05 % OPA (100MLWATER) (10+90). The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark 100ml to get a concentration of 500 µg/ml.

Selection of wavelength for linearity Solutions of 500µg/ml of Enoxaparin sodium were prepared and the solution was scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of Enoxaparin sodium was observed at 232.0 nm. Enoxaparin sodium showed linearity in the concentration range of 5 to 25 µg/ml at their respective maxima. They were scanned in the wavelength range of 200-400 nm and the overlain spectrum was obtained (Fig.-2).

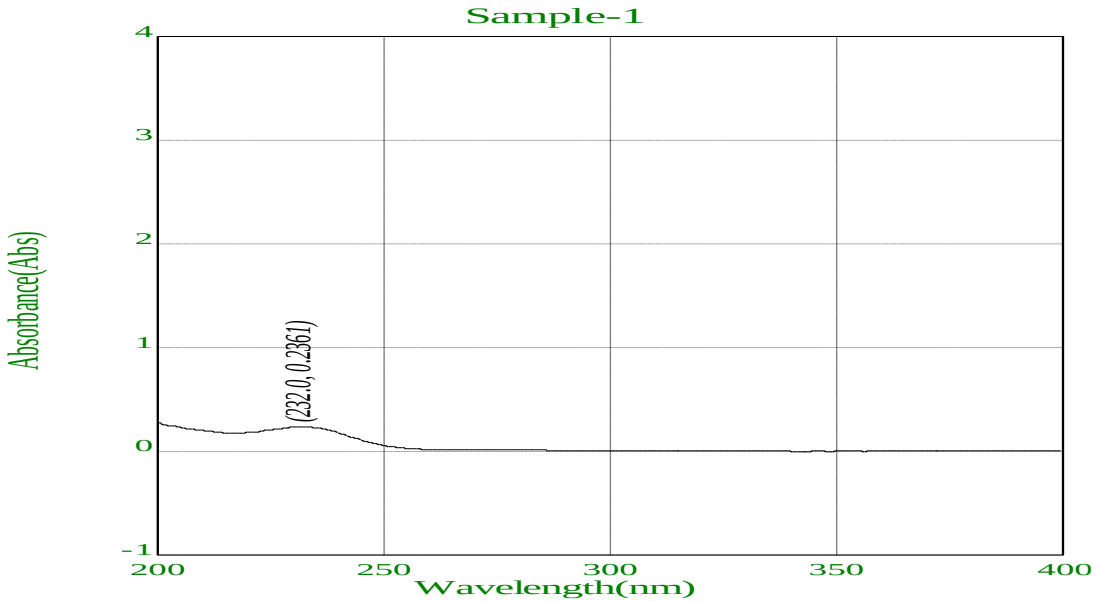


Fig 2: Determination of λ_{max} of Enoxaparin sodium

Method validation

Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results was recorded. The results of linearity are reported in Table 1.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of Enoxaparin sodium to pre-analysed sample solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicates of 5 concentrations levels. The results of linearity are reported in Table 1

Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different

analyst. The value of SD and %RSD are less than 2 indicate the precision of method. Result of precision shown in Table 4.

Analysis of injectable formulation

Xomparin Injection 60mg in 0.6 ml. (0.1ml) was taken in 150 ml volumetric flask. Then 20 ml was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark. After sonication filtration was done through 0.45 µm filter. Filtrate was Further pipette 0.3ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents. (15 µg/ml). the amounts of Enoxaparin per injection were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. injectable Assay for %Label claim for %RSD Calculated, Result was shown in **(Table No. 04)**

Brand Name: XOMPARINM 60 MG IN 0.6 ML

i.e. 500 µgm/ml Enoxaparin ----- STOCK -II

Table 1 : results of linearity of Enxp

Parameter	Enxp
Concentration (µg/ml)	5-25 µg/ml
Correlation coefficient (r^2)	0.999
Slope (m)	110.2
Intercept (C)	6.948

***value of five replicate and five concentrations**

Table 2: Results of recovery study

% Level	**% Mean±SD***
80%	100.62±0.06
100%	100.58±0.06
120%	99.37±0.06

***mean of each 3 reading for RP-HPLC method.**

Table 3.Results of precision

METHOD	Drug	Conc^n (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
		05	545.35±1.29	97.88	548052±0.71	98.29
		15	1624.18±0.68	97.84	1624.15±2.47	97.83
		25	2748.17±2.85	99.43	2748.59±0.76	99.51

***value of 3 replicates and 3concentrations**

Table 4 analysis of marketed formulation

% Label Claim				
Conc	Area I	Amt Found	LC	% Label Claim
15.00	1626.29	14.69457	0.979638	97.96
15.00	1626.34	14.69503	0.979668	97.97
Mean	1626.32	14.69		97.97
SD	0.035	0.000		0.002
%RSD	0.002	0.002		0.002

RESULTS AND DISCUSSION

Attempts were made to develop RP-HPLC method for estimation of Enoxaparin from injection.For the RP - HPLC method,Agilent (Autosampler) GradientSystem DAD Detector and C1 8(Agilent) with 250mm x4.6 mm i.d and 5µm particle size Methanol Water 0.05 % OPA (10:90v/v) pH 3.0 was used as the mobile phase for the method. The detection wavelength was 232 nm and flow rate were 0.8 ml/min. In the developed method, the retention time of Enoxaparin were found to be 6.28min.The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Enoxaparin in bulk drug as well as in formulations.

CONCLUSION:

Simple, rapid, accurate and precise RP-HPLC have been developed and validated for the routine analysis of Enoxaparin in API and injection dosage forms. Both methods are suitable for the determination of enoxaparin in Single-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of injectable dosage forms.

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