

Available online on 25.08.2019 at <http://jddtonline.info>

## Journal of Drug Delivery and Therapeutics

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Research Article

### Development and Validation of Spectrophotometric Method for the Estimation of Enoxaparin sodium in marketed formulation

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#### ABSTRACT

Simple, cost effective, precise and accurate UV-Spectrophotometric method for estimation of Enoxaparin sodium was developed and validated as per ICH guidelines. This Method involves solving of calibration curve based on measurement of absorbance wavelengths 234nm in 0.1 N HCl. The method obeys the Beer's law in the concentration ranges 100-500µg/ml. The developed method was validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. % Recovery for both the drugs were in the range of 99.288-99.632% indicating excellent accuracy with percentage RSD Values less than 2. The method was precise, with a relative standard deviation of less than 2% for all the validation parameters. Thus, method can be used for routine monitoring of drugs in industry for the assay of bulk drugs and commercials.

**Keywords:** UV-Spectrophotometric, Enoxaparin sodium, Validation, ICH guidelines

**Article Info:** Received 11 July 2019; Review Completed 08 Aug 2019; Accepted 17 Aug 2019; Available online 25 August 2019



#### Cite this article as:

Sahu S, Lariya NK, Development and Validation of Spectrophotometric Method for the Estimation of Enoxaparin sodium in marketed formulation, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):1236-1239

DOI: <http://dx.doi.org/10.22270/jddt.v9i4-s.3972>

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#### INTRODUCTION

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-sulfooxoxan-2-yl)oxy-6-(hydroxyl methyl)-3-(sulfoamino)-4-sulfooxoxan-2-yl]oxy-4-hydroxy-5-sulfooxoxane-2-carboxylic acid<sup>1</sup>. The empirical formula of Enoxaparin sodium is C<sub>42</sub>H<sub>59</sub>N<sub>3</sub>Na<sub>4</sub>O<sub>35</sub>S<sub>2</sub> and its molecular weight is 1322.000 g/mol, Brands: Lovenox Injection (150 mg), 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)<sup>2</sup>.

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<sup>3</sup>. Literature survey reveals that very few methods<sup>4-5</sup> 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<sup>6</sup>.

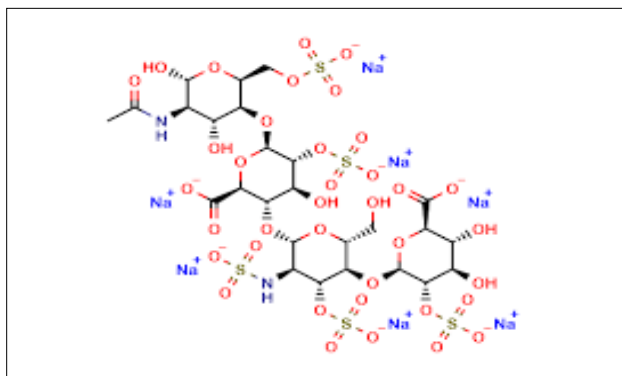


Fig. 1: Structural formula of Enoxaparin sodium

## EXPERIMENTAL

### Reagents and chemicals

Enoxaparin sodium pure drug was obtained as a gift sample from Cipla Private Limited (Goa, India). Lovenox Injection (150 mg), were purchased from local market. Methanol, acetonitrile were procured from Rankem, RFCL Limited, New Delhi, India. Milli Q water was used throughout the study. Other chemicals used were of analytical or HPLC grade.

### Apparatus

Spectral analysis were made on a Labindia spectrophotometer, Model- 3000 Plus, was employed with spectral bandwidth of 1 nm and wavelength accuracy of  $\pm 0.3$  nm with automatic wavelength correction with a pair of 10 mm quartz cells. Glassware used in each procedure were

soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven.

### Method Development

#### Standard stock solution

Accurately weighed Enoxaparin sodium (100 mg) was transferred to a 100 mL volumetric flask, dissolved in 80 mL 0.1 N HCl. The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark 100ml with 0.1N HCl to get a concentration of 1000  $\mu\text{g}/\text{ml}$ .

#### Selection of wavelength for linearity

Solutions of 100 $\mu\text{g}/\text{ml}$  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 234.0 nm. Enoxaparin sodium showed linearity in the concentration range of 100-500  $\mu\text{g}/\text{ml}$  at their respective maxima. They were scanned in the wavelength range of 200-400 nm and the overlain spectrum was obtained (Fig.-2&3).

#### Preparation of calibration curve

1.0ml, 2.0ml, 3.0ml, 4.0ml and 5.0ml from stock solution were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with 0.1N HCl. This gave the solutions of 100 $\mu\text{g}/\text{ml}$ , 200 $\mu\text{g}/\text{ml}$ , 300 $\mu\text{g}/\text{ml}$ , 400 $\mu\text{g}/\text{ml}$  and 500 $\mu\text{g}/\text{ml}$  respectively for Enoxaparin sodium. Triplicate dilutions of drug solutions were prepared and the prepared working solutions of Enoxaparin sodium were scanned 234nm. The absorbance's were recorded and were plotted against the concentrations to obtain their respective calibration curves.

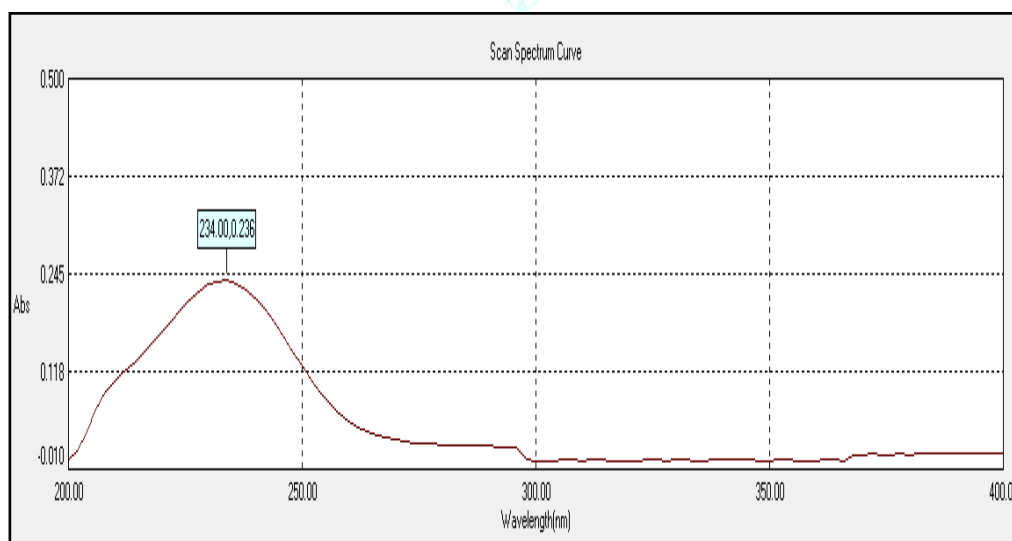
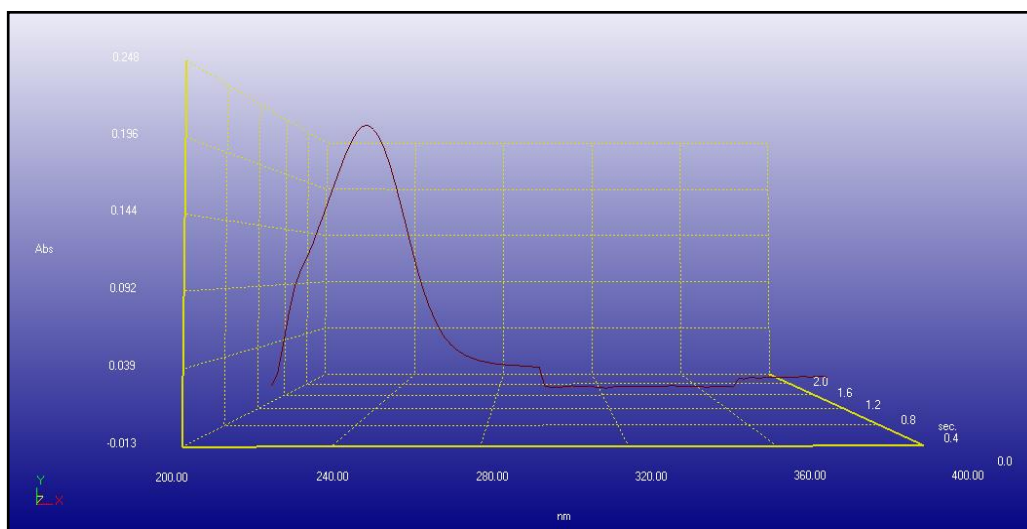


Fig 2: Determination of  $\lambda_{\text{max}}$  of Enoxaparin sodium



**Fig 3: 3D graph of Determination of  $\lambda_{\max}$  of Enoxaparin sodium**

### Methods validation

Validation of the method was carried out in accordance with the International Conference on Harmonization Q2B guidelines 2005<sup>9</sup>.

#### 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 preanalysed 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 replicate 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

Lovenox Injection amount equivalent to 150mg (1.0ml) was taken in 150 ml volumetric flask. Then 5ml of 0.1 N HCl 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 with 0.1 N HCl. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with 0.1 N HCl to get the final concentrations of drug in the working range. The absorbances of final dilutions were observed at selected wavelength and the concentration was

obtained from calibration curve method. The procedure was repeated for five times. Result of Assay shown in Table 5.

**Table 1: Results of linearity of ENXP**

Parameter	Enxp
Concentration ( $\mu\text{g/ml}$ )	100-500
Correlation Coefficient ( $r^2$ )*	0.999
Slope (m)*	0.001
Intercept (c)*	0.002

\*Value of five replicate and five concentrations

**Table 2: Results of recovery study**

% Level	% Mean $\pm$ SD*
80%	99.632 $\pm$ 0.148
100%	99.288 $\pm$ 0.123
120%	99.561 $\pm$ 0.225

\* Value of three replicate and five concentrations

**Table 3: Results of precision**

Parameter	% Mean $\pm$ SD*
Repeatability	99.577 $\pm$ 1.178
Intermediate precision	
Day to day precision	99.266 $\pm$ 1.083
Analyst-to-Analyst	99.270 $\pm$ 1.154
Reproducibility	99.259 $\pm$ 1.082

\* Value of five replicate and five concentrations

**Table 4: Assay of injectable formulation**

Conc. Present ( $\mu\text{g/ml}$ )	% Conc. Found (Mean)
100	99.08
200	99.62
300	99.26
400	99.26
500	99.84

\*Average of three replicate and five concentrations

## RESULTS AND DISCUSSION

Method development by UV-Spectrophotometer is cost effective and time saving as compared to HPLC method of analysis. Thus, for estimation of routine sample of drugs simple, rapid, sensitive and accurate analytical UV methods were utilized which reduces unnecessary tedious sample preparations and use of costly materials. To develop suitable methods of analysis, various solvents were studied. Based on sensitivity of the method and non-toxic behaviour 0.1 N HCl was selected as a solvent for the methods. The method obeys the Beer's law in the concentration ranges 100-500 $\mu$ g/ml with R<sup>2</sup> value 0.999. The recovery was found between 99.288 $\pm$ 0.123 to 99.632 $\pm$ 0.148% with RSD value less than 2. The standard deviation, coefficient of variance and standard error were obtained for Enoxaparin sodium was satisfactorily low. Result of precision at different levels was found to be within acceptable limits (RSD < 2). Thus, the method provides a simple, convenient, rapid and accurate way to determine ENXP in marketed formulation.

## CONCLUSION

The developed UV spectrophotometric method for the estimation of Enoxaparin sodium was found to be simple and useful with high accuracy, precision and repeatability. Sample recoveries in all formulations using the above method was in good agreement with their respective label

claim or theoretical drug content; thus, suggesting the validity of the method and non-interference of formulation excipients in the estimation. In the selected solvent system (0.1 N HCl), drugs were stable. Thus, suggesting that samples need not be estimated immediately after collection. The developed method was found to be stability specific and were validated as per ICH guidelines (1994, 1996 and 2005) and statistical method.

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