Research Article

A simple and rapid RP-HPLC method for the quantitative determination of Enoxaparin Sodium in bulk and injectables

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Abstract

Objective: Aim of present study was to develop a simple, sensitive and rapid RP-HPLC method and validated for the quantitative analysis of Enoxaparin sodium in bulk and injectables. **Material and methods:** A Sunsil C_{18} column (150mm*4.6mm i.d., 5.0µm) with mobile phase KH_2PO_4: Methanol in the ratio of 70:30 v/v adjusted 5.2. UV detection was done at 231nm. **Results and Conclusion:** Linearity was obtained in the concentration range of 5-50 µg/ml. The % recovery of the proposed method for 50%, 100%, 150% was found to be 97.05%-99.14%. The values of %RSD (precision studies) was found to be <2.0%. Limit of detection and quantification was found to be 98µg/ml and 298µg/ml respectively. The developed method was completely validated as per ICH guidelines and satisfactory results were obtained.

Keywords: Enoxaparin sodium, quantitative analysis, RP-HPLC, validation, injectables

Introduction

Enoxaparin sodium belongs to the group of low molecular weight heparin. figure 1 shows the structure of Enoxaparin Sodium. It is official in EP, IP, BP and USP. The IUPAC name is 6-[5-acetamido-4,6-dihydroxy-2-[sulfooxymethyl]oxan-3-yl]oxy-3-[5-[6-carbox-4,5-dihydroxy-3-sulfooxyoxan-2-yl]oxy-6-[hydroxylmethyl]-3-[sulfoamino]-4-sulfooxyoxan-2-yk]oxy-4-hydroxy-5-sulfooxyoxane-2-carboxylic acid (Jain et al., 2013). The molecular formula is ($C_{26}H_{40}N_2O_{36}S_5$) n, and its molecular weight is 1134.9g/mol. It is a white amorphous powder that is freely soluble in water and insoluble in organic solvents (British Pharmacopoeia, 2014). The mechanism of action of enoxaparin sodium is antithrombin dependent (European Pharmacopeia, 2014). Enoxaparin sodium is an anticoagulant that helps prevent blood clots (Babu et al., 2011). It is used to treat or prevent a type of blood clot called deep vein thrombosis (DVT), can lead to blood clots in the lungs (Chengalva et al., 2017). Enoxaparin sodium has greater bioavailability and longer half-life than un fractioned heparin, permitting less frequent subcutaneous administration (Buckley et al., 1992). Enoxaparin Sodium Injections are available in market under the brand name CLEXANE, TENOXA0.6, SANKARIN, EVAPARIN, and many other brand names in the market. In the present study, an attempt was made to develop a simple, precise and accurate method for estimating the drug in the form of a pharmaceutical dosage form and to validate according to International Conference on Harmonization (ICH) guidelines (ICH Q2 (R1); 2005). (Buckley et al., 1992). A study of the literature shows that there are very few methods, such as UV and LC-MS; are available for estimating enoxaparin sodium alone or in combination with other drugs and in its dosage form (Sahu et al., 2019).

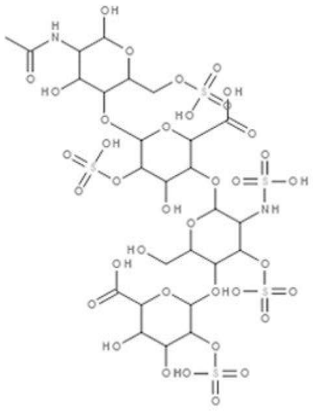

Figure 1. shows the structure of Enoxaparin sodium.

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Materials and methods

Instruments: Shimadzu HPLC (LC-20AD multi-solvent delivery system, SPD-20A UV-Visible detector, LC solution software), Waters HPLC (1525 Binary pump, W2487 detector, 717 PLUS Auto sampler, EMPOWER 3.0).

Chemicals: Enoxaparin sodium was obtained as gift sample from pharmaceutical industry. CLEXANE injection (20mg/0.2ml) was brought from local market. Analytical reagent HPLC grade water was used.

Chromatographic conditions: The isocratic mobile phase consisted of KH_2PO_4: Methanol in the ratio of 70:30 v/v pH 5.2, flowing through the column at constant flow rate 0.6ml/min. A SunsilC_{18} column (150mm*4.6mm i.d., 5.0µm) was used as the stationary phase. 231nm was selected as the detection wavelength for UV-Visible detector.

Method Development:

***Selection of diluent*:** Diluent used for preparation of sample and standard should be compatible and not affect retention and resolution of an alyte. After various trials HPLC grade water was used as diluent.

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

Preparation of working standard solution: From the standard stock solution 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml, 3.0ml, 3.5ml, 4.0ml, 4.5ml, 5.0ml of aliquots were transferred into series of 10ml volumetric flask and diluted with HPLC grade water to get the concentration of 5, 10,15, 20, 25, 30, 35, 40, 45, 50 µg/ml.

Preparation of Enoxaparin sodium sample solution: The CLEXANE injection of Enoxaparin sodium was prepared by taking 20mg/0.2ml in 10ml volumetric flask and diluted with HPLC grade water to obtain 100µg/ml. From the above solution pipette out 2ml in 10ml volumetric flask to obtain 20µg/ml.

Optimization of parameters: Different mobile phase and different ratios were tried for all the solvents but Enoxaparin sodium was found to obtain a peak at 2.7min using KH_2PO_4: Methanol in the ratio of 70:30 v/v pH 5.2, and UV detection at 231nm. The optimum mobile phase ratio was selected on the basis of their ability to obtain peak. Optimized chromatogram of Enoxaparin sodium is shown in figure 2.

Results and discussion

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.

***Specificity*:** No peaks were observed at the retention time of Enoxaparin sodium when blank was injected as shown in figure 3 and chromatogram with standard in figure 3a.

***System suitability*:** The standard solutions were injected for five times and measure the area for all five injections in HPLC. The %RSD for the areas of five injections was found to be within the specified limits. Table 1 shows results of system suitability.

***Linearity*:** The standard solution was injected and the proposed method was found to be linear in the range of 5-50µg/ml with correlation coefficient was 0.9994 (figure 4), slope 748738 and intercept 683538 was shown in table 2.

***Precision*:** The precision of the proposed method was estimated in terms of inter-day and intra-day precision wherein the standard solution was injected for 6 times respectively. The results shown in table 3 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.

***Accuracy*:** The accuracy of the method was determined by performing recovery studies by spiking standard solution to

Table 1. Results of system suitability

Concentration	Retention time	%RSD
20 µg/ml.	2.776 min	0.0000691%

Table 2. Results of quantitative determination of Enoxaparin sodium

S. No.	Parameters	Results
1.	UV detection	231nm
2.	Linearity and range(µg/ml)	5-50 µg/ml
3.	Slope	748738
4.	Correlation coefficient	0.9994
5.	Y-intercept	683538

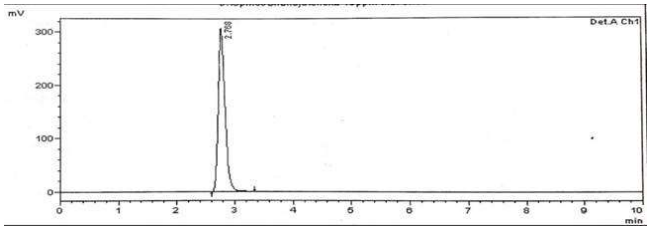

Figure 2. Optimized chromatogram of Enoxaparin sodium

that of sample solution at three different levels i.e., 50%, 100%, 150% was injected. Values of %recovery greater than 95-105% indicate that the proposed method was accurate for the analysis of drug and the results were reported in table 4.

***Robustness*:** The robustness of the proposed method was evaluated by changing flow rate, mobile phase ratio. 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 5.

Limit of detection :(LOD)

LOD = 3.3 x SD/ slope.

The LOD of the proposed method was found to be 98µg/ml.

Limit of Quantitation :(LOQ)

LOQ = 10 x SD/ slope.

The LOQ of the proposed method was found to be 298µg/ml.

***Assay*:** Sample solution and standard solution were injected separately into the system and chromatograms were recorded and drug present was calculated using the below mentioned formula.

%Assay= (sample area/standard area) *100

= (2889218/2992891) *100

= 96.53%.

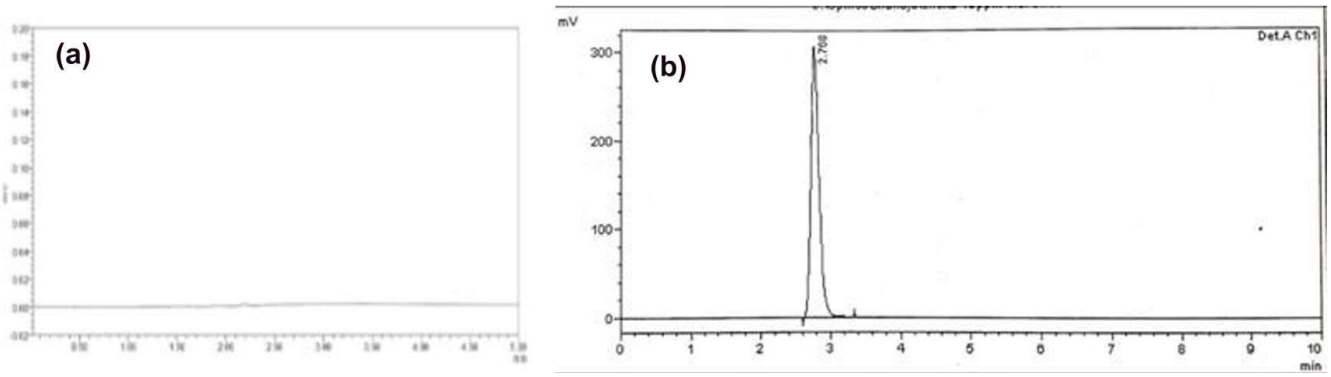

Figure 3: (a) Blank chromatogram (b) Chromatogram of standard Enoxaparin Sodium

Table 3. Results of Intraday and Inter-day precision

Concentration	Retention time	Intraday precision (%RSD)	Retention time	Inter-day precision (%RSD)	
20 µg/ml	2.775 min	0.000039%	2.782	Day 1	Day 2
				0.0000590%	0.0000592%

Table 4. Results of accuracy

Level	Amount of standard added (µg/ml)	Pre-analyzed sample (µg/ml)	Retention time	% Recovery
50%	5 µg/ml	20 µg/ml	2.771 min	97.05%
100%	10 µg/ml	20 µg/ml	2.773 min	98.56
150%	15 µg/ml	20 µg/ml	2.779 min	99.14

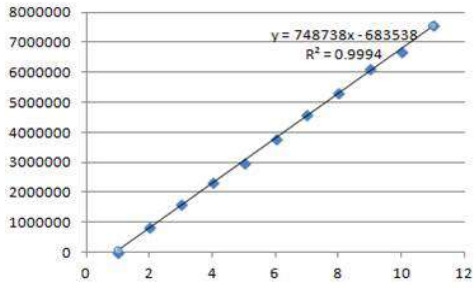

Figure 4. Calibration curve Enoxaparin Sodium

Table 5. Results of robustness

S. No.	Concentration	Retention time	Flow rate	%RSD	Retention time	Mobile phase	%RSD
1.	20 µg/ml	2.821	0.59	0.000069%	2.775	69	0.000065%
2.		2.684	0.61	0.000067%	2.775	71	0.000100%

Conclusion

The RP-HPLC method developed for quantitative analysis is simple and rapid for Enoxaparin sodium in both bulk and in jectables formulation. The developed method was completely validated as per ICH guidelines and satisfactory results were obtained.

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