

Pharmaceutical Analysis of Eptifibatide via Simple, Rapid, Economical UV-Spectrophotometric Methods

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Abstract Eptifibatide is an antiplatelet drug of the glycoprotein IIb/IIIa inhibitor class. Pharmaceutically it is applied to reduce the risk of acute cardiac ischemic events. The present work reveals two simple, rapid and economical UV-Spectrophotometric methods for pharmaceutical analysis of Eptifibatide bulk and in parenteral formulation. The 'Method I' is based on the Zero Order UV-Spectrophotometric determination of drug at its wavelength maximum 218.20 nm and 'Method II' employed First Order Derivative - Area Under Curve (AUC) technique in which the area has been integrated between two wavelengths 220.20 to 237.20 nm. The drug obeyed linearity in the concentration range of 3 - 18 µg/mL with coefficient of correlation; greater than 0.999 in both methods. The amounts of drug determined by both methods are in conformity with label claim. These methods are validated for accuracy, precision and ruggedness with % RSD value less than 2.0.

Keywords: Eptifibatide; UV-Spectrophotometry; First Order Derivative-AUC

1. INTRODUCTION

Eptifibatide is a cyclic Heptapeptide antiplatelet drug of the glycoprotein IIb/IIIa inhibitor class derived from protein found in the venom of the southeastern pygmy rattlesnake Katori, N. et al. (2004). It is used in myocardial infarction and acute coronary syndrome Hantgan, R. et al. (2001). Chemically Eptifibatide is N⁶-(aminoiminomethyl)-N²-(3-mercapto-1-oxopropyl)-L-lysylglycyl-L-α-aspartyl-L-tryptophyl-L-prolyl-L-cysteinamide, cyclic (1→6) disulfide, The Merck Index (2008); the chemical structure of Eptifibatide is as shown in Fig. 1.

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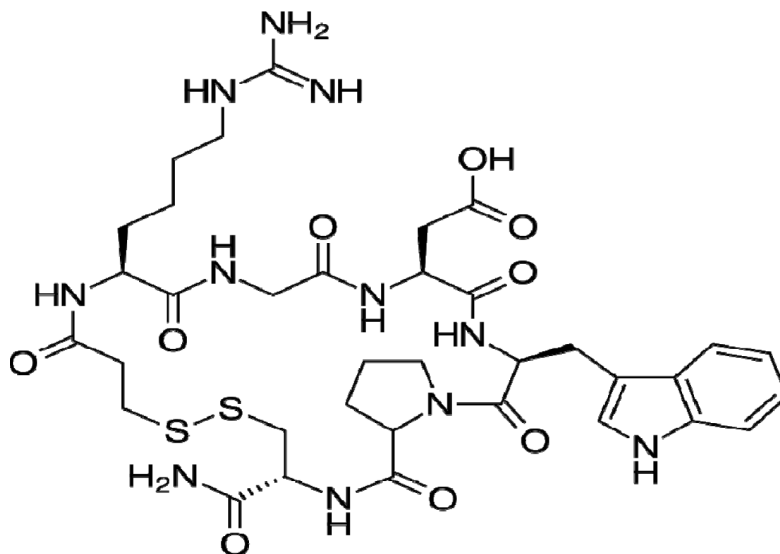


Figure 1: Chemical structure of Eptifibatide.

Literature revealed very few analytical methods such as HPLC Kachhadia, P. (2008) and LC-Tandem Mass Spectroscopy Liu, Z. *et al.* (2009), Yu, X. *et al.* (2010) for determination of Eptifibatide in bulk, pharmaceutical formulation and in human plasma. To our knowledge there is no method for determination of Eptifibatide by UV-Spectrophotometry in bulk and pharmaceutical formulations.

So, the objective of the present investigation was to develop simple, rapid, economical precise and accurate Zero order UV - Spectrophotometric and UV - Spectrophotometric - First Order Derivative- Area under Curve (AUC) methods for determination of Eptifibatide in bulk and in parenteral formulation. The methods thus developed were also subjected to analytical validation as directed by the ICH guidelines (2005).

2. EXPERIMENTAL

2.1 Materials and Equipments

Eptifibatide was obtained as a generous gift sample. Parenteral formulation was purchased from market, containing 20 mg vial (Integrilin). Spectroscopic measurement study was done using UV-Visible Spectrophotometer with software UV Probe 2.21 and spectral bandwidth 1 nm was employed for all samples, using a pair of 10 mm matched quartz cells. Eptifibatide is soluble in distilled water therefore it was selected as a solvent.

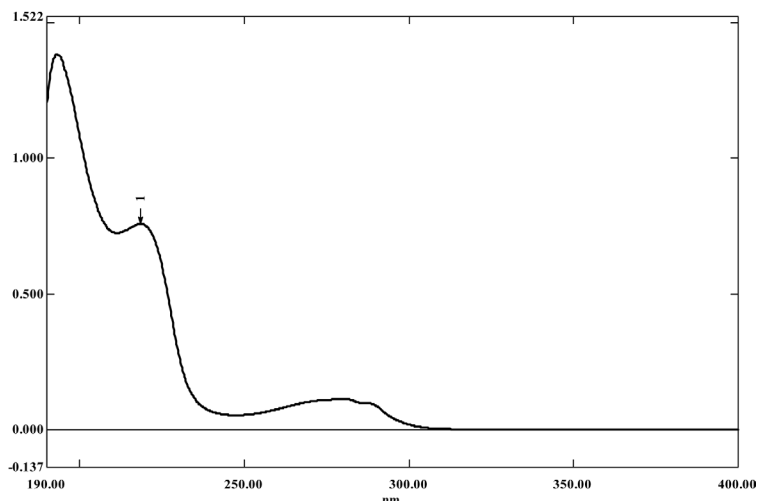


Figure 2: Zero order spectrum of Eptifibatide depicting λ_{max} 218.20 nm in distilled water.

2.2 Preparation of stock standard solution

Stock standard solution was prepared by dissolving 10 mg of anhydrous eptifibatide in 100 mL of distilled water to obtain concentration of 100 $\mu\text{g}/\text{mL}$.

2.3 Selection of analytical wavelengths and study of linearity curves

From the stock standard solution, an appropriate volume 1 ml was further diluted with distilled water to get concentration 10 $\mu\text{g}/\text{mL}$, scanned in the UV- region of 400 – 200 nm. Eptifibatide showed maximum absorbance at wavelength 218.20 nm which was chosen for further studies in Method-I as shown in **Fig. 2**. The aforesaid spectrum was derivatized into first order derivative at $\Delta\lambda$ 2.0, scaling factor 4.0 using UV Probe 2.21 software and the AUC was determined in the wavelength range of 220.20 nm -237.20 nm in Method-II as depicted in **Fig. 3**.

2.4 Preparation of Eptifibatide sample solution and assay

The sample vial containing 20 mg Eptifibatide having concentration 2 mg/ml; An appropriate portion of 5.0 mL was transferred into 100 mL volumetric flask containing 50 ml distilled water and diluted up to the mark with same. The resulting solution was filtered through Whatmann filter paper 0.45 μm . From it, an appropriate volume 0.9 mL was transferred into 10 mL volumetric

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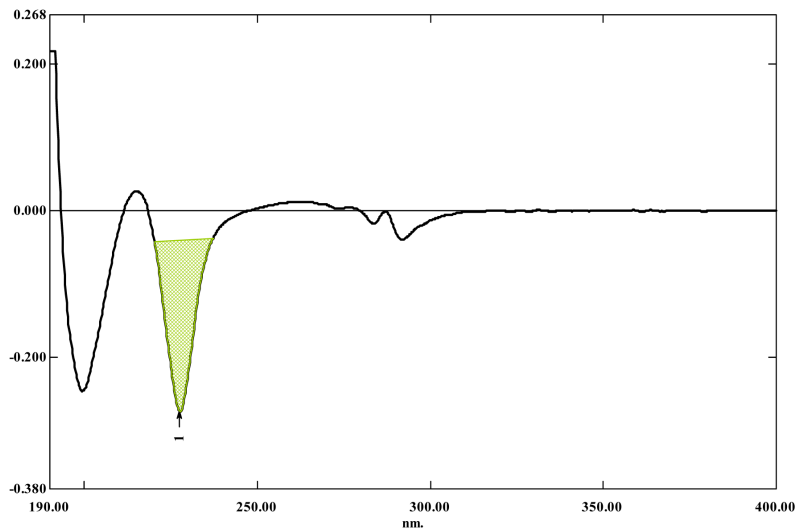


Figure 3: First order derivative UV- Spectrum of Eptifibatide showing selection of Wavelengths between 220.20- 237.80 nm in distilled water.

flask and volume was made up with solvent. In Method-I, the absorbance was studied at the selected wavelength whereas in Method –II, the same spectrum was derivatized into first order and area under curve was recorded between selected wavelengths. The concentrations of the drug were determined using respective linear regression equations.

3. METHOD VALIDATION

3.1 Accuracy

Accuracy of the methods was studied at three different levels i.e. 80%, 100% and 120% levels. To the pre-analyzed sample solution (9 μ g of Eptifibatide), a known amount of stock standards solution of Eptifibatide were at three different levels 80%, 100% and 120%. These solutions were re-analyzed by the proposed methods.

3.2 Precision

For repeatability; six replicates of sample solution were scanned at concentration of 9 μ g/mL. The experiments were performed at three different concentration levels (6, 9 and 12 μ g/mL) and repeated three times in a day to determine intra-day precision and on three different days to determine inter-day precision.

Table 1: Optical Characteristic and Linearity data of Eptifibatide.

Parameter	Method-I	Method-II
Wavelength(s)	218. 20 nm	220.20- 237.20 nm
Linearity [$\mu\text{g/mL}$]	3 - 18	3 - 18
Linearity Equation	$Y = 0.0473 X + 0.0389$	$Y = 0.1278 X + 0.0737$
Slope \pm SD	0.0473 ± 0.0005	0.1278 ± 0.0001
Intercept \pm SD	0.0389 ± 0.0002	0.0737 ± 0.0004
Correlation Coefficient \pm SD	0.999 ± 0.0004	0.999 ± 0.0002

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Table 2: Result for Analysis of Parenteral Formulation (Integrilin).

Drug	Method-I		Method-II	
	Amount found (%) [n= 6]	% RSD	Amount found (%) [n= 6]	% RSD
Eptifibatide Pharmaceutical formulation	99.60	0.78	100.02	0.76

ⁿNumber of determinations

3.3 Sensitivity

The sensitivity of the methods was assessed as Limit of Detection (LOD) and Limit of Quantification (LOQ). To determine LOD and LOQ, the concentration at the low end of the linear range of the calibration plot were analysed. LOD and LOQ were calculated using equation $LOD = 3.3 \times N/B$ and $LOQ = 10 \times N/B$; Where, 'N' is the standard deviation of the peak areas of the drugs ($n = 3$), taken as a measure of noise, and 'B' is the slop of the corresponding calibration plot.

3.4 Ruggedness

Ruggedness of the methods was performed by two different analysts at concentration level 9 $\mu\text{g/mL}$.

4. RESULTS AND DISCUSSION

Eptifibatide showed maximum absorption at 218.20 nm in distilled water and obeyed linearity in the concentration range of 3 -18 $\mu\text{g/mL}$ at their respective

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Table 3: Summary of validation parameters for UV-Spectrophotometric analysis of Eptifibatide.

Parameter	Method-I	Method-II
% Recovery [n= 9]	100.22	99.02
% RSD	0.61	0.74
Sensitivity (μg)		
LOD	0.28	0.59
LOQ	0.85	1.80
Precision [% RSD]		
Intra-day [n=3]	1.44 - 1.66	0.76 -1.55
Inter-day [n =3]	1.36 -1.92	1.36-1.63
Repeatability [n=6]	1.21	1.05
Ruggedness [% RSD]		
Analyst – I [n= 6]	0.82	1.08
Analyst – II [n= 6]	0.66	1.07

wavelengths in both the proposed methods. The optical characteristic and linearity data of both these methods are shown in Table 1.

The amount of the Eptibatide estimated in pharmaceutical formulation using both these methods is shown in Table 2. The amounts estimated were found to be in good agreement with good agreement with label claimed. These methods were validated for accuracy, precision and ruggedness as per ICH guidelines. The accuracy of the methods were assessed at three different levels i.e. 80 %, 100 % and 120 % level and methods were found to be accurate as indicated by low values of % RSD. The precision of the methods were studied as repeatability, intra-day and inter-day precision and the % RSD values were found to be less than 2 indicates methods are precise. Both methods are simple, accurate and rapid can suitably be used for determination of Eptifibatide in Parenteral formulation. Summary of validation parameters is shown in Table 3.

5. CONCLUSION

Both these developed methods are simple, rapid and economical. Accuracy and precision are found within acceptable range. These methods proved to be rugged and can be used for routine analysis of Eptifibatide in bulk and in its parenteral formulation.

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