

**AN ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE
ESTIMATION OF ZOLADEX BY UV & HPLC**

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ABSTRACT

The analytical method development commenced with the exploration of various parameters such as choice of solvent, pH, and detection wavelength for UV spectroscopy, and mobile phase composition, column selection, and flow rate for HPLC. Optimization of these parameters was aimed at achieving maximum sensitivity, selectivity, and resolution for the quantification of Zoladex. The UV method was developed using a simple solvent system and optimized at a wavelength of 280 nm, taking advantage of Zoladex's inherent UV absorbance. On the other hand, HPLC method development involved selecting an appropriate stationary phase, optimizing the mobile phase composition (acetonitrile-water), and adjusting the flow rate to achieve efficient separation of Zoladex from potential interfering substances. Following method development, validation studies were conducted according to ICH guidelines to ensure the reliability and robustness of the developed methods. Parameters assessed during validation included specificity, linearity, accuracy, precision, and robustness. Specificity studies demonstrated the ability of the methods to accurately quantify Zoladex in the presence of potential impurities or degradation products. Linearity was established over a concentration range suitable for the intended use of the methods, ensuring accurate quantification across the expected concentration range of Zoladex in pharmaceutical formulations. Accuracy and precision studies were conducted to evaluate the reliability and reproducibility of the methods, with results demonstrating satisfactory levels of both within acceptable limits.

Key Words: Zoladex (goserelin acetate), UV spectroscopy, high performance liquid chromatography (HPLC), ICH guidelines, quality control, analysis, stationary phase.

INTRODUCTION

Chromatography is the most widely and acceptable technique for analytical method development¹. High-performance liquid chromatography (HPLC) has become the workhorse of the pharmaceutical industries where it is used to identify, characterize, and purify molecules at all stages of a process from R&D to quality assurance and validation². With the advancements in TLC and its increasing applications, High Performance Thin Layer Chromatography (HPTLC) is also accepted for method development³. Assays based on absorption in the ultraviolet and visible region of electromagnetic spectra are also used extensively⁴. Still due to the considerable improvements over last 15 years in UV Spectrophotometric techniques enhancing the reliability and accuracy of technique, it is being preferred in various cases⁵. It has some specific advantages over several other instrumental analytical techniques like its easy routine operation, less sophistication, low-cost maintenance, wide applicability, sample recovery and cheaper procurement⁶.

Analytical method validation is the next important step in justification and acceptability of an analytical method, after method development⁷. It enables scientists to communicate scientifically and effectively on technical matters⁸. Set standards of evaluation procedures for checking compliance and taking remedial measures⁹. However, validation of equipment and analytical methods is necessary, not only due to regulations and accreditation standards, but also as prerequisite in terms of any good analytical practice and should be on going in the form of re-validation with method changes.

MATERIAL AND METHOD

Calibration curve Method:

Determination of λ_{max} of Drug: Standard solution (10 μ g/ml) of pure zoladex was prepared. The pure drug solution was scanned on UV spectrophotometer, which showed maximum absorbance at 278.0 nm.

Preparation of Standard Stock Solution: 10 mg of zoladex was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the diluent (methanol: water, 80:20 v/v), to give a stock solution of 1000 ppm.

Preparation of Working Standard Solution: From stock solutions of zoladex 0.05, 0.1, 0.15, 0.2 and 0.25 ml was taken and diluted up to 10 ml gives standard drug solution of 5, 10, 15, 20, 25 μ g/ml concentration.

Preparation of the Calibration Curves of the Drug: The standard drug solutions were taken area 3 times and the mean area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve. A typical spectrum and the calibration curve were obtained.

Preparation of Analysis of Capsule Formulation: Weight equivalent to 10 mg of zoladex and dissolved with 5 ml solvent methanol:water, 80:20 v/v, in 10 ml Volumetric Flask and sonicate it for 10min by ultrasonicator, after that volume was made up to 10 ml with solvent to obtain concentration of 1000 μ g/ml. from this take 0.1ml and dilute up to 10 ml with methanol and take the area of the sample solutions at 278.0 nm and the concentration of drug in the sample solution was determined by using Regression equation, After obtaining the value of concentration, Calculated the Percentage estimation of drug.

Analytical method development by HPLC:

Mobile Phase Selection: Initially to estimate zoladex number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was methanol:Acetonitrile in the ratio of (50:50v/v). The mobile phase was filtered through 0.45 μ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Selection of wavelength: 100 mg of zoladex was weighed accurately and transferred to a 100 ml volumetric flask, and the volume was adjusted to the mark with the mobile methanol: Acetonitrile in the ratio of (50:50v/v). From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200- 400nm), maximum absorbance was found at Lambda max 278nm.

Selection of Separation Variable: Standard drug solution of zoladex was prepared in different mobile phase and chromatograph was recorded by using different column (5 and 10 μ m) at

different chromatographic condition like different flow rate and temperature. Considering the theoretical facts and after several trials' separation variables were selected which were constant during whole experiment.

System Suitability Parameters: Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of zoladex 10 μ g/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

Preparation of Standard Stock Solution: 10 mg of zoladex was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with methanol to give a stock solution of 1000 ppm.

Preparation of Working Standard Solution: From stock solutions of zoladex 1ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with methanol, gives standard drug solution of 5, 10, 15, 20, 25 μ g/ ml concentration.

Preparation of the Calibration Curves of the Drug: Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Analysis of capsule formulation:

Assay of Capsule formulation: 20 capsules were weighed and ground to a fine powder. Powder equivalent to 10 mg zoladex was weighed and transferred to a 10 ml volumetric flask and volume was made up to 10 ml with methanol to obtain concentration of 1000 μ g/ml. Resultant solution was filtered through Whatmann filter paper. 1 ml of filtrate was taken in 10 ml volumetric flask and volume was made up to 10 ml with diluents (Methanol) to obtain concentration of 100 μ g/ml. Further 1.0 ml of this solution was taken and diluted up to 10 ml obtain final concentration of 10 μ g/ml. The amounts of zoladex in capsule formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with Tablet formulation.

RESULTS AND DISCUSSION

Solubility study:

Table No. 1: Solubility of Zoladex

S. No.	Solvent	Solubility
1	Water	Slightly soluble
2	0.1 NHCl	Insoluble
3	Methanol	Freely Soluble
4	Ethanol	Freely Soluble
5	0.1 NNaOH	insoluble
6	Acetone	soluble

Determination of Amax of Drug: 278.0 nm

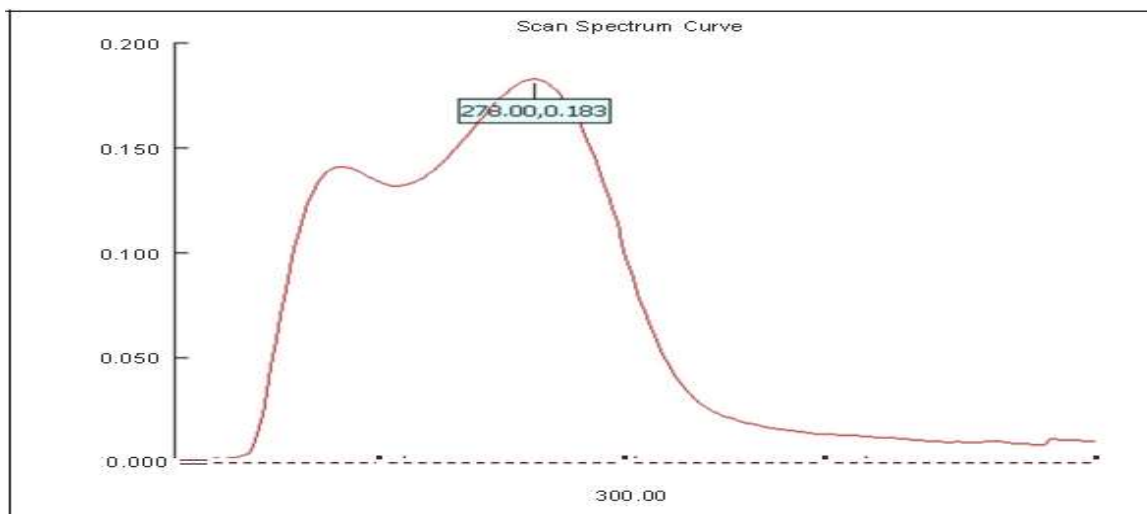


Figure 1: Selection of λ_{max} of Zoladex

Preparation of the calibration curves of the drug using AUC method

Table No. 2: Linearity of Zoladex

Conc.µg/ml	0	5	10	15	20	25
Rep.1	0	4.41	9.39	12.69	15.07	18.80
Rep.2	0	4.46	9.42	12.73	15.09	18.83
Rep.3	0	4.46	9.50	12.80	15.10	18.81
Mean	0	4.46	9.39	12.73	15.09	18.82
S.D.	00	0.005	0.029	0.049	0.016	0.011
R.S.D%	000	0.130	0.320	0.401	0.103	0.063

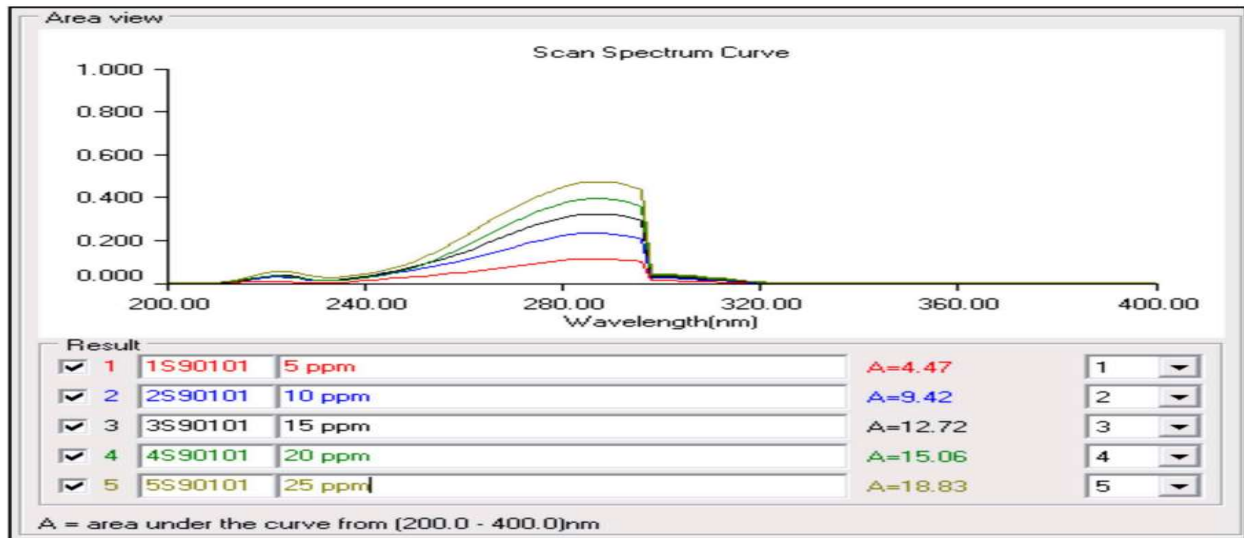


Figure 2: Calibration curves of the drug using AUC method

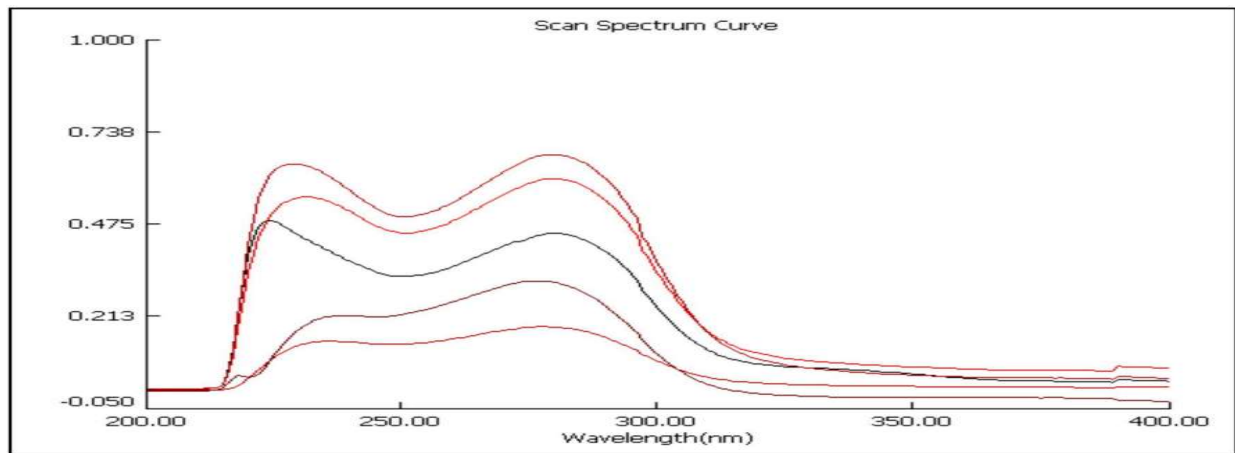


Figure 3: Calibration Curves of the Drug

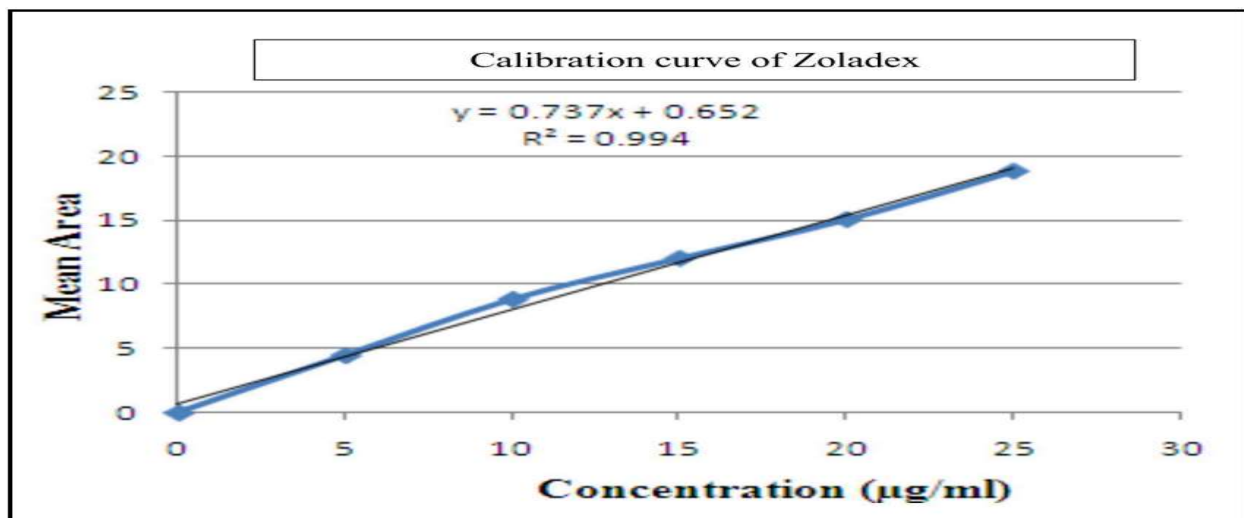


Figure 4: Calibration Curve of Standard Zoladex

Optical parameter:

Table No. 3: Result of Optical Parameter of Zoladex

S. No.	Parameters	Observation
1	Amax	278nm
2	Beer's law limit (µg/mL)	5-25 µg/mL
3	Regression equation	$Y = 0.737 X + 0.652$
4	Correlation Coefficient (r2)	0.999

Analysis of Capsule formulation:

Table No.4: Assay of Capsule Formulation

Brand Name	ZOLADEX	
	Label Claim	% Purity
Danocrine	200	99.95 %

Validation Parameters:

Accuracy:

Table No. 5: Recovery Studies for Accuracy of capsule formulation

Level of Recovery %	80	100	120
Amount Present	10	10	10
	10	10	10
	10	10	10
Amount of Std. Added	8	10	12
	8	10	12
	8	10	12
Amount Recovered	7.98	10.01	11.98
	8.01	9.99	11.99
	7.99	10.02	12.01
% Recovery	99.91	100.067	99.944
	0.191	0.153	0.127
	0.191	0.153	0.127

Table No. 6: Statistical Validation of Recovery Studies

Level of Recovery (%)	Drug	% Recovery	Standard Deviation*	% RSD
80	Zoladex	99.93	0.190	0.195
100	Zoladex	100	0.149	0.155
120	Zoladex	99.99	0.130	0.128

Precision: Repeatability:

Table No. 7: Results of analysis Data of capsule Formulation

Drug	Label claim	Amount found*	Label claim (%)	S.D.	%RSD
Zoladex	200mg	199.2 mg	99.50 %	0.000813	0.148121

Intermediate Precision (Inter-day and Intra-day precision):

Table No. 8: Intra-day and Inter-day precision

Intra-day Precision		Inter-day Precision	
	% Label Claim		% Label Claim
After 1hr	99.19%	First day	98.19
After 2hr	98.51%	Second day	98.00
After 3hr	99.43%	Third day	97.81
Mean	99.10	Mean	98.01
SD	0.365	SD	0.144
%RSD	0.126	%RSD	0.150

Analyst to Analyst:

Table No. 9: Result of Analyst-to-Analyst Precision

Analyst	Label claim	Amount found	Label claim (%)	S.D.	% RSD
1	200mg	199.01 mg	99.9%	0.00083	0.15109
2	200mg	199.50 mg	99.9%	0.00126	0.22551

Result of LOD & LOQ:

Table No. 10: Result of LOD & LOQ

Zoladex	
LOD	LOQ
0.15 µg/mL	0.50 µg/mL

Results of HPLC method

Mobile Phase Selection: Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Methanol: Acetonitrile (50:50 v/v) in the ratio of 50:50. The mobile phase was filtered through 0.45 μ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min

Table No. 11: Mobile Phase selection

Mobile phase	Ratio (v)	Flow rate	Conclusion
Water: Methanol	50:50	1 ml/min	Poor resolution
Methanol: Acetonitrile	50:50	1 ml/min	Most suitable

Selection of Separation Variable

Table No. 12: Selection of Separation Variable

Variable	Condition
Column	
Dimension.	250mm x4.60mm
Particle Size	5 μ
Bonded Phase	Octadecyl silane (C ₁₈)
Mobile Phase	
Methanol	50
ACN	50
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 μ l
Detection wavelength	278.0nm
Retention time Zoladex	3.288+0.3 min

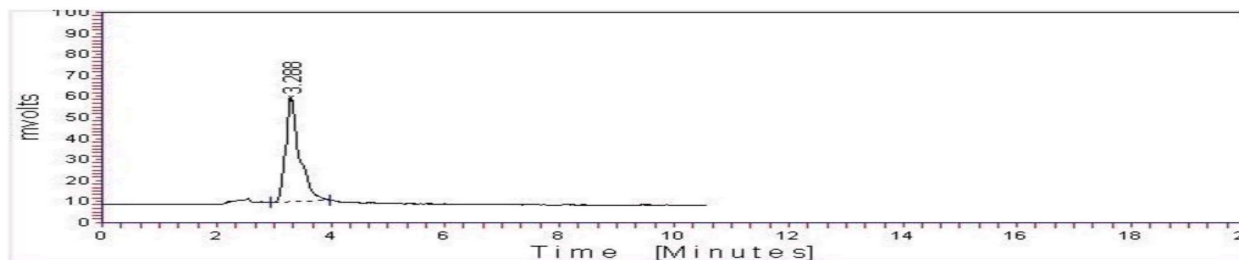


Figure 5: Trial graph of Methanol:Acetonitrile (50:50v/v)

System Suitability Parameters:

Table No. 13: Result of System Suitability Parameters for Zoladex

System suitability Parameters	RT	Arc	Theoretical plates	Tailing factor
Rep-1	3.280	771.195	2595.167	3.301
Rep-2	3.279	779.223	2598.360	3.309
Rep-3	3.289	778.954	2647.658	3.289
Mean	3.287	777.257	2642.420	3.287
S.D.	0.002	4.412	15.342	0.002

Linearity and Calibration Graph:

Table No. 14: Result of Linearity of Zoladex

Std. Conc. µg/ml	0	5	10	15	20	25
1	0	386.578	772.250	1171.5124	1551.290	1931.649
2	0	388.489	782.571	1179.245	1560.923	1941.261
3	0	389.586	780.944	1179.645	1571.455	1939.482
Mean	0.00	387.200	780.249	1174.799	1561.610	1933.459
SD	0.000	0.621	4.399	5.590	8.601	4.890
%RSD	0.000	0.159	0.581	0.480	0.549	0.261

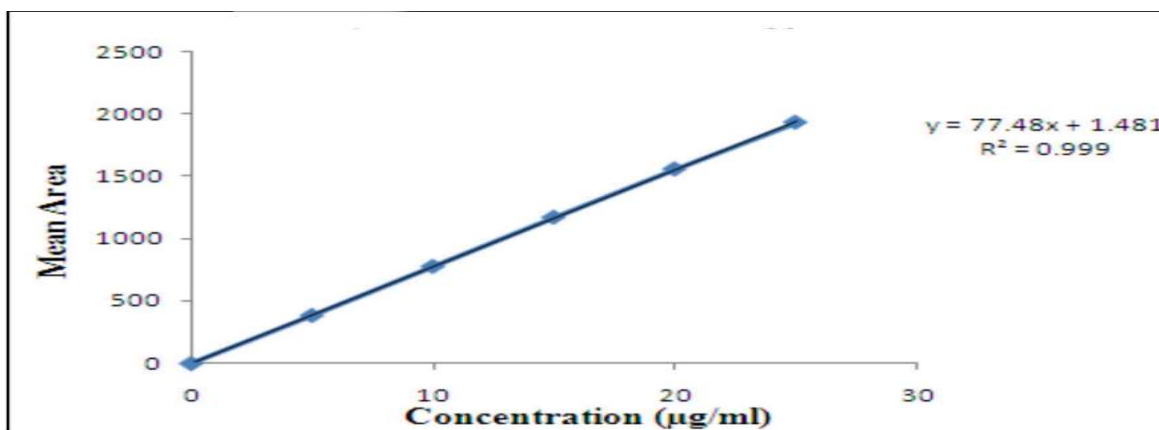


Figure 6: Calibration Graph of Zoladex

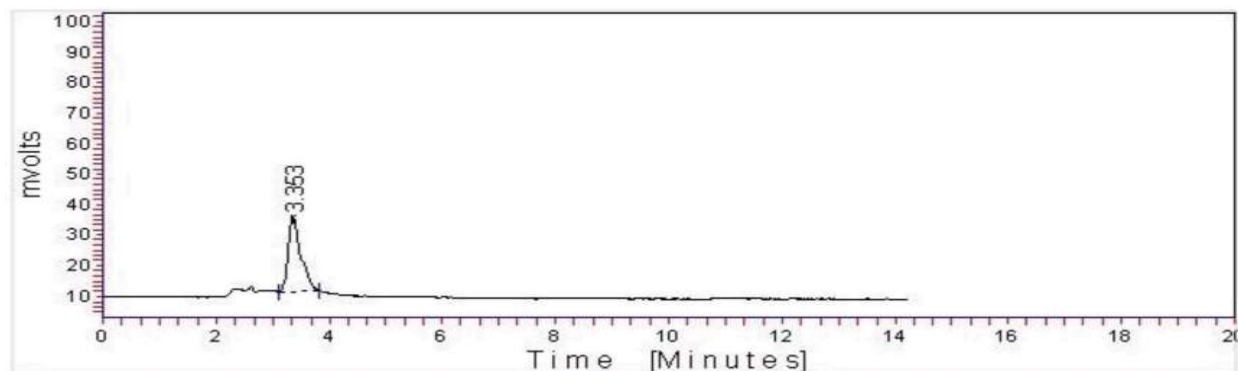


Figure 7: Chromatogram of Zoladex

Assay of Capsule Formulation

Table No. 15: Result of Analysis for Zoladexin Capsule Formulation

StdConc.pg/ml	ZOLADEX
	10
Rep-1	9.98
Rep-2	9.97
Rep-3	10.02
%found*	
Rep-1	99.92
Rep-2	99.81
Rep-3	100.11
Mean	99.929
SD	0.149
%RSD	0.149

Validation of Developed Method

Linearity:

Table No. 16: Response Ratio Data for Linearity of Zoladex

Replicates	Concentration ($\mu\text{g/ml}$)	Mean AUC	Response Ratio
Rep-1	5	385.207	77.041
Rep-2	10	777.257	77.726
Rep-3	15	1170.814	78.054
Rep-4	20	1556.600	77.830
Rep-5	25	1930.464	77.219
Mean = 77.574; S.D. = 0.427; R.S.D. = 0.550			

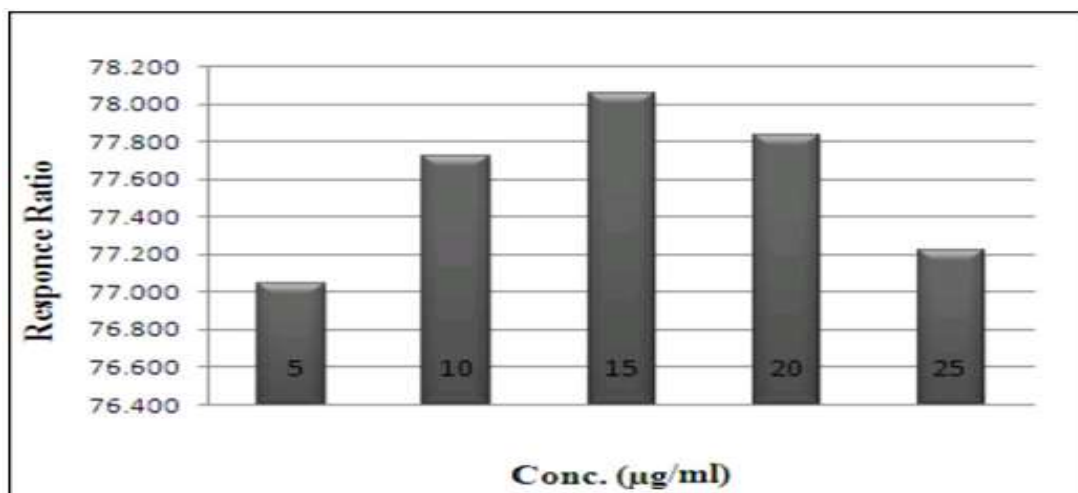


Figure 8: Response Ratio graph for Linearity of Zoladex

Result of Accuracy

Table No. 17: Recovery Studies of Formulation

Level of Recovery (%)	80	100	120
	Zoladex	Zoladex	Zoladex
Amount present (mg)	10	10	10
	10	10	10
	10	10	10
Amount of Std. added (mg)	8	10	12
	8	10	12
	8	10	12
Amount recovered (mg)	8	9.98	11.99
	8.01	9.99	12.02
	8.02	10.03	12.01
%Recovery	100.00	99.80	99.92
	100.13	99.90	100.17
	100.25	100.30	100.08

Table No. 18: Statistical Validation of Recovery Studies

Level of Recovery (%)	Drug	%Recovery	Standard Deviation*	%RSD
80	Zoladex	100.12	0.125	0.125
100	Zoladex	100.00	0.265	0.265
120	Zoladex	100.05	0.127	0.127

Result of precision: Repeatability:

Table No. 19: Results of analysis Data of capsule Formulation

Drug	Label claim	Amount found*	Label claim	S.D.	% RSD
Zoladex	200mg	199.98mg	99.99	0.150	0.158

Intermediate Precision-(Inter-day and Intra-day Precision)

Table No. 20: Intra-day and Inter-day Precision

Intra-day Precision		Inter-day Precision	
	% Label Claim		% Label Claim
	Zoladex		Zoladex
After1hr	99.85	First day	98.90
After 2hr	99.52	Second day	98.50
After 3hr	99.15	Third day	98.10
After4hr	99.05		
After5hr	98.98		
After6hr	98.95		
Mean	99.25	Mean	98.50
SD	0.359	SD	0.400
%RSD	0.362	%RSD	0.406

Analyst to Analyst

Table No. 21: Analyst to Analyst

Analyst	Label claim	Amount found*	Label claim
1	200mg	199.58	99.79
2	200mg	199.25	99.625

Result of Robustness

Table No. 22: Result of Robustness of Formulation

Compound	% RSD in Normal	Changed Condition n= 6	
Temperature		- 5°C	5°C
Zoladex	0.56	0.78	0.75
Flow rate		(-10%)	(+10%)
Zoladex	0.69	0.95	0.98
Mobile phase ratio		-2%	+2%
Zoladex	09	0.99	0.99

CONCLUSION

Analytical techniques are the study of the separation, Identification and quantification of the chemical components of natural and artificial materials. These are applied in both qualitative and quantitative analysis. Qualitative analysis gives an indication of the identity of the chemical species in the sample and quantitative analysis determines the amount of one or more of these components. Analytical techniques used in forensics, bioanalysis, clinical, environmental analysis and materials analysis. Chemical analysis is important in controlling the quality of raw materials, intermediate and finished products. In the present research work, a successful attempt was made for “Validated UV and HPLC method development for the estimation of Zoladex in marketed formulation” which was developed by experimentation based on thorough literature survey and ascertained by statistical parameters of sampling.

Liquid chromatographic system from waters comprising of manual injector, Waters controller pump for constant flow and constant pressure delivery and U.V. detector (Waters 486) connected to data Ace software controlling the instrumentation as well as processing the data generated were used. The isocratic mobile phase consisted of methanol and acetonitrile in the ratio of (50:50v/v) at a flow rate of 1.0ml min⁻¹. AC-18 column (4.6 x 250mm, 5 µm particle size) was used as the stationary phase, 278.0nm was selected as the detection wavelength for UV-vis.detector.

The simplicity, rapidity, accurate and reproducibility of the proposed methods completely fulfill the objective of the research work of estimation of the drug in marketed formulation. Proposed method was found to be linear in the range of 5-25µg/ml Zoladex with the correlation coefficient near to one 0.999 respectively. The validation and the reliability of proposed method were assessed by recovery study. The recovery of added standards (80%, 100% 120%) was near to one for zoladex respectively. The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and % RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in capsule dosage form. The result obtained shows the developed method to be precise, simple, rapid and accurate. Thus, these can be used for routine analysis of zoladex in bulk drug and capsule dosage form. It was thus, concluded that the proposed methods is new, simple, accurate, safe, free from pollution, precise and can be successfully employed in the routine analysis. The simplicity, rapidity reproducibility and economy of the proposed methods

completely fulfill the objective of this research work. The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

CONFLICTS OF INTERESTS

There are no any conflicts of interests.

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