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METHOD DEVELOPMENT AND VALIDATION OF LIRAGLUTIDE IN BULK AND MARKETED DOSAGE FORM BY USING RP-HPLC TECHNOLOGY

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ABSTRACT

Simple, Precise, Economical, Fast and Reliable RP-HPLC Technique has been developed for the estimation of Liraglutide in bulk and pharmaceutical dosage form. The detection was carried out by using a UV detector set at a wavelength of 245nm and 20µl sample was injected. The retention time for Liraglutide was 2.978 min. The peak obtained was symmetrical with tailing factor less than 2 and theoretical plates more than 2000. Isocratic elution at a flow rate of 1.2ml/min was employed on Inertsil - Extend - C18 (250 × 4.6 mm, 5 µm) HPLC column. A mobile phase comprising PHP Buffer (0.1% OPA): MeOH: ACN 50:25:25%V/V (pH 4.2) was developed. Validation experiments were performed to demonstrate linear over the concentration range of 10 - 100µg/ml and get the correlation Regression (r^2) 0.9996, showed good recoveries 98.95 - 101.22 %, the % RSD of Precision studies were found < 2%. The method can be used for quality control assay of Liraglutide. The results were validated statistically as per ICH Q2 R1 guideline and were found to be satisfactory. The proposed methods were successfully applied for the determination of Liraglutide in commercial pharmaceutical dosage form.

Keywords:

Key words:
RP-HPLC, Liraglutide, Method Development, Validation, and ICH guidelines.

INTRODUCTION

Liraglutide is GLP-1RA approved for treatment of type 2 diabetes. The drug profile of this drug is characterized by mild dose-related weight loss of 2-6 kg [1]. Currently, liraglutide is the only GLP-1 RA approved for the treatment of obesity in a dose higher than that approved for the treatment of type2 diabetes [2].

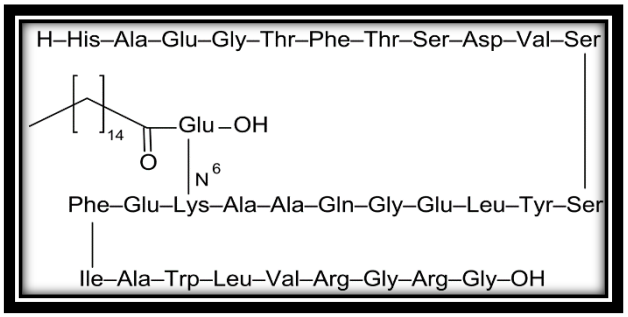


Fig. 1: Chemical Structure of Liraglutide

Hence the method was developed and validated as per ICH guidelines [3, 4]. The literature reveals that various methods for the determination of Liraglutide and pharmaceutical validations among these methods are UV - Spectrophotometric, HPLC & UPLC method for Liraglutide was reported [5-11].

MATERIALS AND METHODS

Equipment and Reagent:

A Shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Younglin RP-HPLC, Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glassware (Borosil®) was used for the validation study.

Reference standard of Liraglutide API was supplied as gift sample by Sura Laboratory, Hyderabad. The commercial formulation was "**Saxenda**" Injection containing 3mg Liraglutide tablet were collected from Mary Medicals in Hyderabad.

Method development:

Preparation of Standard Stock solution:

Stock solution was prepared by diluting 10 mg of drug in sufficient quantity of methanol in separate volumetric flask and volume was made up to 10 ml to get the concentrations 1000 µg/ml for each drug. Dilutions from stock solution were prepared in the range of 10-100 µg/ml. This solution (0.5ml) was further diluted to 10 ml with mobile phase to obtain a working standard stock solution of 50µg/ml for the RP- HPLC method.

Optimization of the chromatographic condition:

Several mobile phases were tried to resolve Liraglutide, but the resolution was not satisfactory. So, modification was made in the above mobile phase. Finally, the system containing PHP Buffer (0.1% OPA): MeOH: ACN 50:25:25%V/V (pH 4.2) as the mobile phase at a flow rate of 1.2ml/min was found to be satisfactory and gave well resolved peak for Liraglutide. The retention time for Liraglutide was 2.978 min. The detection wavelength of Liraglutide was estimated at 245 nm. Complete resolution of the peaks with clear baseline separation was obtained.

Method Validation:

Linearity and range:

Linearity was demonstrated by analyzing six different concentrations of active compounds. Accurately measured standard working solutions of Liraglutide 10, 25, 50, 75, 100µg/ml were prepared in 10ml volumetric flasks and diluted to the mark with mobile phase. Aliquots (20µl) of each solution were injected under the operating chromatographic conditions described above. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area vs. concentrations of Liraglutide Coefficient of correlation was 0.9996.

Accuracy (recovery):

The accuracy of the method was determined by calculating recoveries of Liraglutide by the standard addition method. Known amounts of standard solutions of Liraglutide (50%, 100%, 150%) were added to pre quantified sample solutions of tablets. The amounts of Liraglutide were determined by applying these values to the regression equation of the calibration curve.

Method precision (Repeatability):

The precision of the method was checked by repeatedly injecting (n = 6) solutions of Liraglutide (50µg/ml) for the RP-HPLC method. The accuracy of the method was evaluated by determination of the recovery of Liraglutide on two days at six levels concentration. Tablets sample solutions were spiked with Liraglutide standard solution, corresponding to 50 to 150% of the nominal analytical concentration (25µg/ml). The results showed good recoveries ranging from 98.95 to 101.22%. The mean recovery data obtained for each level as well as for all levels combined were within 2.0% of the label claim for the active substance with an R.S.D. < 2.0%, which satisfied the acceptance criteria set for the study.

Limit of Detection (LOD) & Limit of Quantification (LOQ):

The sample was dissolved by using Mobile Phase and injected until peak was diapered. After 0.053µg/ml dilution Peak was not clearly observed. So, it confirms that 0.03µg/ml limit of Detection (LOD) & 0.1µg/ml Limit of Quantification (LOQ).

Robustness:

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (altered by ± 0.1 ml/min) and mobile phase composition (methanol ± 5%).

RESULT AND DISCUSSION

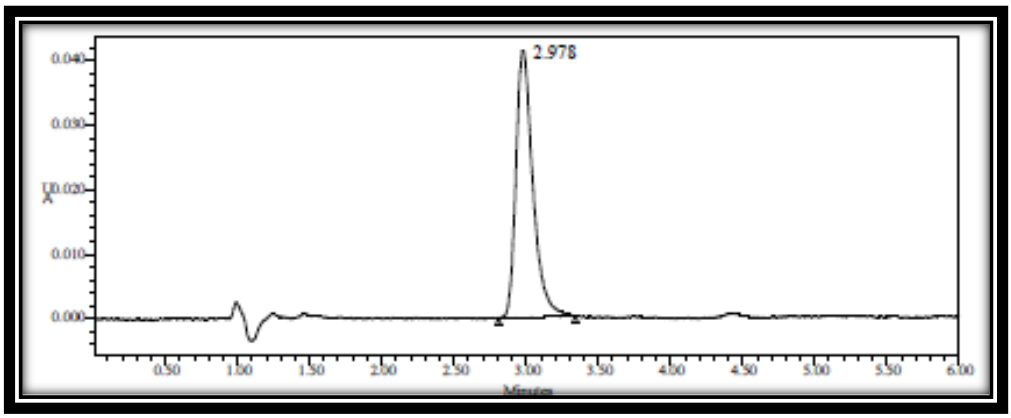
Liraglutide standard having concentration 50µg/ml was scanned in UV- region between 200- 400 nm. λ max of Liraglutide was found to be at 245nm. The Liraglutide peak in the sample was identified by comparing it with the Liraglutide standard and the Retention time was found to be around 2.978 minutes. The peak obtained was

symmetrical with tailing factor less than 2 and theoretical plates more than 2000. The estimation Liraglutide was carried out by RP-HPLC using Mobile phase having a composition of PHP Buffer (pH 4.2; 0.1% OPA): MeOH: ACN (50:25:25%V/V). Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10minutes. The column used was Inertsil - Extend C_{18} (250 × 4.6 mm, 5 µm). Flow rate of Mobile phase was 1.2 ml/min.

System suitability parameters such as %RSD for six replicate injections were found to be 0.166 %, Theoretical Plates - 5617.7, and Tailing Factor - 1.2917. The acceptance criteria of System Suitability is RSD should be no more than 2.0% and the method shows Method Repeatability 0.0807 % which shows that the method is precise. The validation of the developed method shows that the drug stability is well within the limits. System suitability parameters are listed in table.2.

Table No. 1: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	Inertsil-Extend C_{18} (250 × 4.6 mm, 5 µm)
Mobile Phase	PHP Buffer (pH 4.2; 0.1% OPA): MeOH: ACN (50:25:25%V/V)
Flow rate (ml/min)	1.2
Run time (minutes)	10.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	245
Drugs RT (min)	2.978



S. No	Peak Name	R_t	Area	Height	USP Tailing	USP Plate Count
1	Liraglutide	2.978	12135	6587	1.2917	5617.7

Fig. 2: Standard Chromatogram of Liraglutide

Table No. 2: System suitability parameters

Parameter	Liraglutide
Calibration range (µg/ml)	10 – 100
Theoretical plates	5617.7
Tailing factor	1.2917
Correlation Coefficient (r^2)	0.9996
% Recovery	98.95 – 101.22 %
System Suitability % RSD	0.166
Method Repeatability %RSD	0.0807

Table No. 3: Summary of results of Injection Precision parameter for Liraglutide

S. No.	Liraglutide
I.P-1	2410835
I.P-2	2409735
I.P-3	2411035
I.P-4	2410063
I.P-5	2409025
I.P-6	2412072
Mean	2410460.833
SD	1078.169637
% RSD	0.044728776

Table No. 4: Summary of results of Method Precision parameter for Liraglutide

S. No.	inj-1	inj-2	Avg	MEAN	SD	% RSD
MP-1	2409365	2408724	2409044.5			
MP-2	2410654	2410936	2410795			
MP-3	2407394	2406363	2406878.5	2408826.4	1944.4211	0.08072068
MP-4	2410047	2409154	2409600.5			
MP-5	2409853	2410631	2410242			
MP-6	2404439	2408357	2406398			

Table No. 5: Summary results of Accuracy for Liraglutide

Conc.		inj-1	inj-2	inj-3	Mean	% Recovery	STD	% RSD
25ppm	50%	1182464	1196743	1192567	1190591	98.95359	7341.655	0.616639
50ppm	100%	2420762	2422832	2422663	2422086	100.6534	1149.439	0.047457
75ppm	150%	3654063	3658946	3647835	3653615	101.2209	5569.051	0.152426

The linearity of the detector response was found to be linear from 10 to 125µg/ml of target concentration for Liraglutide standard with a correlation coefficient value greater than 0.999. The correlation coefficient of (r2) = 0.999, which shows that the method can produce good response in UV-detector.

Table No. 6: Summary of results of Linearity parameter for Liraglutide

Conc. (µg/ml)	Average Area
10	705560
25	1288524
50	2455048
75	3541523
100	4602442
Slope Intercept	43722x + 245073
r^2	0.9996

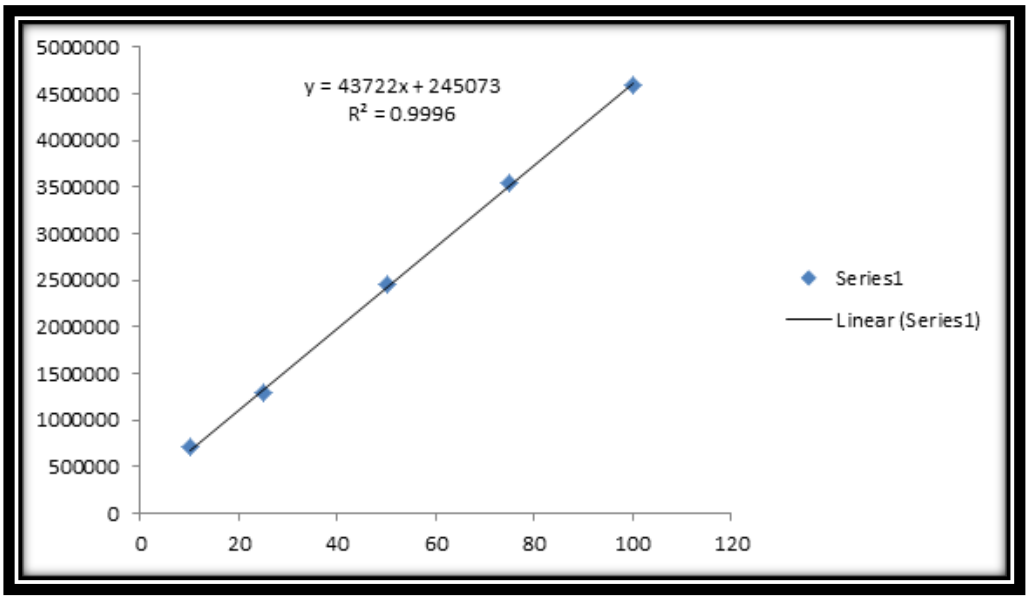


Fig. 3: Linearity Curve of Liraglutide

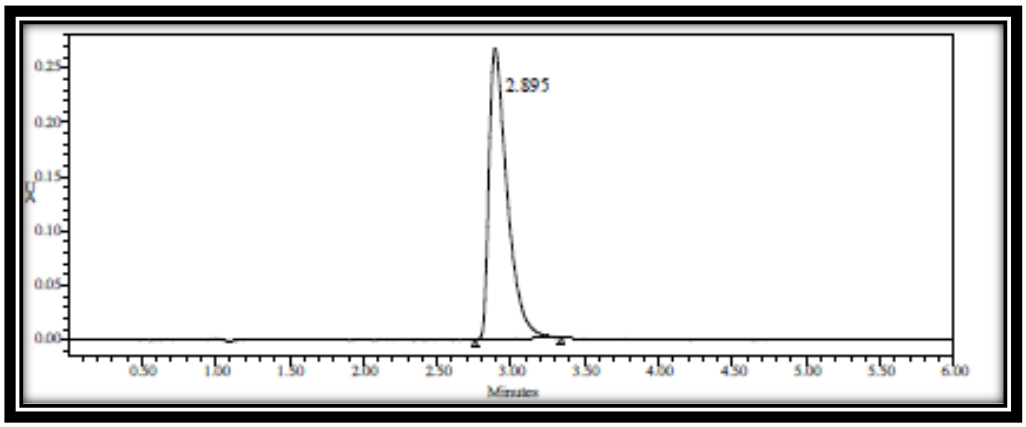


Fig. 4: Chromatogram of Liraglutide (10 µg/ml)

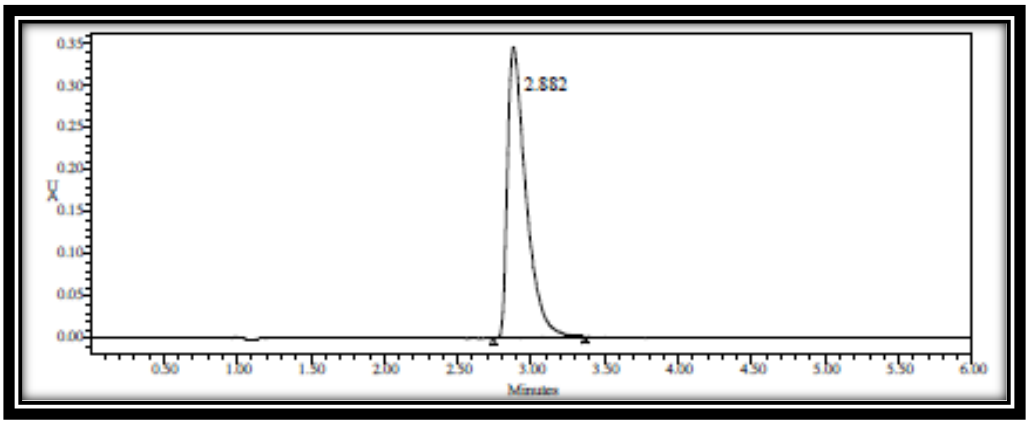


Fig. 5: Chromatogram of Liraglutide (25 µg/ml)

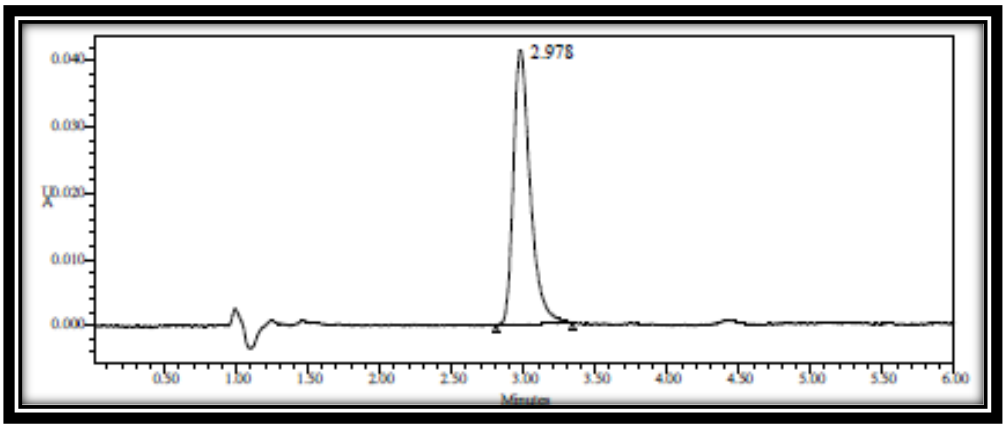


Fig. 6: Chromatogram of Liraglutide (50 µg/ml)

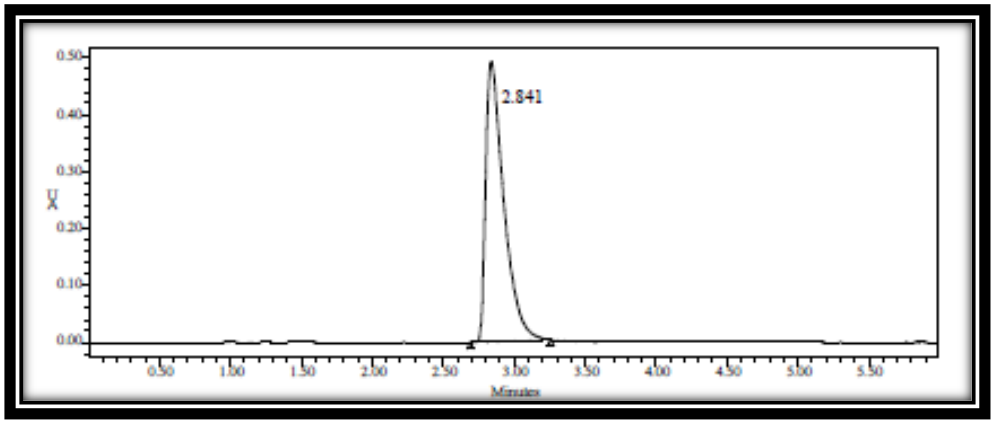


Fig. 7: Chromatogram of Liraglutide (75 µg/ml)

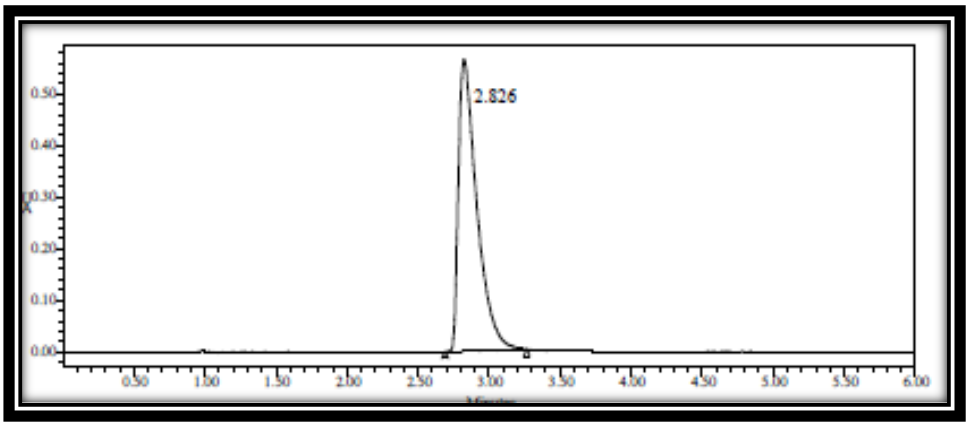


Fig. 8: Chromatogram of Liraglutide (100 µg/ml)

Table No. 7: Summary of results of LOQ for Liraglutide

Injection	Area of Liraglutide (0.1 µg/ml)
Inj-1	4994
Inj-2	4897
Inj-3	4871
Inj-4	4998
Inj-5	4873
Inj-6	4937
Mean	4928.333
SD	57.56967
% RSD	1.168137

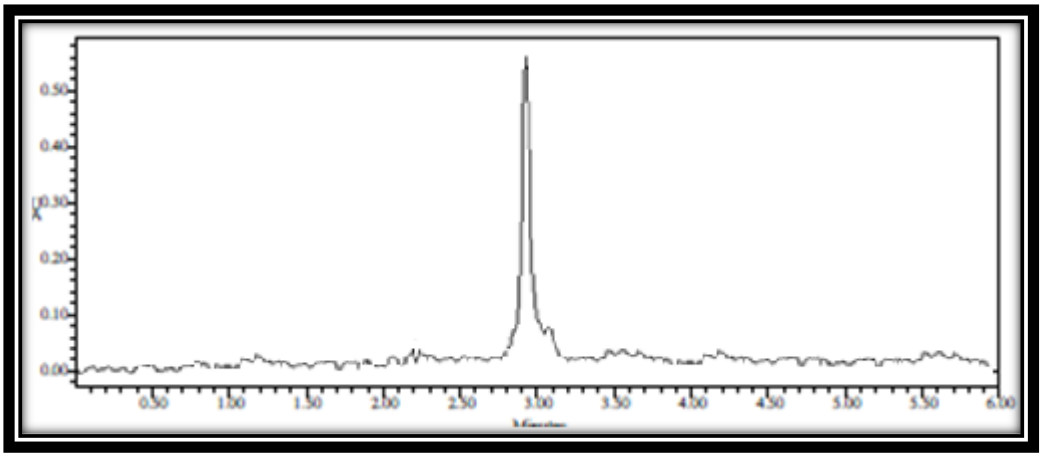


Fig. 9: LOD Chromatogram of Liraglutide

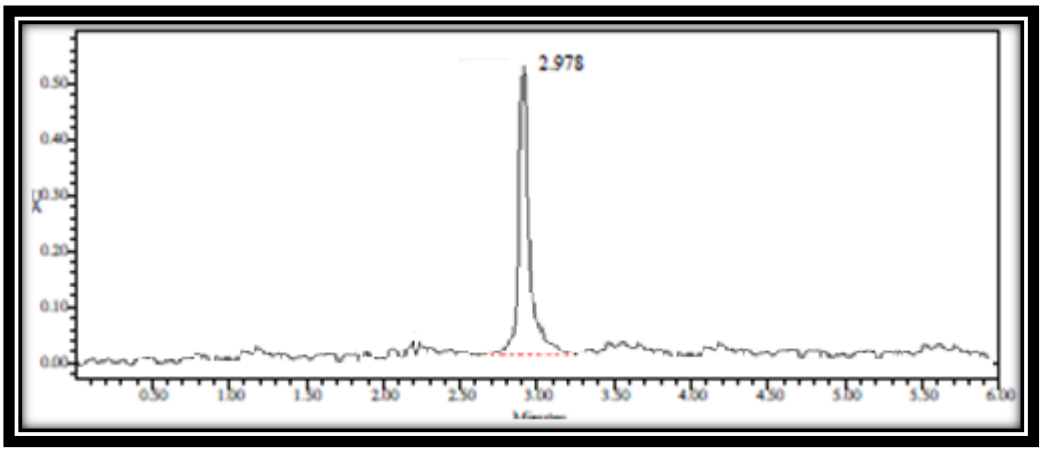


Fig. 10: LOQ Chromatogram of Liraglutide

Table No. 8: Summary of results of Robustness parameter for Liraglutide

Parameters	Adjusted to	Avg. Area[a]	RT	SD	% RSD
	1.1 ml/min	2670501.7	3.27	12826.87	0.48
Flow Rate As per method 1.2 ml/min	**As it is**	2406217.3	2.98	2902.38	0.12
	1.3ml/min	2088153	2.54	7390.12	0.35
	45: 27.5: 27.5	2560872.17	2.75	18999.19	0.74
Mobilephase comp[n] Buffer: MeOH: ACN (50:25:25)	**As it is**	2406217.3	2.98	2902.38	0.12
	55: 22.5: 22.5	2277340.2	3.14	8126.60	0.36

[a]Avg. Area = Six Repeatable injections.

CONCLUSION

RP-HPLC methods for Liraglutide was developed separately in bulk and tablet dosage form by, Absorbance maxima method. Further, RP-HPLC method for the estimation of Liraglutide was in bulk and combined dosage form. The methods were validated as per ICH guidelines. The standard deviation and % RSD calculated for these methods are < 2 %, indicating high degree of precision of the methods. The results of the recovery studies showed the high degree of accuracy of these methods. In conclusion, the developed methods are accurate, precise, robust and selective and can be employed successfully for the estimation of Liraglutide in bulk and pharmaceutical dosage form. Because of cost-effective and minimal maintenance, the present RP-HPLC methods can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of Liraglutide

in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

Conflict of Interest: The authors declare no conflict of interest.

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