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RP-HPLC METHOD FOR DETERMINATION OF ANTI-DIABETIC DRUG LIRAGLUTIDE IN BULK AND TABLET FORMULATION

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ABSTRACT

RP-HPLC method for determination of anti-diabetic drug Liraglutide in bulk and tablet formulation has been developed and validated. A RP-HPLC method was performed on Agilent Zorbax Eclipse XDB C18(100mm×4.6mm, 3.5µm) column using PHP Buffer(10mM): MeOH: ACN 60:20:20%V/V (pH 3.8 Adjusted with 0.1% Ortho-phosphoric acid), as a mobile phase at a flow rate of 1.5ml/min and analytes were monitored at 245nm. The retention time for Liraglutide was found to be 2.66min. The peak obtained was symmetrical with tailing factor less than 2 and theoretical plates more than 2000. The linearity was found in the concentration range 10-60µg/ml for Liraglutide. LOD and LOQ was found to be 0.85µg/ml and 2.60µg/ml. for Liraglutide. The percentage mean recovery at three different levels (80%,100%120%) for Liraglutide was found to be 100.4 -105.37%w/w. The percentage assay of Liraglutide in dosage form was found to be 99.1-99.5%w/w. The method was validated in accordance with ICH guidelines Q2 (R1) and was found to be Specific, Accurate, Precise, Robust and can be successfully applied for routine analysis of Liraglutide in bulk and pharmaceutical dosage form. The developed method has been found suitable for routine analysis of Liraglutide in bulk and tablet formulation.

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INTRODUCTION

Diabetes mellitus is a complex metabolic disease that can have devastating effects on multiple organs in the body. It causes due to the insufficient secretion of insulin. In diabetes blood sugar level increased for prolonged period due to the group of metallic disease. The rising prevalence of diabetes all over the world has gained public concern largely ascribing to its relevant long-term complications. [1]

According to the International Diabetes Federation (IDF), It is estimated that there are approximately 435 million people living with diabetes worldwide and the number will reach over 642 million by 2040. [2,3] Accounting around 90% of all cases, type II diabetes mellitus is major disease it affects the human health and it may lead to cause of death. [4] Insulin and its analogs have played a huge role in the treatment of diabetes, their side effects, including hypoglycaemia, obesity and allergies, cannot be ignored. [5] Hence after long years of research, the discovery of Glucagon- like peptide-1(GLP-1) receptor agonist has significantly helps the diabetic patients, as these drugs not only reduces blood glucose level and maintain the glucose level but also significantly reduces the risk of the side effects.

Liraglutide is a long-acting glucagon-like peptide-1 (GLP-1) analogue developed by Novo Nordisk and approved by the European Medicines Agency (EMA) on July 3, 2009, and by the U.S. Food and Drug Administration (FDA) on January 25, 2010. one of the antidiabetic drug introduced in the market is **VICTOZA**(Liraglutide) injection. [6-8]

Liraglutide long-acting glucagon-like peptide-1 receptor agonist, has used for the treatment of type II diabetes, which reduces the hyperglycaemia, through increased the glucose dependent insulin, inhibiting the glucagon release, delay the gastric emptying and appetite suppression. Liraglutide also plays important role for cardio protection, including reducing the infract size, improving the left ventricular ejection. [9,10]

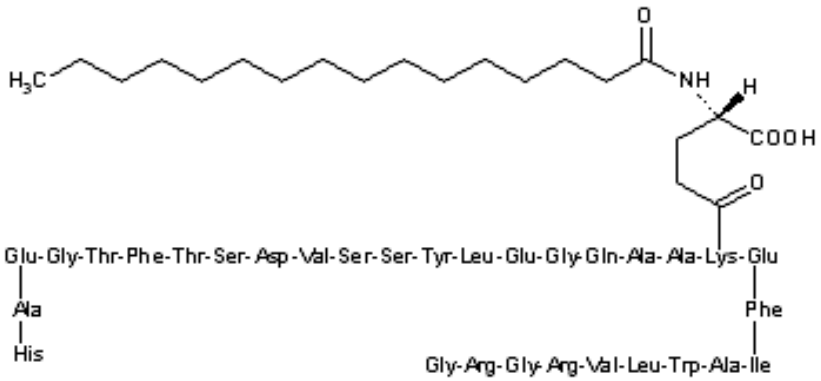

Figure 1: Structure of Liraglutide (LIRA).

Literature survey cites very few RP-HPLC methods reported for the determination of anti-diabetic drug Liraglutide in bulk and tablet formulation.

Hence in this proposed project work, a New RP-HPLC method for determination of anti-diabetic drug Liraglutide in bulk and tablet formulation have been developed and validated as per ICH guidelines Q2 (R1). [11]

MATERIALS

The chemicals and reagents which are used in method development are Acetonitrile (HPLC grade), Methanol (HPLC grade), Phosphate Buffer (Potassium di hydrogen ortho phosphate Mono basic), Ortho phosphoric acid, HPLC grade water.

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITION

An isocratic HPLC Shimadzu LC-2010CHT Liquid Chromatograph was used, Electronic Weighing Balance (Sartorius – TE 214 S), Ultra sonicator (RC Systems – MU 1700), UV–Visible Spectrophotometer (Shimadzu – 1700, Software Version - UVProbe 2.34), Digital pH Meter (Digisun Electronics – 7007), Vacuum Pump (Servewell Instruments Pvt. Ltd.), Supor 200 Membrane Filter, 0.2 µm (Pall India Pvt. Ltd.), Cellulose Acetate Filter, 0.45 µm (Sartorius AG).

Method Development

Standard LIRA solution

Accurately weighed 10mg of LIRA standard and transferred into 10mL volumetric flask, 3-5mL of Mobile phase was added and sonicated for 2 min to dissolve it fully. Make up the volume with Mobile phase to get 1000µg/ml of standard LIRA solution and labelled as **STD Stock A**

Preparation of Buffer (10mM PHP)

Accurately weighed 0.68gm of HPLC grade potassium dihydrogen orthophosphate and transferred into 500mL volumetric flask and added 200mL double distilled water and sonicated for 5 min to dissolve it fully. Finally make up the volume up to 500ml mark with double distilled water.

Preparation of 0.1% OPA

0.1ml of ortho-phosphoric acid was transferred into a 100mL volumetric flask and make up the final volume up to the mark with double distilled water and labelled.

System suitability parameters

The standard solutions of LIRA (30µg/ml) were prepared and analysed six times. Chromatograms were studied for different system suitability parameters such as tailing factor, height equivalent to theoretical plates (HETP) see that whether they comply with the recommended limit.

Method Validation

Method validation of RP-HPLC method was done as per ICH guidelines for parameters like linearity, accuracy, precision, robustness, LOD and LOQ.

Specificity

Solutions of standard and sample were prepared. It was observed that other substances present in the formulation did not interfere with the peak of LIRA and thus the method was specific. The peak purity of LIRA was checked by comparing the spectra at different level viz. peak start, peak apex and peak end position of the spot.

Linearity and range

The linearity of an analytical procedure specifies the results which are directly proportional to the concentration of analyte in the sample. The linearity and range were determined from coefficient of correlation (R2) obtained by plotting AUC vs. concentration.

Accuracy

Accuracy studies were performed at three different levels (80%, 100% and 120%) and the % Recovery of LIRA was calculated and presented below.

Precision

The precision of the method was determined in terms of repeatability and intraday and Interday precisions. Intraday and Interday precision was determined.

Limit of detection and limit of quantification

Detection limit was determined based on the standard deviation of peak area and was calculated by formula LOD= 3.3(Standard deviation/Slope). Also, Quantification limit was determined based on the standard deviation of peak arca and was calculated by formula LOQ 10(Standard deviation/Slope).

Robustness

Few parameters were deliberately varied for study of robustness. The robustness was carried out by changing wavelength, change in saturation time and change mobile phase volume. All these parameters were varied by ±2% and mean, S.D and % RSD were calculated**.** [11]

METHOD

The Column used was Agilent Zorbax XDB C18 (100 mm × 4.6 mm, 3.5µm) and mixture of PHP: MeOH: ACN (10mM) in the ratio 60:20:20% v/v with pH 3.8 was used as the mobile phase and was filtered before through 0.45µ membrane filter. The flow rate of mobile phase was maintained at 1.5ml/min and UV detection was carried out at 245 nm. The standard solutions of LIRA (30 µg/mL) in mobile phase were prepared.

RESULTS

The retention time was found to be 2.66min for LIRA (Fig 1). The developed method was validated as per ICH Q2 (R1) guidelines. For linearity and range, RP-HPLC analysis was performed on series of concentration range for LIRA. The graph was plotted by AUC vs Concentration and the range was determined from the linear part of the graph and was found to be in the concentration ranges of 10-60µg/ml for LIRA with correlation coefficients of 0.999(Fig 2). LOD and LOQ for LIRA were found to be 0.85 and 2.60µg/ml. The %RSD for AUC of the study was found to be less than 2% indicating that the method was stable during Inter and Intraday studies. Accuracy was determined by standard addition method at three different levels. (80%, 100% and 120%) by calculating mean percentage recovery and % recovery at three different levels was found to be 105.37%,102.4%,100.5%w/w for LIRA which was well within the range of 95-110% w/w, hence the method was found to be accurate. The percentage assay for LIRA was found to be 99.15-99.5%w/w respectively which is well within the acceptance criteria of 95-105%w/w. Hence the developed and validated method was found to be specific, accurate, precise, linear and robust and thus can be routinely applied for determination of liraglutide in bulk and pharmaceutical dosage form.

Detection of wavelength

Liraglutide standard solution was scanned from 400-200nm in UV spectrophotometer. And it showed maximum absorbance at 245nm.

Effect of mobile phase composition

Several mobile phase systems were tried to get sharp, symmetrical peak of LIRA in the chromatogram. The observations obtained with the mobile phases tried in different ratios and the chromatograph obtained.

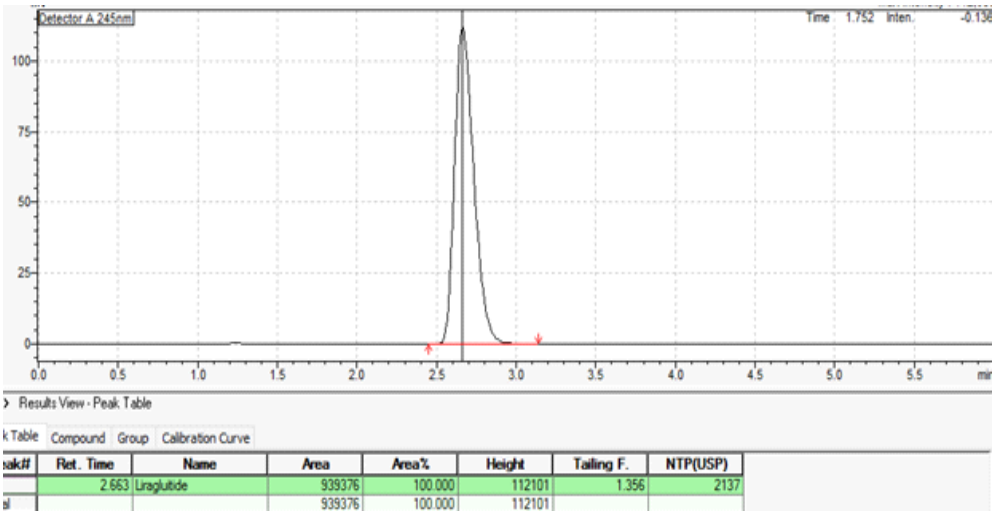

Figure 2: Chromatogram for Liraglutide(30μg/ml) in mobile phase containing PHP: MeOH: ACN (60:20:20% v/v).

System suitability

System suitability was performed to confirm that the system was appropriate for the analysis to be performed. The test was carried out by six replicate injections of standard solutions containing 30 µg/ml LIRA analysing each analyte for its theoretical plates (2137), resolution (2.663 min) and tailing factor (1.356) has represented in the Figure 2.

METHOD VALIDATION

SPECIFICITY

Specificity was determined to see if any interference occurs with that of retention time of this drug. From the chromatogram obtained, there was no extra peak found in chromatogram of blank, standard and sample thus indicating no interference in the retention time of the analytes was found as shown in the Figure 3.

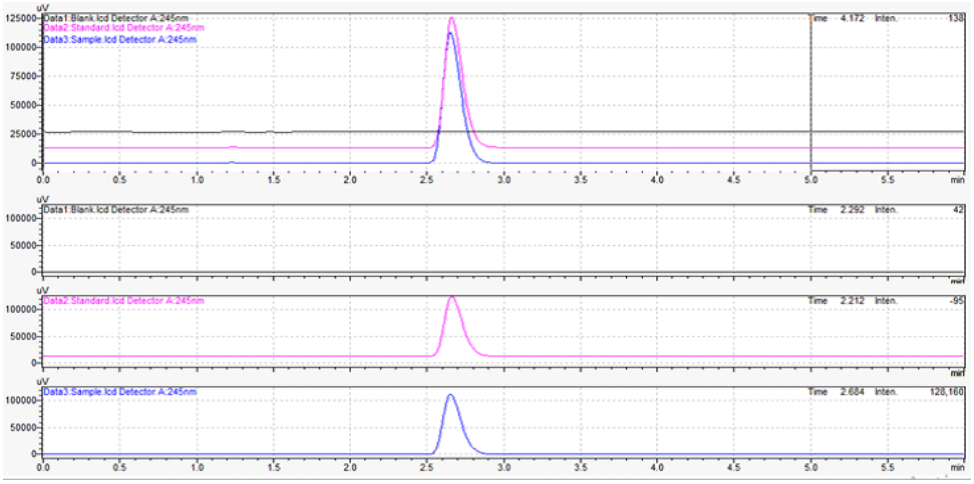

Figure 3: Stacked Chromatogram for specificity studies of LIRAGLUTIDE.

Linearity

The linearity of an analytical procedure specifies the results which are directly proportional to the concentration of analyte in the sample. The linearity and range were determined from coefficient of correlation (R2) obtained by plotting AUC vs. concentration at 245 nm. LIRA was observed to be linear in the range of 10-60µg/ml respectively and correlation coefficient was found to be well within acceptance criteria of NLT 0.999 as presented in Table 1 and 2 and Figure 4 respectively.

Table 1: Linearity of Liraglutide.

Sl. No.	Concentration (µg/ml)	AUC
1	10	316066
2	20	631779
3	30	942303
4	40	1273474
5	50	1582523
6	60	1916729

Table 2 Linear Regression Analysis Data for Calibration Curve of LIRA.

Parameters	Liraglutide
Range(µg/mL)	10-60
Correlation coefficient(R^2)	0.999
Slope	31962
Intercept	8193

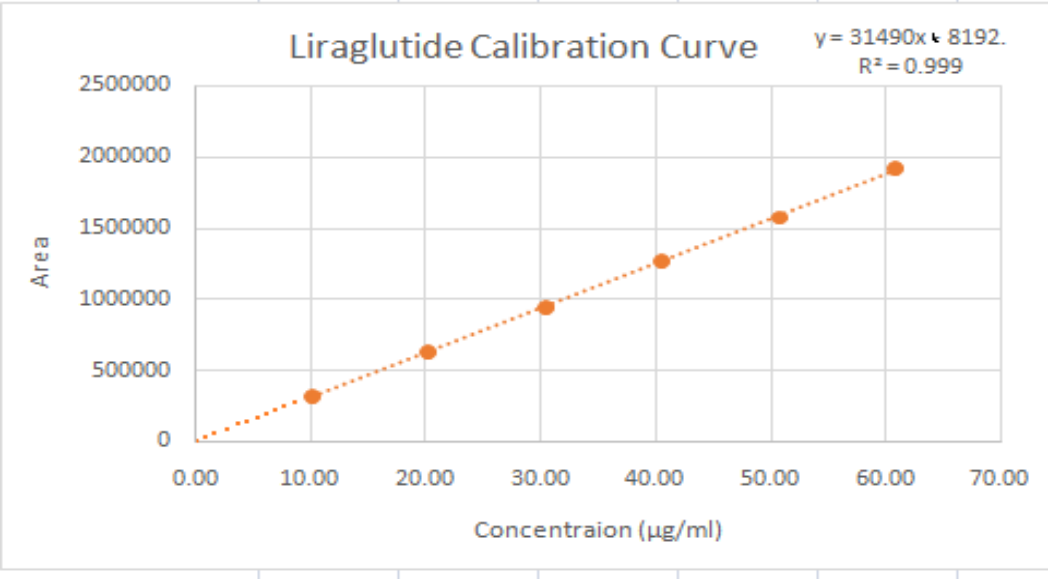

Figure 4: Calibration curve of Liraglutide.

Accuracy

Accuracy studies were performed at three different levels (80%, 100% and 120%) and the % Recovery of LIRA was calculated and presented below. The percentage recovery for LIRA at three different levels (80%,100%,120%) was found to be from100.5%-105.3% for LIRA which is well within the acceptance criteria limits(95-110%) as represented in Table 3 and Figure 5 respectively.

Table 3: Percentage Recovery data for accuracy studies at three different levels.

DRUGS	Conc.of STD (µg/ml) A	Conc.of Sample (µg/ml) B	Total (A+B) (µg/ml)	Conc	Peak Area* forMixture STD+ Sample	Total amount(A+B) from graph (µg/ml)	Recovery of Std (µg/ml)	Recovery of Std
	8	30	38		784760 786231 785750 **785496**	38.43	8.43	105.37
LIRA	10	30	40		930469 939901 942090 **935185**	40.24	10.24	102.4
	12	30	42		1144533 1161172 1139275 **1152853**	42.06	12.06	100.5

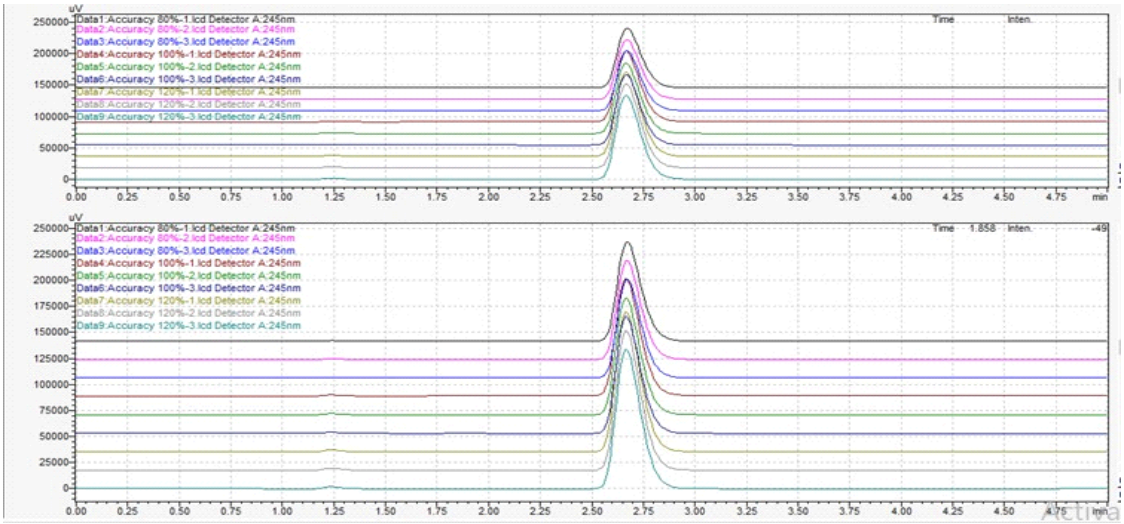

Figure 5: Overlain Chromatogram for accuracy studies of LIRA at three different levels.

Precision

It can be inferred that as the %RSD for Intra-day and Inter- day studies for LIRA was within the acceptance criteria of less than 2%. Hence, it can be concluded that the developed method was found to be precise during intra and inter day studies. It can be inferred that as the %RSD for Intra-day and Inter- day studies for LIRA was within the acceptance criteria of less than 2%. Hence, it can be concluded that the developed method was found to be precise during intra and inter day studies as presented in Table 4.

Table 4: Results for Intra-day and Inter-day precision studies.

Intraday TIME (Hrs) LIRA (30µg/ml)	MEAN AUC (n=3)	Intraday TIME (Days) LIRA (30µg/ml)	MEAN AUC (n=3)
0	930469	1	955968
2	939901	2	956912
4	942090	3	957502
MEAN	937487	MEAN	956794
SD	6175.25	SD	773.778
%RSD	0.658	%RSD	0.080

LOD and LOQ

The lowest amount of LIRA that can be detected and quantified was calculated from the respective calibration curve. The LOD for LIRA was found to be 0.85µg/mL and the LOQ for LIRA was found to be 2.60µg/mL. The results of LOD and LOQ studies are presented in Table 5.

Table 5: Data for LOD and LOQ Studies.

Paramete	LIRA
Mean of y-intercept	8193
SD of slope	31962
LOD(µg/ml)	0.85
LOQ(µg/ml)	2.60

ROBUSTNESS

Robustness was evaluated on the basis of parameters change with small but deliberate variations. The standard solution of LIRA was studied with variation in flow rate, pH and organic phase ratio as represented in Table 6,7 and 8 and as shown in the Figure 6,7 and 8 respectively.

Table 6: Data for robustness for variation in Flow rate (mL/min).

DRUG	Acceptancecriteria	FLOW RATE		
		STD (1.5mL/min)	+3%(1.545mL/min)	-3%(1.455mL/min)
	TF (≤2.0)	1.353	1.3525	1.35
LIRA	TP (≥2000)	2136.5	2164	2105

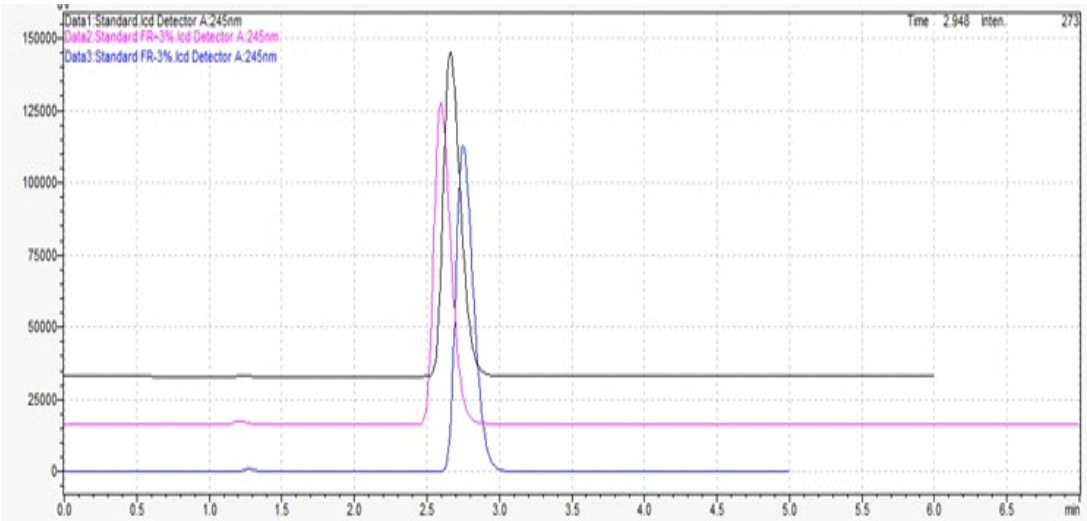

Figure 6: Chromatogram for Robustness studies for Change in Flow rate.

Table 7: Data for robustness Change in pH.

DRUG	Acceptancecriteria	pH		
		STD (3.8)	+3% (3.9)	-3% (3.69)
	TF (≤2.0)	1.354	1.35	1.350
LIRA	TP (≥2000)	2136.5	2072	2149

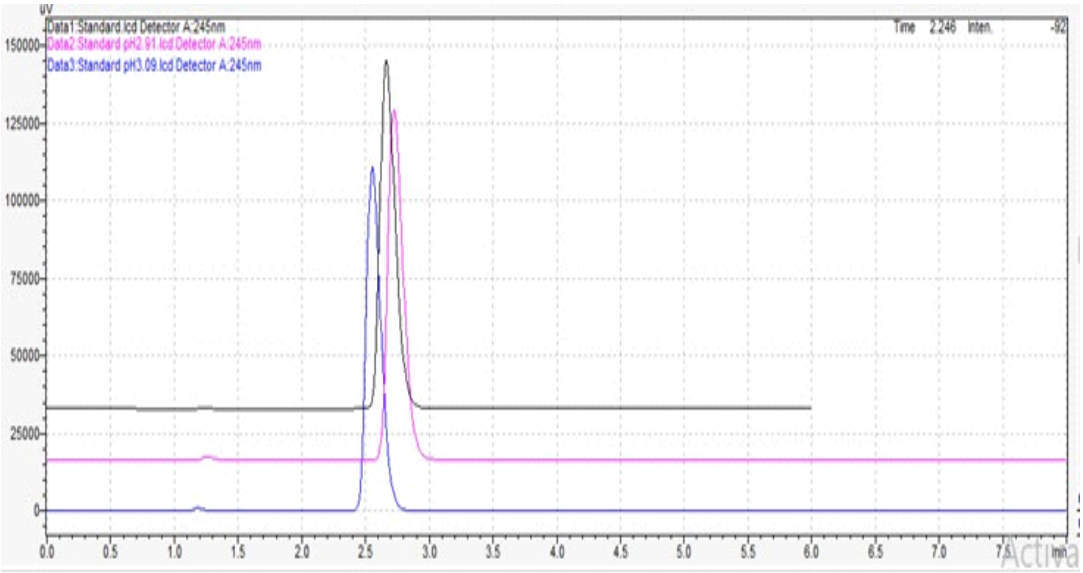

Figure 7: Chromatogram for Robustness studies for Change in pH.

Table 8: Data for robustness variation in Organic phase ratios.

DRUG	Acceptance criteria	ORGANIC PHASE		
		STD (60:20:20)	+3% (61.8:19.1:19.1)	-3% (58.2:20.9:20.9)
LIRA	TF (≤2.0)	1.355	1.354	1.357
	TP (≥2000)	2136.5	2103.5	2164.5

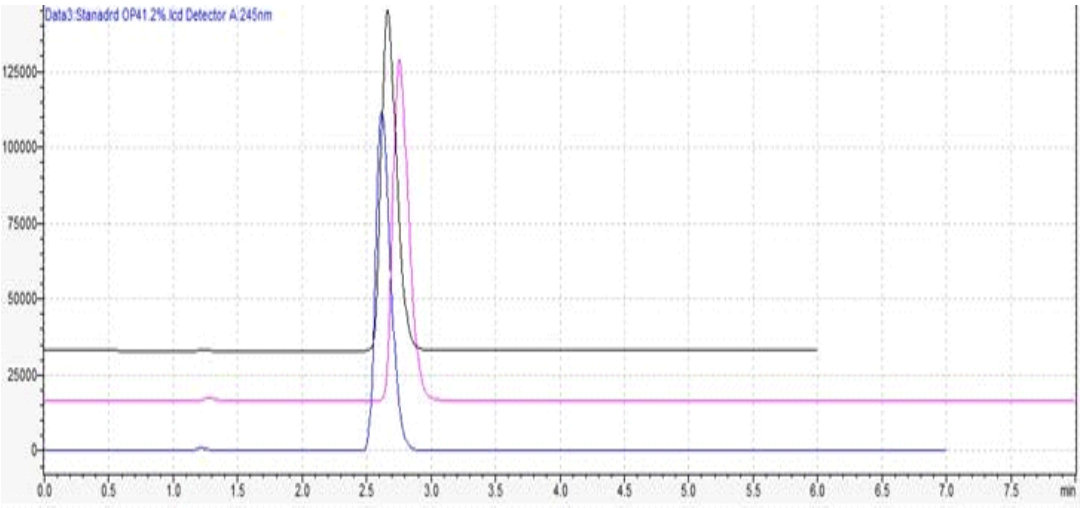

Figure 8: Chromatogram for Robustness studies for Change in Organic phase ratios.

CONCLUSION

The developed method was validated as per ICH Q2 (R1) guidelines. The results obtained for all validation parameters were found within the acceptance criteria which are shown in **Table (2,3).** Thus, the proposed method was found to be specific, accurate, linear, precise, robust and can be successfully applied for routine analysis of liraglutide in pharmaceutical dosage form, simultaneous analysis and can be applied for stability indicating studies.

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ABBREVIATIONS

ACN:	Acetonitrile
AUC:	Area Under Curve
DM:	Diabetes mellitus
EMA:	European Medicines Agency
FDA:	Food Drug Administration
HPLC:	High Performance Liquid Chromatography
IBD:	International Diabetic Federation
ICH:	International Conference on Harmonization
LIRA:	Liraglutide
LOD:	Limit of Detection
LOQ:	Limit of Quantification
µg:	Microgram
µl:	Microlitre
µm:	Micrometre
MeOH:	Methanol
Mg:	Milligram
Min:	Minute
Ml:	Milliliter
Mm:	Millimeter
mM:	Millimole
nm:	Nanometre
OPA:	Orthophosphoric acid
PHP:	Potassium dihydrogen ortho-Phosphate Buffer
RP:	Reverse Phase
RSD:	Relative Standard Deviation
RS:	Resolution
SD:	Standard Deviation
Sl No.:	Serial Number
TF:	Tailing factor
TP:	Theoretical plates
UV:	Ultraviolet
:	Wavelength
%:	Percentage

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