FP 605

Selective and sensitive method for estimation of liraglutide in human plasma using LCMS-8060

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1. Overview

Liraglutide was analyzed in human plasma using a solid phase extraction method in MRM mode on LCMS-8060. The developed method is selective and sensitive for estimation of liraglutide in human plasma

2. Introduction

Liraglutide is a glucagon-like peptide-1 receptor agonist (GLP-1 receptor agonist) also known as incretin mimetics. It works by increasing insulin release from the pancreas and decreases excessive glucagon release(1). Liraglutide is a medication used for treatment of type 2 diabetes or obesity. The prolonged action of liraglutide is achieved by attaching a fatty acid molecule at one position of the GLP-1-(7-37) molecule, enabling it to both self-associate and bind to albumin within the subcutaneous tissue and blood stream. The active GLP-1 is then released from albumin at a slow, consistent rate. Albumin binding also results in slower degradation and reduced renal elimination compared to GLP-1-(7-37).

Following subcutaneous administration, a mean C max of 35 ng/mL was achieved after 8-12 hours of dosing with an absolute bioavailability of 55 %. It indicates that the method required for pharmacokinetic evaluations need to achieve a sensitivity limit of 0.50 ng/mL.

Such method should address many problems posed by peptides viz., poor ionization, non-specific adsorption, carry-over and low extraction recovery.

We have therefore developed a method with high chromatographic resolution and ample sensitivity giving lowest limit of quantification (LLOQ) of 0.50 ng/mL for liraglutide in human plasma using LCMS-8060. Method was developed keeping some key criteria in focus- namely simpler extraction procedure, highly optimized chromatography and enhanced sensitivity. These factors enable selective and high-throughput analysis of liraglutide for the pharmacokinetic investigation.

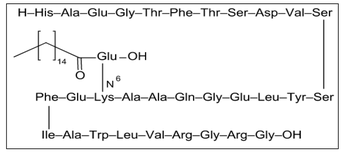

Figure 1. Structure of Liraglutide(2)

3. Materials and methods

3-1. Sample Preparation

• Preparation of calibration curve standards and quality control (QC) samples

Calibration standards for liraglutide were prepared in K_2 ETDA human plasma at concentration levels ranging from 0.50 to 202.70 ng/mL. Quality control samples were prepared at concentration levels between 0.50 to 152.03 ng/mL for LLOQ QC, LQC, MQC and HQC respectively.

• Sample extraction

Six hundred microliters of methanol was added to plasma samples and vortexed to mix for 1 min. After precipitation of proteins, samples were centrifuged for 10 minutes and processed by using solid phase extraction technique. The sample extraction protocol is mentioned below:

Conditioning and equilibration (1mL methanol followed by 1 mL water)
Sample loading
Wash 1 (0.5 mL wash solution 1 x 1 time)
Wash 2 (0.5 mL wash solution 2 x 1 time)
Elution (0.2 mL of elution solution)
SPE eluent was collected into prelabelled RIA vials and vortexed to mix before filling in HPLC vials for direct injection.

3-2. LC-MS/MS analysis

Figure 2. Nexera X2 with LCMS-8060

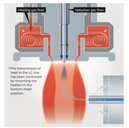

Figure 3. Heated ESI probe

LCMS-8060 triple quadrupole mass spectrometer by Shimadzu (Figure 2), sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

In order to improve ionization efficiency, the newly developed heated ESI probe (Figure 3) combines high-temperature gas with the nebulizer spray, assisting in the desolvation of large droplets and enhancing ionization. This development allows high-sensitive analysis of a wide range of target compounds with considerable reduction in background.

The details of analytical conditions are given in Table 1.

Table 1. Instrument parameters for analysis of Liraglutide

UHPLC condition (Nexera X2)		MS parameters (LCMS-8060)	
Column	Shim-pack velox biphenyl column 100 mm×2.1 mm, 2.7 um.	MS interface	Electro Spray Ionization (ESI)
Mobile phase	A: Buffer B: Acetonitrile	Nitrogen gas flow	Nebulizing gas- 3 L/min; Drying gas- 10 L/min
Flow rate	0.25 mL/min	Zero air flow	Heating gas- 10 L/min
Elution mode	Gradient	MS temp	Desolvation line- 250 °C; Heating block- 400 °C; Interface- 300 °C
Column temp	40 °C		

4. Results

4-1. Selectivity

Selectivity of the method was evaluated by analyzing 6 different lots of blank human plasma and blank plasma spiked with liraglutide at LLOQ level. No significant interference was observed at the retention time and MRM transition of analyte (refer Figure 4).

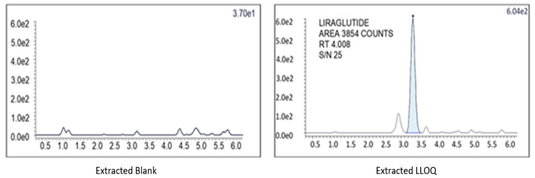

Figure 4. Chromatograms of extracted blank and extracted LLOQ (0.500 ng/mL)

4-2. Linearity

Linearity experiments were conducted for quantitation of liraglutide in human plasma. Calibration curve was plotted from 0.50-200.00 ng/ mL (Table 2). The correlation coefficient (r^2) for liraglutide was found to be > 0.98 as shown in Figures 5. Signal-to-noise ratio (S/N) for LLOQ was found more than 10:1, across 3 PA batches.

Table 2. Representative calibration curve of Liraglutide

Calibration curve data for liraglutide (n=3)

Level	CC1	CC2	CC3	CC4	CC5	CC6	CC7	CC8	CC9	CC10
Nominal conc	0.500	1.000	1.520	4.064	10.160	25.390	50.780	101.560	152.030	202.700
Mean (n=3)	0.503	1.070	1.469	4.175	10.591	25.886	50.073	99.647	159.287	204.988
SD	0.007	0.070	0.091	0.252	1.380	1.354	1.370	1.789	2.049	4.339
% RSD	1.40	6.52	6.19	6.03	13.03	5.23	2.74	1.80	1.29	2.12
% Nominal	100.61	106.97	96.62	102.74	104.24	101.95	98.61	98.12	104.77	101.13

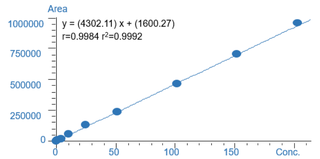

Figure 5. Representative calibration curve of Liraglutide

4-3. Intra-day Precision and accuracy

Intraday precision and accuracy were evaluated using 6 replicates of LLOQQC, LQC, MQC and HQC in single P&A batch. Statistical data is summarized in table 3.

Table 3. Intra-day Precision and Accuracy

QC (n=6)	LLOQQC	LQC	MQC	HQC
Nominal concentration	0.50	1.52	25.39	152.03
PA batch observed concentration (ng/mL)	0.46	1.29	24.79	161.22
	0.61	1.57	23.20	148.50
	0.51	1.38	25.12	146.16
	0.60	1.55	24.05	143.41
	0.47	1.86	24.84	142.15
	0.44	1.57	24.41	154.97
Mean	0.51	1.54	24.40	149.40
STDEV	0.07	0.20	0.70	7.35
% CV	14.52	12.79	2.85	4.92
% Nominal	102.63	101.01	96.10	98.27

4-4. Global Precision and accuracy

Global precision and accuracy were evaluated on 3 PA batches. Precision and accuracy results were found well within the acceptance criteria with % CV < 13.09% and % nominal ranging from 102.40% to 106.78% at LQC, MQC and HQC level. At LLOQQC level, the % CV was found 18.52% and % nominal 109.98% (refer Table 4).

Table 4. Global Precision and Accuracy

QC level (n=18)	Mean Conc.	% CV	% Accuracy
LLOQ QC (0.50 ng/mL)	0.55	18.52	109.98
LQC (1.52 ng/mL)	1.62	13.09	106.78
MQC (25.39 ng/mL)	26.00	7.45	102.40
HQC (152.03 ng/mL)	158.18	12.87	104.04

4-5. Recovery

Recovery experiments was studied at LQC, MQC and HQC level. Recovery was found precise, consistent and reproducible at all levels. Global recovery for liraglutide was found 50.92% (refer Table 5).

Table 5. Global Recovery

Global recovery	Liraglutide
LQC	43.24
MQC	54.54
HQC	54.97
Mean	50.92
SD	6.65
% CV	13.06

4-6. Matrix effect

Matrix effect was studied at LQC and HQC levels. Mean matrix effect was found to be 0.97 for liraglutide. Statistical data for matrix effect is shown in Table 6. The results confirm the suitability of the method for quantitative estimation of liraglutide in human plasma.).

Table 6. Matrix effect

Liraglutide	Post extracted sample	Aqueous sample	Matrix factor	Liraglutide	Post extracted sample	Aqueous sample	Matrix factor
LQC	5,983	6,217	0.96	HQC	5,22,379	5,43,668	0.96
	5,777	5,883	0.98		5,76,332	5,81,895	0.99
	5,372	5,746	0.93		6,06,953	6,36,429	0.95
	5,723	6,024	0.95		5,61,864	6,19,884	0.91
	6,232	6,343	0.98		5,89,440	6,03,813	0.98
	5,936	5,718	1.04		5,50,638	5,81,429	0.95
Mean			0.97				0.96
SD			0.04				0.03
% CV			3.69				3.02

4-7. Carry-over effect

Carry-over effect was evaluated by injecting extracted samples in the sequence of extracted blank, extracted highest calibrator, extracted blank and extracted lowest calibrator. No carryover was observed at the retention time and MRM transition of liraglutide in the extracted blank sample following the highest standard calibrator.

4-8. Other experiments

Based on validation guidelines, method was assessed for following experiments and results were found within acceptance criteria:

- Bench top stability
- Auto sampler stability
- Freeze thaw stability
- Extended batch verification

5. Conclusion

LCMS-8060, along with special sample preparation and optimized chromatography provides a very selective and sensitive method for bioanalysis of liraglutide study samples in human plasma. Ultra-high speed and high-separation analysis was achieved on Nexera X2 UHPLC by using a simple mobile phase at a minimal gradient flow rate of 0.250 mL/min. By providing these ready-to-use solutions, we partner with your labs to achieve desired results in your scientific endeavors.

6. References

1. Liraglutide monograph for professionals. Drugs.com. American Society of Health-System Pharmacists. Retrieved 17 October 2021
2. https://en.wikipedia.org/wiki/File:Liraglutide_structure.svg (accessed October 17, 2021)