

Liraglutide (GLP-1 Analogue) Identification and Quantification in Plasma

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Abstract

A novel liquid chromatography-mass spectrometry (LC-MS) method was developed and validated for the quantification of liraglutide in human plasma. Liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist, is widely used for the treatment of type 2 diabetes and chronic obesity. Sample preparation was performed by protein precipitation followed by solid-phase extraction using Supel™ Swift HLB SPE tubes to achieve efficient matrix clean-up. Chromatographic separation was carried out on a positively charged surface (PCS) BIOshell™ A160 Peptide PCS-C18 U/HPLC column. The method provided robust chromatographic performance and highly accurate detection in complex biological matrices.

Introduction

Liraglutide is a synthetic peptide primarily used for the treatment of type 2 diabetes and chronic obesity. The peptide belongs to the class of drugs known as glucagon-like peptide-1 (GLP-1) receptor agonists.¹ Liraglutide exerts its therapeutic effect by mimicking the activity of the endogenous hormone called GLP-1, which regulates blood glucose levels and appetite. Structurally, liraglutide consists of a 31 amino acid long peptide with a 16-carbon lipid side chain that enables albumin binding, thereby prolonging its half-life in plasma (**Figure 1**). Accurate evaluation of liraglutide concentrations in plasma is therefore important due to its protein binding and stability characteristics.

Human plasma represents a complex biological matrix containing high concentrations of proteins, which can lead to column clogging and ion suppression during LC-

MS analysis. Therefore, it is important to perform suitable sample preparation.² In this study, plasma proteins were first precipitated using an acetonitrile:water mixture, followed by centrifugation of the resulting suspension. Additional matrix cleanup was performed using solid-phase extraction (SPE) with Supel™ Swift HLB tubes. Chromatographic separation was carried out on a superficially porous particle (SPP, Fused-Core® technology) BIOshell™ A160 Peptide PCS-C18 U/HPLC column, which is designed to provide efficient separations of peptides and small proteins. The column incorporates a positively charged surface (PCS) that supports improved peak shapes and enhanced loading capacity for basic peptides, even when lower concentrations of weak-acid mobile phase modifiers are used.

Experimental

Sample Preparation

Liraglutide spiked in human plasma

Liraglutide was dissolved in a 70:30 acetonitrile:water mixture to obtain a stock solution with a concentration of 1 mg/mL. The solution was further diluted with water containing 0.1% formic acid (FA) to prepare spiking solutions with concentrations of 2000 ng/mL, 1000 ng/mL, 800 ng/mL, 600 ng/mL, 400 ng/mL, and 200 ng/mL. The diluted solutions were added to human plasma in a 1:1 ratio (100 µL liraglutide spiking solution to 100 µL plasma). The final concentrations in the SPE extract applied to LC-MS analysis are shown in **Table 1**.

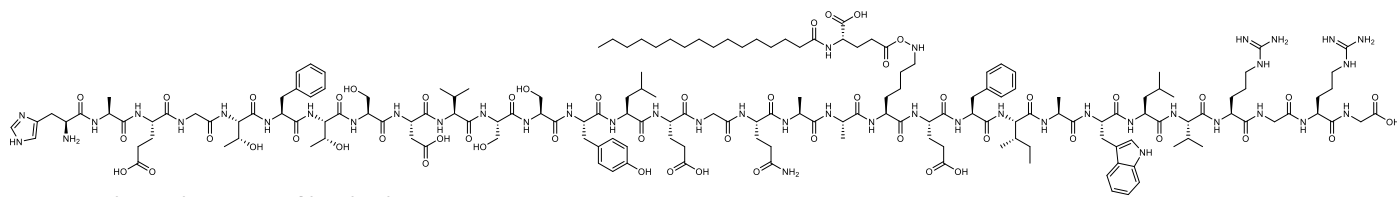


Figure 1. Chemical structure of liraglutide.

Table 1. Final concentrations of spiked liraglutide (after SPE cleanup)

No.	Equivalent concentration in plasma sample (ng/mL)	Concentration after SPE clean up (ng/mL)
1	2000	333.33
2	1000	167.67
3	800	133.33
4	600	100.00
5	400	66.67
6	200	33.33

To the plasma:liraglutide mixture (200 µL), 100 µL acetonitrile was added, resulting in the formation of a white precipitate. The mixture was centrifuged at 10,000 RPM for 10 min at 4 °C. The resulting supernatant was directly loaded onto conditioned SPE tubes.

SPE cleanup

For SPE clean-up, Supel™ Swift HLB SPE tubes (1 mL/30 mg, **57493-U**) were used in combination with a 24-port Visiprep™ vacuum manifold. The cartridges were conditioned with 500 µL water prior to loading the complete supernatant obtained from the centrifugation step described above. A wash step was performed using 500 µL water. Liraglutide was subsequently eluted using 2 x 300 µL acetonitrile:methanol (70:30 v/v) containing 0.1% formic acid (FA) (**Figure 2**).

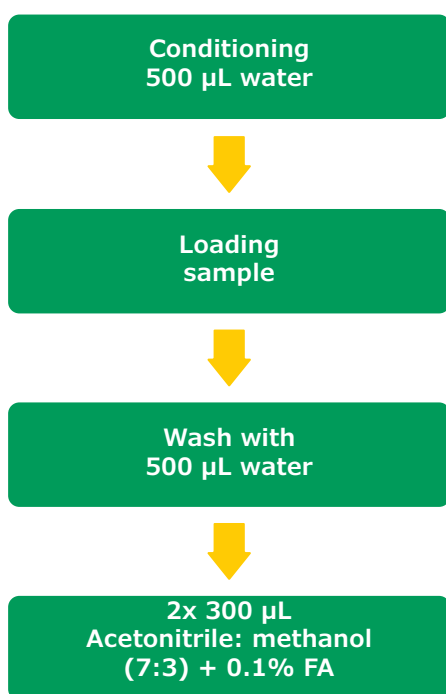


Figure 2. SPE cleanup procedure using Supel™ Swift HLB.

LC-MS Method

Sample analysis by LC-MS was performed using a BIOshell™ A160 Peptide PCS-C18 column (2.7 µm, 150 x 2.1 mm I.D.). Mobile phases containing 0.1% formic acid (FA) in the mobile

phases were used for the chromatographic separation. The LC-MS conditions applied for the analysis of liraglutide in plasma extracts are summarized in **Tables 2 and 3**.

Table 2. LC-MS conditions for analysis of liraglutide on plasma extracts

LC Conditions			
Column:	BIOshell™ A160 Peptide PCS-C18, 2.7 µm, 150 x 2.1 mm (50659-U)		
Mobile phase:	[A] Water + 0.1% formic acid; [B] Acetonitrile + 0.1% formic acid		
	Time (min)	% A	% B
	0	70	30
	8	20	80
	10	70	30
	12	70	30
Column temp.:	60 °C		
Flow rate:	0.5 mL/min		
Detector:	MS/MS (see conditions Table 3)		
Injection:	10 µL		

Table 3. MS conditions for liraglutide analysis

MS Conditions			
Instrument:	Bruker™ QTOF Impact II		
Source type:	Electrospray		
Polarity:	Positive		
Capillary spray voltage:	4.5 kV		
Dry gas temp.:	200 °C		
Dry gas flow:	6 L/min		
Nebulizer:	2 bar		
BBCID:	MS	MS/MS(bbCID)[M	
	isCID	2 ev	5 ev
	Collision Energy	20 ev	70 ev
	Acquisition Time Factor	1.0 x	1.0 x
Expected Liraglutide masses:	3748.9465 u (C ₁₇₂ H ₂₆₅ N ₄₃ O ₅₁); (z=4): 938.2468 u		

Results & Discussion

LC Conditions

The structure of liraglutide, which contains both a hydrophobic palmitic side chain and a polar peptide backbone, presents challenges for chromatographic separation. The selection of a suitable stationary phase is therefore critical to achieve adequate retention and good peak shape. For this reason, a BIOshell™ Peptide PCS-C18 column was employed on which liraglutide eluted at 5.3 min under the selected chromatographic conditions.

The positively charged surface of the Fused-Core® particles consistently provided excellent peak shapes (Figure 3) and allowed for flexible injection volumes ranging from 1 to 10 µL (Figure 4).

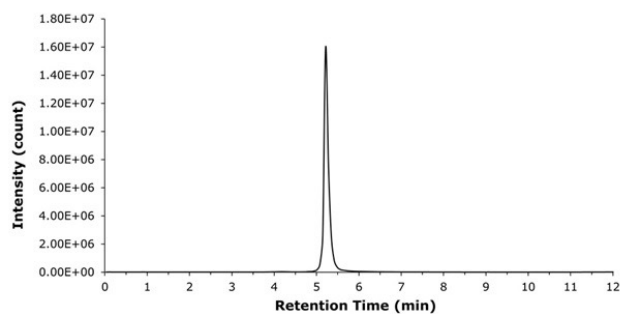


Figure 3. XIC (938.2468 u) chromatogram of liraglutide.

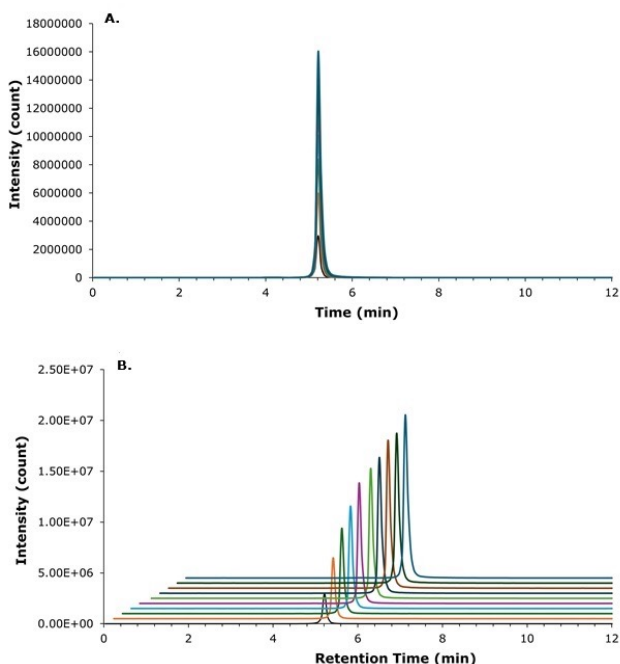


Figure 4. Loading study using injection volumes from 1 to 10 µL (133.3 ng/mL liraglutide extract solution) peak overlay without (A) and with (B) offset.

MS-Detection

Liraglutide, with an exact mass of 3748.9465 u ($C_{172}H_{265}N_{43}O_{51}$), was primarily detected by targeting its quadruply charged ion ($z=4$) at m/z 938.2468 u, which was observed to be one of the most abundant charge states alongside the triply charged ion ($z=3$). Furthermore, broadband collision-induced dissociation (bbCID) fragmentation patterns provided highly accurate confirmation, enabling confident identification of liraglutide even in this complex biological matrix (Figures 5 and 6).

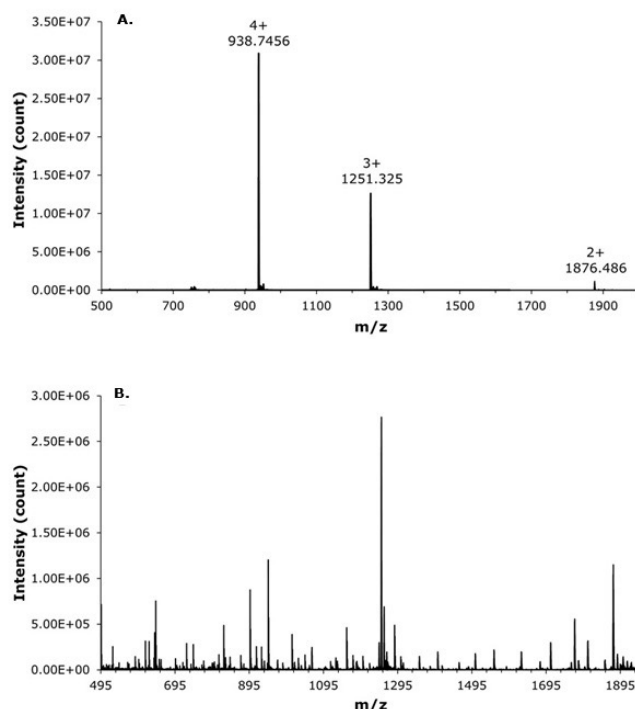


Figure 5. MS spectra of liraglutide masses for (A) MS $z= 4, 3$, and 2 , and (B) MS/MS BBCID spectra.

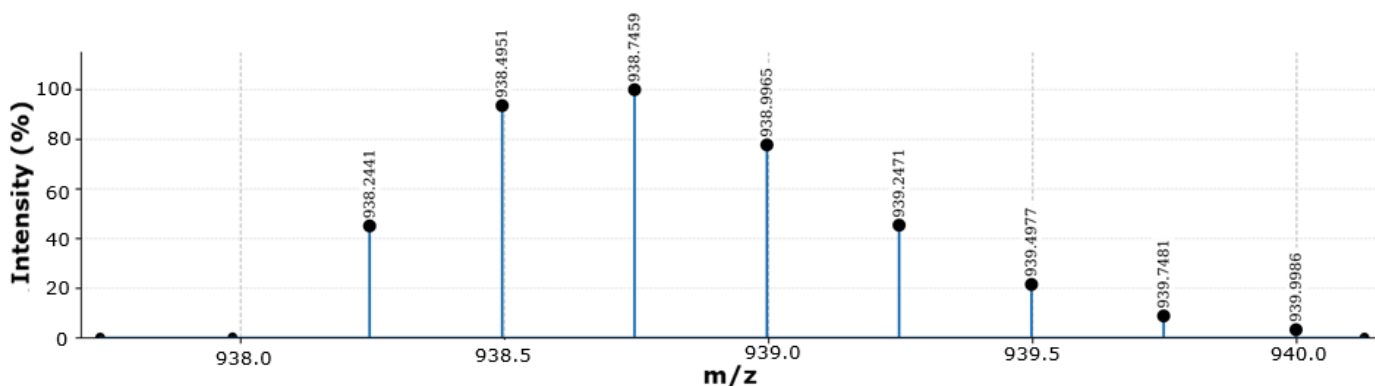


Figure 6. Isotopic pattern of liraglutide $z=4$ in the range of 938.00 to 940.00 m/z .

Purification of spiked liraglutide from plasma samples

To verify the LC-MS method and sample preparation procedure, liraglutide was spiked into human plasma samples. The spiked plasma was purified using a two-step protocol. First, proteins were precipitated by the addition of acetonitrile followed by centrifugation. Second, the resulting supernatant was further purified using Supel™ Swift HLB SPE tubes, which contain a hydrophilic-lipophilic-balanced polymer (Figure 2), and was then directly analysed by LC-MS. Using this procedure, the method sensitivity was determined with a limit of detection (LOD) of 26.62 ng/mL and a limit of quantification (LOQ) of 80.68 ng/mL for the injected solution, corresponding to plasma concentrations of 159.72 and 484,08 ng/mL, respectively. The recovery for the 66.67 ng/mL spike concentration was determined to be 58%.

The spiked samples were further used to generate a matrix-matched calibration curve for the determination of liraglutide in plasma, as shown in Figure 7.

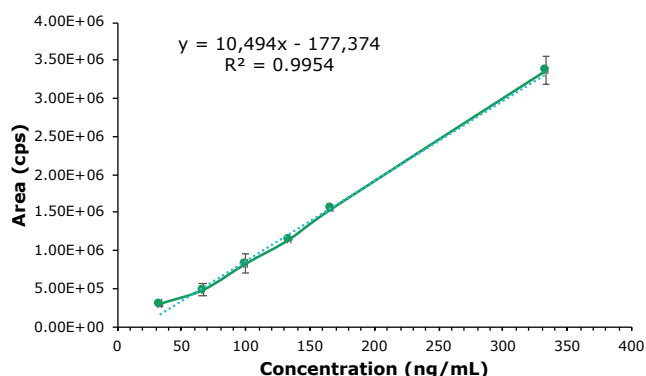


Figure 7. Matrix-matched calibration curve for liraglutide spiked plasma samples (33.33, 66.67, 100, 133.33, 166.67 and 333.33 ng/mL in the final SPE extract solution).

The analytical method provided effective separation of liraglutide from plasma proteins, enabling more accurate peak area determination and improved detection. Liraglutide eluted at a retention time of 5.3 min, while most matrix-related impurities eluted after 7 min (Figure 8).

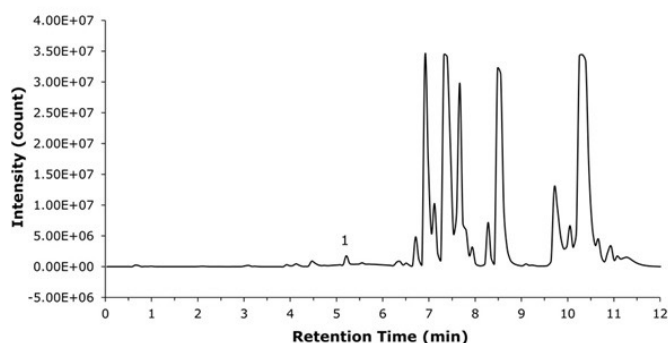


Figure 8. BPC chromatogram of spiked plasma sample. Liraglutide (1) elutes at 5.3 min.

In the extracted ion chromatogram (XIC) of a liraglutide spiked sample, no interferences were detected (Figure 9).

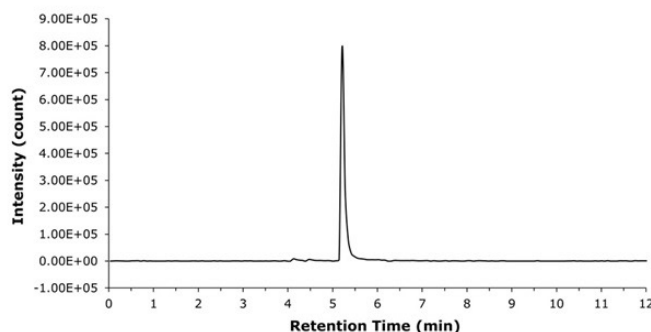


Figure 9. XIC (938.2468 u) chromatogram of liraglutide spiked in plasma sample.

Conclusion

In this study, an LC-MS method for the identification and quantification of liraglutide in human plasma samples was successfully developed. The method incorporates a streamlined purification protocol with protein precipitation followed by SPE clean up using Supel™ Swift HLB tubes containing a hydrophilic-lipophilic balanced polymer sorbent. This approach provided efficient sample preparation and allowed the simultaneous processing of 18 samples using a 24-port manifold. The sample preparation approach also offers potential for further scale-up of sample throughput by automation using 96-well plate formats or dispersive in-pipette tip SPE (DPX).

A key strength of the method lies in its chromatographic performance, which achieved effective separation of liraglutide from complex plasma matrix components. This separation supports reliable detection and accurate quantification of liraglutide by minimizing potential matrix interferences. The straightforward and potentially high-throughput purification protocol, combined with the analytical precision of the LC-MS system employing an efficient Fused-Core® BIOshell™ A160 Peptide PCS-C18 U/HPLC column, makes the method particularly well-suited for routine bioanalytical applications.

References

1. Marso, S. P.; Daniels, G. H.; Brown-Frandsen, K.; Kristensen, P.; Mann, J. F. E.; Nauck, M. A.; Nissen, S. E.; Pocock, S.; Poulter, N. R.; Ravn, L. S.; Steinberg, W. M.; Stockner, M.; Zinman, B.; Bergenstal, R. M.; Buse, J. B. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N. Engl. J. Med.* **2016**, *375* (4), 311–322. DOI: [10.1056/NEJMoa1603827](https://doi.org/10.1056/NEJMoa1603827).
2. Meng, X.; Xu, H.; Zhang, Z.; Fawcett, J. P.; Li, J.; Yang, Y.; Gu, J. Differential mobility spectrometry tandem mass spectrometry with multiple ion monitoring for the bioanalysis of liraglutide. *Anal. Bioanal. Chem.* **2017**, *409* (20), 4885–4891. DOI: [10.1007/s00216-017-0431-6](https://doi.org/10.1007/s00216-017-0431-6).

Featured Products

Description	Cat. No.
HPLC Column and SPE	
BIOshell™ A160 Peptide PCS-C18, 2.7 µm, 150 x 2.1 mm I.D.	50659-U
Supel™ Swift HLB SPE Tubes, 1 mL/30 mg, Pk. 108	57493-U
Visiprep™ SPE Vacuum Manifold, DL (Disposable Liner) 24-port model	57265
Reagents and Solvents	
Water suitable for UHPLC, LiChrosolv®	1.03728
Acetonitrile ≥99.9% (GC), suitable for UHPLC, LiChrosolv®	1.03725
Formic acid, 98%-100%, for LC-MS LiChropur™	5.33002
Liraglutide, ≥95% (HPLC), GLP-1 receptor agonist, Powder or Lyophilized powder or film	SML3925

Related Products

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Supel™ Swift HLB DPX Tip, Universal format, Tip volume 1 mL, box of 96 tips, bed weight 20 mg	52999-U

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