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Research Article

DEVELOPMENT AND VALIDATION OF A SELECTIVE ELECTRO SPRAY IONIZATION – LIQUID CHROMATOGRAPH TANDEM MASS SPECTROMETRY METHOD FOR SIMULTANEOUS DETERMINATION OF SEMAGLUTIDE AND EMPAGLIFLOZIN IN HUMAN PLASMA

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ABSTRACT

Objective: The objective of the study was to develop a method capable of simultaneously estimating semaglutide and empagliflozin used in combination for treating Diabetes.

Methods: A structured protein precipitation extraction technique was used for the estimation of semaglutide and empagliflozin. The two compounds were separated on a Zorbax C18 (50 mm × 2.1 mm, 5 μ Particle size) column, with a positive polarity Electro Spray Ionization on a Liquid chromatograph with Tandem Mass Spectrometry instrument. Verapamil was employed as an internal standard for this estimation, which was carried out through a multiple reaction monitoring method and a gradient program utilizing acetonitrile and 0.1% formic acid in water as mobile phases to achieve a separation in 2.5 min.

Results: The method established was performing linearly over a working range of 1.00–1000 ng/mL for semaglutide (r^2>0.98) and 0.51–500 ng/mL for empagliflozin (r^2>0.98) in human plasma. The validation parameters, including specificity, selectivity, precision, accuracy, recovery, matrix effects and stability, were within acceptable limits. The stability was established in compliance with the International Council for Harmonization guideline M10 on Bioanalytical method validation.

Conclusion: This method was selective and with suitable sensitivity at the 1.00 ng/mL and 0.50 ng/mL Lower Limit of Quantification employed for semaglutide and empagliflozin. It can be utilized for estimation in human plasma and will facilitate further application to bioequivalence and exploratory formulation studies for the combination of these two drugs in pharmaceutical dosage forms

Keywords: Semaglutide, Empagliflozin, Electro spray ionization, Method validation, Mass spectrometer.

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INTRODUCTION

Semaglutide is used in Type 2 diabetes and belongs to the Incretin mimetics classification. It is a receptor agonist for Glucagon-like peptide-1 (GLP-1). It facilitates the release of insulin from the pancreas to counter high blood glucose levels [1]. It is also being employed for weight management due to its anorexic property in addition to the reduction of body fat. Semaglutide cannot be administered in Diabetic Ketoacidosis or Type 1 diabetes. The exploratory studies conducted by Goldenberg and Steen [2] and Li *et al.* [3] indicate that it enhances the growth of β cells in the Pancreas. The United States Food and Drug Administration and the European Medicines Agency in 2017 and 2018 approved subcutaneous once-a-week for Type 2 diabetes treatment [4-10]. Lee *et al.* have developed a related method for quantifying semaglutide in rat brain and plasma [11]. Kapitza *et al.* have studied the effect of semaglutide on combined oral contraceptive medications [12]. Empagliflozin is used in Type 2 diabetes, a Sodium/glucose co-transporter 2 (SGLT2) inhibitor [13-16] that exerts a diuretic effect, resulting in blood pressure and body weight reduction. There are some reported Liquid chromatograph Tandem Mass Spectrometry (LC-MS/MS) methods for determining gliflozins in plasma [17] and in combination with metformin [18], with a lower limit of quantification (LLOQ) of 5 ng/mL. This combination of semaglutide and empagliflozin is important due to the combined effect in reducing blood glucose levels. Although methods for bioanalytical analysis of semaglutide and empagliflozin have been reported individually, no technique has been reported for the simultaneous estimation of both in human plasma using LC-MS/MS.

This bioanalytical method was developed and validated in human plasma for the first time. It can be employed for pilot and pivotal clinical studies for the estimation of semaglutide and empagliflozin. This method validation was carried as per the guidance provided by the International Council for Harmonization (ICH) M10 guidelines applicable for the Bioanalytical method validation and study sample analysis [19].

METHODS

Reagents and chemicals

Acetonitrile and methanol were sourced from Merck (LC-MS grade). Ammonium formate (LC-MS grade), Sigma Aldrich, was used. Milli-Q Water (Type 1) was procured internally from the Millipore Advance water system. Human Plasma and blood lots were procured from Delta Laboratories. Anticoagulant Dipotassium Ethylene Diamine Tetra Acetic Acid was procured from Merck. The Semaglutide, empagliflozin, and verapamil Analytical Standards were procured as gift samples with no commercial value.

Instrumentation

All the weighings were carried out using a microbalance (Mettler Toledo: MX5). The degassing of solutions was performed using an ultrasonicator for 5 min. A Vortex mixer and an Eppendorf centrifuge were used for extraction using the micro centrifuge tubes. An ultra-performance liquid chromatography (LC) (Shimadzu) in tandem with a Sciex 6500+ LC-MS/MS with Analyst Software 1.7 was employed for Chromatographic separation and detection. In this study, we have utilized a positive polarity Multiple Reaction Monitoring (MRM) for quantitation.

Preparation of stock and working solutions for the analytes and internal standard

Semaglutide and empagliflozin (2 mg/mL) stocks were prepared with 10 mg weighings dissolved in a 5 mL volumetric flask. The stocks were prepared independently in Methanol for calibration standards and quality control (QC) samples to overcome weighing differences, which could cause erroneous estimations. Verapamil was used as the Internal standard by dissolving 5 mg in a 5 mL volumetric flask with Methanol to prepare a 1 mg/mL stock. The spiking solutions containing both analytes (20–20000 ng/mL for semaglutide and 10–10000 ng/mL for empagliflozin, respectively) were prepared using a serial dilution process from an intermediate cocktail stock containing both analytes. The dilutions were performed using a 50% methanol in water solution.

Sample preparation

The selected Protein Precipitation (PPT) extraction method gave the best sample clean-up. The calibration samples of semaglutide and empagliflozin in plasma were prepared by spiking 95 μL of the blank human plasma with 5 μL of the standard working solution. A 15 μL of the IS working solution was added, followed by 750 μL of Acetonitrile in a 2 mL microcentrifuge tube. The mixing was carried out on the vortex mixer for 15 min, and then centrifuged at 4000 rpm for 12 min at 8°C. After centrifugation, 600 μL of supernatant was aliquoted into a 1 mL autosampler vial. An Injection volume of 2 μL was employed for the best peak shape and separation.

Chromatographic condition

A reversed-phase chromatographic gradient separation was achieved on a Zorbax C18 (50 mm × 2.1 mm, 5 μ Particle size) at 35°C using mobile phase (A: 5mM Ammonium Formate with 0.1% formic acid and B: Acetonitrile). The autosampler temperature was maintained at 6°C. The LC binary gradient program was employed with a run time of 3 min. The flow rate employed was 0.5 mL/min for the run. All the mobile phases were filtered using a 0.22 μM membrane filter. The gradient program was developed to ensure no matrix effects, which could cause signal suppression.

Mass spectrometric conditions

An Atmospheric Pressure Ionization 6500+ with an Electro Spray Ionization (ESI) interface operated in MRM mode. The semaglutide and empagliflozin were detected and fragmented in positive mode. The instrument was optimized for semaglutide, empagliflozin, and internal standard verapamil during tuning at a concentration of 150 ng/mL prepared in Acetonitrile and water solution (50:50) and infused at a flow rate of 10 μL/min through an infusion pump. The MRM transitions chosen were m/z 1029.1→1302.6 for semaglutide, 451.0→71.1 for empagliflozin, and 455.2→165.3 for verapamil used as Internal Standard. Verapamil was selected as the internal standard due to its seamless ionization in the positive mode and adequate compensation of signal fluctuation. The mass spectrometric conditions were augmented for quantification of semaglutide and empagliflozin using an: ESI probe with a source temperature of 500°C; ion spray voltage of 5500; curtain gas, 20 psi, nebulizing gas (GS1) 50 psi, heater gas (GS2) 40 psi, Declustering Potential (90 eV) and a Collision energy (25 eV). Verapamil was analyzed with a Declustering Potential (80 eV) and Collision Energy (18 eV). The entrance potential and cell exit potential were maintained at 10 eV. Ultra-high-purity inert nitrogen gas was the collision gas employed. The optimized parameters resulted in adequate linearity in the recognized range. The structure of semaglutide and empagliflozin are represented in Figs. 1 and 2 respectively. The full scan mass spectrum of empagliflozin is represented in Fig. 3.

Data analysis

Analyst 1.7 was used for data processing. The calibration curves were constructed using the instrument response (analyte peak area/IS peak area) to the Analyte concentrations using a linear regression model $y = mx + c$, where y denotes the observed area ratio, m is for slope, and c is for intercept, respectively, with a weighting factor $1/x^2$. The acceptance criteria were established to be >0.98 for the coefficient of determination (r^2) with a minimum of 6 non-zero calibration curve standards, that is, at least 75% of the standards should have to be acceptable (±20% bias for a LLOQ and ±15% bias for other standards) for a calibration curve.

Bioanalytical method validation

Calibration and QC samples

Calibration curves were made by spiking 5 μL of spiking solution to 95 μL of blank human plasma. The final concentrations in the plasma samples were 1.00, 2.85, 9.90, 99.0, 302, 470, 770, and 1000 for semaglutide and 0.50, 1.50, 5.30, 53.0, 152, 245, 370, and 500 ng/mL for empagliflozin. The QC samples were set at concentrations of 1.02, 2.90, 485, 735, and 0.51, 1.45, 243, and 368 ng/mL (LLOQ QC, Low QC [LQC], Middle QC [MQC], High QC [HQC]) for semaglutide and empagliflozin.

Preparation of plasma calibration standards and QC samples

The standards were prepared using 95 μL of interference-free blank human plasma and by spiking 5 μL of the respective cocktail working solution.

Calibration curve

A cocktail calibration curve was prepared using 8 non-zero standards encircling the range (1.00–1000 ng/mL) for semaglutide and (0.50–500 ng/mL) for empagliflozin. The linearity assessment was conducted using a weighted ($1/x^2$) least squares regression. The assessment of linearity was across the three tested Precision and accuracy batches. Linearity was assessed by plotting calibration curves (Area ratio versus concentration) in human plasma. The LLOQ standard was included in the calibration curve when the accuracy was ±20%. However, for all other higher standards, the acceptable accuracy was ±15%.

Precision and accuracy

The Precision and Accuracy of this method were evaluated across three days using QC samples at four concentrations LLOQ QC, which was accepted with a %CV across the 6 tested replicates at ≤20 and an Accuracy of 100 ±20%. However, the remaining LQC, MQC and HQC were only considered acceptable when their precision was ≤15% and accuracy was 100±15%.

Specificity and selectivity

The specificity was evaluated using six different plasma lots to investigate the interferences for analytes and the internal standard at their retention time. The shortlisted lots for this study were processed as per the extraction process albeit without analytes or internal standard. The acceptance criteria were set as all blank lots must have <20% and <5% of interference to the LLOQ area response and Internal standard response at their respective retention times. Selectivity was established by employing the same six blank plasma lots. Each of these lots was spiked individually with semaglutide, empagliflozin, and verapamil to ensure there were no cross contributions across the tested analytes.

Recovery

Recoveries were evaluated at three QC levels LQC, MQC and HQC (2.90, 450, and 770 ng/mL for semaglutide and 2.90, 485, and 735 ng/mL for empagliflozin) by comparing the peak area in spiked pooled human plasma samples with those of the analyte spiked in neat solutions.

Matrix effect

The matrix effect, as suggested by Matuszewski *et al*. [20], was superseded by the latest quantitative estimation approach (ICH M10)

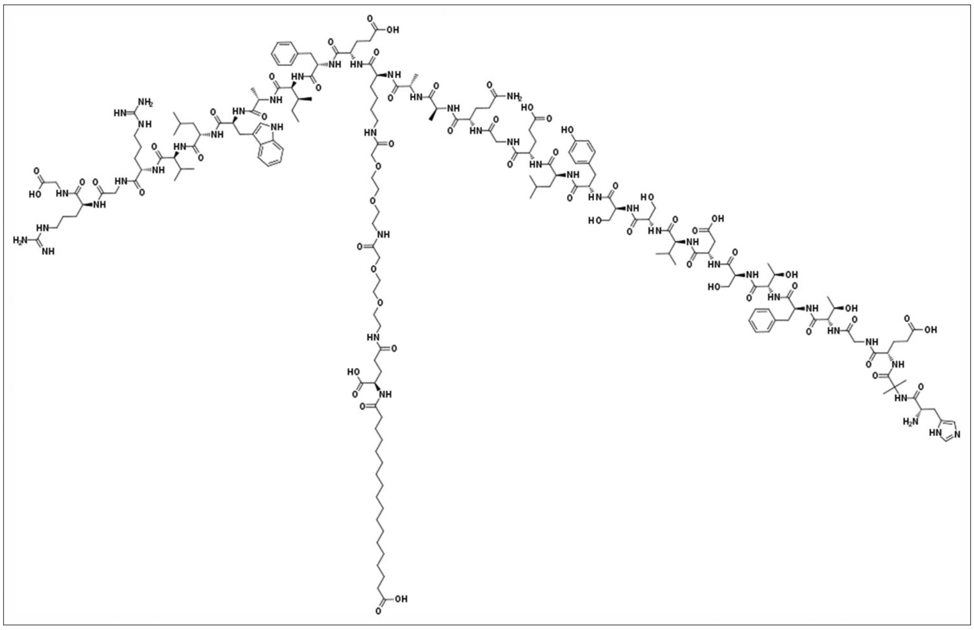

Fig. 1: Structure of semaglutide

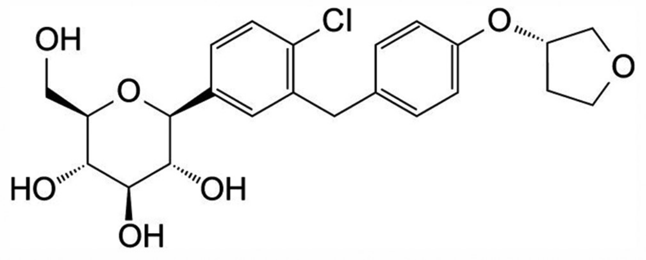

Fig. 2: Structure of empagliflozin

using 6 different interference-free lots, and the accuracy of the six lots was calculated to determine the sturdiness of the method. This ensured co-eluents were devoid of suppressive or enhancing effect on the values obtained.

The evaluation of matrix effect was carried out at the LQC and HQC levels. The selected blank lots were spiked with LQC and HQC and checked for the values. The acceptable criteria for the matrix factor were a mean accuracy of 85–115%.

Stability experiments

The semaglutide and empagliflozin stability in human plasma was assessed by analyzing six replicates of the QC samples at low and high levels in four different exposure conditions as follows; (1) The bench top stability where the spiked samples were kept at room temperature (ambient temperature for >14 h; (2) The autosampler stability at autosampler temperature, that is, 6°C for 22 h, (3) The Freeze thaw stability using five freeze and thaw cycles (24 h for the first cycle, followed by 12 h intervals), and (4)Long term stability for 15 days in the deep freezer at −80°C. Samples were considered stable if the obtained accuracy was within 85–115% of the nominal concentration.

RESULTS AND DISCUSSION

Method optimization

This method aimed to develop a rugged and reproducible bioanalytical method appropriate for simultaneously estimating semaglutide and empagliflozin in a single bioanalytical run on the LC-MS/MS system. During the development of the extraction method, a PPT approach was found to be best suited to maximize the recovery of semaglutide and empagliflozin. The semaglutide method available in human plasma and related pharmacokinetic studies employed an LLOQ of 3 ng/mL previously. For tuning of semaglutide (Molecular weight: 4114 daltons), the [M + 1] positive charge was beyond the mass range for suitable detection in the selected instrument, and hence, a multiply charged prominent [M + 4] precursor ion 1029.1 was selected during tuning in positive mode. The product ion of 1302.6 was the chosen fragment, resulting in the best chromatographic response. Empagliflozin ionization was achieved in positive mode. Empagliflozin was tuned in positive mode with a precursor ion of 451.1. The product of 71.1 and its combination with 451.1 helped in achieving the best response. The MRM parameters for MS/MS determination were optimized to maximize for both analytes. The currently available method can quantify plasma concentrations of semaglutide in human plasma from 3 to 250 ng/mL by Kapitza *et al*. The method validated in this article ensures a wider linear dynamic range from 1 to 1000 ng/mL, which is handy in Single and Multiple ascending dose studies. The currently available method for empagliflozin utilizes an LLOQ of 2 μg/L with a 200 μL processing volume. However, we have employed a mobile phase, which has a heightened sensitivity, helping us to achieve an LLOQ of 0.500 ng/mL. The sample processing volume had been optimized to 100 μL. This is mainly important as lower blood volume can be collected from the subjects during the clinical study.

Calibration curves

The calibration standards were set at standard concentrations of 1.00–1000 ng/mL for semaglutide and 0.50–500 ng/mL empagliflozin. The calibration curve (8 non-zero standards) for both analytes displayed a weighting factor of $1/x^2$ and a linear fit across the selected range. The eight-point calibration curves were plotted for both analytes with linear fit weighting regression factor. The mean correlation coefficient for the selected analytes in human plasma was >0.980. The calibration curve results for both analytes in human plasma are summarized in Table 1, and the calibration curves for semaglutide and empagliflozin are presented in Figs. 4 and 5, respectively.

Specificity and selectivity

Specificity and selectivity for this plasma method were studied in six different lots to determine the extent of endogenous interferences. Each blank lot was processed in two replicates (1 devoid of any spiking and 1 spiked with the spiking solution of LLOQ and Internal Standard). The interference in the blank sample corresponding to each lot was compared against the mean peak response at the LLOQ level and the

Table 1: Precision and accuracy data of calibration curve standards for semaglutide and empagliflozin in human plasma

Matrix	Analyte	Nominal concentration (ng/mL)	Back calculated Conc. (ng/mL)	n	CV (%)	Accuracy (%)	RE (%)
Human plasma	Semaglutide	1.00	0.970	3	13.57	97.3	2.7
		3.00	2.88	3	3.76	96.0	4.0
		10.6	9.57	3	3.19	90.3	9.7
		106	97.3	3	3.89	91.8	8.2
		303	292	3	3.14	96.4	3.6
		485	476	3	2.44	98.1	1.9
		775	788	3	2.04	101.7	-1.7
		1000	1016	3	1.57	101.6	-1.6
	Empagliflozin	0.500	0.51	3	14.18	102.0	-2.0
		1.50	1.39	3	9.13	92.4	7.6
		5.30	5.21	3	3.50	98.3	1.7
		53.0	51.4	3	6.04	97.0	3.0
		152	157	3	7.13	103.7	-3.7
		245	244	3	2.58	99.7	0.3
		370	369	3	1.69	99.7	0.3
		500	493	3	1.14	98.6	1.4

n=3 replicates at each concentration. Data presented in Mean, %CV, and %Accuracy

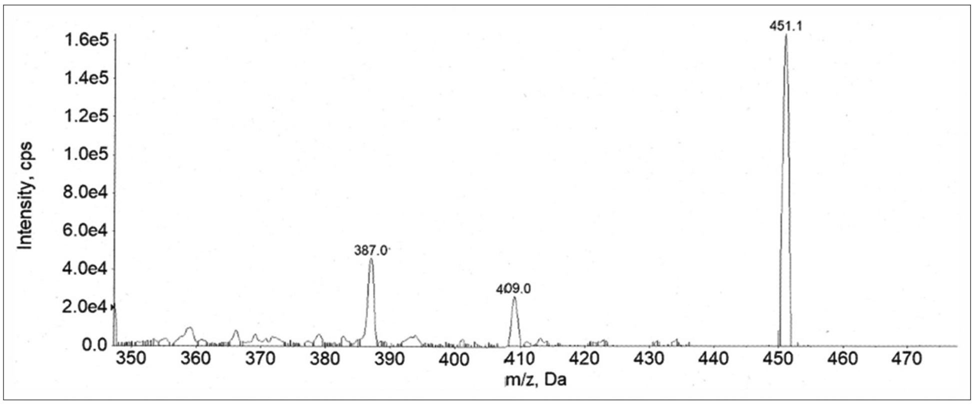

Fig. 3: Full scan mass spectrum of empagliflozin

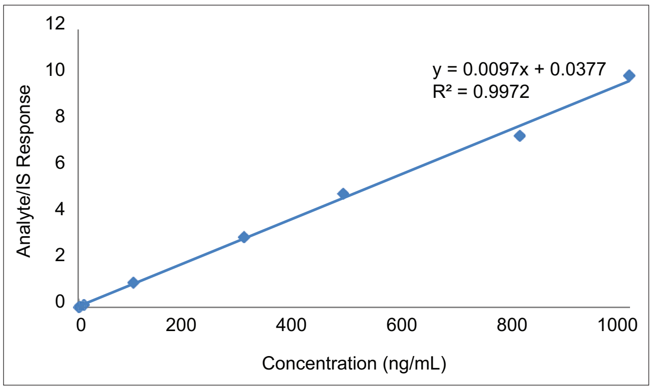

Fig. 4: Linearity plot of semaglutide

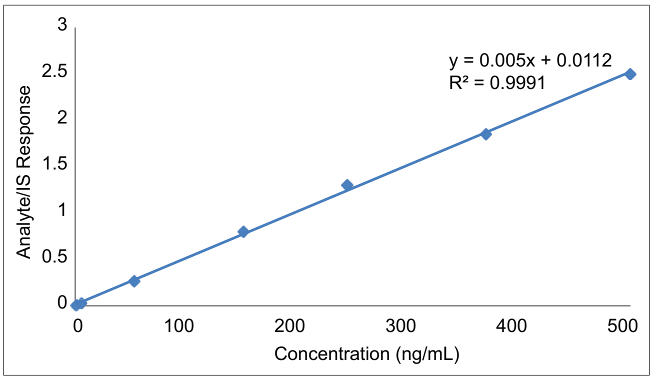

Fig. 5: Linearity plot of empagliflozin

Internal Standard verapamil. No interference was observed at the retention times of semaglutide and empagliflozin, and verapamil in the tested samples. Representative chromatograms for semaglutide and empagliflozin for LLOQ and ULOQ are presented in Figs. 6-11.

Accuracy and precision

The inter-day and intra-day accuracy and precision results for semaglutide in plasma are presented in Tables 2 and 3, respectively. Empagliflozin results are presented in Tables 4 and 5. The precision and accuracy were assessed at the four QC levels using six replicates at each level. QC levels employed were (1.02, 2.90, 485, and 735 ng/mL) for semaglutide and (0.510, 1.45, 243, and 368 ng/mL) for empagliflozin. For semaglutide at each QC level, the intra-day accuracy was between 93.8% and 104.0%. Moreover, precision was between 1.85% and 9.86%. In the case of inter-day, the accuracy was between 94.7% and 102.4% and the precision was between 2.42% and 6.88%.

For empagliflozin at each QC level, the intra-day accuracy was between 92.9% and 98.0% and precision was between 2.71% and 13.26%. In the case of inter-day, the accuracy was between 95.6% and 99.4% and the precision was between 4.33% and 12.56%.

Extraction recovery

As shown in Tables 6 and 7, the extraction recovery for semaglutide was 85.2%, 85.5%, and 90.2% at LQC, MQC, and HQC, respectively. The % CV for semaglutide ranged from 2.87% to 9.16%. Similarly, the extraction

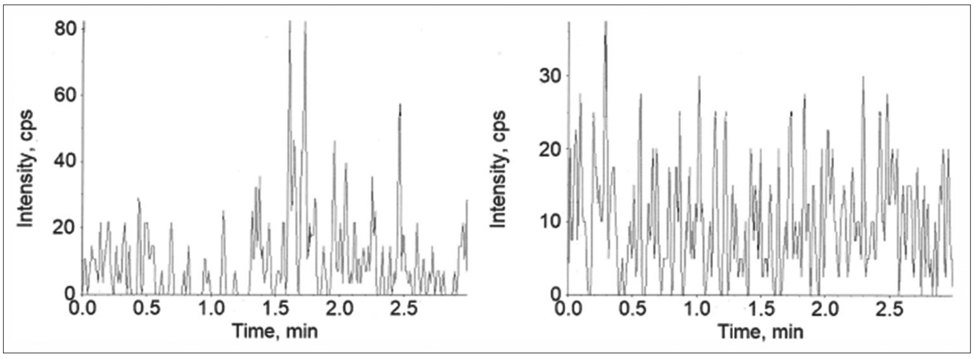

Fig. 6: Extracted blank of semaglutide

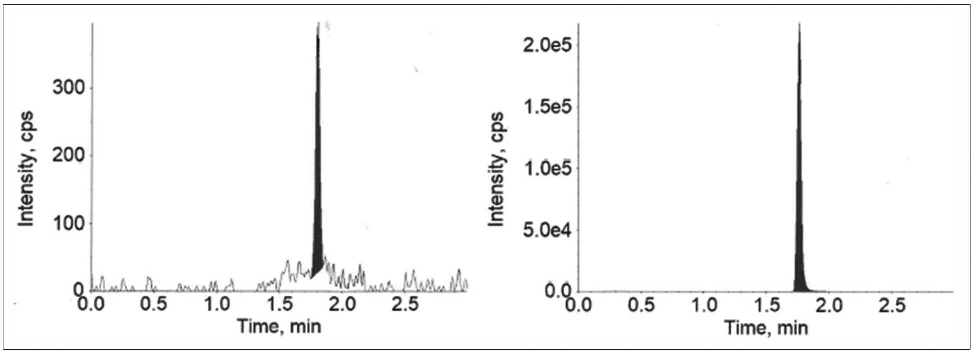

Fig. 7: Extracted lower limit of quantification of semaglutide

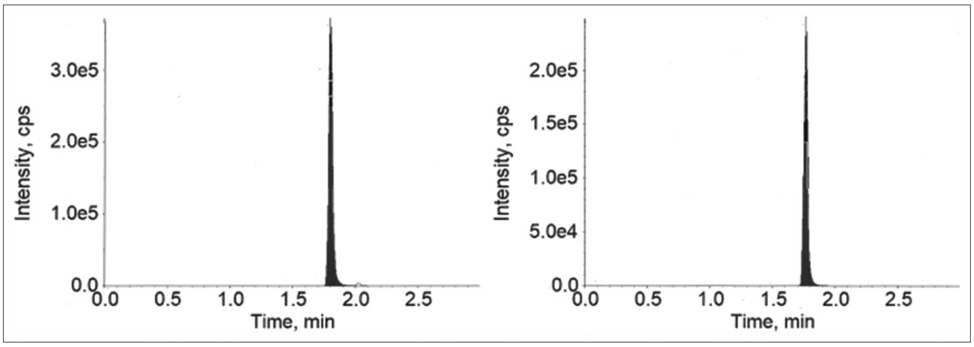

Fig. 8: Extracted upper limit of quantification of semaglutide

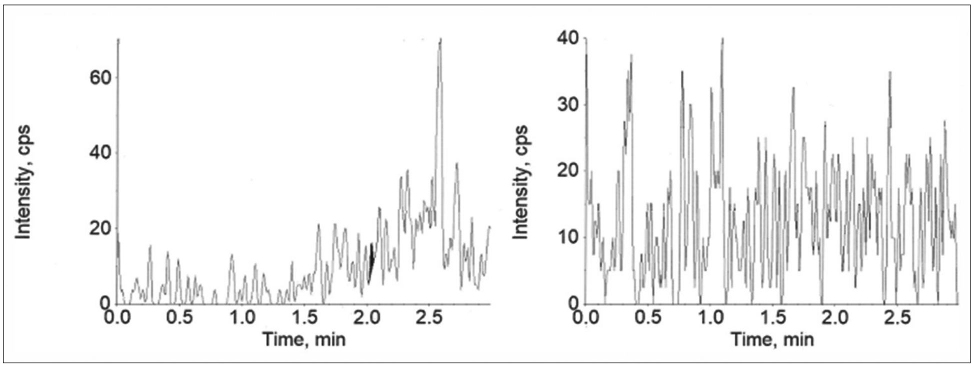

Fig. 9: Extracted blank of empagliflozin

recovery for empagliflozin in human plasma was 86.0%, 85.6%, and 81.7% at LQC, MQC, and HQC, respectively. The % CV for empagliflozin ranged from 5.65% to 12.05%. The recovery of semaglutide and empagliflozin is uniform across the tested levels, and the results from the validation experiments indicate no impact on recovery with a change in concentration.

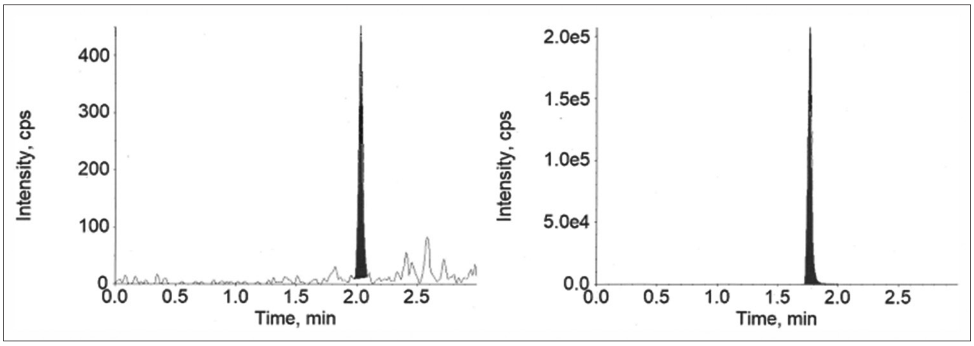

Fig. 10: Extracted lower limit of quantification of empagliflozin

Table 2: Intra-day precision and accuracy for the estimation of semaglutide

QC level	Nominal Conc. (ng/mL)	Back calculated Conc. (ng/mL)	Precision (%)	Accuracy (%)
LLOQ	1.02	1.02±0.10	9.86	100.0
LQC	2.90	2.72±0.15	5.70	93.8
MQC	485	476±13.6	2.85	98.1
HQC	735	764±14.1	1.85	104.0

n=6 replicates in each concentration, Data presented in (mean±SD), LLOQ: Lower limit of quantification, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control

Table 3: Inter-day precision and accuracy for the estimation of semaglutide

QC level	Nominal Conc. (ng/mL)	Back calculated Conc. (ng/mL)	Precision (%)	Accuracy (%)
LLOQ	1.02	1.01±0.06	5.72	98.7
LQC	2.90	2.75±0.19	6.88	94.7
MQC	485	471±17.2	3.65	97.0
HQC	735	753±18.2	2.42	102.4

n=6 replicates in each concentration, Data presented in (mean±SD), LLOQ: Lower limit of quantification, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control

Table 4: Intra-day precision and accuracy for the estimation of empagliflozin

QC level	Nominal Conc. (ng/mL)	Back calculated Conc. (ng/mL)	Precision (%)	Accuracy (%)
LLOQ	0.510	0.474±0.063	13.26	92.9
LQC	1.45	1.38±0.06	4.08	95.2
MQC	243	236±9.51	4.02	97.5
HQC	368	360±9.77	2.71	98.0

n=6 replicates in each concentration, Data presented in (mean±SD), LLOQ: Lower limit of quantification, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control

Table 5: Inter-day precision and accuracy for the estimation of empagliflozin

QC level	Nominal Conc. (ng/mL)	Back calculated Conc. (ng/mL)	Precision (%)	Accuracy (%)
LLOQ	0.510	0.488±0.06	12.56	95.6
LQC	1.45	1.41±0.12	8.17	97.4
MQC	243	239±10.4	4.33	98.6
HQC	368	365±27.8	7.61	99.4

n=6 replicates in each concentration, Data presented in (mean±SD), LLOQ: Lower limit of quantification, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control

Table 6: Extraction recovery of semaglutide in human plasma

Level	Mean recovery (%)	%CV
LQC	85.2	9.16
MQC	85.5	3.98
HQC	90.2	2.87

n=6 replicates in each concentration, Data presented in Mean and %CV, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control

Table 7: Extraction recovery of empagliflozin in human plasma

Concentration (ng/mL)	Mean recovery (%)	%CV
LQC	86.0	12.05
MQC	85.6	5.65
HQC	81.7	11.75

n=6 replicates in each concentration, Data presented in Mean and %CV, LLOQ: Lower limit of quantification, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control

Table 8: Matrix effect of semaglutide in human plasma

Concentration (ng/mL)	Mean accuracy % to measure matrix effect	%CV
2.90	96.3	9.19
735	101.3	2.87

n=6 replicates in each concentration, Data presented in Mean and %CV

Table 9: Matrix effect of empagliflozin in human plasma

Concentration (ng/mL)	Mean accuracy % to measure matrix effect	%CV
1.45	99.9	12.05
368	95.0	11.75

n=6 replicates in each concentration, Data presented in Mean and %CV

Matrix effect

The mean accuracy from the six independent lots to measure the matrix effect was 96.3% and 101.3%, with a CV of 9.19% and 2.87% at LQC and HQC, respectively, for semaglutide, are captured in Table 8. The mean accuracy from the six independent lots to measure the Matrix effect was 99.9% and 95.0%, with a CV of 12.05% and 11.75% at LQC and HQC, respectively, for empagliflozin, are captured in Table 9. The results indicate that the matrix effect is mitigated to ensure no suppression or enhancement of the signal, thereby ensuring accurate estimation.

Stability

The stability of semaglutide and empagliflozin was evaluated in human plasma and the results are presented in Tables 10 and 11, representing that the % degradation is within the acceptable 15%, at room temperature for 15 h, in the autosampler 6°C for 22 h, after five

Table 10: Stability of semaglutide in human plasma

Experimental condition	Sample Conc. (ng/mL)	Human plasma		
		Measured Conc. (ng/mL)	CV (%)	Stability (%)
Bench top stability for 14 h at room temperature 24°C	2.90	2.63±0.11	4.31	90.8
	735	754±6.18	0.82	102.6
Autosampler stability for 22 h at 6°C	2.90	2.54±0.29	11.39	87.6
	735	744±10.80	1.45	101.2
Five freeze thaw cycles (Thawing after 24 h interval after 1st cycle followed by 12 h intervals for subsequent cycles) at−80°C	2.90	2.61±0.12	4.76	90.1
	735	727±13.65	1.88	98.9
Long term stability for 15 days at−80°C	2.90	2.63±0.11	4.31	90.8
	735	729±14.27	1.96	99.2

Measured concentration provided in mean±SD, All stability measurements performed with n=6 replicates

Table 11: Stability of empagliflozin in human plasma

Experimental condition	Sample Conc. (ng/mL)	Human plasma		
		Measured Conc. (ng/mL)	CV (%)	Stability (%)
Bench top stability for 14 h at room temperature 24°C	1.45	1.37±0.08	6.07	94.7
	368	355±19.4	5.45	96.6
Autosampler stability for 22 h at 6°C	1.45	1.34±0.11	8.19	92.1
	368	363±20.8	5.74	98.7
Five freeze-thaw cycles (Thawing after 24 h interval after 1st cycle, followed by 12 h intervals for subsequent cycles) at−80°C	1.45	1.39±0.09	6.40	95.8
	368	359±24.8	6.91	97.6
Long-term stability for 15 days at−80°C	1.45	1.38±0.10	7.23	95.5
	368	361±23.1	6.41	98.3

Measured concentration provided in mean±SD, All stability measurements performed with n=6 replicates

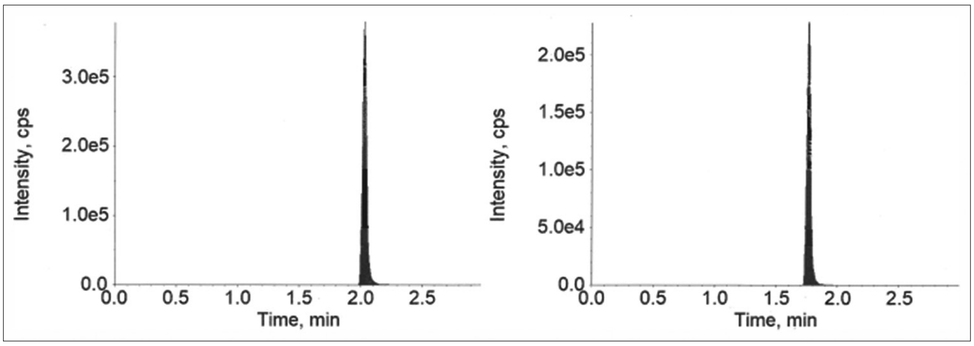

Fig. 11: Extracted upper limit of quantitation of empagliflozin

freeze and thaw cycles and on long storage for 15 days at −80°C in the deep freezer. The stability results indicate that the applicability of the method within the set criteria, as outlined in the guidelines.

CONCLUSION

A robust method was developed in the current study, which was comprehensively validated to quantify two antidiabetic drugs semaglutide and empagliflozin simultaneously in human plasma using a cost-effective LC-MS/MS approach. This method will enable its application to pharmacokinetic studies and a blend of these drugs in pharmaceutical dosage forms. This reproducible extraction technique and the optimized chromatography resulted in the ideal outcomes. The procedure captured is easy to adopt and was designed to ensure easy adaptability for future exploratory studies, early-phase clinical trials and therapeutic drug monitoring. This method could be used as a reference and could be used as a basis to other combinations of GLP-1 receptor agonists and SGLT2 inhibitors.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally to this article.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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