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Research Article

DEVELOPMENT AND VALIDATION OF A SELECTIVE LIQUID CHROMATOGRAPH WITH TANDEM MASS SPECTROMETRY METHOD FOR SIMULTANEOUS ESTIMATION OF SEMAGLUTIDE AND ERTUGLIFLOZIN IN HUMAN PLASMA

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ABSTRACT

Objective: The objective of the study was to develop a method capable of simultaneously estimation of semaglutide and ertugliflozin to support pharmacokinetic and bioequivalence studies.

AQ3 **Methods:** An organized protein precipitation extraction technique was used for estimation of semaglutide and ertugliflozin. The two compounds were separated on an Agilent Zorbax C18 (50 mm × 2.1 mm, 5 µ Particle size) column, using an electro spray ionization with a positive ionization mode on a liquid chromatograph with tandem mass spectrometry instrument. Verapamil was the chosen internal standard for this estimation. The quantification was carried out using 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 2.2 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 Ertugliflozin (r^2>0.98) in human plasma. The validation parameters consisted of specificity, selectivity, precision, accuracy, recovery, matrix effects, and stability, which 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 as the lower limit of quantification employed for semaglutide and Ertugliflozin. It can be utilized for quantification in human plasma and will facilitate the further application to exploratory formulation studies for the combination of these two drugs in pharmaceutical dosage forms.

Keywords: Semaglutide, Ertugliflozin, 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]. Kapitza *et al*. have studied the effect of semaglutide on combined oral contraceptive medications [11]. Ertugliflozin is used in Type 2 diabetes, a sodium/glucose co-transporter 2 (SGLT2) inhibitor [12-18] that exerts a diuretic effect, resulting in blood pressure and body weight reduction. This combination of semaglutide and Ertugliflozin is important due to the combined effect in reducing blood glucose levels. Although methods for bioanalytical analysis of semaglutide and Ertugliflozin have been reported individually, no technique has been reported for the simultaneous estimation of both in human plasma using a liquid chromatograph with tandem mass spectrometry (LC-MS/MS).

This bioanalytical method was developed and validated in human plasma for the 1st time. It can be used for crucial clinical studies for the estimation of semaglutide and ertugliflozin. 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 tetraacetic acid was procured from Merck. The semaglutide, ertugliflozin, 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). An ultrasonicator was used for degassing. 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 ertugliflozin (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 ertugliflozin) were prepared using a serial method using an intermediate mixture containing both analytes. The dilutions were performed using a 50:50 %v/v methanol: water.

Sample preparation

The selected protein precipitation (PPT) extraction method gave the best sample clean-up. The calibration standards and QC samples of semaglutide and ertugliflozin 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 1.5 mL microcentrifuge tube. The mixing was carried out on the vortex mixer for 10 min, and then centrifuged at 4000 rpm for 15 min at 4°C. After centrifugation, 650 µL of supernatant was aliquoted into a 1 mL autosampler vial. An Injection volume of 2 µL was employed for optimum response and chromatography.

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: 5 mM Ammonium Formate with 0.1% formic acid and B: Acetonitrile). The autosampler temperature was maintained at 5°C. The LC binary gradient program was employed with a run time of 2.2 min. The flow rate employed was 0.6 mL/min for the run. All the mobile phases were filtered using a 0.22 µM membrane filter. The gradient program was developed to overcome matrix effects, which are deleterious for chromatography.

Mass spectrometric conditions

AQ4 An atmospheric pressure ionization 6500+with an electro spray ionization (ESI) interface operated in MRM mode. The semaglutide and ertugliflozin were detected and fragmented in positive mode. The instrument was augmented for semaglutide, ertugliflozin, and internal standard verapamil during tuning at a concentration of 100 ng/mL prepared in Acetonitrile and water solution (50:50% v/v) 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, 437.2→328.9 for Ertugliflozin, and 455.2→165.3 for verapamil used as an 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 amplified for quantification of semaglutide and ertugliflozin using an: ESI probe with a source temperature of 500°C; ion spray voltage of 5500; curtain gas, 25 psi, nebulizing gas (GS1) 55 psi, heater gas (GS2) 45 psi, Declustering Potential (90eV) and a Collision energy (15eV). Verapamil was analysed with a Declustering Potential (80eV), and Collision Energy (18eV). The entrance potential and cell exit potential were maintained at 10eV. Ultra-high-purity inert nitrogen gas was the collision gas employed. The optimized parameters resulted in satisfactory linearity in the documented range.

Data analysis

Analyst 1.7.1 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.

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, 3.00, 10.6, 106, 303, 485, 775, and 1000 for semaglutide and 1.00, 3.00, 10.5, 105, 310, 495, 765, and 1000 ng/mL for ertugliflozin. The QC samples were set at concentrations of 1.01, 2.70, 500, and 750 ng/mL (lower limit of quantification QC [LLOQQC], Low QC [LQC], Middle QC [MQC], High QC [HQC]) for semaglutide AQ5 and 1.01, 2.80, 504, and 755 ng/mL for LLOQQC, LQC, MQC, HQC for ertugliflozin.

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 ertugliflozin. 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 3 days using QC samples at four concentrations LLOQQC, which was accepted with a coefficient of variation (%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, although without analytes or an 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, ertugliflozin, and verapamil to ensure there was no contribution of the tested analytes and internal standard to one another.

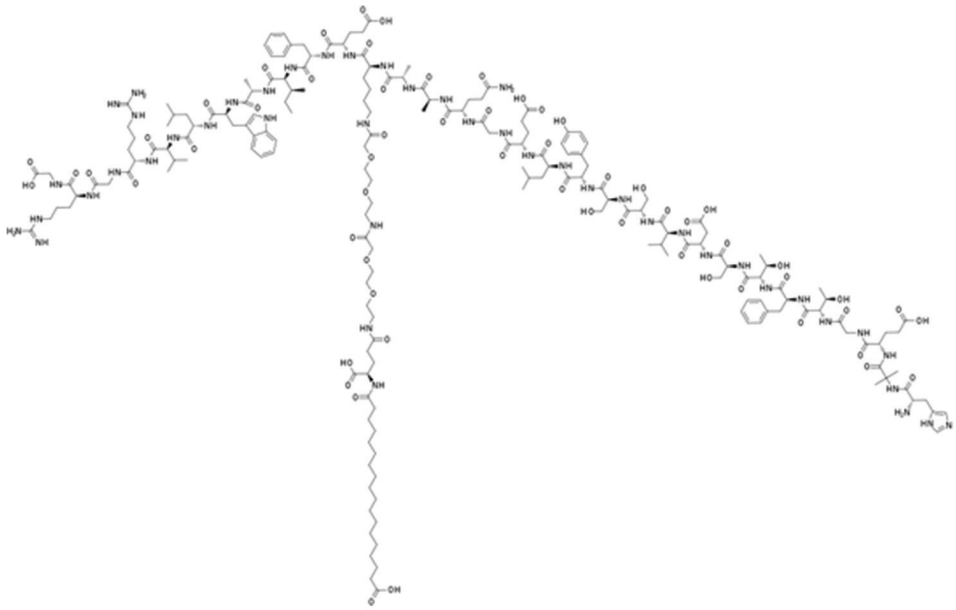
Recovery

Recoveries were evaluated at three QC levels LQC, MQC, and HQC (2.70, 500, and 750 ng/mL for semaglutide and 2.80, 504, and 755 ng/mL for Ertugliflozin) by comparing the peak area in spiked pooled human plasma samples with those of the analyte spiked in neat solutions.

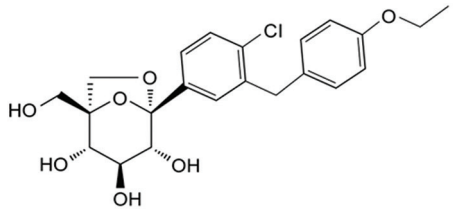
Matrix effect

The latest quantitative estimation approach (ICH M10) using six different interference-free lots, and the accuracy of the six lots was calculated to determine the robustness of the method. This ensured co-eluents were devoid of suppressive or enhancing effect on the values obtained.

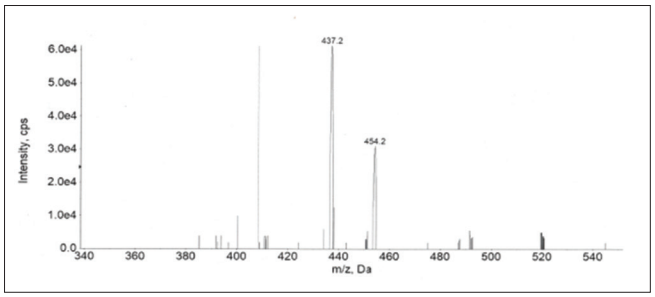
The evaluation of matrix effect was carried out at the LQC and HQC levels. The selected blank lots were spiked with LQC and HQC and



AQ6 **Fig. 1:** **Structure of semaglutide**



AQ6 **Fig. 2:** **Structure of ertugliflozin**



AQ6 **Fig. 3:** **Full scan mass spectrum of ertugliflozin**

checked for the values. The acceptable criteria for the matrix factor were a mean accuracy of 85–115%.

Stability experiments

The semaglutide and ertugliflozin 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 (~25°C) for >12 h; (2) The autosampler stability at autosampler temperature i.e. 4°C for 20 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 18 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 ertugliflozin 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 ertugliflozin. 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 da), 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. Ertugliflozin ionization was achieved in positive mode with a precursor ion of 437.2. The product of 328.9 and its combination with 437.2 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 Ertugliflozin utilizes an LLOQ of 1 µg/L with a 50 µL processing volume. There are also reported methods where the upper limit of quantification (ULOQ) was capped at 500 ng/mL. However, we have employed a mobile phase that has an intensified sensitivity. The sample processing volume had been optimized to 100 µL.

Calibration curves

The calibration standards were set at standard concentrations of 1.00–1000 ng/mL for semaglutide and ertugliflozin. 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 greater than 0.980. The calibration curve results for both analytes in human plasma are summarized in Table 1, and the calibration curves for semaglutide and ertugliflozin are presented in Figs. 4 and 5, respectively.

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

Matrix	Analyte	Nominal concentration (ng/mL)	Back calculated conc. (ng/mL)	n	CV (%)	Accuracy (%)
Human plasma	Semaglutide	1.00	0.96	3	13.51	96.3
		3.02	2.90	3	3.63	95.9
		10.6	9.60	3	3.76	90.6
		106	97.6	3	4.08	92.1
		303	291	3	2.77	96.1
		485	482	3	2.58	99.5
		775	781	3	3.51	100.8
		1000	1016	3	1.57	101.6
	Ertugliflozin	1.00	0.94	3	5.44	94.3
		3.00	2.78	3	9.16	92.6
		10.5	10.4	3	3.47	99.2
		105	103	3	6.04	97.9
		310	314	3	7.13	101.3
		495	488	3	2.58	98.7
		765	738	3	1.69	96.4
		1000	986	3	1.14	98.6

n=3 replicates at each concentration. Data presented in mean, %CV, and %accuracy. %CV: Coefficient of variation

Specificity and selectivity

Specificity and selectivity for this plasma method were considered in six different lots to decide the extent of endogenous interferences. Each blank lot was treated in two replicates (1 lacking spiking and one 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 internal standard verapamil. No interference was observed at the retention times of semaglutide, ertugliflozin, and verapamil in the tested samples. Representative chromatograms for semaglutide and ertugliflozin for LLOQ and ULOQ are presented in Figs. 7, 8, 10, and 11. The carryover was evaluated across all the runs for acceptance of <20% at analyte retention time and <5% at internal standard retention time.

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. Ertugliflozin 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 (1.01, 2.70, 500, and 750 ng/mL) for semaglutide and (1.01, 2.80, 504, and 755 ng/mL) for ertugliflozin. For semaglutide at each QC level, the intra-day accuracy was between 93.1% and 103.4%, and precision was between 2.10% and 9.19%. In the case of inter-day, the accuracy was between 93.4% and 101.2%, and the precision was between 3.32% and 7.74%.

For ertugliflozin at each QC level, the intra-day accuracy was between 95.3% and 99.3%, and precision was between 6.79% and 9.14%. In the case of inter-day, the accuracy was between 95.9% and 99.2%, and the precision was between 4.99% and 6.31%.

Extraction recovery

As shown in Tables 6 and 7, the extraction recovery for semaglutide was 86.6%, 84.4%, and 88.9% at LQC, MQC, and HQC, respectively. The %CV for semaglutide ranged from 3.60% to 9.89%. Similarly, the extraction recovery for ertugliflozin in human plasma was 86.5%, 85.3%, and 82.5% at LQC, MQC, and HQC, respectively. The %CV for ertugliflozin ranged from 8.84% to 12.92%. The recovery of semaglutide and ertugliflozin is uniform across the tested levels, and the results from the validation experiments indicate no impact on recovery with a change in concentration.

Matrix effect

The mean accuracy from the six independent lots to measure the matrix effect was 100.7% and 101.9%, with a CV of 5.70% and 1.85% 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 91.8% and 97.1%, with a CV of 11.05% and 7.66% at LQC and HQC, respectively, for ertugliflozin, 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.

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 (%)
LLOQC	1.01	1.00±0.02	2.10	99.0
LQC	2.70	2.79±0.26	9.19	103.4
MQC	500	466±18.5	3.98	93.1
HQC	750	745±21.4	2.87	99.3

n=6 replicates in each concentration. Data presented in (mean±SD). LLOQC: ???, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control, QC: Quality control, SD: Standard deviation AQ7

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 (%)
LLOQC	1.01	0.99±0.08	7.57	98.5
LQC	2.70	2.73±0.21	7.74	101.2
MQC	500	467±23.9	5.13	93.4
HQC	750	748±24.9	3.32	99.8

n=18 (6 replicates over 3 days) in each concentration. Data presented in (mean±SD). LLOQC: ???, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control, QC: Quality control, SD: Standard deviation AQ7

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

QC level	Nominal Conc. (ng/mL)	Back calculated Conc. (ng/mL)	Precision (%)	Accuracy (%)
LLOQC	1.01	0.95±0.09	9.14	94.4
LQC	2.80	2.78±0.19	6.79	99.3
MQC	504	481±34.4	7.17	95.3
HQC	755	731±64.4	8.81	96.8

n=6 replicates in each concentration. Data presented in (mean±SD). LLOQC: ???, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control, QC: Quality control, SD: Standard deviation AQ7

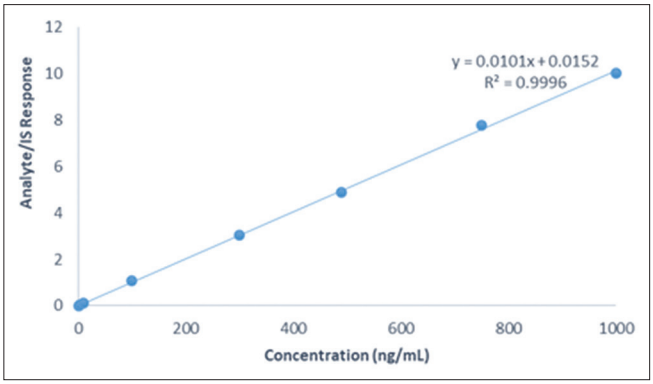


Fig. 4: Linearity plot of semaglutide

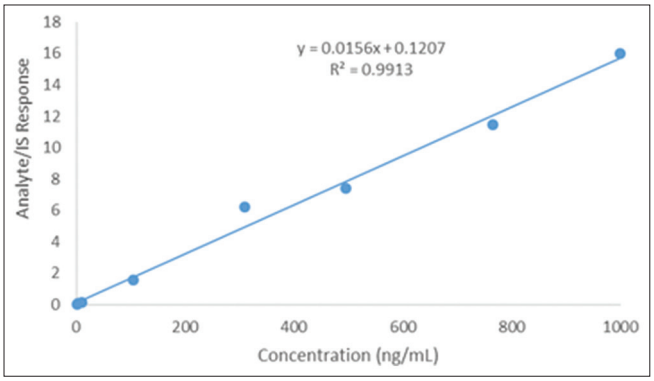
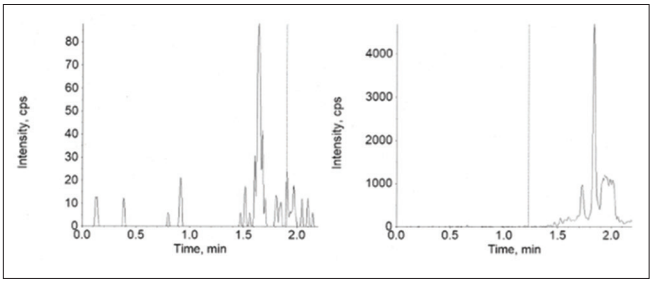


Fig. 5: Linearity plot of ertugliflozin



AQ6 **Fig. 6: Extracted blank of semaglutide**

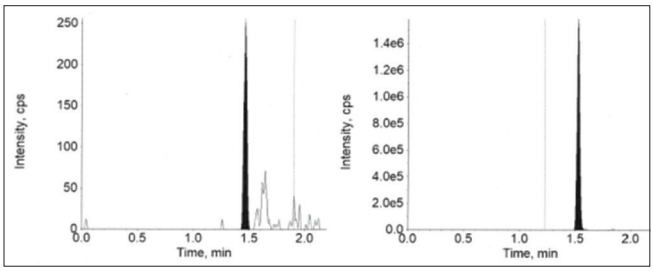


Fig. 7: Extracted lower limit of quantification of semaglutide

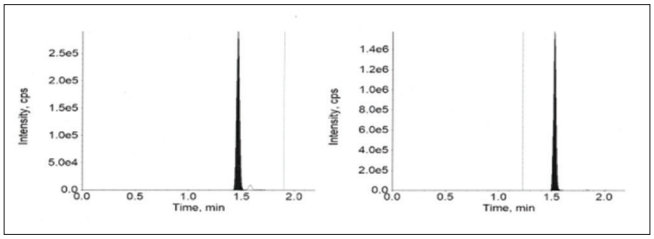


Fig. 8: Extracted upper limit of quantification of semaglutide

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

QC level	Nominal conc. (ng/mL)	Back calculated conc. (ng/mL)	Precision (%)	Accuracy (%)
LLOQC	1.01	0.99±0.05	5.51	97.6
LQC	2.80	2.78±0.15	5.29	99.2
MQC	504	483±30.5	6.31	95.9
HQC	755	749±37.3	4.99	99.1

n=18 (6 replicates over 3 days) in each concentration. Data presented in (mean±SD). LLOQC: ???, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control, QC: Quality control, SD: Standard deviation AQ7

Table 6: Extraction recovery of semaglutide in human plasma

Level	Mean recovery (%)	%CV
LQC	86.6	9.89
MQC	84.4	8.07
HQC	88.9	3.60

n=6 replicates in each concentration. Data presented in mean and %CV. %CV: Coefficient of variation, LQC: Low quality control, MQC: Middle quality control, HQC: High quality control

Table 7: Extraction recovery of ertugliflozin in human plasma

Concentration (ng/mL)	Mean recovery (%)	%CV
LQC	86.5	12.92
MQC	85.3	10.41
HQC	82.5	8.84

n=6 replicates in each concentration. Data presented in mean and %CV. %CV: Coefficient of variation, 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
2.70	100.7	5.70
750	101.9	1.85

n=6 replicates in each concentration. Data presented in mean and %CV. %CV: Coefficient of variation

Table 9: Matrix effect of ertugliflozin in human plasma

Concentration (ng/mL)	Mean accuracy % to measure matrix effect	%CV
2.80	91.8	11.05
755	97.1	7.66

n=6 replicates in each concentration. Data presented in mean and %CV. %CV: Coefficient of variation

Stability

The stability of semaglutide and ertugliflozin 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 13 h, in the autosampler 4°C for 20 h, after five freeze and thaw cycles and on long storage for 18 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.

Table 10: Stability of semaglutide in human plasma

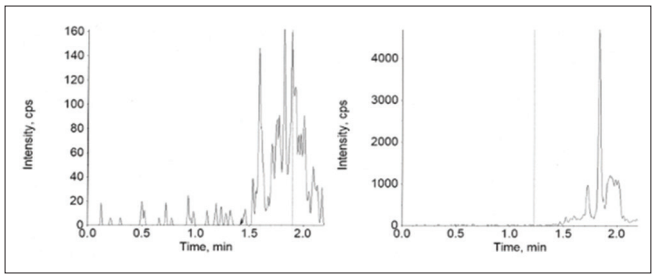
Experimental condition	Sample conc. (ng/mL)	Measured Conc. (ng/mL)	CV (%)	Stability (%)
		Human plasma		
Bench top stability for 13 h at room temperature 24°C	2.70	2.69±0.14	5.16	99.8
	750	757±20.1	2.66	100.9
Autosampler stability for 20 h at 4°C	2.70	2.59±0.32	12.31	95.7
	750	740±11.1	1.50	98.6
5 freeze thaw cycles at −80°C	2.70	2.51±0.28	11.16	93.1
	750	725±12.8	1.76	96.7
Long term stability for 18 days at −80°C	2.70	2.53±0.32	12.83	93.6
	750	716±35.1	4.91	95.4

Measured concentration provided in mean±SD. All stability measurements performed with n=6 replicates. CV: Coefficient of variation, SD: Standard deviation

Table 11: Stability of ertugliflozin in human plasma

Experimental condition	Sample conc. (ng/mL)	Measured conc. (ng/mL)	CV (%)	Stability (%)
		Human plasma		
Bench top stability for 13 h at room temperature 24°C	2.80	2.58±0.32	12.28	92.3
	755	718±82.3	11.47	95.0
Autosampler stability for 20 h at 4°C	2.80	2.82±0.16	5.58	100.6
	755	743±86.4	11.62	98.5
5 freeze thaw cyclesat −80°C	2.80	2.63±0.33	12.34	94.0
	755	763±19.9	2.61	101.1
Long-term stability for 18 days at −80°C	2.80	2.59±0.30	11.54	92.6
	755	751±27.3	3.63	99.4

Measured concentration provided in mean±SD. All stability measurements performed with n=6 replicates. CV: Coefficient of variation, SD: Standard deviation



AQ6

Fig. 9: Extracted blank of ertugliflozin

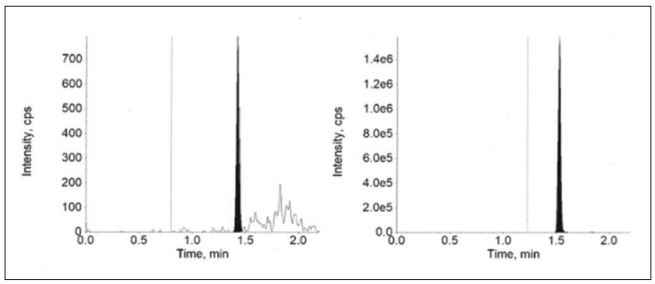


Fig. 10: Extracted lower limit of quantification of ertugliflozin

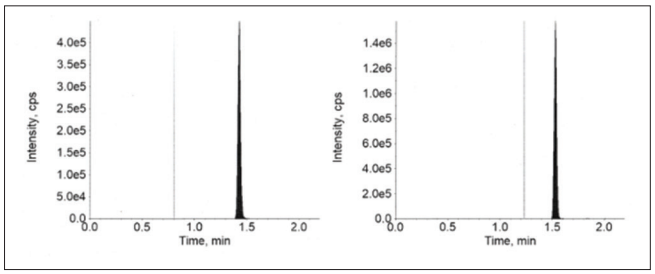


Fig. 11: Extracted upper limit of quantification of ertugliflozin

CONCLUSION

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