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Review Article

A Comprehensive Review of Analytical Methods for the Determination of Semaglutide in Pharmaceutical and Biological Samples

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ABSTRACT

Semaglutide is one of the most potent glucagon-like peptide (GLP-1) analogs, has widely been used to treat type 2 diabetes mellitus and obesity. The development of reliable analytical methods for semaglutide is essential for evaluating its purity, potency, stability, quality, and pharmacokinetic properties. This review provides an overview of various analytical methods used for the identification and quantification of semaglutide in pharmaceutical formulations and biological samples. The methods discussed include reverse-phase ultra-performance liquid chromatography (RP-UPLC), reverse-phase high-performance liquid chromatography (RP-HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS), liquid chromatography-high-resolution mass spectrometry (LC-HRMS), and ultra-performance liquid chromatography coupled with high-resolution selected ion monitoring (UPLC-HR-SIM). RP-UPLC and RP-HPLC methods have demonstrated satisfactory performance in terms of linearity, accuracy, precision, specificity, and robustness, making them suitable for routine pharmaceutical quality control and stability-indicating studies. Forced degradation studies have also been performed to evaluate the stability of semaglutide under acidic, alkaline, oxidative, thermal, and photolytic conditions. Advanced mass spectrometric techniques offer high selectivity and sensitivity for the quantification of semaglutide in biological matrices, supporting pharmacokinetic investigations and studies of its distribution in different tissues. Further improvements in sensitivity, efficacy, and cost-effectiveness may facilitate the development of robust analytical procedures for pharmaceutical quality control and bioanalytical investigations of semaglutide.

INTRODUCTION

Diabetes is one of the most common metabolic and civilization diseases. Polydipsia, dry skin,

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frequent urination, fatigue, blurred vision, unexplained weight loss and even depression can be symptoms of excessively high levels of sugar in human blood stream.

Dysfunctional secretion of pancreatic hormone insulin plays a major role in this disorder. A deficiency of insulin or a lack of sensitivity of peripheral receptors to insulin leads to hypoglycemia. Glucose is overproduced and underutilized as an energy source. These are several types of diabetes

The most common forms include:

- 1) Type-1
- 2) Type-2
- 3) Gestational diabetes

Type-1

Type -1 diabetes (also known as juvenile diabetes) occurs in children and teenagers. Initial symptoms appear very quickly and progress rapidly. This form of diabetes has an autoimmune cause. Endogenous antibodies damage pancreatic beta cells that are responsible for producing insulin. An insufficient amount of this hormone in the blood stream leads to hyperglycemia. Diabetes uses natural or synthetic insulin as the only method of medicinal treatment.

Type-2

Type -2 diabetes usually appears in adults. A lack of physical activity, obesity, diet high in sugar content are the main contributing factors to elevated levels of sugar in blood. This type of diabetes is connected with insulin resistance. Insulin resistance occurs when human cells respond inadequately to insulin, impairing their ability to efficiently absorb and store glucose from

the blood stream. Treatment begins with lifestyle modifications, followed by antidiabetic drugs and insulin.

Gestational diabetes

It can develop during pregnancy between 33% to 70 % of gestational diabetes is diagnosed in early pregnancy (early GD is defined by hypoglycemia occurring before 20 weeks of gestation). Early gestational diabetes is associated with worse pregnancy outcomes compared to women diagnosed with late gestational diabetes (hyperglycemia typically from 24 weeks to 28 weeks of gestation). In most circumstances the condition resolves after childbirth.

Glucagon-like peptide-1 (GLP-1) agonists are an innovative class of drugs approved to treat type 2 diabetes mellitus. Semaglutide (C187H291N45O59) is an analog that is 94% similar to human GLP-1. Two amino acid substitutions and one acylation were made as a major structural modification to avoid dipeptidyl peptidase-4 (DPP-4) cleavage and to increase its affinity for albumin.

In fact, GLP-1 cannot be used for clinical purposes as it is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) enzymes. There are three important modifications in semaglutide that allow its clinical use: the substitution of the amino acid alanine with the alpha-aminoisobutyric acid at position 8, substitution of lysine with arginine at position 34, and the attachment of a linker and a C18 di-acid chain at position 26. All these modifications allow strong binding to albumin, which reduces its renal clearance and degradation by DPP-4 enzymes. Thanks to these modifications, the half-life of semaglutide is about a week, compared to the few minutes of GLP-1.



It is a drug which is available as injectable as well as oral dosage form. Once weekly subcutaneous injectable semaglutide (Ozempic) was approved by the US food and administration (FDA) in 2017 and by the European medicines Agency (EMA) in 2018 for the treatment of type 2 diabetes. Subsequently, a daily oral tablet of semaglutide (Rybelsus) was approved in 2019 by the FDA and in 2020 by EMA. In 2021, the US FDA also approved semaglutide subcutaneous injection (Wegovy) for chronic weight management in patients with obesity (or) overweight, which was the first since 2014.

Therapeutic peptides including glucagon-like peptide-1 (GLP-1) receptor agonists are emerging as a new effective generation of therapeutic because of the advancements in modification chemistry and analytical technologies and rapid progression in proteomics. Moreover, peptide drug development has expanded with recent advances in structural biology, recombinant biologics, and new synthetic technologies. Peptide-based therapeutics has also attracted attention because of their exceptional specificity, minimal immunogenicity, low toxicity, and therapeutic efficacy. Since 2000, more than 30 non-insulin peptide drugs have been approved worldwide. Nonetheless, quantitative peptide bioanalysis remains a challenge due to their high molecular weight, complex structures, significant endogenous interference, low in vivo concentrations, and susceptibility to non-specific binding. These aspects of peptides can lead to substantial loss during preparation and increase the risk of matrix interference.

MECHANISM OF ACTION

Initially intended to control blood glucose levels in patients with type 2 diabetes, semaglutide, a potent glucagon-like peptide 1 analogue, has been established as an effective weight loss treatment

by controlling appetite. Integrating the latest clinical trials, semaglutide in patients with or without diabetes presents significant therapeutic efficacy in ameliorating cardiometabolic risk factors and physical functioning, independent of body weight reduction. Semaglutide may modulate adipose tissue browning, which enhances human metabolism and exhibits possible benefits in skeletal muscle degeneration, accelerated by obesity and ageing. This may be attributed to anti-inflammatory, mitochondrial biogenesis, antioxidant and autophagy-regulating effects. However, most of the supporting evidence on the mechanistic actions of semaglutide is preclinical, demonstrated in rodents and not actually confirmed in humans, therefore warranting caution in the interpretation.

PHARMACOKINETICS OF SEMAGLUTIDE

Absorption

Absolute bioavailability of semaglutide is 89%. Maximum concentration of semaglutide is reached 1-3 days post dose.

Similar exposure is achieved with subcutaneous administration of semaglutide in the abdomen, thigh, or upper arm.

In patients with type 2 diabetes, semaglutide exposure increases in a dose-proportional manner for once-weekly doses of 0.5 mg, 1 mg, and 2 mg. Steady-state exposure is achieved following 4-5 weeks of once-weekly administration. In patients with type 2 diabetes, the mean population-PK estimated steady-state concentrations following once weekly subcutaneous administration of 0.5 mg and 1 mg semaglutide were approximately 65.0 ng/ml and 123.0 ng/ml, respectively. In the trial comparing semaglutide 1mg and 2mg, the



mean steady state concentrations were 111.1 ng/ml and 222.1 ng/ml, respectively.

Distribution

The mean apparent volume of distribution of semaglutide following subcutaneous administration in patients with type 2 diabetes is approximately 12.5L. Semaglutide is extensively bound to plasma albumin(>99%).

Elimination

The apparent clearance of semaglutide in patients with type 2 diabetes is approximately 0.05 L/h. With an elimination half-life of approximately 1 week, semaglutide will be present in the circulation for about 5 weeks after the last dose.

PHARMACODYNAMICS OF SEMAGLUTIDE

Semaglutide lowers fasting and postprandial blood glucose and reduces body weight. All pharmacodynamic evaluations were performed after 12 weeks of treatment (including dose escalation) at steady state with semaglutide 1 mg.

Fasting and Postprandial Glucose

Semaglutide reduces fasting and postprandial glucose concentrations. In patients with type 2 diabetes, treatment with semaglutide 1 mg resulted in reductions in glucose in terms of absolute change from baseline and relative reduction compared to placebo of 29 mg/dL (22%) for fasting glucose, 74 mg/dL (36%) for 2-hour postprandial glucose, and 30 mg/dL (22%) for mean 24-hour glucose concentration.

Insulin Secretion

Both first-and second-phase insulin secretion are increased in patients with type 2 diabetes treated with OZEMPIC compared with placebo.

Glucagon Secretion

Semaglutide lowers the fasting and postprandial glucagon concentrations. In patients with type 2 diabetes, treatment with semaglutide resulted in the following relative reductions in glucagon to placebo, fasting glucagon (8%), postprandial glucagon response (14-15%), and mean 24 hour glucagon concentration (12%).

ANALYTICAL METHODS FOR SEMAGLUTIDE

The analytical methods used to evaluate Semaglutide, providing an overview of different analytical methods and emphasizing their applications in pharmaceutical analysis.

1. RP-UPLC Method:

The objective of the study was to develop was to develop RP-UPLC method for the determination of purity of semaglutide in bulk and pharmaceutical dosage form.

This method was developed by SUBHA HARIKA PENMETSA and RAJA SUNDARARAJAN in the year 19-october-2018.

The chromatographic separation was achieved which acquity BEH C18 [50mm × 1.6mm] 1.8µm column thermostated at 30°C with mobile phases containing 0.01 N potassium dihydrogen phosphate [3.2 pH] : Acetonitrile in the ratio of 50:50 v/v. The flow rate was maintained at 0.4 ml/min and injection volume was found to be 0.50 µL. The detection was done at 292nm using TUV detector. The retention time was found to be 1.026 min. Linearity study was carried out between 25% to 150% concentration levels and R² value was



found to be as 0.999. The % RSD of repeatability and intermediate precision was found to be 0.5 and 0.6 µg/ml. The LOD and LOQ were found to be 0.086 µg/ml and 0.261 µg/ml, respectively. Forced degradation was carried out under various stress conditions to demonstrate the stability-indicating capability of the developed UPLC method. The proposed method was found to be simple, precise, accurate and validated according to the international conference on Harmonization guidelines.

2. RP-HPLC + Forced Degradation :

The objective is for the development of a specific and simple method to trace semaglutide presence in active pharma ingredient and pharma dosages .

This method was developed by ARUN KUMAR KUNA, GANAPATY S, RADHA GV in the year 2019-april 17.

As part of the study on semaglutide drug solvents of HPLC instrument (empower software) with PDA detector ultrasonicator (make : labman) and PH meter are used .The method was optimized with mobile phase with a composition of buffer and solvent were of 60:40 % V/V flow maintained was 1.0 ml/min, the injection volume of 10ml run time was 150×4.6 mm, 5m. Results for the developed method are accurate and specific. The detection wavelength was 292 nm, the retention time for semaglutide was 2.689 min, linearity resulted with $r_2=0.9998\%$ RSP for precision was 1.0% mean recovery for accuracy was in the range of 99.73 to 100.29, This study report is for industrial application of determining semaglutide presence in pharma ingredient and dosages.

3. Stability – Indicating RP-HPLC :

A new specific, economic and selective, accurate, precise and robustness Reverse Phase High

Performance Liquid Chromatography was developed for the quantification of semaglutide in pharmaceutical substances and product.

This method was developed by MERUGU MANASA , VIJEY AANANDHI M in the year 2021.

Chromatographic separation was achieved by C18 column [Azilent C18 150×4.6mm] is used as stationary phase and 0.01 N potassium dihydrogen ortho phosphate: acetonitrile [50:50] used as a mobile phase at a flow rate of 0.1 ml/min and monitored at 230nm. The run time was 5min. The retention time of semaglutide was found to be 2.222 min. To fulfill international conference on Harmonisation requirements developed method was validated. Validation parameters include system suitability, specificity, linearity, accuracy, precision, intermediate precision and robustness. Calibration curve was linear over the concentration range of 7.42 µg/ml from regression analysis relative correlation coefficient (R^2) value was found to be 0.999. Accuracy and precision results were within the limits. LOD and LOQ were found to be 0.007 µg/ml and 0.022 µg/ml respectively. Semaglutide was subjected to the stress conditions like acidic, basic, oxidative, photolytic and thermal conditions. In acidic, alkaline and peroxide stress conditions. In acidic, alkaline, and oxidative conditions degradant peaks were observed but there is no interference with semaglutide peak in all accelerated conditions, hence proving the stability indicating and specific nature of method. Assay result was found to be 99.99% w/w, thus it is proving that the method can also be applied for the estimation of semaglutide in pharmaceutical drug products.

4. LC-MS/MS method:

The objective is for the development of a specific and simple method to trace semaglutide presence



in active pharmaceutical ingredients and pharmaceutical dosages.

This method was developed by Tae suk lee, Eun ji park, Minkyu chol, Hyun seok on, Yejin An, Taehyung kim, Tae soyoung shin and developed in the year 28 march 2023.

This study aimed to develop a novel liquid chromatograph tandem mass spectroscopy [LC-MS/MS] analysis of semaglutide in both plasma and brain to characterize the pharmacokinetics and brain distribution in rats. Semaglutide was extracted by simple protein precipitation with methanol from plasma and by solid phase extraction from brain tissue. Liraglutide was used as an internal standard. Gradient elution profiles with mobile phase comprising 0.1% formic acid in water and acetonitrile were used for chromatographic separation, The lower limit of qualification [LLOQ] of the LC-MS/MS assay was 0.5mg/ml for both eat plasma and brain. Intra & inter day accuracy ranged 89.20 – 109.50% in the plasma and 92.00 – 105.00% in the brain. Precision was within 8.92% in the plasma &7.44% in the brain.

Sprague – Dawley rats were given semaglutide by intravenous [IV 0.02 MG/KG] & subcutaneous [sc 0.1&0.2mg/kg] injection. Plasma concentration of semaglutide showed a muti exponential decline with an average half – life of 7.22-9.26 hours in rats. The subcutaneous bioavailability of semaglutide was 76.65 – 82.85%. The brain tissue of plasma partition coefficient (Kp) value of semaglutide was estimated as <0.01. Among the different regions of the brain semaglutide concentrations were significantly higher in the hypothalamus. The analytical method and pharmacokinetic information may be helpful towards a better understanding of the effect of semaglutide in the brain and further development of GLP – 1 analogues for various brain disease.

5. LC-HRMS METHOD:

This method was developed by N. Arabouche, A. Blanchot, J.S.Raul, P. Kintz in the year 15 june 2024.

In this study the author have developed and validated a method for the identification and quantification of semaglutide in the whole blood using a LC-HRMS.

After the addition of the internal standard (bovine insulin), the blood was subjected to precipitation using a mix oof acetonitrile/methanol (70:30 , v:v). The validation procedure demonstrated an acceptable linearity between 28500µg/ml LOD and LOQ were 1and 2µg/ml respectively .Intra and inter day precision were below 20% at three concentrations. The method was successfully applied to blood samples of 3 diabetic patients under treatment of semaglutide. The samples tested positive with concentrations ranging from 31 to 70µg/ml. Which fall with in in the limits of therapeutic blood concentrations described in the literature.

6. UPLC-HR-SIM Method :

The method was developed by xinyi jiang, jong zi, meng yu, yiran zhao,xiangyi wang, yanhe zhou, xinmiao Guo, jiuming he , jianpeng huang in the year 2025.

To develop a highly sensitive and interference resistant ultra performance liquid chromatography (UPLC) coupled with high resolution selected ion monitoring (HR-SIM) mass spectrometry method for the qualification of semaglutide in biological samples and to apply it for pharmacokinetic analysis.

METHOD: The UPLC-HR-SLM method was utilized to quantify semaglutide in beagle plasma requiring minimal sample pretreatment . The



method demonstrated a lower limit of quantification (LLOQ) of 5.0 µg/ml for semaglutide in beagle plasma.

RESULTS: Following intravenous (IV) administration at a dose of 0.030 mg/kg, the plasma concentration of semaglutide in beagles exhibited a multi exponential decay pattern with an average elimination half-life ($t_{1/2}$) of 44.90 ± 11.45 h.

YEAR	AUTHORS	ANALYTICAL METHODS	COLUMN	MOBILE PHASE	DETECTION	LOD	LOQ
2018	S.H. Penmetsa, Raja. S	RP-UPLC	Acquity BEH C18, 50×1.6 mm, 1.8 µm	0.01 N KH ₂ PO ₄ Ph3.2:ACN 50:50	TUV, 292 nm	0.086 µg/ml	0.261 µg/ml
2019	A.k. Kuna, S. Ganapathi, G. V. Radha	RP-HPLC + forced degradation	Discovery C18, 150 × 4.6 mm, 5 µm	0.1 % OPA buffer : ACN 60:40	PDA, 292 nm	0.08 µg/ml	0.26 µg/ml
2021	M. Manasa, Vijey M. Aanandhi	Stability-indicating RP-HPLC	Agilent C18, 150 × 4.6 mm, 5 µm	0.01 N KH ₂ PO ₄ : ACN 50:50	UV, 230 nm	0.007 µg/ml	0.022 µg/ml
2023	Tae Suk Lee et al.	LC-MS/MS	C18 peptide-analysis column*	0.1% formic acid in water : ACN, gradient	MS/MS	NR	0.5 ng/ml
2024	N. Arbouche, A. Blanchot, J.S. Raul, P. Kintz	LC-HRMS	LC-HRMS column system	ACN : methanol 70:30 for precipitation ; LC mobile phase used for HRMS	HRMS	1 ng/ml	2 ng/ml
2025	Xinyi Jiang et al.	UPLC-HR-SIM-MS	Acquity Premier Peptide CSH C18, 2.1 × 50mm, 1.7 µm	0.1 % formic acid in water : 0.1% formic acid in ACN 30:70	HR-SIM, m/z 1029.4575	NR	5 ng/ml

CONCLUSION

The analytical methods developed for the estimation of semaglutide demonstrate the importance of reliable, sensitive, accurate, and precise analytical techniques in pharmaceutical analysis. Various chromatographic techniques,

including RP-UPLC, RP-HPLC, LC-MS/MS, LC-HRMS, and UPLC-HR-SIM, have been employed for the identification, quantification formulations and biological samples.

Among these methods, RP-UPLC and RP-HPLC are useful for routine pharmaceutical quality



control, assay determination, impurity profiling, and stability-indicating studies. Forced degradation studies help evaluate the stability of semaglutide under different stress conditions. In contrast, advanced mass spectrometric techniques, such as LC-MS/MS, LC-HRMS, and UPLC-HR-SIM, provide enhanced selectivity and sensitivity for quantifying semaglutide in biological matrices, including plasma, whole blood, and brain tissue. These methods also support pharmacokinetic studies and investigations of drug distribution.

The reviewed studies demonstrate that method validation parameters, including linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ), are essential for ensuring analytical reliability. However, the suitability of each method depends on its intended application, sample matrix, required sensitivity, and regulatory requirements.

In conclusion, the development and validation of appropriate analytical methods, are essential for ensuring the quality, safety, efficacy, and consistency of semaglutide pharmaceutical products and for understanding its pharmacokinetic behavior. Further research should focus on improving analytical sensitivity, reducing sample preparation time, minimizing solvent consumption, and developing robust, cost-effective methods suitable for routine quality control and biological analysis.

REFERENCES

1. Whalenk, Finkel R, Panavelil TA, editors. Lippincott illustrated reviews: pharmacology. 6th edition. Philadelphia: wolters Kluwer; 2015. ISBN:- 978-1-4511-9177-6. Pg.no:- 335-337
2. Papakonstantinou I, Tsioufis K, katsi V. Spotlight on the mechanism of action of semaglutide. *Curr Issues Mol Biol.* 2024;46(12):14514-14541.doi:10.3390/cimb46120872.
3. Yang XD, Yang YY. Clinical pharmacokinetics of semaglutide: a systematic review. *Drug Des Devel Ther.* 2024;18:2555-2570.doi:10.2147/DDDT.S470826
4. Eswarudu MM, Krishna PS, Shannu S, Prasanna BL, Kumar PT, Afrin S, et al. A review on analytical profile of semaglutide in pharmaceutical dosage forms and biological matrices. *Indo Am J Pharm Sci.* 2023;10(11): 75-80
5. Bharmare NS, Dugaije TR, Gangurde SB, Bhamare KS, Taro YV, Bargude AJ, et al. Analytical review of semaglutide. *Int J Res Publ Rev.* 2023;4(1):1266-1271.
6. Penmetsa SH, Sundararajan R. Method development and validation of RP-UPLC method for the determination of semaglutide in bulk and pharmaceutical dosage form. *Int J Res Anal Rev.* 2018;5(4):i534-i543.
7. Kumar MA, Shanmugasundaram P. Development of a validated questionnaire to assess attitude of patient towards self-management among uncontrolled diabetic population in Tamil Nadu. *Int J Res Pharm Sci.* 2019;10(2):1089-1095.
8. Manasa M, Aanandhi MV. Stability indicating method development and validation of semaglutide by RP-HPLC in pharmaceutical substance and pharmaceutical product. *Res J Pharm Technol.* 2021;14(3):1385-1389.
9. Lee TS, Park EJ, Choi M, oh HS, An Y, Kim T, et al. Novel LC-MS/MS analysis of the GLP-1 analog semaglutide with its application to pharmacokinetics and brain distribution studies in rats. *J chromatogr B analyt Technol Biomed Life Sci.* 2023; 1221:123688.
10. Arbouche N, Blanchot A, raul JS, Kintz P. Semaglutide and health risk: development and validation of a LC-HRMS method for testing semaglutide in whole blood and application to real cases. *J Chromatogr B.* 2024;1241-124187.



11. Jiang X, Li T, Yu M, Zhao Y, Wang X, Zhou Y, et al. Development and validation of UPLC-HR-SIM to quantify semaglutide for its pharmacokinetic study. *Intell Pharm.* 2025;3(2):143-149.

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