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An Overview on Recent Analytical Methodologies for the Determination of Antiobesity Glucagon like Peptides-1 Receptor Agonists

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ABSTRACT

Recently, glucagon-like peptide 1 (GLP-1) has received significant attention in the treatment of obesity and diabetes due to its potent incretin effect. Unique unimolecular peptides with several functions have surfaced as one of the most promising therapeutic strategies to improve metabolic efficiency and return body weight to normal. The US Food and Drug Administration (FDA) has currently approved the following GLP-1-based medications: liraglutide, semaglutide, and tirzepatide for long term management of obesity. This paper investigates published analytical techniques that have been documented in the literature thus far for measuring anti-obesity tides in pharmaceutical formulations and biological samples. These encompass a range of methodologies, such as capillary electrophoresis, spectrophotometry, liquid chromatography-electrospray ionization-tandem mass spectrometry, high-performance thin layer chromatography, and electrochemical approaches.

Keywords: *glucagon-like peptide 1, liraglutide, semaglutide, tirzepatide, analytical overview*

1. Introduction

Obesity is a global health emergency that negatively impacts every organ system, exacerbating illness and driving up medical expenses. Individuals with obesity who are not responding to lifestyle therapy should be referred to approved pharmacological treatment modalities, medical devices, and/or bariatric surgery. The discovery of innovative therapeutics for the treatment of obesity is imperative due to the progression of severe obesity in the patient population and associated co-morbidities (Srivastava & Apovian, 2018). A class of medications known as GLP-1R agonists targets the action of incretin hormones; these hormones are primarily mediated by receptors found

in the nerves, islets, heart, lung, skin, and other organs.

Clinical trials and animal studies have shown that GLP-1R agonists are superior in treating or preventing obesity. GLP-1R agonists are therefore promising medications for the treatment of obesity (Wang et al., 2023). GLP-1, a significant incretin hormone in humans, functions through a variety of pathways, including increased (glucose-dependent) insulin secretion, decreased hepatic gluconeogenesis, and suppression of glucagon release. It also results in decreased energy intake, less appetite, and delayed stomach emptying. As a result, a number of GLP-1 receptor agonists, including, liraglutide, semaglutide and tirzepatide were created to function similarly to GLP-1 (Mahapatra, Karuppasamy, & Sahoo, 2022).

Laboratory-applied chromatographic techniques for the quantitative analysis of biological samples and drug compounds during all phases of drug development for research and quality control include spectrophotometry, spectrofluorimetry, liquid chromatography (LC) and high performance liquid chromatography (HPLC).

The current study discusses the analytical methods utilized to identify the most often prescribed anti-obesity drugs, including liraglutide, semaglutide, and tirzepatide (Fig 1), either alone or in combination with other drugs in their pharmaceutical and biological matrices. Records for simultaneous estimations with metabolites, impurities, and other drug members are also included.

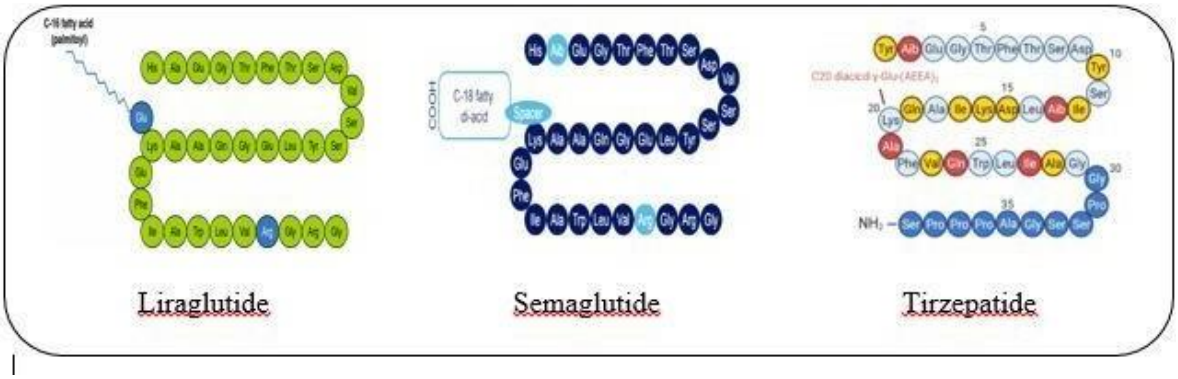

Figure 1. Chemical Structure for Liraglutide, Semaglutide, and Tirzepatide.

2- Analytical review of liraglutide

Liquid chromatography–tandem mass spectrometry and HPLC methods are the most prevalent analytical techniques for assessments of liraglutide.

Since GC offers such a high degree of accuracy when it comes to measuring minute components, it has really been the most employed method. However, ageing of standards, overlapping signals, and baseline drift can seriously impair GC accuracy. Additionally, sample derivatization is typically needed for GC studies, which lengthens the analytical period.

Liraglutide characterisation uses HPLC analysis less frequently than GC analysis, however, HPLC analysis takes less time and does not require sample derivatization. Furthermore, this method works better for blend analysis than GC.

Dong *et al.* (Dong, Gu, Wei, Si, & Liu, 2018) developed the first method for quantifying liraglutide in rat plasma using human insulin as internal standard. C18 column was used for the chromatographic separation of liraglutide, and the mobile phases were acetonitrile and 0.1% formic acid (A) and water and 0.1% formic acid (B). Using multiple-reaction monitoring (MRM) mode and positive ion electrospray ionization, detection was applied.

Subsequently, Giri *et al.* (Giri, Kukreja, Sharma, & Shah, 2023) proposed stability- indicating reversed phase-liquid chromatographic method for detecting liraglutide in bulk active pharmaceutical component. On a C18 liquid chromatography column, liraglutide was chromatographically separated from its degradation products using gradient elution mode and ammonium formate buffer (pH 3.0) as the mobile phase. A flow rate of 0.7 milliliters per minute was used for the elution. At 215 nm, liraglutide and its degradants were detected.

Afterward, two liquid chromatography–tandem mass spectrometry methodologies were developed for detecting liraglutide simultaneously with insulin degludec in rat plasma. In the first method, samples were eluted using C_{18} column with 1000/20/1 water/acetonitrile/formic acid (v/v/v) and 1000/1 acetonitrile/formic acid (v/v) as the mobile phase (Zhai et al., 2020). The second one also utilized a C_{18} column as a stationary phase. Water and acetonitrile with 0.1% formic acid under gradient elution was used as mobile phase. Positive electrospray ionization multiple reaction monitoring (MRM) mode was used for detection by the mass spectrometer (Ziebarth, Diedrich, Machado, & Mainardes, 2024).

Liquid chromatography-mass spectrometry was also used to determine impurity profiles, revealing possible degradation pathways and identify degradation products of stressed drug samples from different suppliers (Chavoshi *et al*., 2024).

The previously reported methods are summarized in the Table 1.

Table 1: Summery of the reported chromatographic techniques available in literature for determination of liraglutide in different matrices.

Matrix	Stationary phase	Mobile Phase	Detection	Ref.
liraglutide in rat plasma	C18	acetonitrile and 0.1% formic acid (A) and water and 0.1% formic acid (B)	multiple-reaction monitoring (MRM) mode and positive ion electrospray ionization	(Dong, Gu, Wei, Si, & Liu, 2018)
liraglutide in bulk active pharmaceutical component	C18	gradient elution mode and ammonium formate buffer (pH 3.0) as the mobile phase	UV detection at 215 nm	Giri, Kukreja, Sharma, & Shah, 2023

liraglutide simultaneously with insulin degludec in rat plasma	C18	1000/20/1 water/acetonitrile/formic acid (v/v/v) and 1000/1 acetonitrile/formic acid (v/v)	Positive electrospray ionization multiple reaction monitoring	Zhai et al., 2020
		Water and acetonitrile with 0.1% formic acid under gradient elution		Ziebarth, Diedrich, Machado, & Mainardes, 2024

3- Analytical review of Semaglutide

Two reversed phase liquid chromatographic methods were developed for determination of semaglutide in bulk and pharmaceutical dosage forms. The first method was applied using C 18 column thermostated at 30°C with mobile phases containing 0.01N 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 detection was done at 292 nm using UV detector (Penmetsa & Sundararajan, 2018). Secondly, Manasa (Manasa & Aanandhi, 2021) utilized C18 column as stationary phase and 0.01N Potassium dihyrogen ortho phosphate: Acetonitrile (50: 50) as a mobile phase at a flow rate of 1.0 mL/min and monitored at 230nm.

Stability indicating assay (SIAM) spectroscopic and chromatographic methods were applied for determination of semaglutide. UV-Visible spectroscopy, HPLC and UPLC techniques were applied using 0.01N Potassium dihydrogen orthophosphate: acetonitrile (61:39) as solvent system. Method- I was developed at the maximum absorption wavelength of 230nm method. Method-II: C18 column was selected as stationary phase. The flow rate and detector wavelength selected were 0.9 mL/min and 230 nm respectively. Method-III: C18 column was selected as stationary phase at 0.5 ml/min flow rate. The detection wavelength was selected at 230 nm (Manasa, 2021). Another SIAM method was developed by Merugu *et al*. (Merugu & Vijey Aanandhi, 2021) using Quality By Design (QBD) approach. The selected Stationary phase was C18, potassium dihydrogen orthophosphate (pH 2) and methanol used as mobile phase in the ratio of 61.2: 38.8 at flow rate of 0.98ml/min. Detection was carried out at the wavelength 230 nm.

Dissolution testing for semaglutide was conducted using RP-UPLC method. C18 column was utilized as stationary phase and 0.01N Potassium dihydrogen ortho phosphate: Acetonitrile (60:40) was selected as mobile phase. The run time was 1.2 min at 0.5 ml/min flow rate. The retention time of semaglutide recorded as 0.89 min at the detection wavelength of 230nm (Manasa & Aanandhi, 2022).

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique was applied for analysis of semaglutide in both plasma and brain to characterize the pharmacokinetics and brain distribution in rats. Liraglutide was used as an internal standard. Gradient elution profiles with mobile phases comprising 0.1 % formic acid in water and acetonitrile were used for chromatographic separation (Lee *et al*., 2023).

Spectrophotometric and spectrofluorometric methodologies were also developed for characterization of semaglutide in bulk and pharmaceutical dosage form. For the first method, semaglutide was detected at

absorption maximum of 293nm (Penmetsa & Sundararajan, 2019). Spectrofluorometric method was developed for semaglutide detection in spiked human plasma. The developed method was based on measuring the native fluorescence of semaglutide in ethanol at

294.8 nm after being excited at 216 nm (Mansour, El-Masry, El-Sherbiny, & Moustafa, 2024).
The previously mentioned chromatographic methods are summarized in Table 2.

Table 2: Summery of the reported chromatographic techniques available in literature for determination of semaglutide in different matrices.

Matrix	Stationary phase	Mobile Phase	Detection	Ref.
Semaglutide in bulk and pharmaceutical dosage forms	C 18 column	0.01N potassium dihydrogen phosphate (3.2 pH): acetonitrile in the ratio of 50:50 v/v.	UV detector 292 nm	Penmetsa & Sundararajan, 2018
		0.01N Potassium dihyrogen ortho phosphate: Acetonitrile (50: 50)	UV detector 230 nm	Manasa & Aanandhi, 2021
Stability indicating assay method	C 18 column	0.01N Potassium dihydrogen orthophosphate: acetonitrile (61:39) as solvent system	UV detector 230 nm	Manasa, 2021
Stability indicating assay method	C 18 column	potassium dihydrogen orthophosphate (pH 2) and methanol used as mobile phase in the ratio of 61.2: 38.8	UV detector 230 nm	Merugu & Vijey Aanandhi, 2021
Dissolution testing for semaglutide	C 18 column	0.01N Potassium dihydrogen ortho phosphate: Acetonitrile (60:40)	UV detector 230 nm	Manasa & Aanandhi, 2022
semaglutide in both plasma and brain	C 18 column	Gradient elution profiles with mobile phases comprising 0.1 % formic acid in water and acetonitrile		Lee *et al*., 2023

4- Analytical review of Tirzepatide

Due to the recent approval of tirzepatide, there is only one spectrofluorometric method was published for its detection. Direct, selective and label-free spectrofluorometric method was proposed and validated for determination of tirzepatide in their pure form, newly approved pharmaceuticals and spiked human plasma. The developed method was based on measuring the native fluorescence of TIR in ethanol at 303 nm after being

excited at 225 nm (Mansour et al., 2024).

5- Final consideration

The present review incorporates outline of various published methods and techniques used in quantifying the recent approved glucagon-like peptide 1 agonists prescribed for management of obesity. Several analytical techniques have been utilized focusing mainly on liquid chromatography mass spectroscopy due to its high sensitivity and selectivity.

The review would help the analytical chemists to recognize the important solvents and how to combine them for his particular set of analytical laboratory instruments. The advantages of one technique over another can be learned by analytical chemists with the help of the appropriate comparisons offered in the published records. Review comprises records of comparative research of one or more compounds in the same class, in addition to records of individual drug profiles. The most effective set of parameters intended to result in a more affordable study and a quicker turnaround time for a reliable analytical process.

Disclosure

There is no conflict of interest to disclose.

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