Capillary-Scale LC-MS of Semaglutide Using the Axcend Focus LC® Coupled to a MassTech MTE30

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Abstract

Semaglutide belongs to a class of medications known as glucagon-like peptide-1 receptor agonists (GLP-1 RAs). It mimics the naturally occurring GLP-1 peptide hormone that is released in the gut in response to eating and has been used for many years in the treatment of Type-2 diabetes. More recently, it has also been found effective in treating obesity. Modern semaglutide treatments for obesity are in very high demand, which has led to shortages in treatment and circulation of counterfeit products. Rapid detection, identification, and differentiation of authentic and illegal semaglutide is increasingly of forensic interest. Structural identification is commonly accomplished using LC-MS or LC-MS/MS to avoid matrix interferences and to elucidate definitive structural information. Traditional LC and MS instrumentation requires significant laboratory space, electrical power, and gas supply, making them unsuitable for point-of-need applications. In this application note, a portable LC (Axcend Focus LC) coupled to a portable MS (MassTech MTE30) with electrospray ionization (ESI) source was used for the determination of semaglutide.

Materials and Methods

A sample of 10 µg/mL semaglutide (i.e., Wegovy) was prepared in 50:50 water/acetonitrile with 0.1% formic acid. This sample was prepared fresh on the day of analysis to avoid potential degradation.

Chromatographic Conditions

Column	10 cm x 0.15 mm i.d. Phenomenex Kinetex 2.6 µm C18 particles
Mobile phase	A) Water + 0.1% formic acid B) Acetonitrile + 0.1% formic acid
Flow rate	1 µL/min
Method	Linear gradient from 5-95% B over 5 min with an isocratic hold at 95% B for 5 min
Injection volume	40 nL
Temperature	Ambient

Mass Spectrometer Settings

ESI voltage	+2.2 kV
Capillary temperature	210˚C
Injection temperature	210˚C
Mass range	800 – 1,400 m/z
AGC target	100,000

Results and Discussion

A representative chromatogram and mass spectrum obtained from LC-MS analysis of semaglutide are shown in Figure 1. Two masses at 1,372 m/z and 1,029 m/z correspond to semaglutide in the M+3H and M+4H charge states, respectively. This is a simple demonstration of the use of the Axcend Focus LC coupled to a MassTech MTE30 MS for point-of-need analysis of semaglutide. A similar approach could be easily applied for detection and identification of other related legal and illegal products.

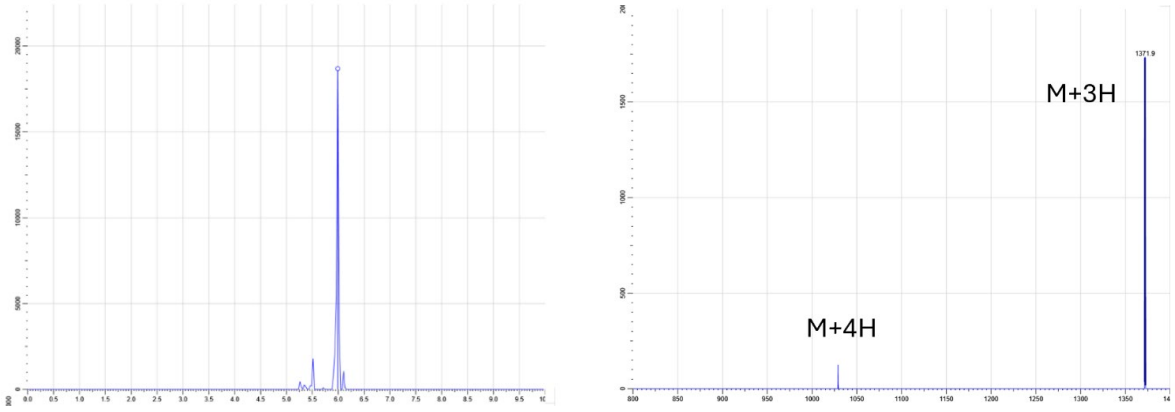

Figure 1. Extracted ion chromatogram (m/z 1,372) of semaglutide (left) and associated mass spectrum (right).