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Application News

High Performance Liquid Chromatograph

Quantitative Determination of Semaglutide and Preservative in Semaglutide Injection by High Performance Liquid Chromatography

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User Benefits

- This method enables simultaneous quantification of both the active ingredient and the preservative in semaglutide injection using a single sample injection.
- The method demonstrates excellent repeatability and is simple to operate.

■ Introduction

Semaglutide is a long-acting glucagon-like peptide-1 (GLP-1) analog that exerts its effects by activating insulin receptors, promoting insulin secretion, and suppressing glucagon secretion. It is primarily used in the treatment of type 2 diabetes and has also shown potential in weight management. In multi-dose formulations of semaglutide injection, a certain concentration of phenol is added as a preservative to inhibit microbial growth. The Chinese Pharmacopoeia sets specific requirements for preservative content in pharmaceutical preparations, with phenol typically maintained at around 0.5 %. Currently, there is limited published literature on analytical methods for semaglutide formulations. Therefore, developing a validated analytical method for quality control of semaglutide injection is of significant practical value in ensuring product quality and enhancing medication safety and consistency for patients.

This article establishes a high-performance liquid chromatographic (HPLC) method for the determination of semaglutide and the preservative phenol in semaglutide injection. The method has been fully validated and is provided here as a reference for relevant professionals.

■ Sample Preparation and Analytical Conditions

1.1 Preparation of Standard Solutions

Stock Solution: Accurately weigh appropriate amounts of phenol and semaglutide reference standards. Dissolve and dilute with water to obtain a mixed stock solution of 5.5 mg/mL phenol and 1.22 mg/mL semaglutide.

Phenol and Semaglutide Standard Solutions: Prepare serial dilutions of the mixed stock solution with water to obtain the concentrations listed in Table 1.

Table 1 Concentrations of Mixed Standard Solutions (µg/mL)

Compound	Mixed Standard Solution of Semaglutide and Phenol					
	S1	S2	S3	S4	S5	S6
Semaglutide	6.09	12.18	24.36	60.9	121.8	243.6
Phenol	27.5	55	110	275	550	1100

1.2 Sample Preparation

Pipette an appropriate amount of semaglutide injection, dilute 50-fold with water, and mix well before analysis.

1.3 Instruments

The experiment was conducted using the Shimadzu Nexera™ LC-40D X3 high-performance liquid chromatograph. The system configuration is as follows:

System Controller	: SCL-40
Degassing Unit	: DGU-405
Solvent Delivery Pump	: LC-40D X3 × 2
Autosampler	: SIL-40C X3
Column Oven	: CTO-40C
Detector	: SPD-M40
Chromatography Workstation	: LabSolutions Ver. 5.106

1.4 Analytical Conditions

Column	: Shim-pack™ GIST C18 (100 mm × 2.1 mm I.D., 2 µm) Shimadzu (Shanghai) Global Laboratory Consumables Co., Ltd. P/N :227-30001-04
Mobile Phase	: Mobile Phase A: 0.1 % trifluoroacetic acid in water Mobile Phase B: 0.1 % trifluoroacetic acid in acetonitrile
Column Temperature	: 35 °C
Flowrate	: 0.3 mL/min
Detection Wavelength	: 280 nm
Injection Volume	: 5 µL
Elution Mode	: Gradient elution starting with 20 % Mobile Phase B. See Table 2 for the time program.

Table 2 Gradient Elution Program

Time	Module	Command	Value
2.00	Pump	B.Conc	20
10.00	Pump	B.Conc	70
10.10	Pump	B.Conc	90
12.00	Pump	B.Conc	90
12.10	Pump	B.conc	20
16.00	Controller	Stop	

■ Results and Discussion

2.1 Chromatograms of Semaglutide and Phenol Standard Solutions

According to the analytical conditions described in Section 1.4, the standard solution containing 27.5 µg/mL of phenol and 6.09 µg/mL of semaglutide was analyzed. The resulting chromatogram is shown in Fig. 1, and details of the compounds are listed in Table 3.

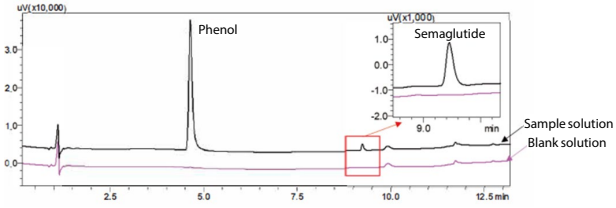

Fig. 1 Chromatogram of Standard Solution
(Phenol: 27.5 µg/mL, Semaglutide: 6.09 µg/mL)

Table 3 Compound Information in the Mixed Standard Solution

No.	Compound	CAS No.	Retention Time (min)
1	Phenol	108-95-2	4.644
2	Semaglutide	910463-68-2	9.212

2.2 Calibration Curve

Using the analytical conditions described in Section 1.4, standard solutions of semaglutide and phenol were analyzed. Calibration curves were generated using the external standard method, with concentration (µg/mL) on the x-axis and peak area on the y-axis. The calibration curves are shown in Fig. 2. The linear equations, correlation coefficients, and accuracy values are listed in Table 4.

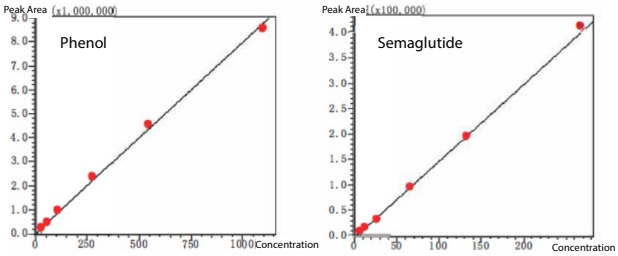

Fig. 2 Calibration Curves for Phenol and Semaglutide

Table 4 Calibration Curve Data for Phenol and Semaglutide

Compound	Linear Range (µg/mL)	Correlation Coefficient	Accuracy (%)	LOD (µg/mL)	LOQ (µg/mL)
Phenol	27.5 - 1100	0.9993	91.5 - 105.1	0.03	0.09
Semaglutide	6.09 - 243.6	0.9994	90.2 - 105.2	0.16	0.47

2.3 Instrument Repeatability

Under the analytical conditions specified in Section 1.4, the lowest concentration standard solution from the calibration curve (phenol: 27.5 µg/mL, semaglutide: 6.09 µg/mL) was analyzed six times. The relative standard deviation (RSD) of the retention time was 0.08 % for phenol and 0.05 % for semaglutide, while the RSD for peak area was 0.15 % and 0.69 %, respectively, demonstrating good accuracy. The chromatograms are shown in Fig. 3.

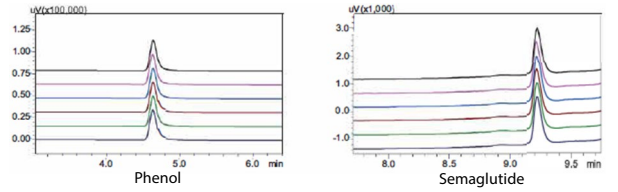

Fig. 3 Chromatograms for Phenol and Semaglutide

2.4 Assay of Sample Content and Spike Recovery Test

The sample was prepared according to the method described in Section 1.2 and analyzed. The measured contents were 1.41 mg/mL for semaglutide and 5.76 mg/mL for phenol. According to the sample label, the indicated content is 1.34 mg/mL for semaglutide and 5.50 mg/mL for phenol. Calculated values correspond to 104.7 % and 105.0 % of the labeled amounts, respectively. Given that the sample volume is 3 mL, the total content of semaglutide is 4.02 mg. According to the pharmacopeial requirement for preparations with a labeled amount under 0.1 g, the actual measured value must fall within 90.0 % to 110.0 % of the labeled content. The results of this experiment are compliant with the pharmacopeial specification.

A spike and recovery test was conducted on the above sample. The semaglutide was spiked at 0.31, 1.22, and 6.10 mg/mL, and phenol at 1.38, 5.50, and 27.50 mg/mL. The recovery results are shown in Table 5.

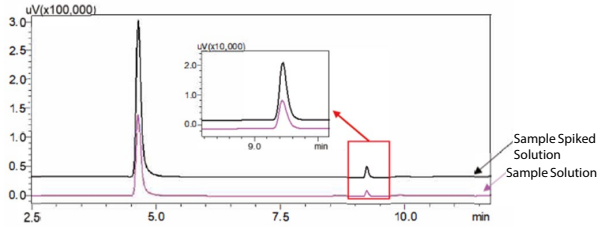

Fig. 4 Chromatograms of Actual Sample Solution and Spiked Sample Solution (Spiked Concentrations: Phenol 5.50 mg/mL, Semaglutide 1.22 mg/mL)

Table 5 Calibration Curve Data for Phenol and Semaglutide

Compound	Labeled Amount	Measured Value	Accuracy (%)	Spike Level	Post-Spike Concentration	Recovery (%)
Phenol	5.50	5.76	104.7	1.38	7.67	109.2
				5.50	11.99	112.7
				27.50	33.56	105.2
Semaglutide	1.34	1.41	105.0	0.31	1.68	97.1
				1.22	2.64	100.7
				6.10	7.55	102.8

■ Conclusion

This study established an HPLC method for the quantitative determination of semaglutide, the main active ingredient, and phenol, the preservative, in semaglutide injection. The analytical results demonstrate that the method offers high accuracy, excellent repeatability, and is simple to operate, making it suitable for the routine quality control of semaglutide injections.

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