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Ion Chromatograph HIC-ESP, HIC-NS

Application News

Determination of Counter Ions of Synthetic Peptides Using Ion Chromatograph

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User Benefits

- The purity of synthetic peptides can be confirmed using ion chromatograph.
- Trifluoroacetic acid and chloride ions can be determined simultaneously.
- The system can be selected according to the purpose, such as a suppressor type when used in conjunction with high-sensitivity analysis such as the impurity measurement of inorganic anions in pharmaceuticals, or a non-suppressor type with a simple equipment configuration when focusing on cost performance.

■ Introduction

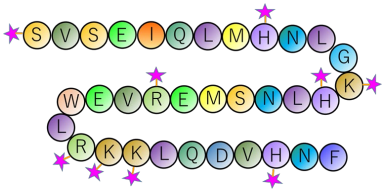
The middle molecule drug is attracting attention as a next generation drug discovery modality. The middle molecule drugs have a smaller molecular weight than biopharmaceuticals, and there are oligonucleotide therapeutics using oligonucleotides and peptide therapeutics with peptide skeletons. Among them, peptide therapeutics have the advantage that they can be produced at low cost, easily taken into cells due to their small molecular weight, and can suppress degradation when taken into the body by adopting a specific conformation.

Since trifluoroacetic acid (TFA) is used to extract the synthesized peptides from the stationary phase, the recovered peptides are converted into peptide TFA salts in which TFA is ionically bonded. The weight of the lyophilized peptide includes the weight of this TFA, which greatly affects the actual peptide content. In addition, TFA may affect bioavailability, and it is necessary to replace it with a salt such as hydrochloride. Therefore, it is essential to quantify the counter ion to confirm the purity of synthetic peptides.

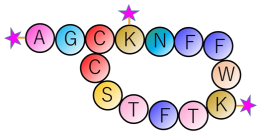
In this paper, we report an example of counter ion analysis using an ion chromatograph (IC) using crude purified linear and cyclic synthetic peptides.

■ Peptide of Interest

The amino acid sequences of the two synthetic peptides (PTH and Somatostatin) are shown in Fig. 1. (Asterisks indicate the N-terminal end of the peptide and the side chain of the basic amino acid where the counter ion is attached.)



PTH (parathyroid hormone (1-34))



Somatostatin

★— The N-terminal end of the peptide and the side chain of the basic amino acid where the counter ion is attached.

Fig.1 Amino acid sequence of the peptide to be measured

■ Suppressor-type Ion Chromatograph

The suppressor-type ion chromatograph is equipped with a suppressor to improve sensitivity by reducing the background conductivity contained in the eluent, and is compatible with high-sensitivity analysis of the order of µg/L.

Fig.2 is a flow chart of a suppressor ion chromatograph (Shimadzu HIC-ESP). The analytical conditions are shown in Table1, and the chromatogram of 10 mg/L trifluoroacetic acid (TFA) and 5 mg/L chloride (Cl) ion mixed standard solution is shown in Fig. 3.

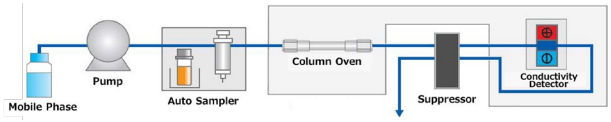


Fig. 2 Flow chart of a suppressor ion chromatograph HIC-ESP

Table 1 Analytical Conditions (HIC-ESP)

Column	: Shim-pack™ IC-SA2*1 (250 mm×4.0 mm I.D., 9 µm)
	: Shim-pack IC-SA2(G)*2 (10 mm × 4.6 mm I.D., 9 µm)
Mobile Phase	: 12.0 mmol/L Sodium Hydrogen Carbonate
	0.6 mmol/L Sodium Carbonate
Flow Rate	: 1.0 mL/min
Column Temp.	: 25 °C
Injection Vol.	: 5 µL
Detection	: Conductivity

*1 P/N : 228-38983-91, *2 P/N : 228-38983-92

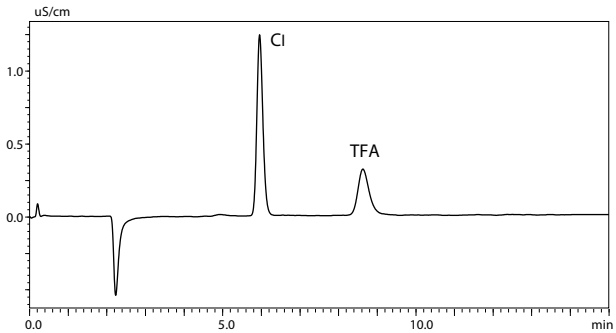


Fig. 3 Chromatograms of standard mixture (HIC-ESP)

■ Non-suppressor-type Ion Chromatograph

The non-suppressor-type ion chromatograph is an ion chromatograph without a suppressor, and is a system with excellent cost performance corresponding to the mg/L order.

Fig.4 is a flow chart of a non-suppressor ion chromatograph (Shimadzu HIC-NS). The analytical conditions are shown in Table2, and the chromatogram of Cl ion and TFA (10 mg/L each) mixed standard solution is shown in Fig. 4.

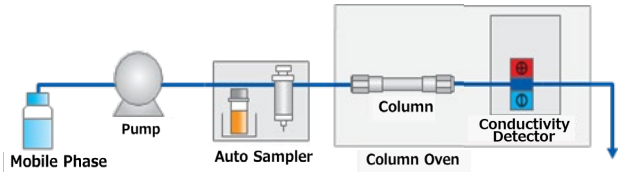


Fig. 4 Flow chart of a non-suppressor ion chromatograph HIC-NS

Table 2 Analytical Conditions (HIC-NS)

Column	: Shim-pack IC-A3*3 (250 mm×4.6 mm I.D., 5 μm)
	: Shim-pack IC-GA3*4 (10 mm × 4.6 mm I.D., 5 μm)
Mobile Phase	: 8.0 mmol/L *p*-Hydroxybenzoic Acid
	3.2 mmol/L Bis-Tris*5
	50 mmol/L Boric Acid
	50 mL/L Acetonitrile
Flow Rate	: 1.2 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 50 μL
Detection	: Conductivity

*3 P/N : 228-31076-91, *4 P/N : 228-31076-92
*5 Bis-(2-hydroxyethyl) iminotris(hydroxymethyl) methane

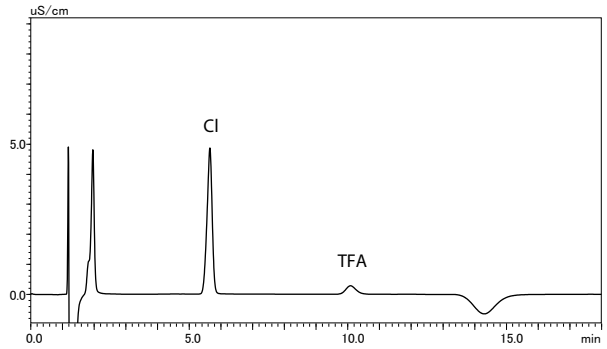


Fig. 5 Chromatograms of standard mixture (HIC-NS)

■ Analysis of Synthetic Peptides

TFA and Cl ion were determined using PTH (Bachem AG code: H-4835.001) and Somatostatin (Bachem AG code: H-1490.005) as real samples. The calibration point was set at 3~4 points over a concentration range of approximately 20 times, centering on the quantitative value.

Peptides that were replaced to Cl ion were quantified using HPLC, and the quantified values were used to calculate counter ion concentrations.

Analysis of TFA

PTH and somatostatin were each dissolved in ultrapure water to determine TFA. The chromatogram of PTH is shown in Fig. 6, the chromatogram of somatostatin is shown in Fig. 7, and the quantitative results of TFA are shown in Table 3. We confirmed that the ratio between the actual quantitative value and the theoretical TFA value calculated from the structure of each peptide was 0.9~1.2, which was almost the same. Similar results were obtained for both the non-suppressor and suppressor systems.

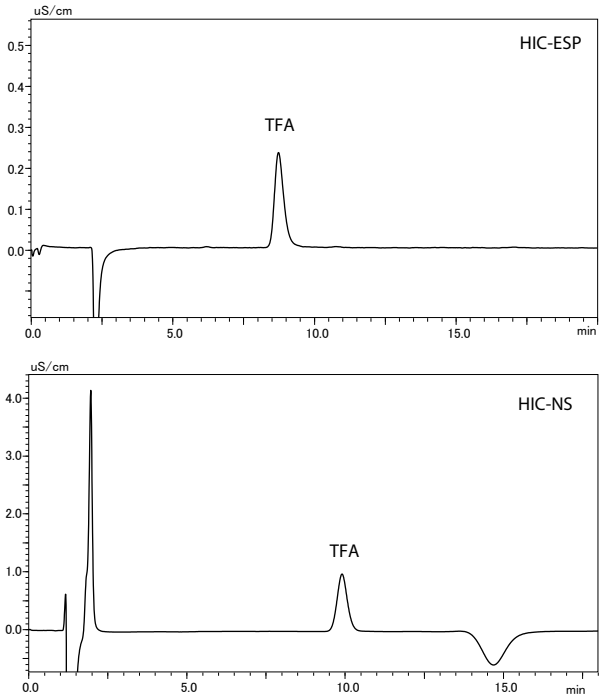


Fig. 6 Chromatograms of PTH (upper : HIC-ESP, lower : HIC-NS)

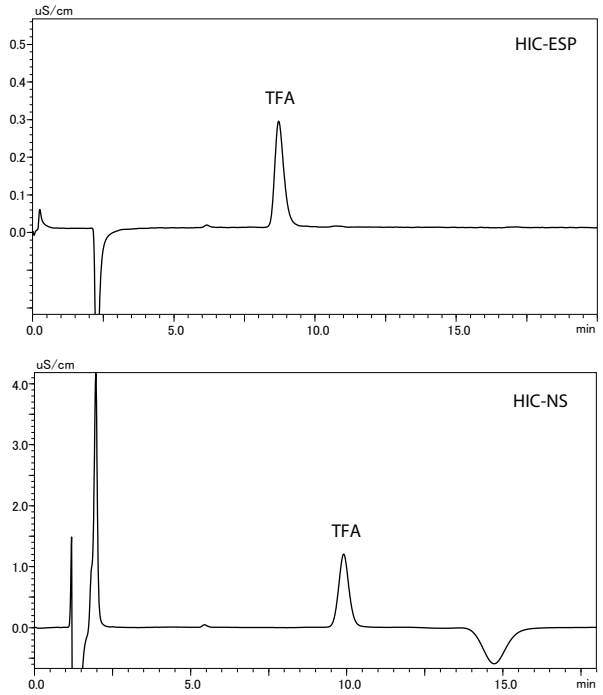


Fig. 7 Chromatograms of Somatostatin (upper : HIC-ESP, lower : HIC-NS)

Table 3 Results of TFA (converted to undiluted solution)

	Counter ion number	Sample conc. (mmol/L)	Theoretical conc. of TFA*6 (mmol/L)	suppressor-type		
				Quantitative value (mmol/L)	TFA ratio	
					/Peptide	Quantitative value /Theoretical value
PTH	9	0.2142	1.9281	1.70	7.94	0.9
Somatostatin	3	0.5564	1.6693	2.06	3.7	1.2

	Counter ion number	Sample conc. (mmol/L)	Theoretical conc. of TFA*6 (mmol/L)	non-suppressor-type		
				Quantitative value (mmol/L)	TFA ratio	
					/Peptide	Quantitative value /Theoretical value
PTH	9	0.2142	1.9281	1.685	7.87	0.9
Somatostatin	3	0.5564	1.6693	2.04	3.67	1.2

*6 Theoretical conc. of TFA = Sample conc. × Counter ion number

Analysis of Cl ion

PTH and somatostatin were each dissolved in ultrapure water, hydrochloric acid was added and freeze-dried. They were dissolved in ultrapure water and analyzed for Cl ion. The chromatogram of PTH Cl salt is shown in Fig. 8, the chromatogram of Somatostatin Cl salt is shown in Fig. 9, and the determination result of Cl ion is shown in Table 4. It was confirmed that the ratio of the quantitative value to the theoretical value of Cl ion was approximately equal to 1.1 for both peptides. Similar results were obtained for both the non-suppressor and suppressor systems.

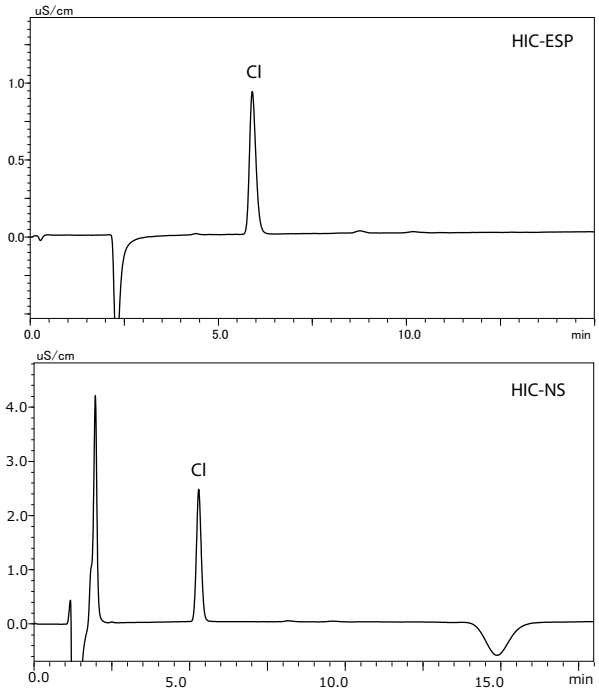


Fig. 8 Chromatograms of PTH (upper : HIC-ESP, lower : HIC-NS)

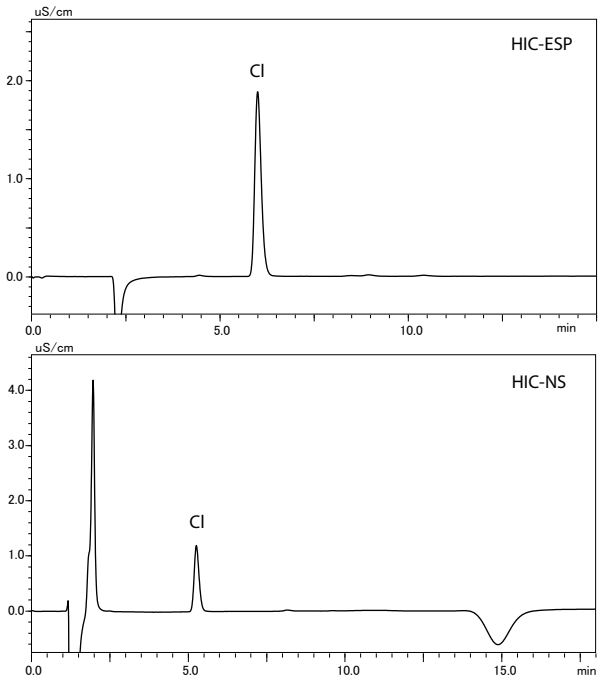


Fig. 9 Chromatograms of Somatostatin (upper : HIC-ESP, lower : HIC-NS)

Table 4 Results of Cl ion

	Counter ion number	Sample conc. *7 (mmol/L)	Theoretical conc. of Cl ion*8 (mmol/L)	suppressor-type		
				Quantitative value*9 (mmol/L)	Cl ion ratio	
					/Peptide	Quantitative value /Theoretical value
PTH	9	0.014	0.126	0.146	10.07	1.1
Somatostatin	3	0.0213	0.0639	0.072	3.15	1.1

	Counter ion number	Sample conc. *7 (mmol/L)	Theoretical conc. of Cl ion*8 (mmol/L)	non-suppressor-type		
				Quantitative value*9 (mmol/L)	Cl ion ratio	
					/Peptide	Quantitative value /Theoretical value
PTH	9	0.014	0.126	0.144	10.29	1.1
Somatostatin	3	0.0213	0.0639	0.072	3.43	1.1

*7 Value determined by HPLC of the redissolved solution
*8 Theoretical Conc. of Cl ion = Sample conc. × Counter ion number
*9 After correction of operating blanks from pretreatment environment

■ Conclusion

In order to confirm the purity of synthetic peptides, counter ions were quantified using IC. It was confirmed that both the suppressor-type and the non-suppressor-type ICs can obtain quantitative values similar to theoretical values calculated from peptide structures. Therefore, when performing this analysis, it is possible to select a system according to the purpose, such as the suppressor-type system when combined with high-sensitivity analysis such as the impurity measurement of inorganic anions in pharmaceuticals, or the non-suppressor-type system with a simple equipment configuration when focusing on cost performance.

From these results, we confirmed that multiple counter ions can be quantified stably without depending on differences in the amino acid sequence or chain length of peptides. This method is a useful method for confirming the purity of synthetic peptides, because the amount of synthesized peptides can be accurately calculated by determining the quantitative value of counter ions.

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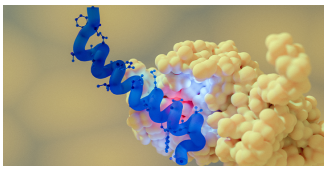

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