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Application News

Cation Non-Suppressor Ion Chromatograph

Analysis of Tris(Hydroxymethyl)Aminomethane in Oligonucleotide Active Pharmaceutical Ingredient (API) Using Ion Chromatography

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User Benefits

- Simultaneous separation of Tris(hydroxymethyl)aminomethane and general inorganic cations using ion chromatograph can be performed by addition of two types of crown compounds to the eluent.
- The elution time can be shortened by adding acetonitrile to the eluent containing two different crown compounds.

■ Introduction

In recent years, there is increasing importance of quality control for medium-molecular-weight drugs involving oligonucleotide therapeutics according to their development progress. Tris(hydroxymethyl)aminomethane (referred to as Tris hereinafter) is a common component in buffer solutions used in life sciences and plays an important role in the manufacturing process of oligonucleotide therapeutics. However, its absence in final product is preferred because it is considered an impurity. Verification of residual Tris levels may be required from a pharmaceutical quality assurance perspective whereas it is not regulated yet.

Currently, analytical methods for Tris are limited and titration method is often used. However, this method is not suitable for precise quantification of trace amounts of target compounds. Furthermore, solutions containing cations such as sodium in addition to Tris are commonly used in oligonucleotide synthesis and manufacturing processes. Therefore, simultaneous separation of Tris and these coexisting cations is preferable for accurate and reliable quantification. This article introduces analysis of Tris using ion chromatography.

■ Analysis of mixed standard solution

Fig. 1 shows the chromatogram of the mixed standard solution of Tris and six cations (lithium, sodium, ammonium, potassium, magnesium, calcium). Crown compounds 18-crown-6 and 15-crown-5 were added to improve the separation of Tris from sodium. The analytical conditions (hereafter referred to as "analytical conditions 1") are shown in Table 1.

A crown compound has a cavity in its molecular structure (Fig. 2) exhibiting different chemical properties inside and outside of the ring. It possesses hydrophobic property derived from carbon structure outside of the ring and anionic properties derived from oxygen inside of the ring. When specific cations are incorporated into the cavity, the entire inclusion complex exhibits hydrophobicity, providing elution controlling by varying type and amount of added crown compound.

Cation analysis eluent containing 18-crown-6 is commonly known. Separation of Tris and sodium is difficult with the eluent of 5 mmol/L 18-crown-6. While separation can be achieved by adding higher concentration of 18-crown-6, the elution times of magnesium, potassium, and calcium become too long. Therefore, by adding 5 mmol/L 15-crown-5 to the eluent in addition to 5 mmol/L 18-crown-6, separation of sodium and Tris can be accomplished by delaying the elution time of only sodium without extending the overall analysis time.

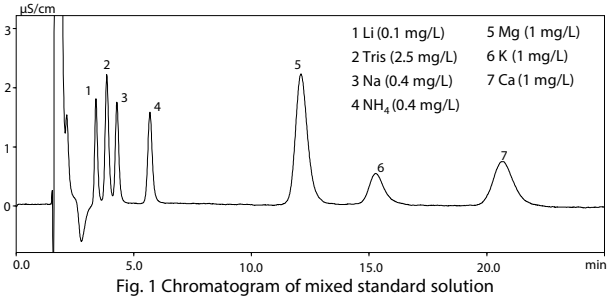

Fig. 1 Chromatogram of mixed standard solution

Table 1 Analytical conditions 1

System	: HIC-NS
Column	: Shim-pack™ IC-C4 (150 mm × 4.6 mm I.D., 7 μm) *1
Guard Column	: Shim-pack IC-C4(G) (10 mm × 4.6 mm I.D., 7 μm) *2
Eluent	: 2.5 mmol/L Methanesulfonic acid, 5 mmol/L 18-Crown-6, 5 mmol/L 15-Crown-5 *3
Flow Rate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 50 μL
Detection	: Conductivity

*1 P/N : 228-38983-91
*2 P/N : 228-38983-92
*3 1.25 mL of 2 mmol/L methanesulfonic acid, 1.322 g of 18 Crown -6, and 992 μL of 15 Crown -5 were added to 1 L of ultrapure water.

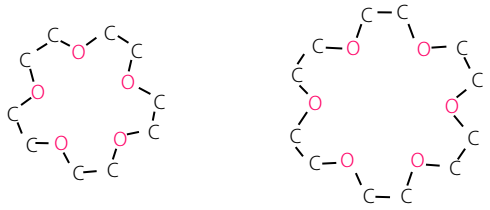

Fig. 2 Structural formulae of crown compounds
Left: 15-crown-5 Right: 18-crown-6

■ Linearities and repeatabilities from mixed standard solution

The mixed standard solution was prepared at the concentrations shown in Table 2. Table 3 shows the results for the linearities and peak area repeatabilities of the mixed standard solutions.

The linearity for each cation was excellent, higher than 0.999 for all. Furthermore, good repeatability of each peak area was obtained through six times consecutive analyses of the mixed standard solution (STD3).

Table 2 Concentrations of mixed standard solutions

Unit: mg/L

	STD1	STD2	STD3	STD4	STD5
Li	0.02	0.05	0.1	0.2	0.4
Tris	0.5	1.25	2.5	5	10
Na	0.08	0.2	0.4	0.8	1.6
NH_4	0.08	0.2	0.4	0.8	1.6
Mg	0.2	0.5	1	2	4
K	0.2	0.5	1	2	4
Ca	0.2	0.5	1	2	4

Table 3 Linearities and peak area repeatabilities (STD3)

Target cation	Linearity (r^2)	Area (%RSD)
Li	0.9998	0.35
Tris	0.9999	0.52
Na	0.9994	1.75
NH_4	0.9999	1.49
Mg	0.9999	2.12
K	0.9999	1.02
Ca	0.9998	1.18

■ Analysis of oligonucleotide API

The oligonucleotide API sample solution was prepared with ultrapure water to make concentration of 600 mg/L, filtered, and then subjected to HPLC analysis. Only approximately 30 mg/L of sodium was detected in the sample solution, and Tris was not detected. Tris was added to this sample to make concentration of 3 mg/L (equivalent to 0.5% based on the weight of the oligonucleotide API), and a spike and recovery test was performed. The chromatogram is shown in Fig. 3. The spike and recovery rate for Tris was 94.3%.

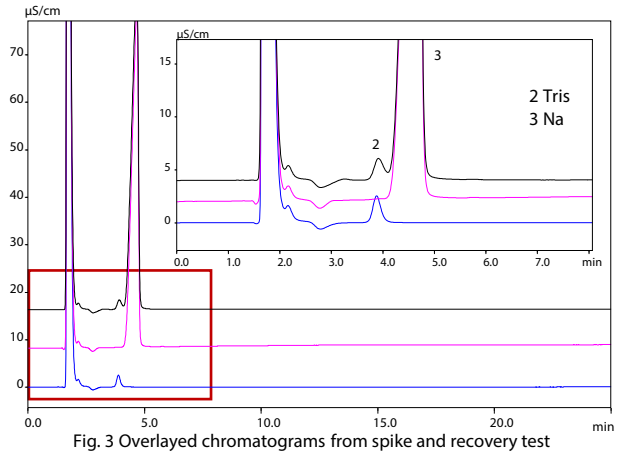

Fig. 3 Overlayed chromatograms from spike and recovery test
Black: spiked sample （3 mg/L Tris addition）
Magenta: non-spiked sample
Blue: Tris (3 mg/L)

■ Addition of organic solvent to eluent

Adding acetonitrile to the eluent can accelerate the elution of the cations targeted in this article. Fig. 4 shows a comparison of chromatograms obtained under the “analytical conditions 1” and the analytical conditions (hereafter referred to as “analytical conditions 2”), where acetonitrile was added to the eluent. The “analytical conditions 2” are also shown in Table 4. Under the “analytical conditions 2”, calcium ions can be eluted within 20 min.

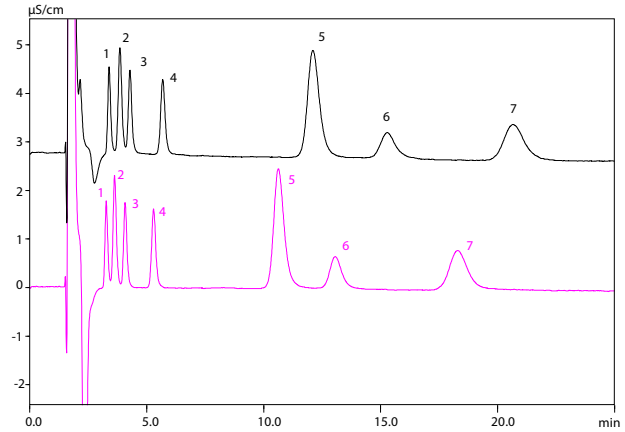

Fig. 4 Overlayed chromatograms of mixed standard solution
Black: Analytical conditions 1
Magenta: Analytical conditions 2

1 Li (0.1 mg/L), 2 Tris (2.5 mg/L), 3 Na (0.4 mg/L) , 4 NH_4 (0.4 mg/L),
5 Mg (1 mg/L), 6 K (1 mg/L), 7 Ca (1 mg/L)

Table 4 Analytical conditions 2

System	: HIC-NS
Column	: Shim-pack™ IC-C4 (150 mm × 4.6 mm I.D., 7 μm)
Guard Column	: Shim-pack IC-C4(G) (10 mm × 4.6 mm I.D., 7 μm)
Eluent	: 2.5 mmol/L Methanesulfonic acid, 5 mmol/L 18-Crown-6, 5 mmol/L 15-Crown-5/Acetonitrile=95:5
Flow Rate	: 1.0 mL/min
Column Temp.	: 40 ˚C
Injection Vol.	: 50 μL
Detection	: Conductivity

■ Conclusion

This article presents simultaneous analysis of Tris and six common cations using an eluent containing crown compounds. Even when oligonucleotide API contains high concentration of sodium, Tris can be successfully separated from sodium. The analytical conditions can be applied to the simultaneous individual quantification of six common cations and Tris as well.

Furthermore, adding organic solvents to the eluent can accelerate the analysis.

For general cation analysis without Tris, please refer to Application News 01-00274-JP.

<Acknowledgments>

We extend our heartfelt gratitude to Juzen Chemical Co., Ltd. for their invaluable cooperation in providing samples for the development of this application.

<Related Applications>

1. ノンサプレッサ方式イオンクロマトグラフ HIC-NSによる陽イオン分析例 Application News No. 01-00274-JP

01-01065-EN First Edition: Mar. 2026

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