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Application News

No.L457

High Performance Liquid Chromatography

Ion Analysis in Drugs (Part 4) Determination of Counterions (Anions) by Ion Chromatography

In Application News No.L387, we introduced examples of impurity ion analysis and counterion analysis in pharmaceuticals using ion chromatography. Typically, a variety of counterions are used to selecting the optimum salts in the development stage of a pharmaceutical product because the physicochemical and pharmacokinetic properties associated with the active pharmaceutical ingredients (API) will vary depending on differences in counterions. Furthermore, as inorganic substances such as catalysts and ions used during synthetic process may affect such properties as solubility and stability, it is very important to conduct analysis of ionic contaminants. In order to eliminate the impurities from the synthesized API, HPLC is commonly used for fractionation and preparative purification. For the preparation of mobile phase, acetic acid, formic acid, trifluoroacetic acid, and their salts are used as additives for easier post-processing. Additionally, in the solid phase synthesis of peptides, trifluoroacetic acid or its salt is used for the isolation of the synthesized peptide from the solid phase, so trifluoroacetate becomes the counterion for the peptide that is the principle component. In many cases, after that, salt replacement by means of acetate or chloride ions takes place before use. Consequently, such ions can become counterions or impurity ions in the API.
Here, we introduce some examples of the analysis of acetate, formate, chloride, and trifluoroacetate ions in a pharmaceutical product.

Analysis of Trace Amounts of Anions

A low-concentration analysis of acetate, formate, chloride, and trifluoroacetate (TFA) ions was carried out. Fig. 1 shows the chromatogram that was obtained from the standard solution. The retention time and the area repeatability (n = 6) are shown in Table 1, and the analytical conditions used are shown in Table 2.

Table 1 Repeatability

	Conc (mg/L)	R.T. %RSD	Area %RSD
Acetate	2.5	0.05	0.54
Formate	2.5	0.04	0.68
Chloride	0.5	0.03	0.78
TFA	10	0.02	0.85

Table 2 Analytical Conditions

Column	: Shim-pack IC-A3 (150 mm L.×4.6 mm I.D.)
Mobile Phase	: A : 8.0 mmol/L *p*-Hydroxybenzoic acid 3.2 mmol/L Bis-Tris 50 mmol/L Boric acid B : Acetonitrile A : B = 95:5 (v/v)
Flowrate	: 1.2 mL/min
Column Temp.	: 40 ˚C
Injection Volume	: 50 µL
Detection	: Conductivity (Non-suppressor mode)

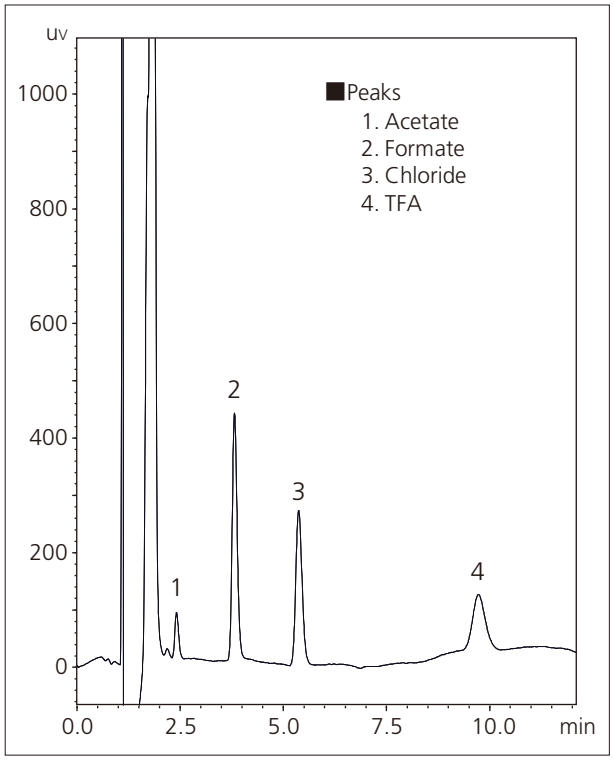

Fig. 1 Chromatogram of a Four-Anion Standard Mixture

Linearity of Calibration Curves

Four standard mixtures in the following concentration ranges were prepared: acetate and formate ions 2.5 to 20 mg/L, chloride ion 0.5 to 4 mg/L, and TFA ion 5 to 40 mg/L. Then measurements were done using an electroconductivity detector to create calibration curves, which are shown in Fig. 2.

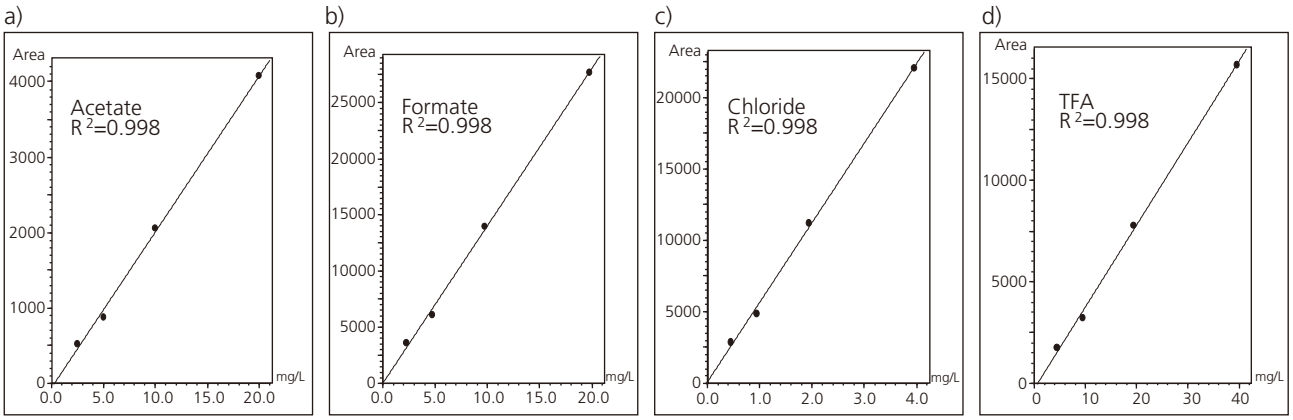

Fig. 2 Linearity of Calibration Curves
a) Acetate, b) Formate, c) Chloride, d) TFA

Analysis of Counterions

In the example shown in Fig. 3, a standard solution of hydroxocobalamin containing acetate (70 mg/L : 0.05 mmol/L) is analyzed. The quantitative value obtained for the counterions (acetate ions) was 3.5 mg/L (0.06 mmol/L). The mole ratio between the principal component and counterions was hydroxocobalamin : acetate ion = 1:1.2.
In the example shown in Fig. 4, a standard solution of angiotensin I containing TFA (65 mg/L) is analyzed. The quantitative value obtained for (TFA ions) was 23 mg/L (0.2 mmol/L).

The mole ratio between the principal component and counterions was angiotensin I : TFA ion = 1:6.2.
Furthermore, repeated analysis of each of the standard solutions resulted in an area repeatability (n = 6) that was favorable, with acetate at 0.52 % in hydroxocobalamin and TFA at 1.15 % in angiotensin I.
The conditions of the analysis are shown in Table 2.

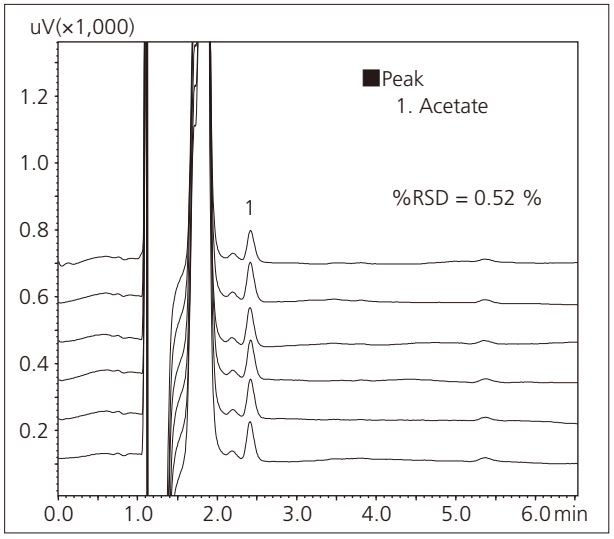

Fig. 3 Chromatogram of Hydroxocobalamin Acetate

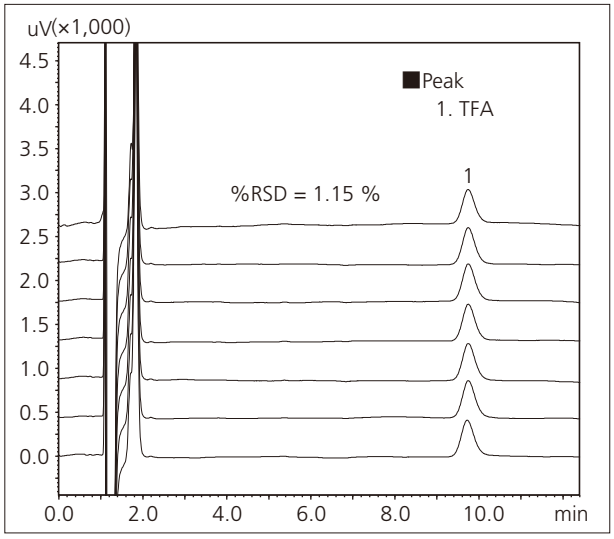

Fig. 4 Chromatogram of Angiotensin I TFA Salt

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