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Application News

No.L504

High Performance Liquid Chromatography

Analysis of Ions in Drugs (Part 5)
Analysis of Organic Acid Counterions by Ion Exclusion Chromatography

The physicochemical and pharmacokinetic properties of active pharmaceutical ingredients change depending on the counterion used, and during drug development various counterions are tested to select the optimum salt.
Residual inorganic impurities from catalysts or ions used during synthesis can also affect product solubility and stability, so it is extremely important that ion impurities are identified.
Application News No. L457 described an example analysis of chloride, formic acid, acetic acid, and trifluoroacetic acid ions present in drugs using ion chromatography. As mentioned above, drugs can contain multiple different counterions and it is sometimes difficult to separate completely the peaks of these ions using ion chromatography.
We describe an example analysis that uses ion exclusion chromatography to increase separation selectivity for formic acid, acetic acid, fumaric acid, and maleic acid, which are organic acids frequently used for drug counterions.

Standard Solution Analysis

In ion exclusion chromatography, retention strength is mainly determined by the degree of disassociation of the solute ion, and separation is performed based on the quick elution of strong acids that are unable to enter packing material pores due to a strong electrical repulsive force, and the delayed elution of weak acids that are able to enter packing material pores due to a weak electrical repulsive force.
We analyzed a 4-component standard solution containing acetic acid, formic acid, fumaric acid, and maleic acid using two ion exclusion columns.
The analytical conditions are shown in Table 1. A UV-VIS detector was used, and an aqueous solution of perchloric acid was used as the mobile phase because it is a poor absorber of UV. The results obtained after injecting 10 µL of standard solution are shown in Fig. 1.

The number of columns (i.e., the size of the separating space) and column temperature are important factors in the separation of multi-component mixtures of organic acids using ion exclusion chromatography.
Consequently, two columns and a column temperature of 50 ˚C were used for the analysis of formic acid, acetic acid, fumaric acid, and maleic acid.

Table 1 Analytical Conditions

Column	: Shim-pack SCR-102H 2 columns (300 mm L. × 8.0 mm I.D.)
Mobile Phase	: 5.0 mmol/L Perchloric Acid aq. solution
Flowrate	: 0.8 mL/min
Column Temp.	: 50 ˚C
Injection Volume	: 10 µL
Detection	: UV-VIS detector (SPD-20A) at 210 nm

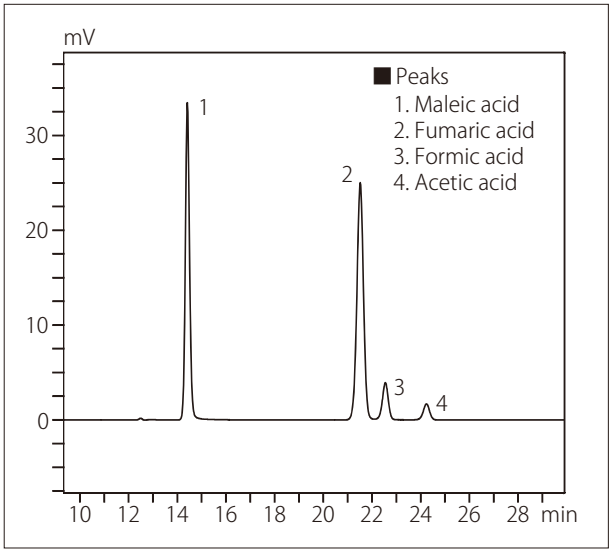

Fig. 1 Chromatogram of Standard Solution

Calibration Curve Linearity

A standard sample of fumaric acid ions was used to prepare four standard concentrations in the range of 6.25 to 50 mg/L. The calibration curve created using these standard concentrations and a UV-VIS detector is shown in Fig. 2. As shown in Fig. 2, good linearity was obtained.

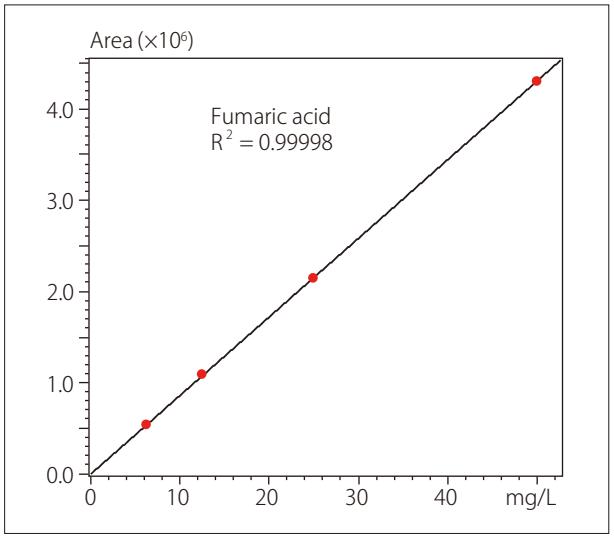

Fig. 2 Calibration Curve Linearity

Analysis of Counterions

The structural formula of the antihistamine drug clemastine fumarate is shown in Fig. 3, and an example analysis of a standard solution of clemastine fumarate (50 mg/L: 0.11 mmol/L) is shown in Fig. 4. The analytical conditions used are identical to those shown in Table 1.

The results of quantitative analysis of the fumaric acid ions present in the clemastine fumarate standard solution are shown in Table 2. Quantitative analysis showed the presence of 13.20 mg/L (0.029 mmol/L) of fumaric acid ions, which is a good recovery rate of 104.6 % when compared to the theoretical value of 12.62 mg/L.

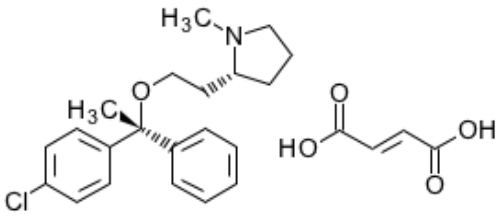

Fig. 3 Structural Formula of Clemastine Fumarate

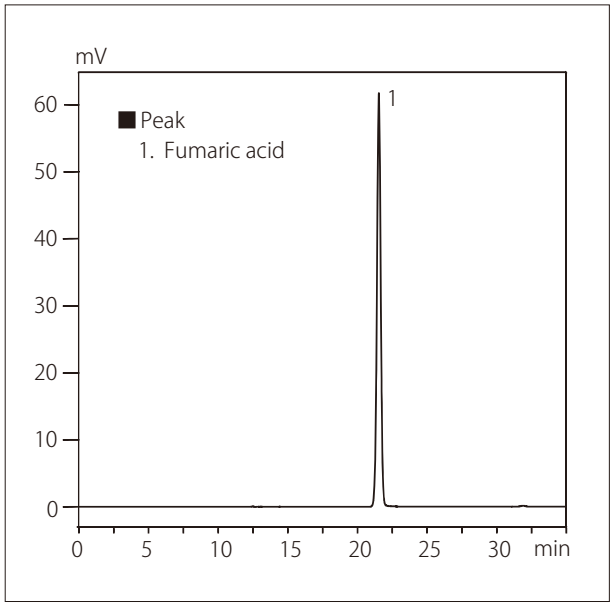

Fig. 4 Chromatogram of Clemastine Fumarate

Table 2 Results of Quantitative Analysis of Fumaric Acid Ions

Quantitative Value [mg/L]	13.20
Theoretical Value [mg/L]	12.62
Recovery Rate %	104.6

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