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Application News

No.L461

High Performance Liquid Chromatography

Analysis of Impurities in Pharmaceuticals Using Newly Developed "Shim-pack MAqC-ODS Ⅰ" Column

Many pharmaceutical products consist of basic compounds. Pharmaceutical impurities such as unreacted starting materials, by-products and decomposition products are usually highly polar basic components as well. Since retention is weak when analyzing these compounds in the reversed-phase mode, mobile phases with added ion-pair reagents have typically been used. However, when using an ion-pair reagent, the additional time required for column equilibration and the difficulties associated with gradient elution have also been a challenge.

Since the newly developed Shim-pack MAqC-ODS Ⅰ is an ODS column with cation-exchange properties, it specifically permits the retention of basic compounds. Thus, even highly polar basic compounds can be retained using a mobile phase that does not include an ion-pair reagent. Gradient elution also can be used to achieve improved sensitivity, as well as to save time in the simultaneous analysis of highly polar basic compounds and other components.

Further, considering the case in which LC/MS analysis is used for analysis of an impurity peak, if a sodium alkyl sulfonate is included as the non-volatile ion-pair reagent, which is not uncommon, there is a danger that precipitates might adhere to surfaces such as the LC/MS interface. There is also a concern that the background signal might increase due to residual ion-pair reagent in the flow line when switching to another mode of separation. In this case, thorough, time-consuming rinsing would be required.

Here, it is demonstrated that LC/MS measurement using the Shim-pack MAqC-ODS Ⅰ is indeed possible by desalting using an automated pretreatment system such as the Co-Sense for LC/MS. Analysis of famotidine is also introduced as an example of analysis of impurity in pharmaceuticals using the Shim-pack MAqC-ODS Ⅰ.

Analysis of Famotidine in Accordance with the Japanese Pharmacopoeia Sixteenth Edition

First, analysis of famotidine was conducted according to the procedure specified in the Sixteenth Edition of the Japanese Pharmacopoeia. For the column, a typical ODS column, the Shim-pack VP-ODS, was used. The structural formula of famotidine is shown in Fig. 1. The analytical conditions that were used are shown in Table 1, and the obtained chromatogram is shown in Fig. 2. The standard solution, consisting of 100 mg/L famotidine, was prepared according to the pharmacopoeia, and the flowrate was adjusted so that famotidine would be eluted at about 6 minutes as specified in the pharmacopoeia.

Table 1 Analytical Conditions

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase	: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust to pH 3.0 with acetic acid, and add water to make 1000 mL. To this solution add 240 mL of acetonitrile and 40 mL of methanol.
Flowrate	: 0.45 mL/min *Adjust the flowrate so that retention time of famotidine is about 6 minutes.
Column Temp.	: 25 ˚C
Detection	: SPD-M20A at 254 nm
Injection Vol.	: 5 µL

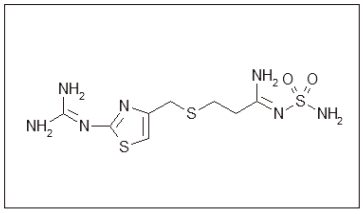

Fig. 1 Structure of Famotidine

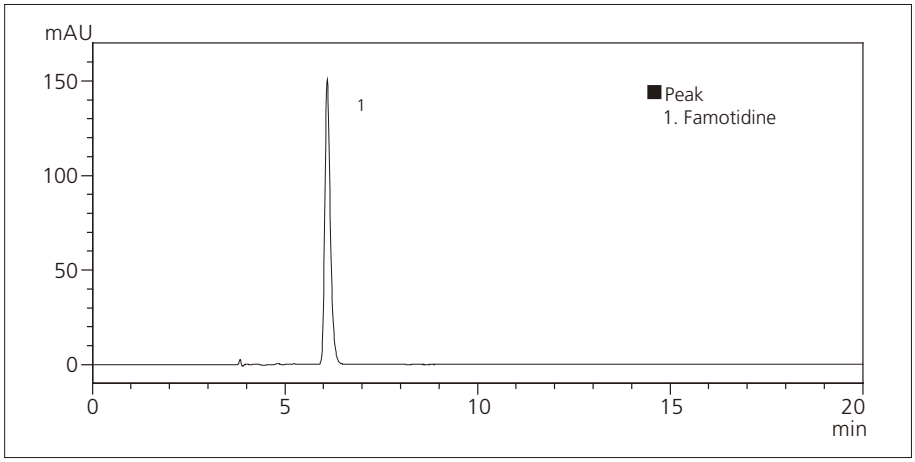

Fig. 2 Chromatogram of Famotidine (Analysis in accordance with the Japanese Pharmacopoeia Sixteenth Edition)

Analysis of Famotidine Using Shim-pack MAqC-ODS Ⅰ

Next, analysis of famotidine was conducted using the Shim-pack MAqC-ODS Ⅰ column. The analytical conditions that were used are shown in Table 2.
The famotidine sample was prepared at a high concentration (1000 mg/L) so as to obtain more pronounced impurity peaks. Preparation consisted of dissolving the famotidine in a small amount of dilute hydrochloric acid and adjusting it with water. The analytical results obtained using the Shim-pack MAqC-ODS Ⅰ are shown in Fig. 3.
For comparison, the results of analysis using the Shim-pack VP-ODS column are shown in Fig. 4. To adjust the retention time of famotidine, the flowrate was set to 0.5 mL/min, while the rest of the analytical conditions remained the same as those shown in Table 1.
Comparing Fig. 3 and 4, many more contaminants were separated in a short period of time when using the Shim-pack MAqC-ODS Ⅰ, which supports gradient elution.

Table 2 Analytical Conditions

Column	: Shim-pack MAqC-ODS Ⅰ (150 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase	: A) 10 mmol/L phosphate (sodium) buffer (pH 2.5) B) Acetonitrile
Time Program	: B Conc. 8 % (0 min) → 8 % (5 min) → 50 % (12 min) → 8 % (12-20 min)
Flowrate	: 1.0 mL/min
Column Temp.	: 25 ˚C
Detection	: SPD-M20A at 254 nm
Injection Vol.	: 5 µL

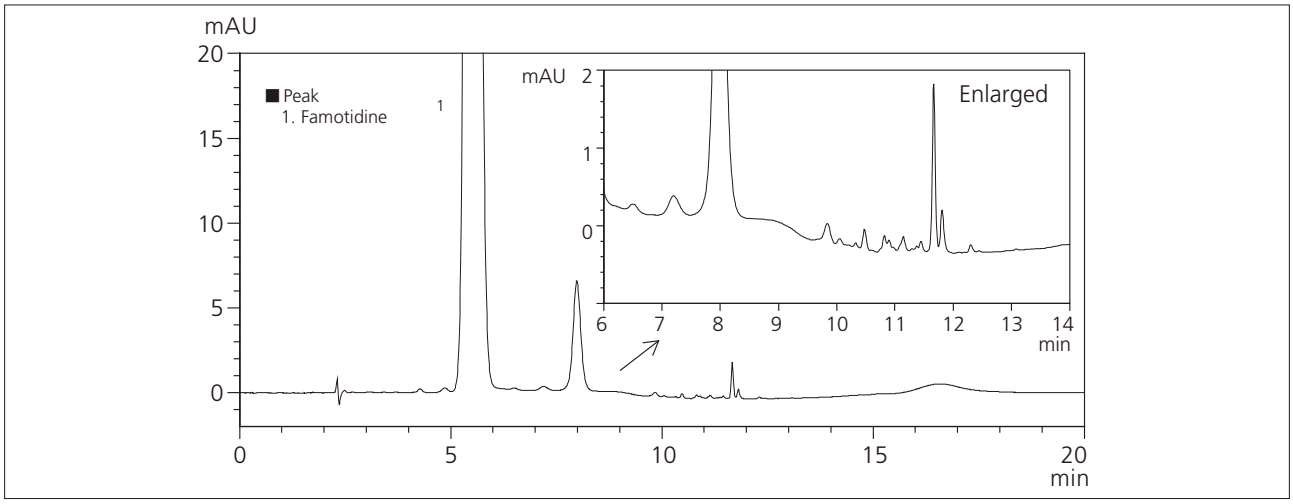

Fig. 3 Chromatogram of Famotidine (1000 mg/L) Using Shim-pack MAqC-ODS Ⅰ

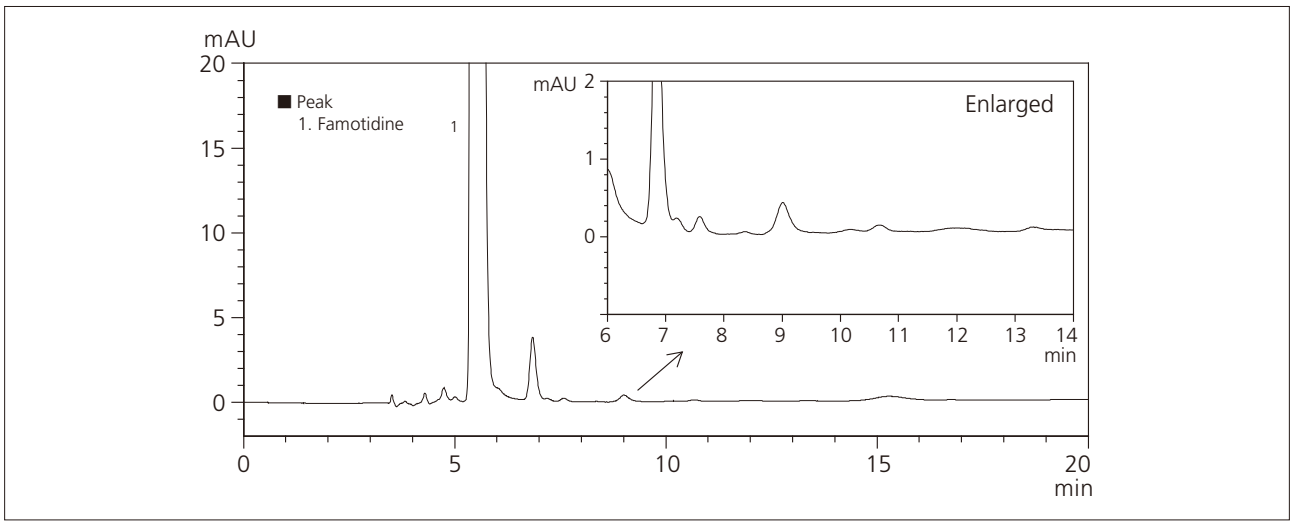

Fig. 4 Chromatogram of Famotidine (1000 mg/L) Using Shim-pack VP-ODS

The Shim-pack MAqC-ODS Ⅰ was developed jointly with Eisai Co., Ltd.

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