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Application News

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High Performance Liquid Chromatography

Impurity Analysis of Pharmaceutical Products Using Next-Generation LC Column "Shim-pack Arata™ C18"

When basic compounds are analyzed in a column packed with general octadecyl silyl (hereinafter, ODS) silica gel, it is known that analytical accuracy is affected by the peak shape. Although a large number of ODS columns with high separation performance have been commercialized in recent years, tailing or other peak shape abnormalities sometimes occur with columns for basic compounds due to the physical properties of the target compound. As an additional problem, the long time required for column equilibration is an issue under low ionic strength acidic mobile phase conditions, for example, when using 0.1 % formic acid in water. Because retention time will change over time if column equilibration is inadequate, stable resolution becomes impossible. The Shim-pack Arata C18 column was developed to solve these many problems. Since a satisfactory peak shape can be obtained with a simple system mobile phase, even with ionic compounds, higher analytical accuracy can be expected.

This article demonstrates that basic compounds and acidic compounds can be analyzed stably while maintaining a satisfactory peak shape by using the Shim-pack Arata C18 column, and introduces an example of application to impurity analysis of pharmaceuticals.

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Analysis of Basic Compounds and Acidic Compounds

Basic compounds and acidic compounds were analyzed using a low ionic strength organic acid mobile phase (0.1 % formic acid in water), which tends to cause deterioration of the peak shape of basic compounds. The tricyclic antidepressant amitriptyline was used as the basic compound, and benzoic acid was used as the acidic compound. The standard solution was prepared so that concentration of each compound in the mobile phase was 100 mg/L, and was analyzed under the conditions shown in Table 1.

Fig. 1 shows the obtained chromatograph. The symmetry factors of the peaks were 1.01 for amitriptyline and 1.00 for benzoic acid, indicating that satisfactory peak shapes could be obtained for both the basic compound and the acidic compound.

Table 1 Analytical Conditions

System	:	Nexera™ X2
Column	:	Shim-pack Arata C18 (75 mm L. × 3.0 mm I.D., 2.2 µm) (Figs. 1 and 3) Typical ODS (75 mm L. × 3.0 mm I.D., sub-2 µm) (Fig. 2)
Mobile Phase	:	0.1 % Formic acid in water/Acetonitrile = 70/30(v/v)
Flow Rate	:	0.4 mL/min
Injection Vol.	:	1 µL
Column Temp.	:	40 °C
Detection	:	SPD-M30A at 254 nm (Fig. 1) SPD-M30A at 280 nm (Figs. 2 and 3)

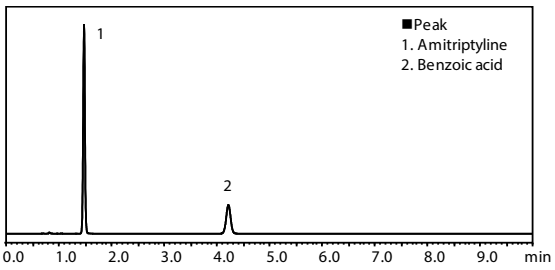

Fig. 1 Chromatograph of Basic Compound (Amitriptyline) and Acidic Compound (Benzoic Acid) by Shim-pack Arata C18 Column

Change of Retention Time and Symmetry Factor with Equilibration Time

With general ODS columns, excessive time may be required until retention time (RT) stabilizes, i.e., until equilibration is achieved, when the analysis is conducted using with a low ionic strength organic acid mobile phase such as 0.1 % formic acid in water. Therefore, the time required for equilibration under a low ionic strength organic acid mobile phase condition was evaluated with dextromethorphan and amitriptyline, which are basic drugs.

<Analytical Method>

The columns used were a general ODS column (new column, shipping solvent: acetonitrile) and a Shim-pack Arata C18 column (new column, shipping solvent: acetonitrile). The columns were equilibrated with the mobile phase without conditioning, and the standard solution was analyzed at set times. The analytical conditions are shown in Table 1. The standard solution was prepared with the mobile phase so that the concentration of each component was 100 mg/L.

<Results>

The transitions of the chromatograms when using the general ODS column and the Shim-pack Arata C18 column are shown in Fig. 2 and Fig. 3, respectively.

With the general ODS column, RT had not stabilized even after an equilibration time of approximately 720 min, and the symmetry factors of dextromethorphan and amitriptyline were 2.49 and 3.90, respectively. In contrast, with the Shim-pack Arata C18 column, RT became uniform at about 30 min after the start of equilibration, and the symmetry factors of dextromethorphan and amitriptyline were 1.19 and 1.07, respectively. After this point, there were no significant changes in either RT or the symmetry factor. These results demonstrate that the Shim-pack Arata C18 column enables quick equilibration while maintaining excellent peak shapes even when using a low ionic strength organic acid mobile phase. Based on this performance, the Shim-pack Arata C18 column is expected to provide outstanding stability in LC/MS (/MS) analysis.

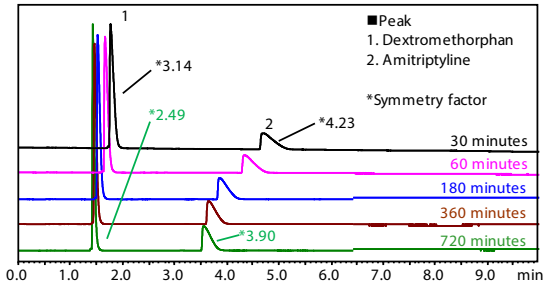

Fig. 2 Transition of Chromatogram of Dextromethorphan and Amitriptyline with General ODS Column

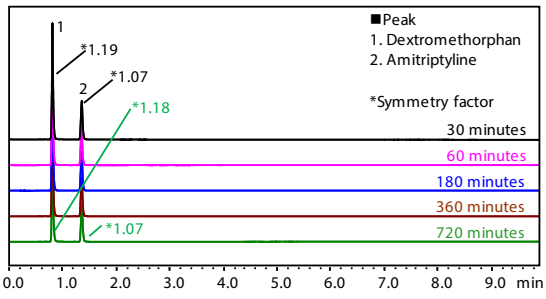

Fig. 3 Transition of Chromatogram of Dextromethorphan and Amitriptyline with Shim-pack Arata C18 Column

Application to Impurity Testing of Drug Substances

Because impurity testing of drug substances is one critical type of test in the pharmaceutical product manufacturing process, high reliability and analytical accuracy are demanded in high performance liquid chromatography (HPLC) columns used in those tests.

<Analytical Method>

Amitriptyline prepared with the mobile phase to obtain a concentration of 100 mg/L was used as the model sample, and 0.1 % phosphoric acid in water was used as the mobile phase. The time until equilibration of a general ODS column and a Shim-pack Arata C18 was compared. Table 2 shows the analytical conditions. The organic solvent ratio was adjusted depending on the column so as to obtain the same main peak retention times.

Table 2 Analytical Conditions

System	:	Nexera X2
Column	:	Typical ODS (75 mm L. × 3.0 mm I.D., sub-2 µm) (Fig. 4) Shim-pack Arata C18 (75 mm L. × 3.0 mm I.D., 2.2 µm) (Fig. 5)
Mobile Phase	:	0.1 % Phosphoric acid in water/Acetonitrile = 70/30 (v/v) (Fig. 4) = 76/24 (v/v) (Fig. 5)
Flow Rate	:	0.4 mL/min
Injection Vol.	:	1 µL
Column Temp.	:	40 °C
Detection	:	SPD-M30A at 210 nm

<Results>

Fig. 4 shows a comparison of the chromatograms at 5 h and 12 h after the start of equilibration for the general ODS column. Retention of the peak of the main component, amitriptyline, and the peak of impurity 1 at around 3 min were weak. On the other hand, retention of the peaks of impurities 2 to 4 eluted at around 4 to 5 min tended to be strong. Furthermore, the peak of impurity 5 eluted at approximately 5.5 min at 5 h after the start of equilibration overlapped the main peak. Thus, the general ODS column required a long time to stabilize after the start of equilibration, and resolution also varied, as retention behavior differed depending on the substance.

Fig. 5 shows a comparison of the chromatographs at 5 h and 12 h after the start of equilibration for the Shim-pack Arata C18. RT of both the main peak and the impurity peaks coincided. Moreover, the Shim-pack Arata C18 not only displayed fast and stable column equilibration performance, but also maintained that performance for an extended time after equilibration.

The Shim-pack Arata C18 column enables stable analysis and does not affect the reliability of the HPLC impurity testing method.

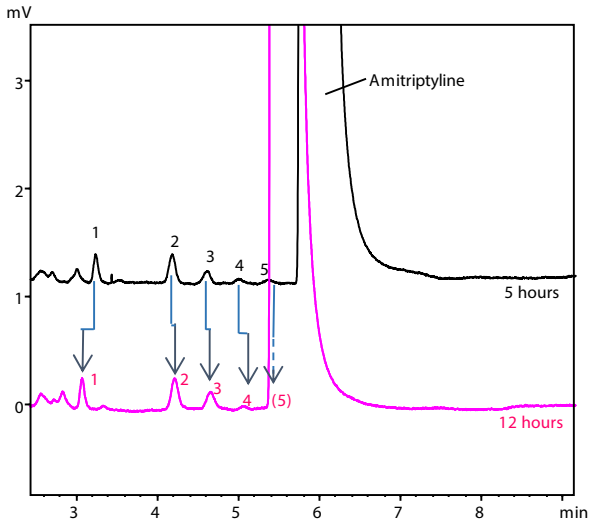

Fig. 4 Enlarged Chromatograms after Equilibration for 5 h and 12 h Using General ODS Column

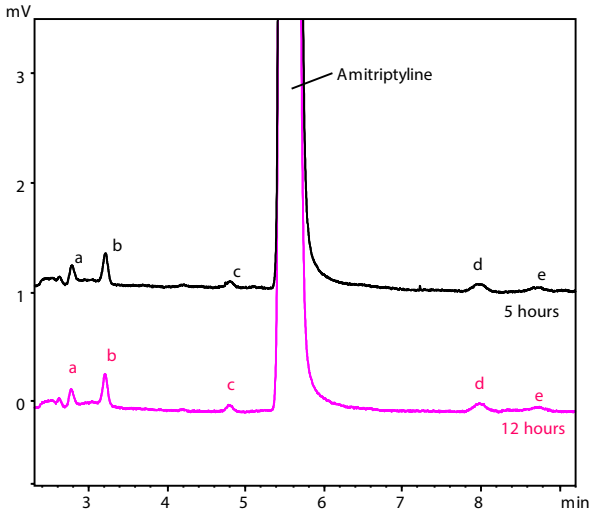

Fig. 5 Enlarged Chromatograms after Equilibration for 5 h and 12 h Using Shim-pack Arata C18

Conclusion

As introduced in this article, it was possible to analyze both basic compounds and acidic compounds with satisfactory peak shapes with a low ionic strength acidic mobile phase (0.1 % formic acid in water) by using the newly-developed Shim-pack Arata C18 column, and stable analysis was also possible in a short time. Highly reliable HPLC impurity testing in quality control of pharmaceuticals is possible by using the Shim-pack Arata C18 column.

* Shim-pack Arta C18 is classified as USP column category L1.

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