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Effect of Inertness on ODS column for HPLC or LC-MS/MS

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ODS columns are most commonly used for HPLC separation because a wide variety of compounds from hydrophobic to hydrophilic ones can be retained and separated by ODS columns. Consequently, a vast number of ODS columns are commercially available, but their characteristics vary with each column.

Among them, many HPLC users in recent years, particularly researchers in pharmaceutical companies, frequently prefer well end-capped and inert ODS columns rather than conventional ones. In this note, benefits of inert ODS columns are described.

Experimental

In the adsorption test for coffee sample, inner diameter, length and particle size of the used columns were 4.6 mm, 150 mm, and 5 μm, respectively. Loading of coffee sample was carried out by injecting 100 μL of coffee with an autosampler into an HPLC system where acetonitrile flows with 1.0 mL/min. Rinsing process for coffee-loaded columns was performed by flowing acetonitrile with 1.0 mL/min for 10 min.

In the adsorption test for basic and acidic compounds, inner diameter, length and particle size of the used columns were 2.1 mm, 150 mm, and 3 μm, respectively. Other conditions were specified in figure captions.

In the sensitivity test for oxine copper, length and particle size of the used columns were 2.1 mm, 150 mm, and 3 μm, respectively. Other conditions were specified in figure captions.

Results and Discussion

First, a simple example using coffee as a sample is shown. Coffee melanoidin is brown heterogeneous polymer present in coffee. Its components are not clarified yet, but it is thought to contain several ionic compounds.
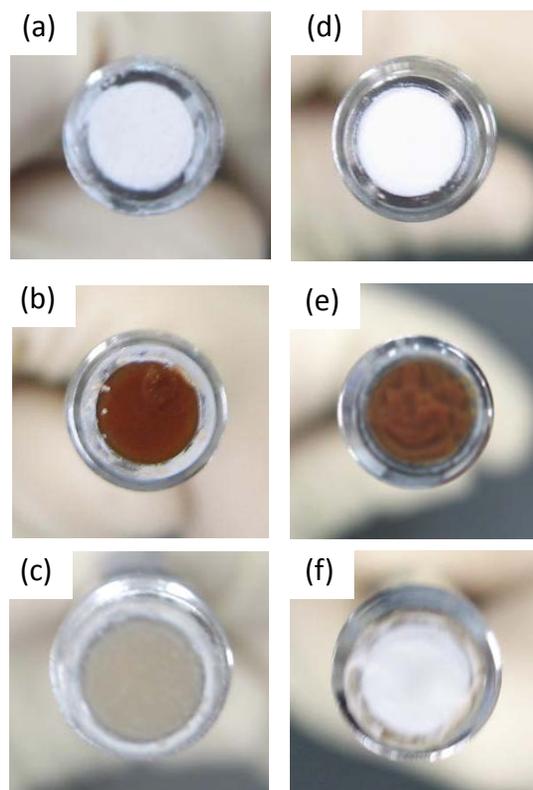

Figure 1: Picture of packed particle in column head taken by removing column joint.
(a) Conventional column A before loading coffee sample.
(b) Conventional column A just after loading coffee sample.
(c) Conventional column A after the rinsing process.
(d) InertSustain C18 before loading coffee sample.
(e) InertSustain C18 just after loading coffee sample.
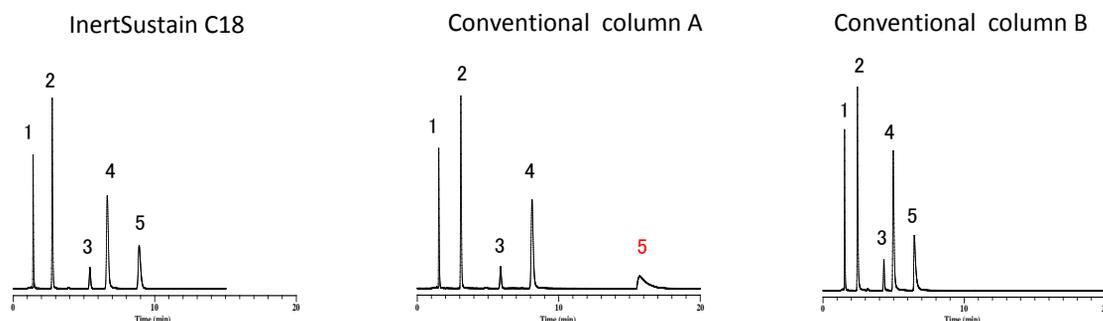(f) InertSustain C18 after the rinsing process.

Figure 2: Chromatograms of basic compounds obtained with three ODS columns. Peaks; 1. Urasil, 2. Pyridine, 3. Phenol, 4. Berberine chloride, 5. Dextromethorphan. Composition of mobile phase was acetonitrile:25 mM phosphate buffer （pH 7.0) = 30/70. Flow rate was 0.2 mL/min. Column temperature was set at 40°C. Detection was performed by UV absorvance with 230 nm.

InertSustain C18   Conventional column A   Conventional column B
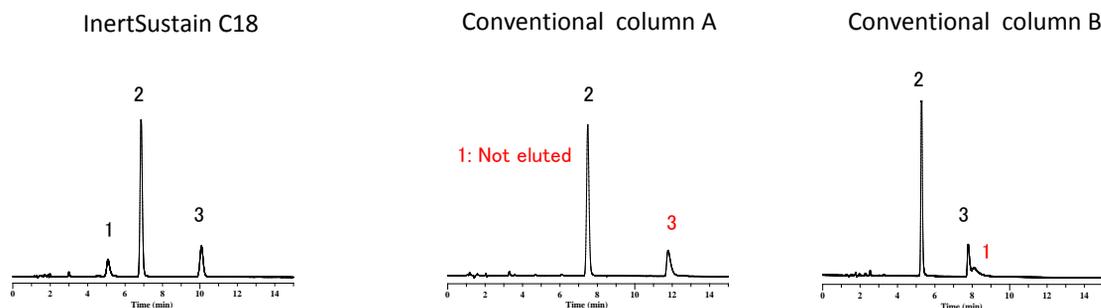
Figure 3: Chromatograms of acidic compounds obtained with three ODS columns. Peaks; 1. Brilliant Blue FCF, 2. Phenol, 3. Salicylic acid. Composition of mobile phase was acetonitrile:0.1 % phosphoric acid aqeous solution = 25/75. Flow rate was 0.2 mL/min. Column temperature was set at 40°C. Detection was performed by UV absorvance with 254 nm.

Adsorbed brownish compounds were observed on the surface of conventional ODS column even after rising process because its end-capping is not sufficient and non-specific adsorption occurred (Figure 1(c)). On the other hand, in case highly end-capped ODS column such as InertSustain C18, the brownish compounds could be removed from the column surface by the rinsing process InertSustain C18 (Figure 1(f)). From this result, it can be said that the more inert ODS column is, the longer it keeps good condition.

This phenomenon makes a significant effect also on chromatograms (Figure 2 and Figure 3). As for InertSustain C18, excellent peak shape was obtained for not only highly basic compounds containing tertily amino group such as dextromethorphan but also highly acidic compounds containing sulfonate group such as Brilliant Blue FCF. In case of conventional column B, a bad result was shown for acidic compounds although good peak shape was offered for basic compounds. Thus, selection of ODS column should be careful because peak shape diffes from one column to another even under the same condition.

If peak is not sharp, quantification, particularly in low concentration, becomes quite difficult. Figure 4 shows chromatograms of oxine copper obtained with two ODS columns coupled with the same LC/MS/MS system. Highly end-capped ODS column provides good peak shape and enables detection or quantitation at lower concentration rather than conventional ODS columns.

InertSustain C18   Conventional column C

Figure 4: Chromatograms of oxine copper obtained with two ODS columns. Mobile phase A is acetonitrile, and mobilephase B is 0.1 % formic acid aqeous solution. Linear gradient elution was carried out as follows; A 5% and B 95% at 0 min, A 100% and B 0% at 38 min. Flow rate was 0.2 mL/min. Oxine copper concentration of injected sample solution was 10 ng/mL, and injection volume was 5 uL. Column temperature was set at 40°C. Detection was performed with a 4000 Q Trap MS/MS (AB Sciex), and MRM transition of 145.7/74.8 was monitored.

Conclusion

As for ODS column, which is commonly used for HPLC and LC/MS/MS, its inertness has an influence not only on peak shape but also detection sensitivity and durability. It is recommended to use inert ODS column which provides good peak shape for both basic and acidic compo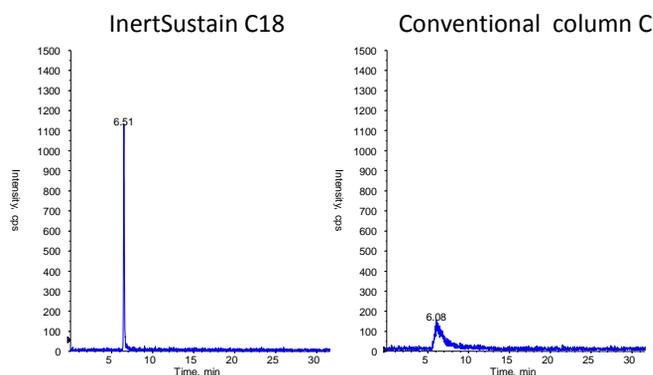unds such as InertSustain C18, as your first choice column.