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Excellence in Science

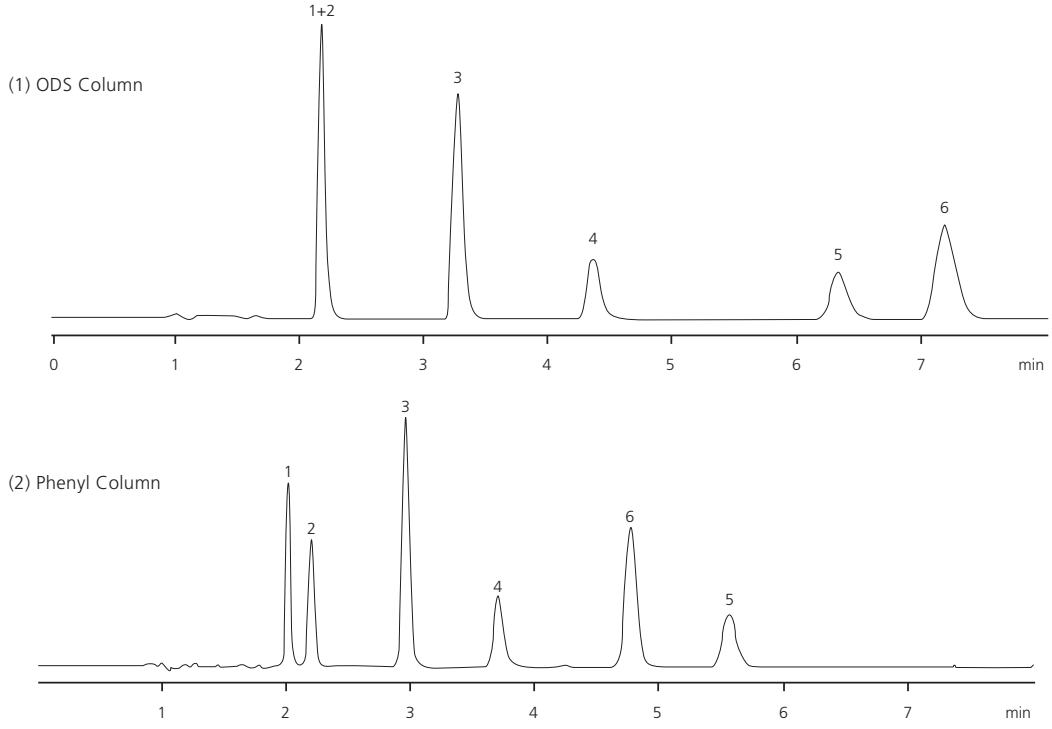
Technical Report

Using a Phenyl Column When Separation with C18 (ODS) Is Insufficient

1. C18 (ODS) Column

The C18 column is an ODS type of column that is marketed by instrument and column manufacturers, in which octadecyl groups are bonded to a silica base to provide wide separation applicability. An appropriate ODS column is typically the first type selected from the various types available at the start of a reverse-phase analysis. However, in the process of investigating the mobile phase conditions, there are cases in which the separation conditions cannot be improved. Selecting a different ODS column or exchanging it with a C8 column to improve separation is also possible, but even then, it may not be possible to achieve sufficient separation.

One possible solution in such a case is to use a phenyl column. Fig. 1 illustrates such a case in which switching to a phenyl column successfully separates peak 1 (indoprofen) from peak 2 (ethyl paraben), that were not sufficiently separated using an ODS column, without even changing the mobile phase conditions.



Column : (1) Luna C18(2), 150 mmL. × 4.6 mmI.D., 5 µm
(2) Luna Phenyl-Hexyl, 150 mmL. × 4.6 mmI.D., 5 µm
Mobile phase : 20 mM Potassium phosphate (pH 2.5) / Acetonitrile = 50 / 50 (v/v)
Sample : 1. Indoprofen
2. Ethyl paraben
3. Naproxen
4. Diflunisal
5. Indomethacin
6. Ibuprofen

Fig. 1 Difference in Separation Patterns Between ODS Column and Phenyl Column (1)

2. Basic Structure and Characteristics of the Phenyl Column

Separation with the ODS column is achieved by hydrophobic interaction enabled by the C18 alkyl chain, as shown in Fig. 3. Since the degree of the hydrophobicity is dependent on the amount of carbon, retention will probably be weak if the alkyl chain is short, as shown in the C8 column of Fig. 4.

On the other hand, separation using the phenyl column is conducted via the π electron, which in this case utilizes the π-π interaction between the phenyl group π electron and the analyte's π electron.

Rather than the phenyl group being bound directly to the base material (silica) as the basic structure, an alkyl chain is interposed as a spacer.

Introduced below are a standard phenyl column, which utilizes a C3 chain as the spacer, and other phenyl columns containing unique chemical bonds

(1) Phenyl Propyl Group

Fig. 5 (1) shows the structure of a standard phenyl column in which the propyl group (C3 chain) acts as the spacer. It features high selectivity of aromatics due to the π-π interactivity. One advantage of this is the ability to replace the ODS column with the phenyl column to obtain improved separation without changing the mobile phase (see Fig. 2). In addition, in this example, the elution order of peak 1 (butyl benzyl phthalate) and peak 2 (dibutyl phthalate) is reversed.

(2) Phenyl Hexyl Group

Fig. 5 (2) shows the structure of a phenyl column in which a hexyl group (C6 chain) serves as a spacer. The analysis shown in Fig. 1 (2) was conducted using this column. Since the π-π interaction effect is bolstered by the hydrophobic action of the C6 chain, it is effective in cases where retention is weak using the typical phenyl propyl column.

Further, we have introduced the Gemini C6-Phenyl column, a phenyl hexyl column in which the base silica particle surface is coated with Si-CH_2-Si (methylene cross-linked). Since the Si-CH_2 layer protects the base material to enhance the inactivity of the surface, the durability under alkali conditions is greatly improved even though it is a silica-based column (applicable pH range: 1–12). The data of Fig. 6 was obtained using a mobile phase at a pH of 10.5. The advantage of using the Gemini C6-Phenyl is the ability to conduct analysis of highly alkaline compounds containing a π electron using a high pH mobile phase to effectively suppress ionization.

(3) Pentafluorophenyl (PFP) Group

As illustrated in Fig. 5 (3), by introducing fluorine, π-π interaction and hydrophobic interaction, as well as other interactions such as dipole-dipole interaction and hydrogen bonding are induced to display retention behavior unlike other solid phases, permitting separation due to slight differences in physical properties.

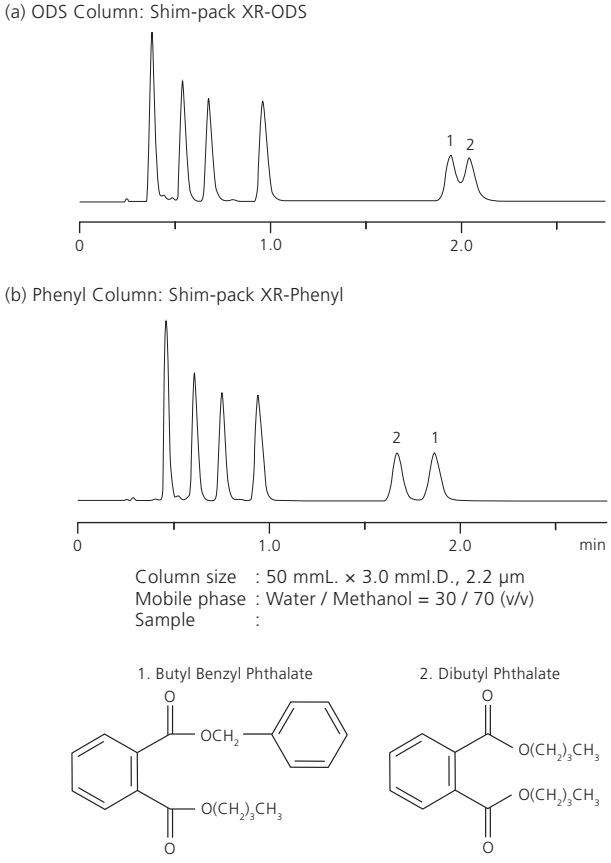


Fig. 2 Difference in Separation Patterns Between ODS Column and Phenyl Column (2)

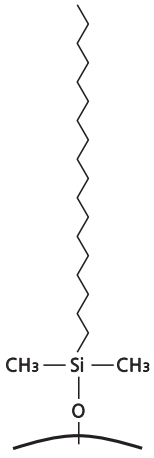


Fig. 3 C18 (ODS) Column

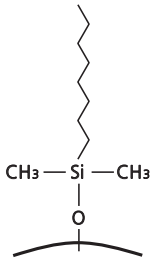
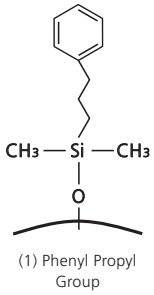
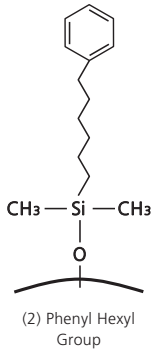


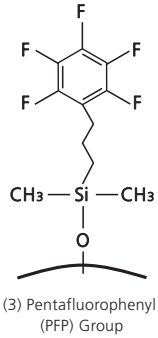
Fig. 4 C8 Column



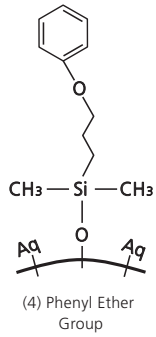
(1) Phenyl Propyl Group



(2) Phenyl Hexyl Group



(3) Pentafluorophenyl (PFP) Group



(4) Phenyl Ether Group

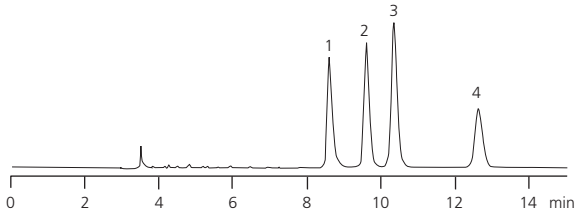
Fig. 5 Phenyl Columns

One feature of this column is its high selectivity with respect to isomers. It is effective for separation of positional isomers which is difficult with the C18 column. Fig. 7 shows an example in which separation is achieved using the simple mobile phase conditions of water/methanol = 50/50.

(4) Phenyl Ether Group

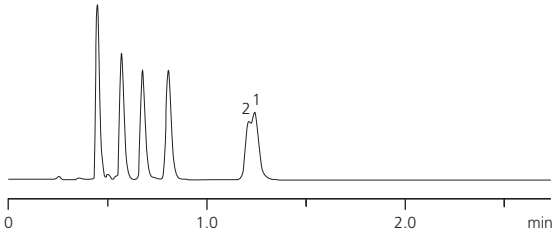
As shown in Fig. 5 (4), this is a column where phenyl ether is adopted. The aromaticity and π-π interaction are further strengthened due to the ether bond.

Despite the difficulty in achieving retention of high-polarity compounds in reverse-phase analysis, an ion pair reagent, for example, can be used as a remedial measure. In contrast to this, the Synergi Polar-RP column of Fig. 5 (4) exhibits strong retention with respect to polarized compounds due to endcapping of the polar functional group, so resolution of this problem can be expected without requiring any great change in the mobile phase conditions (*see* Fig. 9). And, if use of an ion pair reagent can be avoided, it can also be used in LCMS analysis, making this column applicable over a wide analysis range.



Column : Gemini C6-Phenyl 150 mmL. × 4.6 mmI.D., 5 µm
Mobile phase : 10 mM Ammonium Bicarbonate (pH 10.5) / Acetonitrile / Methanol = 30 / 35 / 35 (v/v/v)
Sample : 1. Imipramine (pKa 9.5)
2. Nortriptyline (pKa 9.7)
3. Amitriptyline (pKa 9.4)
4. Clomipramine (pKa 9.5)

Fig. 6 Reverse-Phase Analysis that Effectively Uses Mobile Phase pH (pH 10.5)



Column : Shim-pack XR-Phenyl 50 mmL. × 3.0 mmI.D., 2.2 µm
Mobile phase : Water / Acetonitrile = 40 / 60 (v/v)
Sample : 1. Butyl Benzyl Phthalate
2. Dibutyl Phthalate

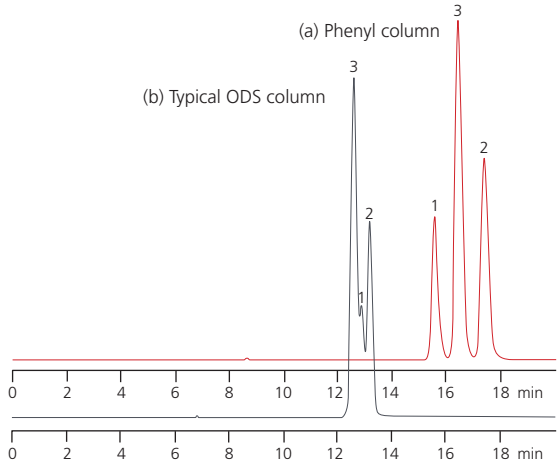
Fig. 8 Peak Behavior Due to Acetonitrile Mobile Phase

3. Regarding Phenyl Columns

The point when selecting a phenyl column is that rather than using acetonitrile as the mobile phase, methanol is used. This is because acetonitrile possesses a π electron (triple bond), which would weaken the π electron possessed by the phenyl column.

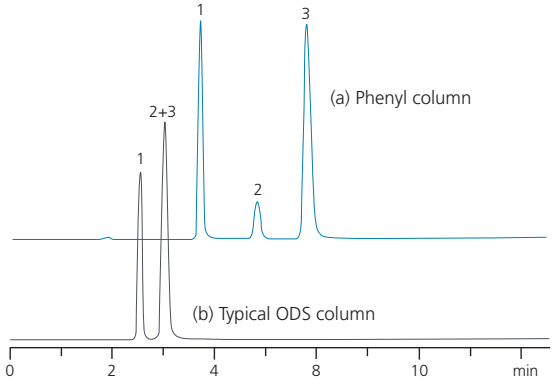
This is illustrated by the type of separation that can be expected with methanol as the mobile phase, shown in Fig. 2 (b), as compared with that using acetonitrile, shown in the analysis example of Fig. 8.

However, care is required due to the increased pressure during solvent delivery after switching to methanol as the mobile phase.



Column : (a) Luna PFP(2), 150 mmL. × 4.6 mmI.D., 3 µm
(b) ODS column, 150 mmL. × 4.6 mmI.D., 3 µm
Mobile phase : Water / Methanol = 50 / 50 (v/v)
Sample : 1. *o*-Methylacetophenone
2. *m*-Methylacetophenone
3. *p*-Methylacetophenone

Fig. 7 Improved Separation with Luna PFP (2)



Column : (a) Synergi Polar-RP, 150 mmL. × 4.6 mmI.D., 4 µm
(b) ODS column, 150 mmL. × 4.6 mmI.D., 3 µm
Mobile phase : 20 mM Potassuim phosphate (pH 7.0) / Acetonitrile = 80 / 20 (v/v)
Sample : 1. Famotidine
2. Cimetidine
3. Ranitidine

Fig. 9 Separation Improvement with Synergi Polar-RP

4. Lineup of Phenyl Columns

The typical phenyl columns used in Fig. 5 (1)–(4) are listed in Table 1. When changing from an ODS column, if the column to be selected will be of the same size (length, inside diameter), the effect of the phenyl column can be clearly predicted.

In the case of ultra-high-speed analysis, since columns for ultra-high-speed analysis are also available, as shown in Table 1, higher-speed analysis is possible just as in the case of ODS columns.

Fractionating columns are also available. Please contact the appropriate representative as listed below.

Table 1 Typical Phenyl Columns

	Chemical Bond Group	Characteristics	Column Name	Particle Size (µm)	Pressure Resistance (MPa)	pH Range	Application Field	
							High Speed Analysis	Conventional Analysis
(1)	Phenyl Propyl Group	- Standard phenyl column - High aromaticity	Shim-pack XR-Phenyl	2.2	35	2.0–7.5	◉	◉
			Shim-pack CLC-Phenyl	5.0	40	2.0–7.5	–	◉
(2)	Phenyl Hexyl Group	- Moderate hydrophobic retention - When retention is weak with C3 phenyl - High aromaticity	Luna Phenyl-Hexyl	3.0	25	1.5–10.0	○	◉
				5.0			–	◉
		- For developing method for wide pH range (1–12) - Superior durability under low pH and high pH conditions	Gemini C6-Phenyl	3.0	25	1.0–12.0	○	◉
				5.0			–	◉
(3)	Pentafluorophenyl (PFP) Group	- Strong polarity selectivity (especially with halogen compounds) - For analysis of fluorinated drugs and Taxanes - For separation of structural isomers not possible with ODS - Kinetex adopts Core-Shell. For ultra-high-speed analysis	Luna PFP (2)	3.0	25	1.5–8.0	○	◉
				5.0			–	◉
			Kinetex PFP	1.7	100	1.5–8.0	◉	◉
				2.6	60			
(4)	Phenyl Ether Group	- Can be used under 100 % aqueous mobile phase conditions - For simultaneous analysis of unchanged substances and metabolites	Synergi Polar-RP	2.5	40	1.5–7.0	◉	◉
				4.0	25		–	◉

◉: Best; ○: OK; – : Unsuitable

Fig. 10 Triple Quadrupole Ultra High Performance LC/MS/MS System Nexera + LCMS-8030

Fig. 11 Single Quadrupole Ultra High Performance LC/MS System Nexera + LCMS-2020

(Note) Kinetex, Luna, Synergi, and Gemini are trademarks or registered trademarks of Phenomenex Inc. in the United States and other countries.

(Note) Reference materials
- Shimadzu Application News No. L402 High Speed, High Resolution Analysis (Part 32) Analysis of Phthalates Using Shim-pack XR Series Columns

(Note) Regarding columns that support high pH, be sure to confirm the applicable pH range of the LC system.

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