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Technical Report

Enhancing Selectivity in Reversed-Phase Analysis Through Effective Utilization of Mobile Phase pH

CoreFocus

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Abstract:

When analyzing ionizable compounds, the pH of the mobile phase plays a crucial role in improving peak shape and separation. However, conventional silica-based columns have limitations in utilizing high-pH mobile phases. Additionally, while polymer-based columns can operate across a wide pH range, they have drawbacks such as lower mechanical strength and column efficiency. In this technical report, we present examples of measurements conducted using mobile phases with various pH levels, employing the Shim-pack Scepter and Shim-pack NovaCore columns—organic silica hybrid columns that combine the advantages of silica-based columns with exceptional high-pH durability.

Keywords: Shim-pack™ series, Shim-pack Scepter™ series, Shim-pack NovaCore series, reversed phase chromatography, C18, ODS, high pH durability

1. Focusing on Mobile Phase pH May Lead to Improved Selectivity

In reversed-phase analysis, it is important to consider the ionization of compounds to improve peak shape and separation. This is because phenomena such as those described in (1) to (3) below, caused by ionized compounds, can be factors that negatively impact the chromatograms.

(1) Ionized compounds have higher polarity compared to their non-ionized state, resulting in weaker retention.
(2) When ionized and unionized states coexist, the peak shape becomes broad and asymmetric because of differences in hydrophobic interactions.
(3) Ionized compounds are more likely to interact with residual silanol groups on the packing material, leading to poor peak shape and decreased reproducibility.

Ionizable compounds, especially weakly acidic or basic ones, have varying ionization ratios depending on pH of the mobile phase (see Figure 1). Therefore, adjusting the pH is crucial for avoiding the conditions described in (1) to (3) and for improving peak shape and separation.

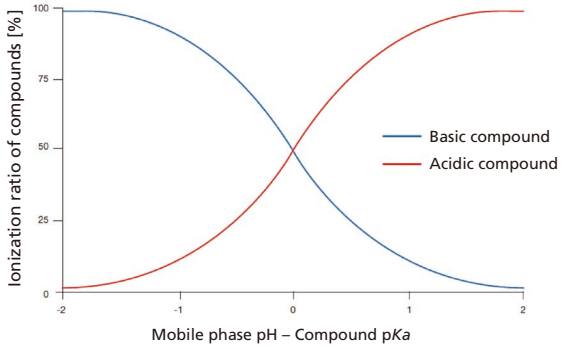


Figure 1: Relationship Between Ionization and Mobile Phase pH

2. The Relationship Between Compound Ionization and Mobile Phase pH

Figure 1 shows the relationship between the p*Ka* of the compound and the pH of the mobile phase. The horizontal axis represents the value obtained by subtracting the p*Ka* of the compound from the pH of the mobile phase.

In general, the ionization ratio of weakly acidic compounds is known to become 1 % (nearly neutral) when the pH is 2 below their p*Ka*. Conversely, when the pH is 2 above the p*Ka*, the ionization ratio becomes 99 % (nearly ionized). On the other hand, weakly basic compounds are known to ionize 99 % (nearly ionized) when the pH is 2 below their p*Ka*. Conversely, when the pH is 2 above the p*Ka*, the ionization ratio becomes 1 % (nearly neutral).

3. Suppression of Ionization in Basic Compounds

In the case of basic compounds, the pH of the mobile phase must be high in order to suppress ionization. However, in general, traditional silica-based columns such as C18 with silica gel as the base particle may begin to disintegrate when the pH exceeds 7, so a high-pH mobile phase may not be usable. On the other hand, polymer-based particles have the advantage of being usable over a wide pH range from 1~14, but their mechanical strength and separation efficiency are lower than silica-based columns.

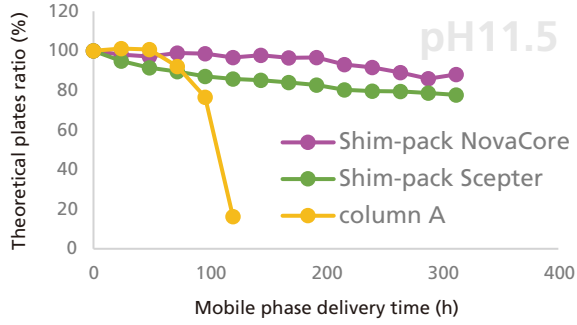
Therefore, it is ideal to have a column that combines the advantages of silica-based and polymer-based packing material. The Shim-pack Scepter and Shim-pack NovaCore meet these needs (see Table 1).

1 Analytical & Measuring Instruments Division

4. Organic Silica Hybrid Columns in the Shim-pack Series

The fully porous Shim-pack Scepter and the core-shell Shim-pack NovaCore columns use organic silica hybrid material for the base particles to maintain the high physical strength of traditional silica-based columns while significantly improving their durability under high pH conditions.

Figure 2 shows the change in column performance when a high-pH mobile phase is delivered for an extended period. This confirms the high pH durability of Shim-pack Scepter and Shim-pack NovaCore compared to column A, a traditional silica-based column.



Columns : Shim-pack NovaCore C18-HB (150 mm L. × 4.6 mm I.D., 5 µm)
Shim-pack Scepter C18-120 (150 mm L. × 4.6 mm I.D., 5 µm)
column A (150 mm L. × 4.6 mm I.D., 5 µm)

Mobile phase : 50 mmol/L
Triethylamine (pH11.5) / methanol=90/10 (v/v)

Figure 2: Changes in Theoretical Plates During Continuous Flow of High-pH Mobile Phase

5. The Behavior of Acidic, Neutral, and Basic Compounds Under Various pH Conditions

Let's look at the behavior of acidic, neutral, and basic compounds at various pH of mobile phase. Figure 3 shows the general advantages and disadvantages of three states based on the relationship between the p*Ka* of compounds and the pH of the mobile phase: p*Ka*>pH, p*Ka*=pH, and p*Ka*<pH.

Figure 4 shows the chromatograms of naproxen (acidic compound), butyrophenone (neutral compound), and amitriptyline (basic compound) with mobile phase conditions from pH2.5 to pH10.1 using Shim-pack Scepter. Butyrophenone has a constant retention time at all pH conditions, but Naproxen has a shorter retention time with increasing pH, and Amitriptyline has a longer retention time with increasing pH.

6. Application Examples Using Organic Silica Columns from the Shim-pack Series

Figure 5 shows chromatograms obtained using Shim-pack Scepter and Shim-pack NovaCore.

Example A analyzed basic compounds under acidic conditions, Example B analyzed acidic/neutral/basic compounds under acidic conditions, and Example C analyzed basic compounds under alkaline conditions. For a typical silica-based column, the peak may be generally broad under the condition of Example A. While for Example B, some compounds may have co-eluted or distorted peak shapes. For an alkaline mobile phase, such as Example C, the pH of a typical silica-based column may exceed the specifications and cannot be used. However, the Shim-pack Scepter and Shim-pack NovaCore showed good separation and peak shape under all conditions of Examples A, B, and C.

It is clear that Shim-pack Scepter and Shim-pack NovaCore can not only withstand a range of mobile phase pH conditions, but will also obtain excellent separation results under each condition.

7. Compatibility with LC-MS and LC-MS/MS

In reversed phase analysis with an MS detector, it is often necessary to include a buffer as part of the mobile phase to improve ionization efficiency. When selecting a suitable buffer solution for target compounds, several conditions must be considered. First, a buffer solution with a p*Ka* close to the desired pH should be selected. Next, ionic strength and ion pairing characteristics should be examined, while also considering compatibility with LC/MS and LC/MS/MS systems as needed. In Table 2, buffers marked with "●" are applicable to MS.

As shown in Figure 5, Shim-pack Scepter and Shim-pack NovaCore are suitable for LC/MS (LC/MS/MS) because they show excellent separation when the mobile phase contains highly volatile solvents such as formic acid.

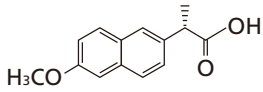
Table 1: Features of Silica-based, Polymer-based, Shim-pack Scepter, and Shim-pack NovaCore Columns

	Silica-based Columns	Polymer-based Columns	Shim-pack Scepter Shim-pack NovaCore
Mechanical Strength	☑	☐	☑
Column Efficiency	☑	☐	☑
Reproducibility	☑	☐	☑
pH Range	☐ (Generally, 2~7)	☑ (Generally, 1~14)	☑ (1~12)
Chemical Inertness	☐	☑	☑

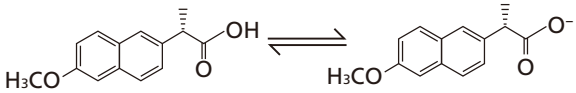
Acidic compound

Ex: Naproxen p*Ka* 4.5

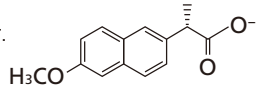
1. The p*Ka* of the compound > the pH of mobile phase
   -> The compound is not ionized.
   《Advantage》
   Retention becomes stronger.
   Peaks become sharper.
   Interactions caused by ions are suppressed.
   《Disadvantage》
   When the retention time is too long, peak shapes may become broad.



2. The p*Ka* of the compound = the pH of mobile phase
   -> Ionized compounds and non-ionized compounds coexist.
   《Advantage》
   None
   《Disadvantage》
   Peak shapes become broad and asymmetric.
   They are affected by interactions caused by ions.



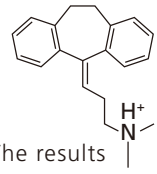
3. The p*Ka* of the compound < the pH of mobile phase
   -> The compound is ionized.
   《Advantage》
   Compared to ②, the peaks become sharper.
   《Disadvantage》
   Retention becomes weaker.
   In MS analysis, it may overlap with ion suppression. The results are significantly affected by interactions caused by ions.



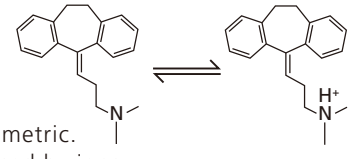
Basic compound

Ex: Amitriptyline p*Ka* 9.4

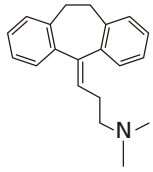
1. The p*Ka* of the compound > the pH of mobile phase
   -> The compound is ionized.
   《Advantage》
   Compared to ②, the peaks become sharper.
   《Disadvantage》
   Retention becomes weaker.
   In MS analysis, it may overlap with ion suppression. The results are significantly affected by interactions caused by ions.



2. The p*Ka* of the compound = the pH of mobile phase
   -> Ionized compounds and non-ionized compounds coexist.
   《Advantage》
   None
   《Disadvantage》
   Peak shapes become broad and asymmetric.
   They are affected by interactions caused by ions.



3. The p*Ka* of the compound < the pH of mobile phase
   -> The compound is not ionized.
   《Advantage》
   Retention becomes stronger.
   Peaks become sharper.
   Interactions caused by ions are suppressed.
   《Disadvantage》
   When the retention time is very long, peak shapes may become broad.



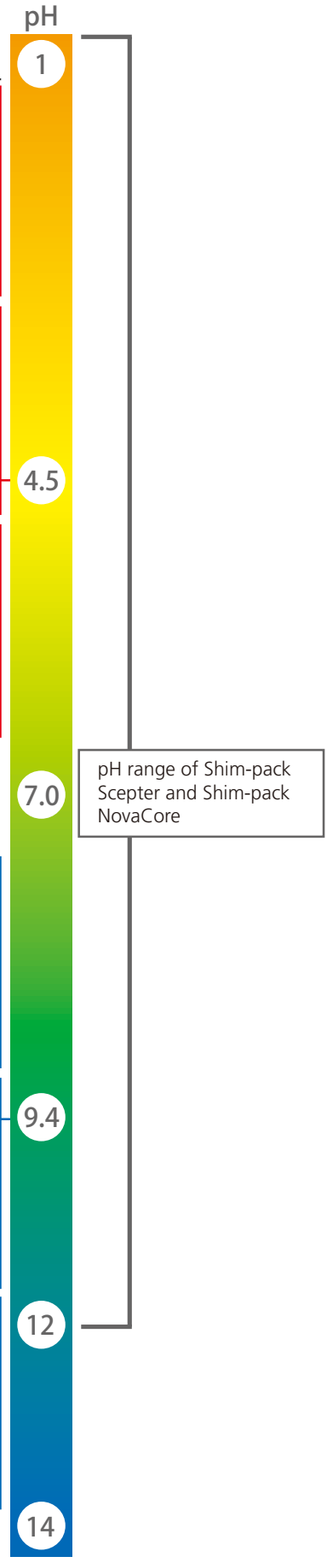
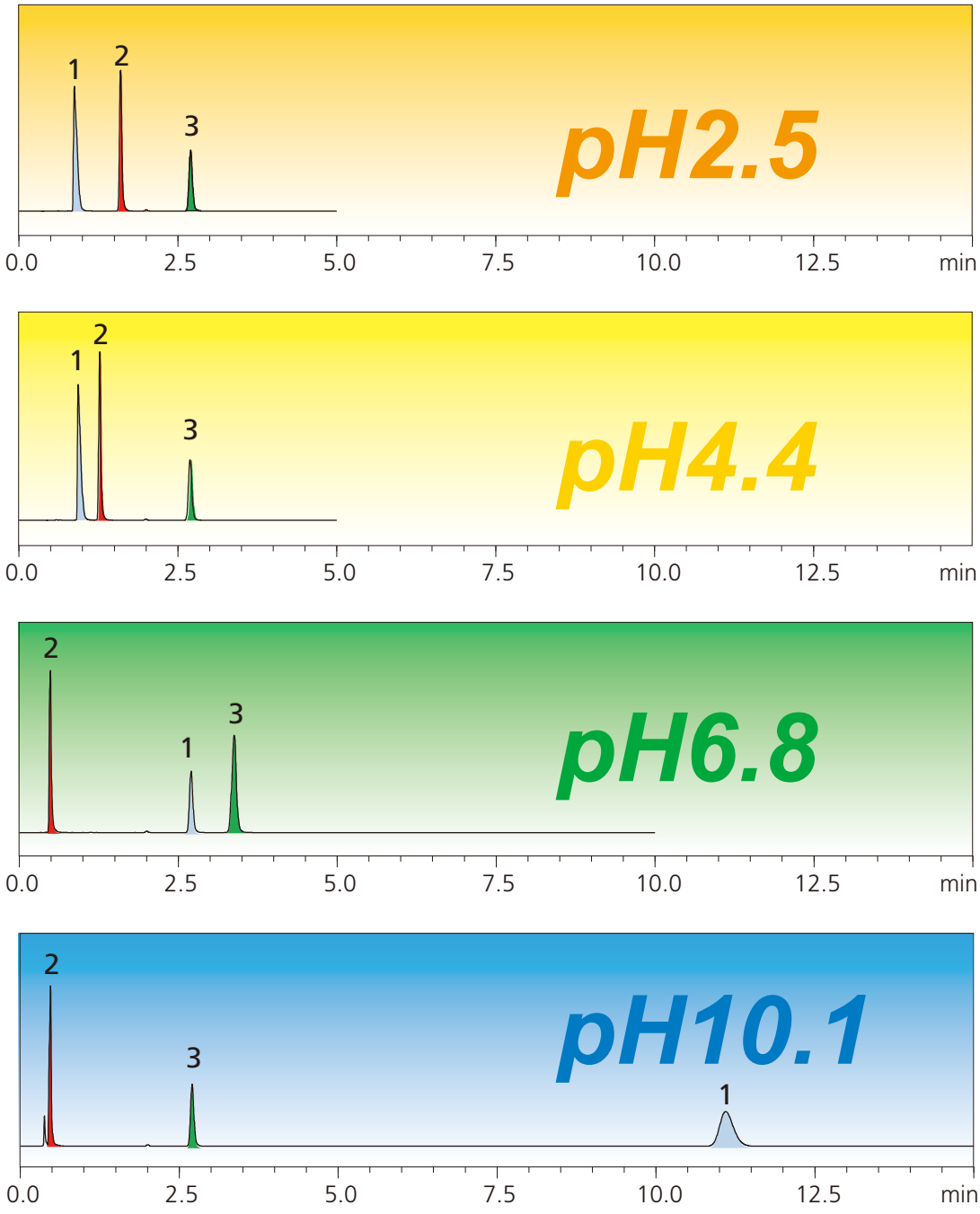


Figure 3: Mobile Phase pH and Selectivity of Acidic/Basic Compounds



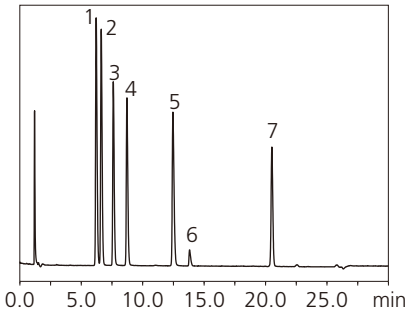
1: Amitriptyline (basic compound), 2: Naproxen (acidic compound) , 3: Butyrophenone (neutral compound)

Conditions

Column	: Shim-pack Scepter C18-120 (100 mm L. × 2.1 mm I.D., 3 µm)
Column temp.	: 40 ˚C
Mobile phase	: A1: 20 mmol/L phosphate (Na) buffer; pH2.5 A2: 20 mmol/L Acetate (Na) buffer; pH4.4 A3: 20 mmol/L phosphate (Na) buffer; pH6.7 A4: 20 mmol/L carbonate (NH4) buffer; pH10.1 B: Acetonitrile A/B = 50/50 (v/v)
Flow rate	: 0.5 mL/min
Detection	: UV 254 nm

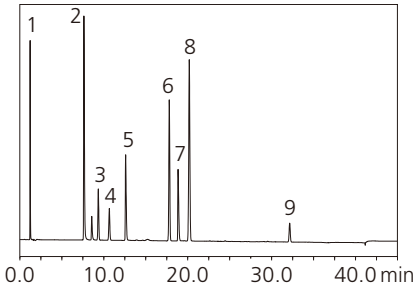
Figure 4: Analysis Examples of Acidic Compound (Naproxen), Neutral Compound (Butyrophenone), and Basic Compound (Amitriptyline) at Various pH Levels

A. Analysis of Basic Compounds Under Acidic Conditions



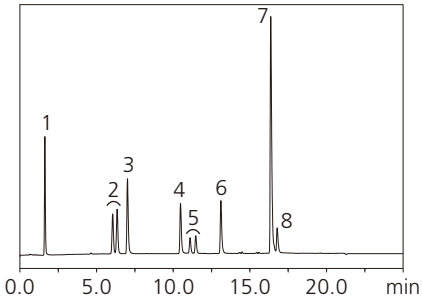
Column : Shim-pack Scepter C18-120 (150 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase : A ; 0.1 % Formic Acid in Water
B ; 0.1 % Formic Acid in Acetonitrile
Time Program : B Conc. 15 % (0 min) → 45 % (25 min) → 15 % (25 ~ 30 min)
Flow Rate : 1.5 mL/min
Column Temp. : Ambient
Detection : UV 254 nm
Sample : 1. Tripelennamine
2. Pyrilamine
3. Chlorpheniramine
4. Brompheniramine
5. Chloropyramine
6. Diphenhydramine
7. Loratadine

B. Analysis of Acidic, Neutral, and Basic Compounds Under Acidic Conditions



Column : Shim-pack Scepter C18-120 (150 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase : A ; 0.1 % Formic Acid in Water
B ; 0.1 % Formic Acid in Acetonitrile
Time Program : B Conc. 5 % (0 min) → 80 % (40 min) → 5 % (40 ~ 45 min)
Flow Rate : 1.5 mL/min
Column Temp. : Ambient
Detection : UV 254 nm
Sample : 1. 4-Pyridinecarboxylic acid
2. Quinidine
3. Benzyl alcohol
4. Phenol
5. Triprolidine
6. 3-Methyl-4-nitrobenzoic acid
7. Nortriptyline
8. 5-Methylsalicylaldehyde
9. Hexanophenone

C. Analysis of Basic Compounds Under Alkaline Conditions



Column : Shim-pack NovaCore C18-HB (150 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase : A ; 10 mmol/L Ammonium Bicarbonate pH10.41
B ; Methanol
Time Program : B Conc. 15 % (0 min) → 80 % (20 min) → 15 % (20 ~ 25 min)
Flow Rate : 1.5 mL/min
Column Temp. : Ambient
Detection : UV 230 nm
Sample : 1. Sotalol
2. Labetalol (Diastereoisomeric Pair)
3. Atenolol
4. Pindolol
5. Nadolol (Diastereoisomeric Pair)
6. Metoprolol
7. Propranolol
8. Alprenolol

Figure 5: Application Examples Using Shim-pack Scepter and Shim-pack NovaCore

Table 2: p*Ka*, pH, and Compatibility with Mass Spectrometry for Each Buffer [1, 2]

Buffer	p*Ka*	Buffer Range (pH)	MS Compatible
Trifluoracetic acid	< 2*	< 2.5	●
Phosphoric acid (pK_1)	2.1	1.1 – 3.1	
Formic acid	3.8	2.8 – 4.8	●
Acetic acid	4.8	3.8 – 5.8	●
Carbonate (pK_1)	6.4	5.4 – 7.4	●
Phosphate (pK_2)	7.2	6.2 – 8.2	
Triethanolamine	7.8	6.8 – 8.8	●
Diethanolamine	8.9	7.9 – 9.9	●
Ammonia	9.2	8.2 – 10.2	●
Ethanolamine	9.5	8.5 – 10.5	●
Carbonate (pK_2)	10.3	9.3 – 11.3	●
Diethylamine	10.5	9.5 – 11.5	●
Triethylamine	11.0	10.0 – 12.0	●
Phosphate (pK_3)	12.3	11.3 – 13.3	

* TFA can be used at low concentrations for LC/MS applications, but it may affect MS sensitivity.

8. Shim-pack Scepter: Recommended as a First Choice

The Shim-pack Scepter lineup offers a wide variety of column chemistries with different separation selectivities, including seven reversed-phase types and one HILIC type. This makes it highly effective for method development and scouting, allowing users to select the most suitable column for each application (see Figure 6).

Additionally, the broad range of particle sizes and column dimensions enables seamless method transfer between UHPLC, analytical HPLC, and preparative HPLC. Furthermore, the lineup includes two types of column bodies designed to minimize adsorption during the analysis of biopharmaceuticals and other compounds, allowing users to choose the appropriate column body based on their analytical conditions (see Figure 7).

Because the Shim-pack Scepter series can be used in such a wide range of applications, we recommend the Shim-pack Scepter series as first-choice columns for method development.

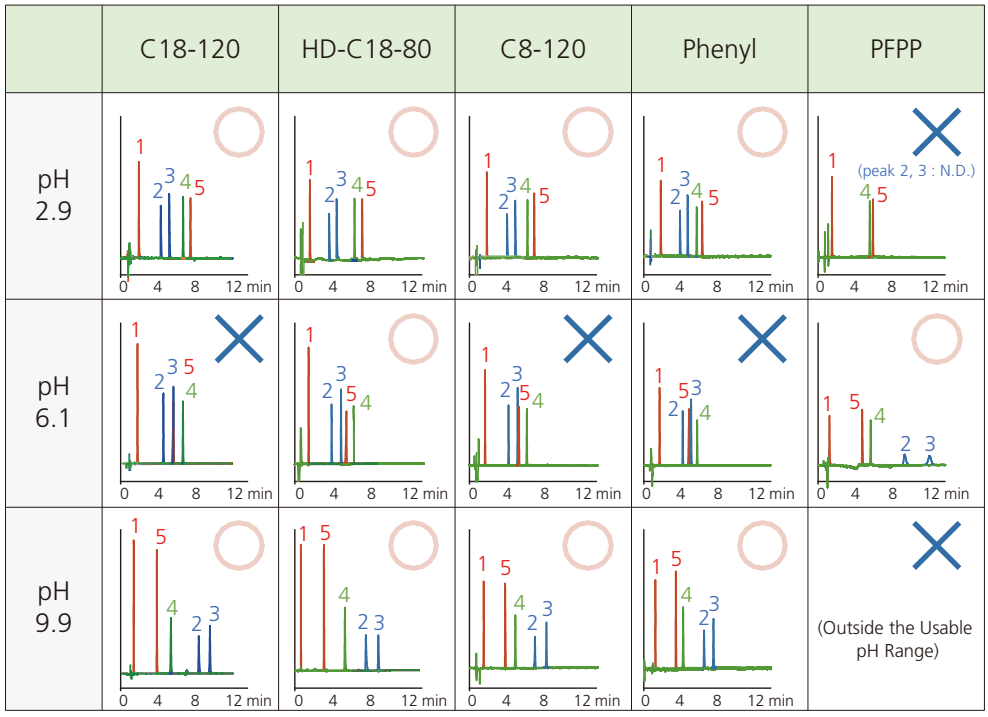


Figure 6: Comparison of Analysis Results Under Gradient Conditions Using Acetonitrile as the Organic Solvent

Table 3: Shim-pack Scepter Series Overview

Shim-pack Scepter	Reversed phase							HILIC
	C18-120	C18-300	HD-C18	C8-120	C4-300	Phenyl	PEPP	Diol-HILIC
Functional Group	Trifunctional bond C18			Trifunctional bond C8	Trifunctional bond C4	Trifunctional bond Phenylbutyl	Trifunctional bond Pentafluorophenylpropyl	Trifunctional bond Dihydroxypropyl
	General-purpose type		High functional group density type					
Base Material	Organic silica hybrid							
Particle Size	1.9 µm, 3 µm, 5 µm							
Pore Size	12 nm	30 nm	8 nm	12 nm	30 nm	12 nm		
Endcapping	Proprietary						None	
Operating pH Range	1-12				1-10		1-8	2-10
100 % aqueous compatible	○	○	×	×	○	○	○	-
USP Category	L1	L1	L1	L7	L26	L11	L43	L20

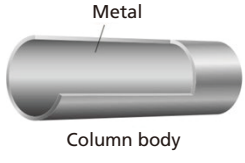
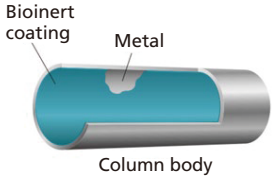
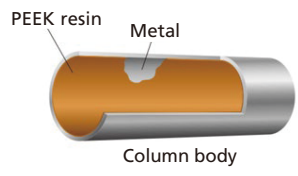
	Shim-pack Scepter	Shim-pack Scepter Claris	Shim-pack Scepter [metal-free]
			
Body Wetted Materials	Stainless steel	Bioinert coating	PEEK
Frit Wetted Materials	Stainless steel	Bioinert coating	PEEK

Figure 7: Column Bodies in the Shim-pack Scepter Series

9. Shim-pack NovaCore: Ideal for High-Speed and High-Resolution Analysis

Shim-pack NovaCore is a core-shell column designed with a non-porous core and porous layers on the exterior, making it ideal for high-speed and high-resolution separations. In core-shell columns, analytes diffuse only into the thin porous outer layer, enabling efficient separation. Compared to fully porous columns, where analytes penetrate deep into the interior of the packing material, Shim-pack NovaCore achieves sharper peaks in a shorter amount of time (see Figure 8).

Figure 9 shows a comparison between Shim-pack NovaCore and a competitor's fully porous column with the same particle size. The results confirm that Shim-pack NovaCore offers reduced retention times. This shorter analysis time not only decreases mobile phase consumption but also contributes to cost savings and reduced environmental impact.

Moreover, Shim-pack NovaCore offers a wide range of column sizes and particle diameters, enabling rapid and efficient method transfer from general-purpose analysis to high-speed analysis (see Figure 10).

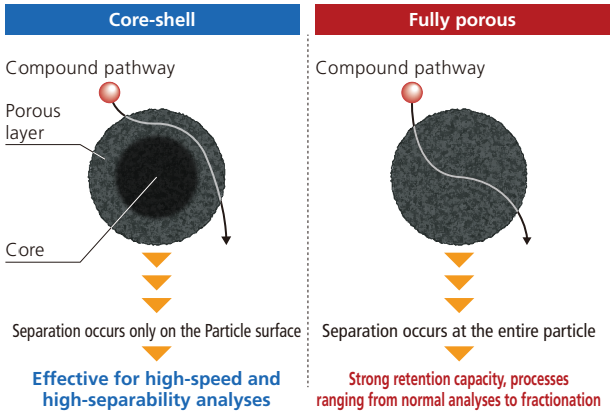
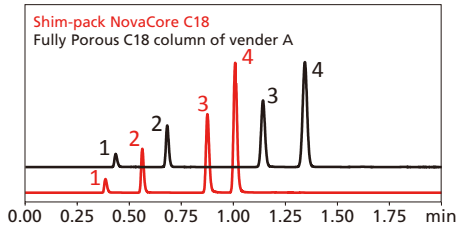
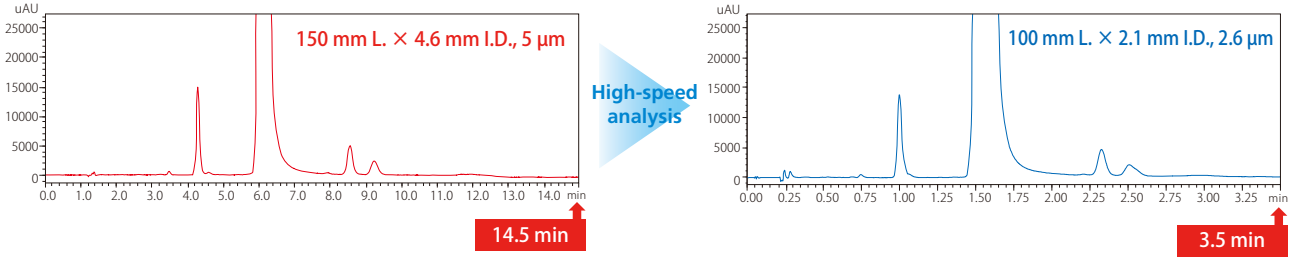


Figure 8: Diagram of Core-Shell and Fully Porous Characteristics



Column : Shim-pack NovaCore C18-HB (100 mm L. × 2.1 mm I.D., 1.7 µm)
column of vender A (100 mm L. × 2.1 mm I.D., 1.7 µm)
Mobile phase: Acetonitrile/Water=70/30 (v/v)

Figure 9: Comparison Between Shim-pack NovaCore (Core-Shell Column) and Competitor Column (Fully Porous C18 Column)



Column : Shim-pack NovaCore C18-HB (150 mm L. × 4.6 mm I.D., 5 µm)
Shim-pack NovaCore C18-HB (100 mm L. × 2.1 mm I.D., 2.6 µm)
Mobile phase : 25 mmol/L Phosphate (Na) buffer (pH2.5) / Acetonitrile = 65/35 (v/v)
Flow rate : 4.6 mm I.D. : 1.0 mL/min
2.1 mm I.D. : 0.8 mL/min

Figure 10: Examples of High-Speed Analysis Using Shim-pack NovaCore

Reference

1) Practical HPLC Method Development; L.R. Snyder, JJ Kirkland, and JL Glajch, Wiley Interscience, 1997
2) Introduction to Protein and Peptide HPLC; TP Bradshaw,Phenomenex, 1998

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