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Technical Report

Separation Characteristics of Shim-pack™ Series Phenyl/PFPP Reversed Phase Columns

CoreFocus

Tomoaki Shimpo[1], Tomoha Somano[1]

Abstract:

Reversed phase chromatography is the most commonly used HPLC separation technique for a wide range of applications. Hydrophobic interactions are the dominant separation mechanism in this mode. Due to the diversity of potentially applicable compounds, it is safe to say that a majority of HPLC analysis work throughout the world is performed using the reversed phase mode. Technical Report "Separation Characteristics of the Shim-pack Reversed Phase Column Series—Reversed Phase Columns C4/C8/C18" (C190-E305) described the separation characteristics of columns modified mainly with C18 carbon chains, which is typically the first choice when selecting a reversed phase column. However, this article describes the separation characteristics of columns modified with phenyl or PFPP groups, which have alternate selectivity compared to alkyl phases, and are often considered the second or third choice for reversed phase columns. We also includes suggestions for deciding which of the Shim-pack series columns to use for different applications.

Keywords: Shim-pack Series, reversed phase chromatography, C18, ODS, C8, C4, Phenyl, Phenyl-Hexyl, Biphenyl, PFPP (PFP, F5), Pentafluorophenylpropyl, Tanaka Test

1. Introduction
Columns Used for Reversed Phase Mode

As mentioned in Technical Report "Separation Characteristics of the Shim-pack Reversed Phase Column Series—Reversed Phase Columns C4/C8/C18" (C190-E305), Section "1-1. Reversed Phase Mode," reversed phase mode is the most commonly used HPLC separation method. Columns used for reversed phase mode and referred to as "reversed phase columns" are packed with particles having a base material such as a polymer, silica, or organic silica hybrid, that is modified with various carbon length alkyl chains or other functional groups.

Typical ligands bonded to the column base material are as follows:

- C30
- C18
- C8
- C4
- C1
- Phenyl
- PFPP (Pentafluorophenylpropyl)

etc.

C18 columns are the most common type of reversed phase column. The base material is chemically modified with an octadecylsilyl (ODS) group with 18 carbons (C) in the chain, so they are also called ODS columns. A C18 column is a good starting phase for method development, but if inadequate separation is achieved using a C18 column, then an alternate phase is used to improve the results.

The phenyl and PFPP columns discussed in this article are often considered as a second or third choice reversed phase column, and can be useful in cases where it is difficult to achieve adequate separation using a C18 column or when a major change from the separation results achieved with a C18 column is desired. Figure 1 shows the differences in separation behavior using reversed phase columns with different functional groups, and Figure 2 shows the structures of the compounds used in the analysis. As seen in Figure 1, a change in selectivity occurs with a different functional group of the reversed phase column. In comparing C4, C8, and C18 phases that differ in the length of their carbon chains, it is evident that the shorter the carbon chain the shorter the retention time for hydrophobic compounds. On the other hand, chromatograms obtained with phenyl and PFPP columns can differ significantly from chromatograms obtained with a C18 column, especially with changes in compound elution order.

Because phenyl and PFPP columns have different interactions than the more commonly used C18 columns, they can be helpful for achieving better resolution than with a C18 column. This article describes using the C18 column assessment method[1)] to uniformly assess phenyl and PFPP columns in addition to Shim-pack C18 columns. In particular, the article describes the differences in the separation selectivity and other characteristics of Shim-pack series reversed phase phenyl and PFPP columns and discusses when they should be used instead of a C18 column.

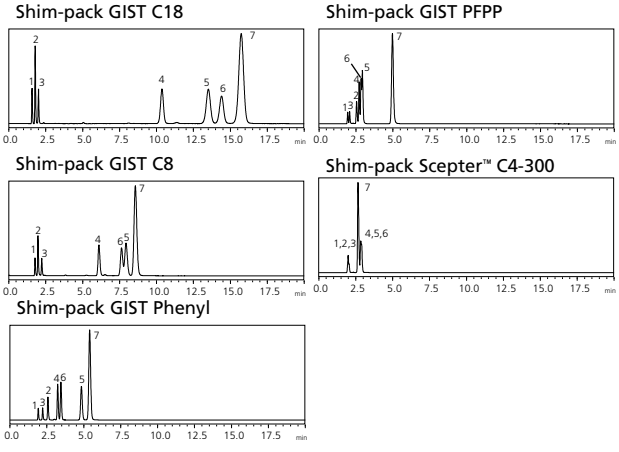


Figure 1: Examples of the Differences in Separation Behavior Due to the Functional Groups in Reversed Phase Columns

1 Analytical & Measuring Instruments Division

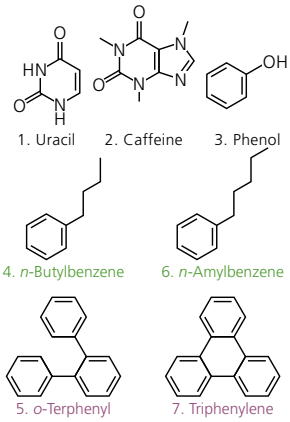


Figure 2: Structural Formulas for Compounds Used in Figure 1 Analysis (Same Analytical Conditions as Table 1)

2. Test Conditions and Evaluative Indices

The test conditions were implemented with reference to the comparative evaluation method for reversed phase C18 columns known as the Tanaka Test method, which is shown in Table 1.[1)]

Table 1: Analysis Conditions

System	: Nexera™ X2
Column	: 150 mm × 4.6 mm I.D., 5 µm
Mobile Phase	: (1) Water : Methanol = 20 : 80
	(2) Water : Methanol = 70 : 30
Flow Rate	: 1.0 mL/min
Column Temp.	: 40 ˚C
Injection Vol.	: 1 µL
Detection	: 254 nm
Sample	: 1. Uracil
	2. Caffeine
	3. Phenol
	4. *n*-Butylbenzene
	5. *O*-Terphenyl
	6. *n*-Amylbenzene
	7. Triphenylene
Vial	: TORAST™ Vial * (Shimadzu GLC)

* P/N: GLCTV-801 (vial) + GLCTV-803 (cap)

As described in the previous article (C190-E305), the Tanaka Test Method involves calculating four attributes for each column. The basic characteristics of the respective attributes are shown in Table 2. Note that the retention index *k* for each compound was calculated from the retention time *t* for each compound with the retention time for Uracil used as *t0*.

Table 2: Explanation of the Attributes in the Tanaka Test Method

Attribute	Index	Mobile Phase Conditions	Explanation
Hydrophobic Retention Capacity	*k* 6.Amylbenzene	Mobile phase (1) (Refer to Table 1.)	Indicates the strength of the retention capacity for hydrophobic compounds
Responsiveness to Hydrophobic Differences	*k* 6.Amylbenzene / *k* 4.Butylbenzene	Mobile phase (1) (Refer to Table 1.)	Indicates the level of the capacity to recognize differences in hydrophobicity between compounds
Responsiveness to Structure	*k* 7.Triphenylene / *k* 5.*o*-Terphenyl	Mobile phase (1) (Refer to Table 1.)	Indicates the level of the capacity to recognize differences between planar structures and three-dimensional structures
Hydrogen Bonding Capacity	*k* 2.Caffeine / *k* 3.Phenol	Mobile phase (2) (Refer to Table 1.)	Indicates the level of hydrogen bonding capacity

A general explanation and a list of cautions regarding these attributes are shown below. In terms of cautions, the analysis conditions in Table 1, which made use of the Tanaka Test Method as a reference, are generally used in evaluations of C18 columns. Also, each attribute indicates the general features of the various columns. The strength of the interaction between compounds and columns varies according to compounds, mobile phase conditions, and other analysis conditions. This means that these features are not expressed in the same way, regardless of the analysis.

■ Hydrophobic Retention Capacity:

This indicates the strength of the retention capacity for hydrophobic compounds, specifically for low molecular weight compounds under water and methanol conditions. In general, the strength of the retention capacity for hydrophobic compounds is proportional to the number of carbons in the column and the specific surface area. However, if a mobile phase with π electrons such as acetonitrile is used, the interaction between the column and the compounds will change, and the retention capacity could change significantly depending on the types of column and compound. For more details about retention difference depending on organic solvents, refer to section 4-2-2 in this article. Furthermore, PFPP columns can retain basic compounds more strongly than C18 columns in some cases and actual retention can be weaker for some types of compounds, even if they have a relatively high hydrophobic retention capacity compared to other columns. For more details about the PFPP column retention of basic compounds, refer to section 4-3 in this article.

■ Responsiveness to Hydrophobic Differences:

This indicates the level of the phase to recognize differences in hydrophobicity between compounds. Columns with a high responsiveness to hydrophobic differences demonstrate a difference in elution time between amyl benzene and butyl benzene. In particular, the level of recognition of hydrophobic differences between compounds in the low molecular weight region is shown under water and methanol conditions. The responsiveness to hydrophobic differences is related to factors such as the length of carbon chains and the quantity of functional groups.

■ Responsiveness to Structure:

This indicates the ability of the column to recognize differences between planar structures and three-dimensional structures. The greater the expression of the difference in elution time between *o*-terphenyl and triphenylene, the higher the responsiveness to structure. In general, this is related to the functional group bonding density and the functional group bonding treatment method. For example, in columns with a high density of octadecyl groups, triphenylene compounds with planar structures fit between carbon chains, resulting in stronger retention, but *o*-terphenyl compounds with bulky three-dimensional structures cannot fit easily between carbon chains, which results in comparatively weak retention.[2)]

■ Hydrogen Bonding Capacity:

This indicates the level of hydrogen bonding capacity. Reversed phase columns, which can have silica or organic silica hybrid base materials, tend to have a lower hydrogen bonding capacity (less interaction with analytes) compared to columns with fewer residual silanol groups. For this reason, the value tends to be higher for reversed phase columns without endcapping. Note that in this article, the value for hydrogen bonding capacity is not necessarily related to the strength of the retention capacity for hydrophilic compounds.

In terms of the characteristics of each column, spider charts based on these four attributes are presented starting on page 5 of this article. Note that these spider charts were calculated from more than 30 test results for reversed phase columns in the Shim-pack lineup.

One note of caution in the explanation of these spider charts is that the size of the area occupied in the spider chart does not necessarily indicate the level of performance by that column. For example, the larger the area occupied in the spider chart, the more likely it is for multiple interactions to have an impact on separation. As such, even if all the column attributes are high, this does not mean that favorable separation is achieved. On the contrary, predictions about separation behavior are difficult, and the desired degree of separation is not always obtainable.

The attributes and spider charts shown in this article are not characteristics that are necessarily demonstrated in all applications. They should instead be treated as general column characteristics based on their performance with the designated probe compounds, and used as a reference. Users are encouraged to evaluate multiple columns based on their specific analytes and method conditions.

3. Overview of Shim-pack Phenyl and PFPP Columns and Tanaka Test Results

This section provides an overview of Shim-pack phenyl and PFPP columns and describes Tanaka Test results. Shim-pack reversed phase column specifications are shown in Table 5.

3-1. Overview of Shim-pack Phenyl and PFPP Columns

Table 5 provides an overview of phenyl and PFPP columns available in the Shim-pack product line as compared to C18 columns.

3-1-1. Shim-pack Phenyl Columns

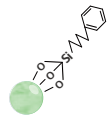
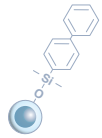
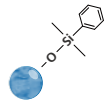
Phenyl columns are classified based on differences in the type of phenyl group(s) used in the stationary phase. Shim-pack phenyl columns are listed by type in Table 3.

The reason phenyl and C18 columns have different separation selectivity is said to be due to the π electrons in phenyl groups.[2] Consequently, phenyl columns that have π electrons exhibit π-π and multiple other interactions with compounds, which result in significantly different retention behavior than C18 columns.[2)-5)] This results in the following phenyl column characteristics compared to C18 columns:

- Useful for separating compounds with long conjugated systems
- Useful for separating aromatic compounds
- Useful for separating isomers

Among Shim-pack phenyl columns, Shim-pack Velox™ biphenyl columns exhibit particularly strong π-π interactions.

Table 3: Comparison of Shim-pack Reversed Phase Phenyl Columns

Illustration of Stationary Phase	 Example: Shim-pack Scepter Phenyl-120	 Example: Shim-pack Velox Biphenyl	 Example: Shim-pack GIST Phenyl
Type	Alkyl phenyl type	Biphenyl type	Direct phenyl type
Characteristics	Phenyl column with carbon chain	Column with biphenyl groups	Column with phenyl groups directly attached to column packing material
Strength of Hydrophobic Retention Capacity Compared to C18	Somewhat weaker	Somewhat weaker	Weaker
Strength of π-π Interactions Compared to C18	Somewhat stronger	Strongest	Stronger
Applicable Shim-pack Columns	• Shim-pack VP Phenyl (Phenyl-propyl) • Shim-pack Scepter Phenyl-120 (Phenyl-butyl) • Shim-pack GIST Phenyl-Hexyl (Phenyl-hexyl)	Shim-pack Velox Biphenyl	Shim-pack GIST Phenyl

3-1-2. Shim-pack PFPP Columns

The Shim-pack series includes three types of PFPP columns. All three types consist of a pentafluorophenylpropyl (PFPP) ligand bonded to either fully porous silica (GIST), superficially porous silica (Velox) or organic silica hybrid (Scepter) base material.

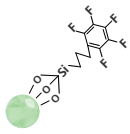
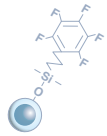
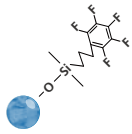
Shim-pack PFPP columns are shown in Table 4. The presence of fluorine atoms with high electronegativity in PFPP columns can skew the electric charge of the stationary phase[6)-7)]. PFPP columns can exhibit a wide variety of interactions, such as dipole-dipole interactions with compounds, dipole-ion interactions, phenyl group π-π interactions, and hydrogen bonding effects[6)-7)]. Therefore, the separation behavior of the PFPP type of reversed phase column stationary phase can be among the most difficult to understand. With a tendency to generate dipole moments, it can sometimes achieve good separation for isomers that are difficult to separate using a C18 column. Of the three types of Shim-pack PFPP columns offered, the Shim-pack GIST PFPP column is treated with endcapping, whereas the other two types do not include endcapping.

PFPP columns without endcapping exhibit stronger dipole-dipole and dipole-ion interactions. For example, the column type without endcapping tends to provide stronger retention of basic compounds and other cationic samples or positively polarized samples.[8)-11)]

PFPP columns offer the following characteristics compared to C18 columns:

- Increases retention of basic compounds that are difficult to retain using a C18 column.
- Useful for separating aromatic compounds
- Useful for separating halogen compounds
- Useful for separating isomers

Table 4: Shim-pack Reversed Phase PFPP Columns

Illustration of Stationary Phase			
Column Name	Shim-pack Scepter PFPP-120	Shim-pack Velox PFPP	Shim-pack GIST PFPP
Strength of Hydrophobic Retention Capacity Compared to C18	Weaker	Weaker	Weaker
Base Material	Fully porous organic silica hybrid	Supercially porous silica (core shell silica)	Fully porous silica
Endcapping	No	No	Yes

3-2. Tanaka Test Results Using Shim-pack Phenyl and PFPP Columns

This section describes the results of the Tanaka Test for Shim-pack reversed phase columns, and the features of each column. All column specifications are shown in Table 5.

Chromatograms for each column under mobile phase (1) conditions, and spider charts for their four attributes are shown in this article starting on page 5 (Figure 3-14). Overall, phenyl and PFPP columns contain less carbon than C18 columns, so they tend to have lower hydrophobic retention capacity and lower responsiveness to hydrophobic differences. However, despite having lower hydrophobicity than C18 columns, phenyl and PFPP columns can provide better retention or separation performance than C18 columns in some cases, depending on compound properties, due to the superior retention of compounds with long conjugate systems by phenyl columns or the superior retention of basic compounds by PFPP columns.

The other features of these columns that are not expressed by the spider charts are noted beside the spider charts for each column.

Table 5: List of Specifications for Shim-pack Reversed Phase Columns

Column Base Packing Material	Column Series Name	Functional Group	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Content (%)	Endcapping	Operating pH Range	100 % Aqueous Mobile Phase Compatible
Fully porous organic silica hybrid	Shim-pack Scepter	C18-120	1.9, 3, 5	120	360	20*	Yes	1-12	○
		C18-300	1.9, 3, 5	300	N.D.	N.D.	Yes	1-12	○
		HD-C18-80	1.9, 3, 5	80	430	25*	Yes	1-12	
		C8-120	1.9, 3, 5	120	360	17*	Yes	1-12	
		Phenyl-120	1.9, 3, 5	120	360	17*	Yes	1-10	○
		PFPP-120	1.9, 3, 5	120	360	15*	No	1-8	○
		C4-300	1.9, 3, 5	300	N.D.	N.D.	Yes	1-10	○
Fully porous silica	Shim-pack Arata	C18	2.2, 5	120	340	17	Yes	2-7.5	○
	Shim-pack GIST	C18	2, 3, 5	100	350	14	Yes	1-10	
		C18-AQ	1.9, 3, 5	100	350	13	Yes	1-10	○
		C8	2, 3, 5	100	350	8	Yes	1-10	○
		Phenyl	2, 3, 5	100	350	10	No	2-7.5	○
		Phenyl-Hexyl	3, 5	100	350	9	Yes	1-10	○
		PFPP	3, 5	100	350	10	Yes	2-7.5	○
	Shim-pack GISS	C18	1.9, 3, 5	200	200	9	Yes	1-10	○
		C8 (metal free body only)	1.9, 3, 5	200	200	6	Yes	1-10	○
	Shim-pack GIS	C18	2,3,4,5,10	100	450	15	Yes	2-7.5	
		C18-P	3, 5	100	450	29	No	2-7.5	
		RP-Shield	5	100	450	9	No	2-7.5	○
	Shim-pack GWS	C18	5	100	450	9.5	Yes	2-7.5	
	Shim-pack VP**	C18	5	120	410	20	Yes	2-7.5	
		C8	5	120	410	12.5	Yes	2-7.5	
		Phenyl	5	120	410	12.3	Yes	2-7.5	
	Shim-pack MAqC	ODS I	5	120	N.D.	13	Yes	2-4	
	Shim-pack FC	ODS	3	120	315	18	Yes	1.5-9	
Core shell silica	Shim-pack Velox	C18	1.8, 2.7, 5	90	125, 130, 100	9, 7, 5	Yes	2-8	
		SP-C18	1.8, 2.7, 5	90	125, 130, 100	7, 7, 5	No	1-8	
		Biphenyl	1.8, 2.7, 5	90	125, 130, 100	7, 7, 5	Yes	1.5-8	○
		PFPP	1.8, 2.7, 5	90	125, 130, 100	4, 4, 3	No	2-8	○

* Includes the percentage carbon content from the organic silica hybrid base material.

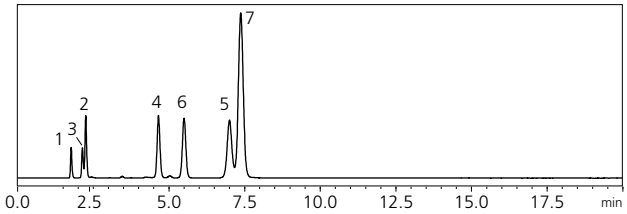
** The Shim-pack XR series is the high-speed analysis column series in the Shim-pack VP series.

Shim-pack Scepter Series

Phenyl Column

Shim-pack Scepter Phenyl-120

Fully porous organic silica hybrid | Prep size available



Features:
- Uses a highly chemically durable organic silica hybrid base material.
- Phenyl-butyl group modifications provide higher pH resistance than other phenyl columns.
- Hydrophobic interactions and π-π interactions result in a column with well-balanced separation.
- Useful for retaining compounds with long conjugated systems or separating isomers
- A reversed phase phenyl column with high pH resistance and available in three column body types for use with a wide range of analysis conditions

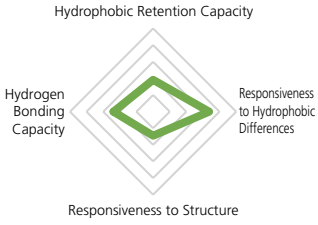
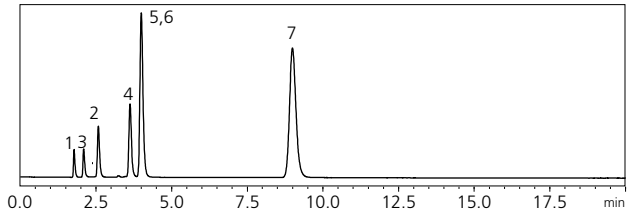


Figure 3: Results for the Shim-pack Scepter Phenyl-120

PFPP Column

Shim-pack Scepter PFPP -120

Fully porous organic silica hybrid | Prep size available



Features:
- Uses a highly chemically durable organic silica hybrid base material.
- PFPP group modifications provide a column with the highest hydrophobic retention capacity of all Shim-pack PFPP columns.
- The lack of endcapping provides strong dipole-dipole interactions by increasing surface silanol activity.
- Useful for separating isomers, strongly retaining basic compounds, etc.
- A reversed phase PFPP column available in three column body types for use with a wide range of analysis conditions.

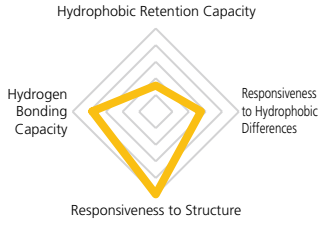
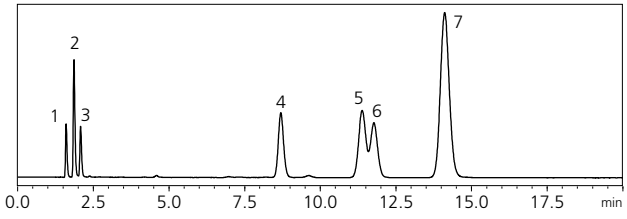


Figure 4: Results for the Shim-pack Scepter PFPP-120

C18 Column

Shim-pack Scepter C18-120

Fully porous organic silica hybrid | Prep size available



Features:
- Uses a highly chemically durable organic silica hybrid base material.
- A first-choice general-purpose C18 column
- A reversed phase C18 column that can be used in a variety of circumstances, with three column bodies and high pH resistance
- A first-choice column for reversed phase nucleic acid pharmaceuticals analysis

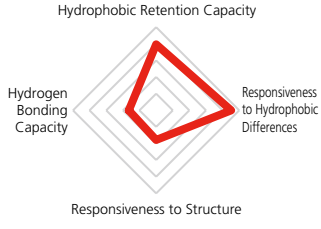


Figure 5: Results for the Shim-pack Scepter C18-120

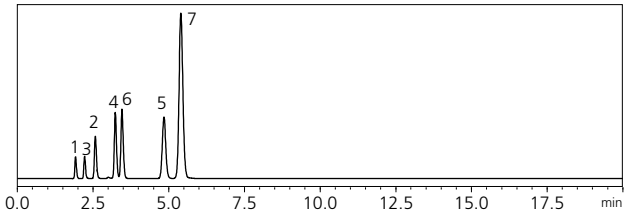
*1. These test results will not necessarily be obtained in all applications.
*2. If you would like preparative size in a column series that does not list "Prep size available," contact your local Shimadzu representative.

Shim-pack GIST Series

Phenyl Columns

Shim-pack GIST Phenyl

Fully porous silica | Prep size available



Features:
- This column uses a highly inert silica base material.
- The base material is directly modified with phenyl groups.
- The column exhibits fewer hydrophobic interactions and more π-π interactions than phenyl columns with carbon chain bonds.
- Useful for retaining compounds with long conjugated systems or separating isomers

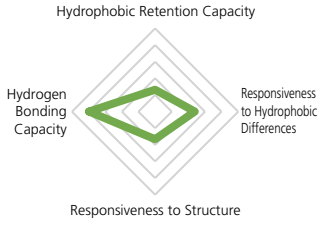
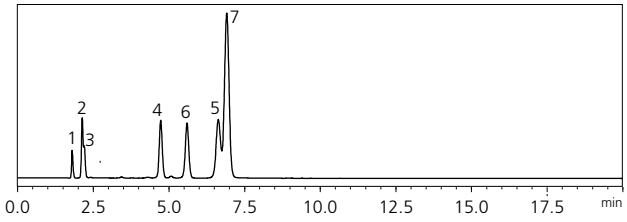


Figure 6: Results for the Shim-pack GIST Phenyl

Shim-pack GIST Phenyl-Hexyl

Fully porous silica | Prep size available



Features:
- This column uses a highly inert silica base material.
- The base material is modified with phenyl-hexyl groups to provide higher pH resistance than other phenyl columns.
- Hydrophobic interactions and π-π interactions result in a column with well-balanced separation.
- Useful for retaining compounds with long conjugated systems or separating isomers

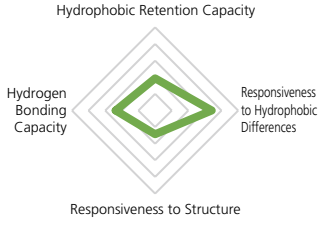
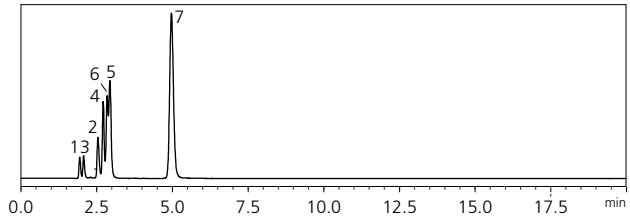


Figure 7: Results for the Shim-pack GIST Phenyl-Hexyl

PFPP Column

Shim-pack GIST PFPP

Fully porous silica | Prep size available



Features:
- This column uses a highly inert silica base material.
- Endcapping treatment provides a more appropriate level of dipole-dipole interactions than other PFPP columns.
- Useful for cases where adsorption to the column during PFPP column use is a concern
- Useful for separating isomers, strongly retaining basic compounds, etc.

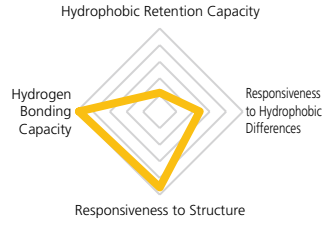
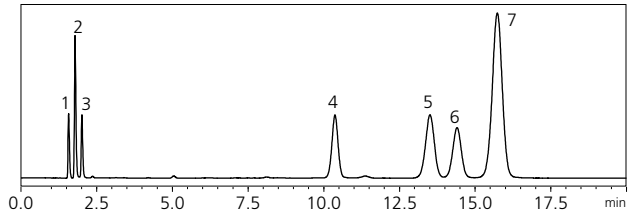


Figure 8: Results for the Shim-pack GIST PFPP

C18 Column

Shim-pack GIST C18

Fully porous silica | Prep size available



Features:
- Extended pH range (1 to 10) compared to other fully porous C18 columns, with a highly inert silica base material
- A first-choice general-purpose C18 column
- Of all the Shim-pack reversed phase columns, this one has the highest values for hydrophobic retention capacity and responsiveness to hydrophobic differences.

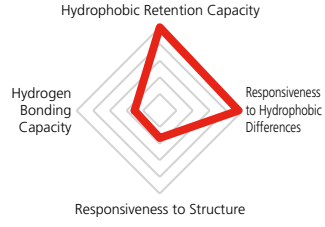


Figure 9: Results for the Shim-pack GIST C18

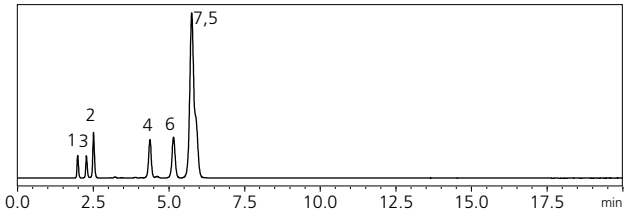
*1. These test results will not necessarily be obtained in all applications.
*2. If you would like preparative size in a column series that does not list "Prep size available," contact your local Shimadzu representative.

Shim-pack VP Series

Phenyl Column

Shim-pack VP-Phenyl

Fully porous silica



Features:

- Uses a standard silica base material meeting the strictest quality controls.
- Phenyl-propyl group bonding provides hydrophobic interactions and π-π interactions that result in a column with well-balanced separation.
- Shim-pack XR-Phenyl columns are suitable for high-speed analysis applications.

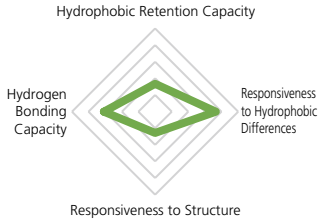
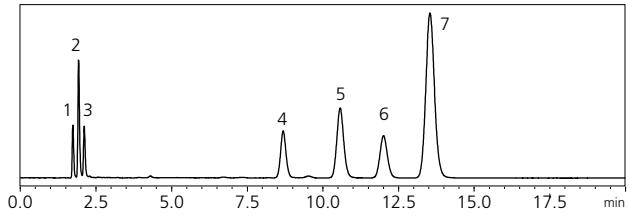


Figure 10: Results for the Shim-pack VP-Phenyl

C18 Column

Shim-pack VP-ODS

Fully porous silica



Features:

- Uses a standard silica base material meeting the strictest quality controls.
- This standard C18 column has a good balance of attribute values and is optimal for standard analyses.
- The Shim-pack XR ODS, ODS II, and ODS III columns are suitable for the high-speed analysis applications.

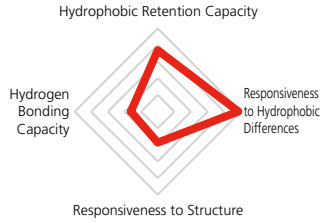


Figure 11: Results for the Shim-pack VP-ODS

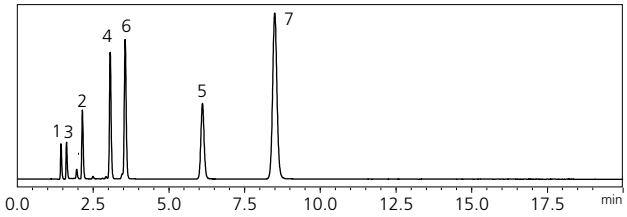
*1. These test results will not necessarily be obtained in all applications.
*2. If you would like preparative size in a column series that does not list "Prep size available," contact your local Shimadzu representative.

Shim-pack Velox Series

Phenyl Column

Shim-pack Velox Biphenyl

Core shell silica



Features:
- Uses a core shell silica base material.
- This core-shell silica column modified with biphenyl groups is recommended for high-speed analysis.
- It provides stronger π-π, π-CH, and additional interactions than other Shim-pack phenyl columns, which is useful for significantly changing the separation selectivity from C18 columns.
- Useful for retaining compounds with long conjugated systems or separating isomers

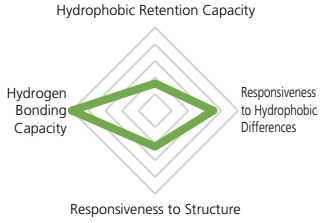
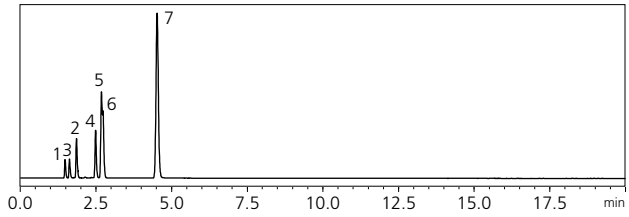


Figure 12: Results for the Shim-pack Velox Biphenyl

PFPP Column

Shim-pack Velox PFPP

Core shell silica



Features:
- Uses a core shell silica base material.
- This core-shell silica column modified with PFPP groups is useful for high-speed analysis.
- The lack of endcapping provides strong dipole-dipole interactions by increasing surface silanol activity.
- Useful for separating isomers, strongly retaining basic compounds, etc.

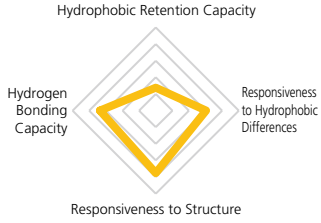
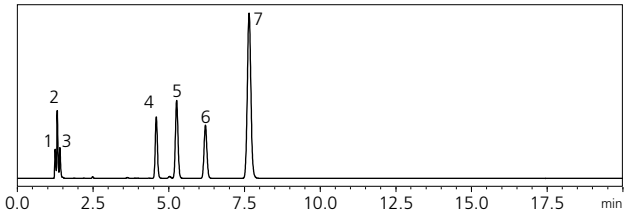


Figure 13: Results for the Shim-pack Velox PFPP

C18 Column

Shim-pack Velox C18

Core shell silica



Features:
- Uses a core shell silica base material.
- This is a general-purpose C18 core shell silica column, and is recommended for high-speed analysis
- Of all the Shim-pack columns, this one has balanced separation selectivity including low hydrogen bonding capacity, and acts as a general-use reversed phase C18 column

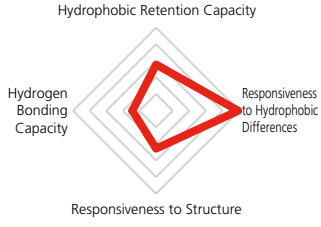


Figure 14: Results for the Shim-pack Velox C18

*1. These test results will not necessarily be obtained in all applications.
*2. If you would like preparative size in a column series that does not list "Prep size available," contact your local Shimadzu representative.

Figures 15-18 show the comparison of four attributes for the most common Shim-pack columns. Tanaka Test results indicate that both phenyl and PFPP columns have lower hydrophobic retention capacity and responsiveness to hydrophobic differences values than C18 columns (Figure 15 and 16). That means hydrophobic compounds elute sooner and the packing material surfaces are less hydrophobic than C18 columns, which makes them adjust to water-based solvents more quickly.

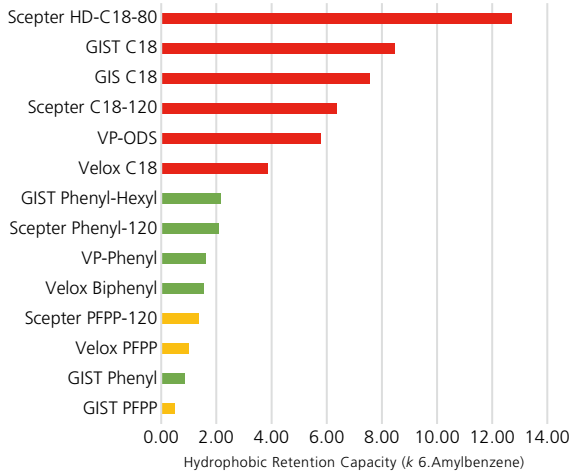


Figure 15: Comparison of Hydrophobic Retention Capacity for Principal Shim-pack Columns

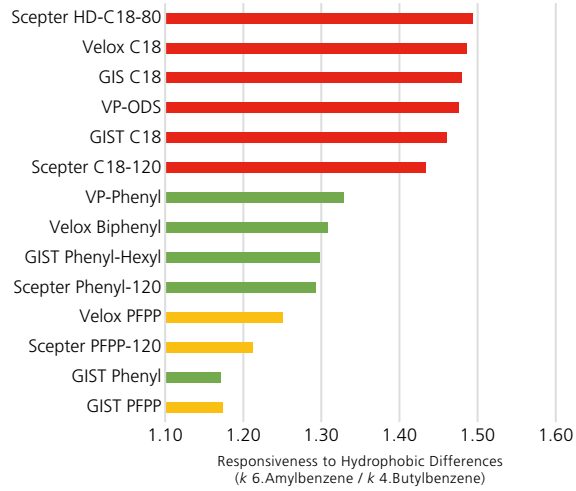


Figure 16: Comparison of Responsiveness to Hydrophobic Differences for Principal Shim-pack Columns

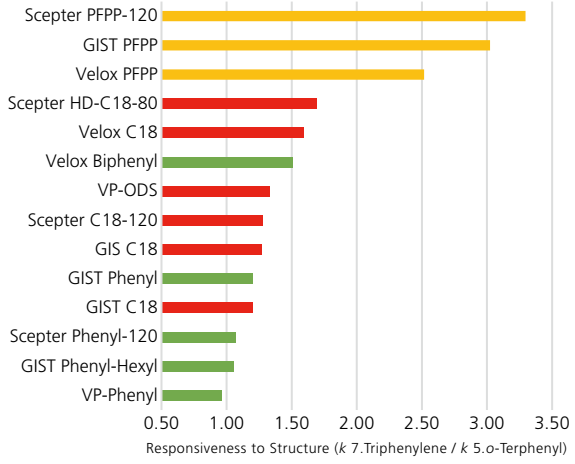


Figure 17: Comparison of Responsiveness to Structure for Principal Shim-pack Columns

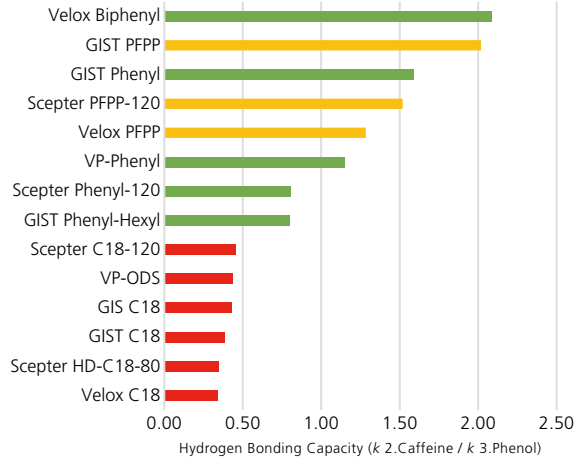


Figure 18: Comparison of Hydrogen Bonding Capacity for Principal Shim-pack Columns

C18 columns Phenyl columns PFPP columns

A comparison of basic hydrophobicity properties indicated in these results shows that alkyl-phenyl and biphenyl type columns with higher carbon content tend to be more hydrophobic. Therefore, alkyl-phenyl type columns are useful for achieving separation with a better balance between hydrophobic interactions and π-π interactions than other phenyl-type columns. In contrast, biphenyl-type columns offer an appropriate level of responsiveness to structure, with strong π-π interactions from two benzene rings. Consequently, they are useful for applications such as analysis of aromatic isomers that are difficult to separate using a C18 column. Meanwhile, PFPP columns offer lower hydrophobicity and higher responsiveness to structure and hydrogen bonding capacity than other reversed phase columns, providing different separation behavior than phenyl columns. The electron-acceptance of fluorine in the stationary phase of PFPP columns generates an electron shift that results in a stronger dipole-dipole interaction with compounds than C18 columns. In addition, silanol groups on the silica surface determined by whether or not PFPP columns are endcapped can also affect separation. Consequently, PFPP columns can be useful for significantly changing separation behavior (selectivity) from C18 results or for increasing the retention strength for basic compounds.

The next section describes the unique separation characteristics of phenyl and PFPP columns that cannot be described based on these results.

4. Characteristics of Phenyl and PFPP Columns

This section describes three characteristics of phenyl and PFPP columns that distinguish them from more commonly used C18 reversed phase columns.

(1) The organic solvent type has a large effect on separation in some cases (phenyl and PFPP columns.)
(2) Responsiveness to isomers is superior in some cases (phenyl and PFPP columns.)
(3) Retention of polar basic compounds is superior in some cases (PFPP columns.)

4-1. Effect of Organic Solvents

Phenyl PFPP

The interactions between compounds and phenyl or PFPP columns differ depending on the type of organic solvent, and the impact on separation behavior is greater than with C18 columns in some cases.[4)]

Figure 19 shows chromatograms obtained from analyzing uracil, caffeine, and phenol using a C18, phenyl, and PFPP column with the analysis conditions shown in Table 6. The upper chromatogram (pink line) in each figure was obtained using acetonitrile, and the lower chromatogram (black line) was obtained using methanol.

In general, when equal ratios of acetonitrile and methanol are mixed with water, the acetonitrile-based mobile phase is said to provide higher elution strength (less retention) in reversed phase mode.[12)-13)]

The respective chromatograms shown in Figure 19 indicate that using acetonitrile results in overall shorter retention times, but the retention time of each compound differs significantly for each column. For example, in terms of the elution order, the elution order of peaks 2 and 3 from a C18 column remained unchanged even after switching to the organic solvent. However, the intervals between the peaks changed significantly when using a phenyl column, and the elution order was reversed when using a PFPP column, after switching to the organic solvent from acetonitrile to methanol.

Methanol and acetonitrile are protic and aprotic organic solvents, respectively, with different chemical properties. Due to their respective properties, the magnitude of various interactions offered by phenyl and PFPP columns will vary depending on the properties of the organic solvent used. Therefore, when using phenyl or PFPP columns, changing the type of organic solvent used can provide an effective way to optimize separation.

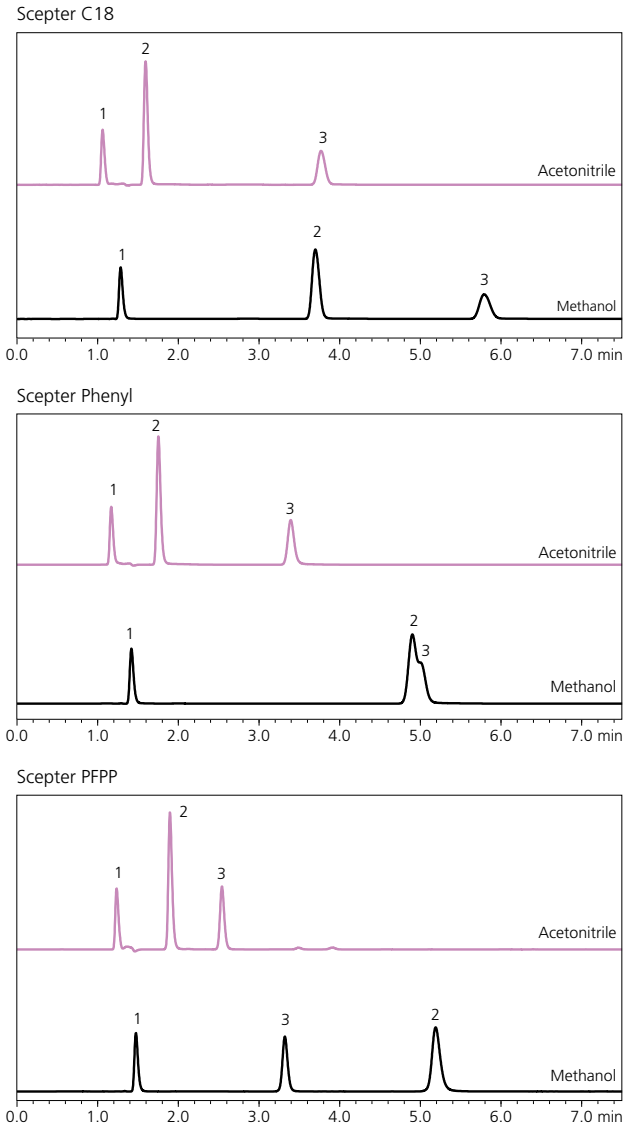


Figure 19: Comparison of Separation Behavior for Two Types of Organic Solvents

Table 6: Analysis Conditions

Column	: Shim-pack Columns (100 mm x 3.0 mm I.D., 1.9 µm)
Mobile Phase	: Water/ Methanol or Acetonitrile = 70 : 30 (v/v)
Flow Rate	: 0.42 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 1 µL
Detection	: UV 254 nm
Sample	: 1. Uracil
	2. Caffeine
	3. Phenol
Vial	: SHIMADZU LabTotal™ Vial for LC/LCMS, P/N : 227-34001-01

4-2. Cases of Superior Responsiveness to Isomers

Phenyl PFPP

Both phenyl and PFPP columns can be more effective than C18 columns for separating isomers, such as compounds with non-enantiomeric aromatic rings or double bonds.

4-2-1. Examples of Separating Isomers

(1) Example of Using a Phenyl Column to Separate Isomers

Phenyl

The following example uses a Shim-pack Velox Biphenyl column to separate diastereomers of PTAD-derivatized vitamin D3 metabolites. Figure 20 shows an example of PTAD-derivatized vitamin D3 metabolite separation, and Figure 21 shows the structures of the compounds used in the analysis. Corresponding analysis conditions are shown in Table 7. The figure shows that the Shim-pack Velox Biphenyl column successfully separated all 4 compounds, which was not possible using the Shim-pack Scepter C18, (typically recommended as the first-choice C18 column,) or the Shim-pack GIS C18-P, which is a high-density C18 column that offers excellent separation of structural isomers.

In particular, the biphenyl column exhibited stronger π-π interactions than the other phenyl columns, due to the two benzene rings. The two adjacent benzene rings make it effective in some cases for isomers that are difficult to separate using other C18 or phenyl columns.

Table 7: Analysis Conditions

System	: LCMS™-8050
Column	: Shim-pack Velox Biphenyl (50 mm x 2.1 mm I.D., 1.8 µm), P/N : 227-32013-02
Mobile Phase	: A) 0.1% Formic acid in water B) 0.1% Formic acid in methanol
Time Program	: B conc. 70% (0 min) → 80% (6-8 min) →70% (8.1-10 min)
Flow Rate	: 0.3 mL/min
Column Temp.	: 30 °C
Injection Vol.	: 5 µL
Detection	: LC-MS/MS (ESI positive, MRM)
Sample	: 1. 3-epi-25(OH)D3-PTAD (6R-isomer) 2. 25(OH)D3-PTAD (6S-isomer) 3. 25(OH)D3-PTAD (6R-isomer) 4. 3-epi-25(OH)D3-PTAD (6S-isomer)
Vial	: SHIMADZU LabTotal Vial for LC/LCMS, P/N : 227-34001-01

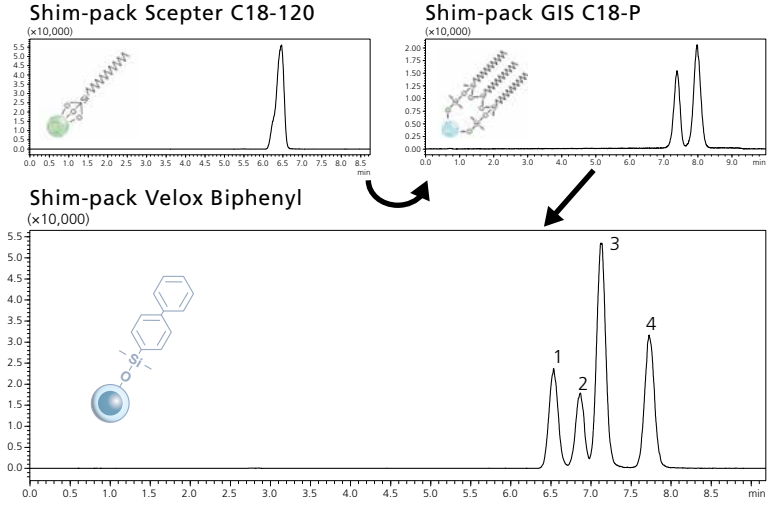
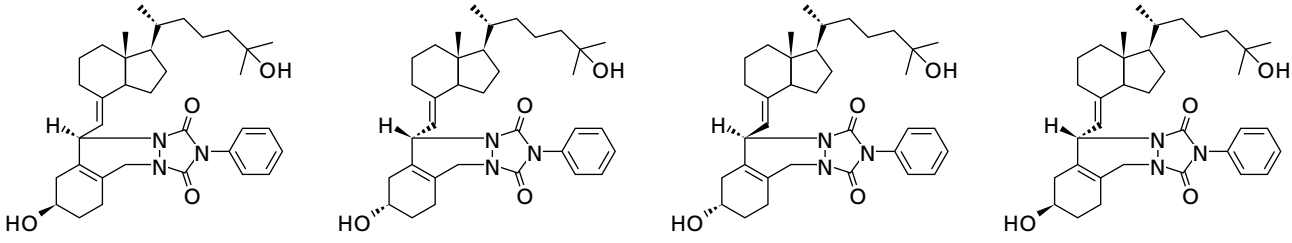


Figure 20: Analysis of PTAD-Derivatized Vitamin D3 Metabolites Using a Shim-pack Velox Biphenyl Column



1. 3-epi-25(OH)D3-PTAD (6*R*-isomer)
2. 25(OH)D3-PTAD (6*R*-isomer)
3. 25(OH)D3-PTAD (6*S*-isomer)
4. 3-epi-25(OH)D3-PTAD (6*S*-isomer)

Figure 21: Structural Formulas of PTAD-Derivatized Vitamin D3 Metabolites

(2) Example of Using a PFPP Column to Separate Isomers (from Application News L533)

PFPP

This example uses a Shim-pack Scepter PFPP-120 column for the analysis of DNPH-derivatized aldehydes.

Figure 22 shows the chromatogram of six DNPH-derivatized aldehydes, which are classified as offensive odors, and frequently tested for air quality monitoring. Figure 23 shows the structures of the compounds used in the analysis, with the method conditions shown in Table 8. The PFPP column produced good separation between structural isomers *n*-butyraldehyde and *iso*-butyraldehyde and structural isomers *iso*-valeraldehyde and *n*-valeraldehyde.

Table 8: Analysis Conditions

System	: Prominence™-i Plus
Column	: Shim-pack Scepter PFPP-120 (150 mm × 4.6 mm I.D., 3 µm), PN : 227-31057-05
Mobile Phase	: A: Water B: Methanol/Acetonitrile = 8/2 (v/v)
Time Program	: B.CONC. 20% (0 min)→55% (5 min)→60% (25 min)→60% (25-35 min)→20% (35-40 min)
Flow Rate	: 1.0 mL/min
Column Temp.	: 35 °C
Injection Vol.	: 20 µL
Detection	: 360 nm (D_2 Lamp)
Mixer Volume	: 0.5 mL

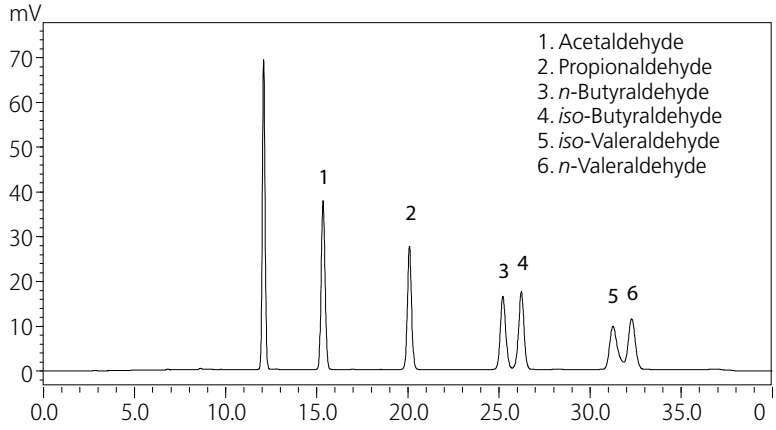
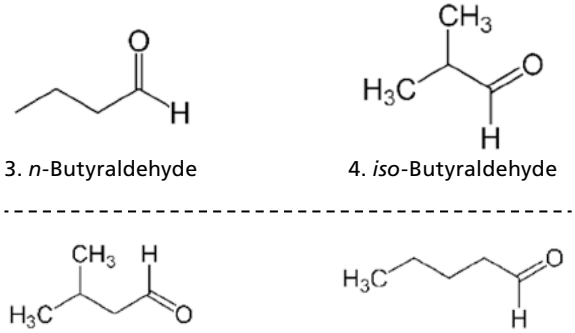


Figure 22: Chromatogram of Standard Mixture Solution (1.0 µg/mL Concentration of Each Aldehyde)



3. *n*-Butyraldehyde

4. *iso*-Butyraldehyde

5. *iso*-Valeraldehyde

6. *n*-Valeraldehyde

Figure 23: Structural Formulas for Two Types of Butyraldehyde and Two Types of Valeraldehyde Isomers

To view Application News L533, click here.

Table 9 shows information about isomer separation for which phenyl and PFPP columns are effective.[14] In particular, it can be useful to try phenyl and PFPP reversed phase columns for separating isomers such as cis/trans isomers or diastereomers. It is presumably because PFPP columns especially recognize changes more easily, such as slight changes in the functional groups of aromatic compounds, based on the various interactions provided by the stationary phase.[15]

Table 9: Isomer Separation Suitable for Phenyl or PFPP Columns*

			Example	Solutions
Isomers	Structural Isomers - Compounds with identical molecular formulas but different molecular structures	Functional group isomers	Alcohols vs ethers, aldehydes vs ketones, or other compounds with different functional groups	Generally can be analyzed using a typical C18 column, etc.
		Positional isomers	*o*-cresol, *m*-cresol, *p*-cresol, or other base structures with functional groups attached at different positions.	• C18 columns with enhanced functionality • Phenyl columns • PFPP columns
	Stereoisomers - Molecules with identical molecular formulas and structures, but with different three-dimensional configurations	Geometric isomers	cis/trans isomers, etc.	• Phenyl columns (especially biphenyl) • PFPP columns
		Optical isomers	Diastereomers	
			Enantiomers (L-amino acids, D-amino acids, etc.)	• Chiral columns for optical isomers • Sample derivatization etc.

* The information shown in this table is not necessarily applicable for all applications and does not guarantee analytical results.

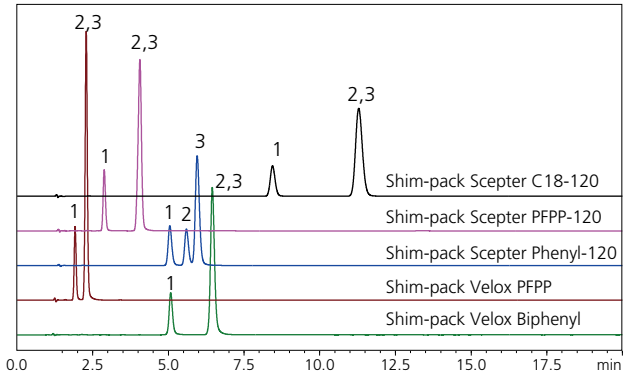
4-2-2. Examples of Isomer Separation with Different Organic Solvents

As described in section 4-1, acetonitrile and methanol have different characteristics that affect selectivity in the separation. However, organic solvent selection is also important for achieving good separation of positional and other isomers.

Figure 24 shows an example of analyzing terphenyl positional isomers using two types of organic solvents and five types of columns, and Figure 25 shows the structures of the compounds used in the analysis. The method conditions are shown in Table 10. The upper chromatograms were obtained using methanol and the lower chromatograms using acetonitrile.

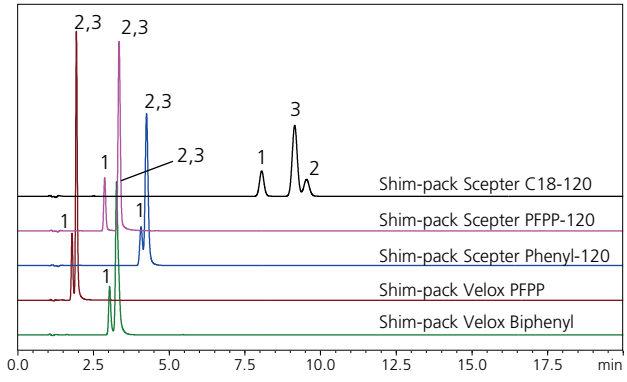
When using methanol, positional isomer separation can be achieved using an alkyl-phenyl type Shim-pack Scepter Phenyl-120 column, but when using acetonitrile, only the Shim-pack Scepter C18-120 column could resolve all 3 positional isomers. The triple bond in acetonitrile introduces *π-π* interactions between the analyte and the mobile phase which compete with and tend to relatively weaken *π-π* interactions between phenyl and PFPP columns and compounds but relatively strengthen the C18 column hydrophobicity. On the other hand, methanol does not affect *π-π* interactions between the column and compounds, so if methanol is used with a phenyl or PFPP column, hydrophobic and *π-π* interactions with the stationary phase will act on compounds, resulting in increased retention[4]. Consequently, changes in the interactions that contribute to separation based on the type of organic solvent or column used presumably will also change separation patterns.

In summary, although phenyl and PFPP columns may offer better isomer separation than C18 columns, they can result in changes in retention and selectivity depending on the type of column or mobile phase used. Therefore, in order to achieve good isomer separation, it is important to consider various types of columns and mobile phases.



Using Methanol

(For analysis conditions, refer to Table 10. Mobile phase: (1) 0.1 % formic acid in water / 0.1 % formic acid in methanol = 20:80 (v/v))



Using Acetonitrile

(For analysis conditions, refer to Table 10. Mobile phase : (2) 0.1 % formic acid in water / 0.1 % formic acid in Acetonitrile = 30:70 (v/v))

Figure 24: Comparison of Terphenyl Positional Isomer Separation Using Different Organic Solvents and Five Types of Columns

1. *o*-Terphenyl

2. *p*-Terphenyl

3. *m*-Terphenyl

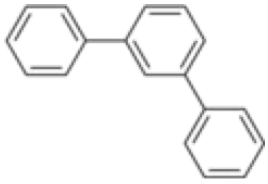


Figure 25: Structural Formulas of Terphenyl Positional Isomers

Table 10: Analysis Conditions

System	: Nexera X3
Column	: Shim-pack columns (100 mm × 2.0 mm I.D., 3 µm)
Mobile Phase	: (1) 0.1% formic acid in water / 0.1% formic acid in methanol = 20 : 80 (v/v) (2) 0.1% formic acid in water / 0.1% formic acid in Acetonitrile = 30 : 70 (v/v)
Flow Rate	: 0.2 mL/min
Column Temp.	: 40 ˚C
Injection Vol.	: 1 µL
Vial	: TORAST-H Vial, P/N :370-04300-01
Detection	: UV 254 nm

4-3. Cases of Superior Retention of Polar Basic Compounds

PFPP

PFPP columns are reversed phase columns that offer superior retention of basic polar compounds.

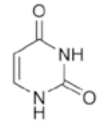
Changes in PFPP column separation behavior due to differences in organic solvent ratios are compared to other reversed phase phenyl or alkyl columns. The analysis conditions and column types are shown in Table 11. A mixture of the acidic, neutral, basic, and amphoteric compounds shown in Figure 26 was used for test samples. Compound retention indices were calculated based on a *t0* marker (uracil) and a marker for each of the 4 components in the mixture sample (with celecoxib as a marker for the acidic compound, testosterone for the neutral compound, nortriptyline for the basic compound, and tyrosine for the amphoteric compound.) Plots of acetonitrile ratios for calculated retention indices are shown smoothed with Microsoft Excel in Figure 27.

Table 11: Analysis Conditions

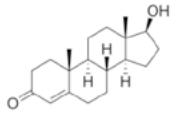
System	: Nexera X3, LCMS-2050
Column	: •Shim-pack columns (100 mm × 2.0 mm I.D., 3 µm) •Shim-pack Scepter C18-120 (fully porous C18) •Shim-pack Scepter Phenyl-120 (fully porous Alkyl-phenyl) •Shim-pack Velox Biphenyl (superficially porous Biphenyl) •Shim-pack Scepter PFPP-120 (fully porous PFPP) •Shim-pack GIST PFPP (fully porous PFPP) •Shim-pack Velox PFPP (superficially porous PFPP)
Mobile Phase	: (1) 0.1% Formic acid in Water / 0.1% Formic acid in Acetonitrile = 70 : 30, 50 : 50, 30 : 70, 10 : 90 (v/v)
Flow Rate	: 0.45 mL/min
Column Temp.	: 40 ˚C
Injection Vol.	: 1 µL
Vial	: TORAST-H Vial, P/N :370-04300-01

MS Conditions

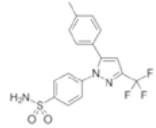
Ionization	: DUIS™, Positive mode
Mode	: SIM
Drying Gas Flow	: 5.0 L/min
Heating Gas Flow	: 7.0 L/min
Desolvation Temp.	: 450 ˚C
Nebulizing Gas Flow:	2.0 L/min



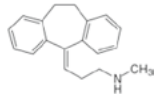
1. Uracil (*t0* marker)



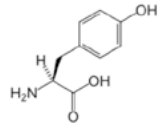
2. Testosterone
(neutral compound marker)



3. Celecoxib
(acidic compound marker)



4. Nortriptyline
(basic compound marker)



5. Tyrosine
(amphoteric compound marker)

Figure 26: Structural Formulas for Compounds Used

The results in Figure 27 indicate that PFPP columns offer the following two features compared to other reversed phase stationary phase columns.

- Strong retention of basic compounds
- Retention of basic and amphoteric compounds increases when acetonitrile ratios reach 70 % or higher.

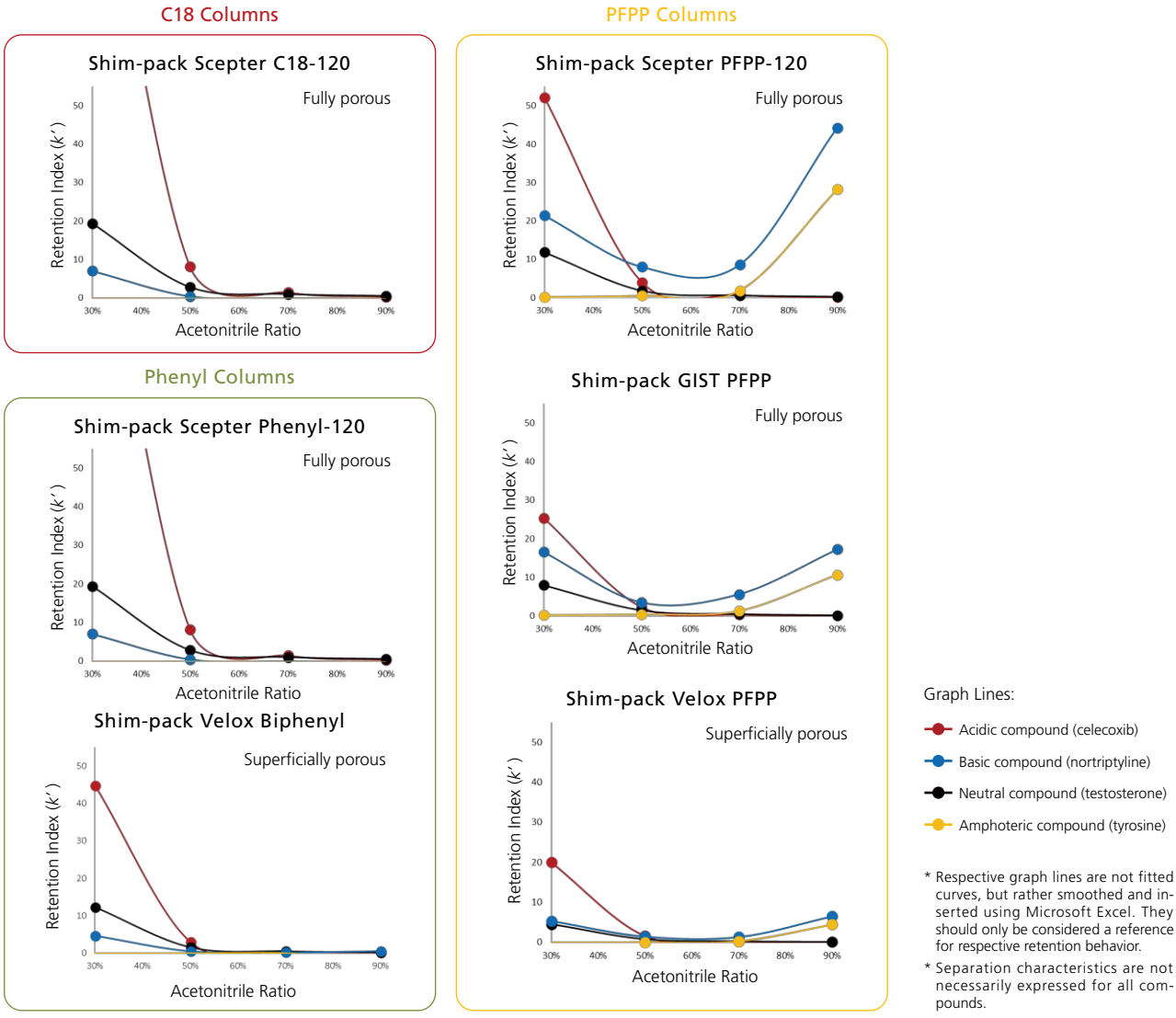


Figure 27: Comparison of Retention Indices for Respective Columns Using Four Types of Compound Markers

In general, for reversed phase mode, the higher the organic solvent ratio, the higher the elution capacity of the mobile phase and the shorter the retention time. That is also the case for C18 or phenyl columns in the above results, with the retention indices for all compound markers decreasing with higher acetonitrile ratios. However, for the PFPP column, the retention of basic compounds and amphoteric compounds increases when the acetonitrile concetration is 70 % and higher.

This is due to an increase in electrostatic interactions and stronger retention of cationic compounds as the ratio of acetonitrile increases in the mobile phase.

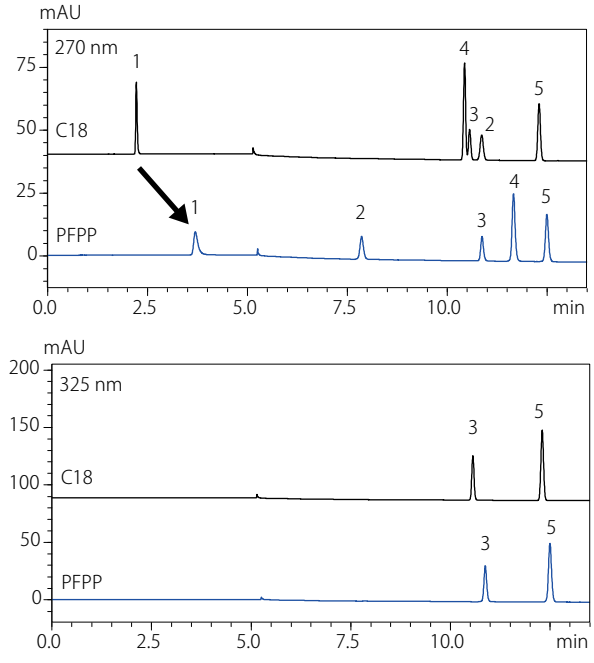
Such phenomena, where the retention of cationic compounds strengthens as the acetonitrile ratio is increased when using acetonitrile with a water-based solvent, are often interpreted as being due to the HILIC mode separation mechanism. However, the retention of uracil used as a *t0* marker, or other nucleic acids used as a retention index for hydrophilic compounds in a previous study of HILIC column characteristics[16], does not increase with increasing acetonitrile ratio for the PFPP columns. Therefore, during PFPP column separation, in addition to interactions due to hydrophobic properties, presumably electrostatic interactions are also strengthening or weakening separation depending on the organic solvent ratio.[9),17)-18)]

Even among Shim-pack PFPP columns, the basic compound retention capacity can change depending on whether the base materials is silica or an organic silica hybrid. Additionally, differences in the specific surface area or porosity of the base material itself, or the presence or absence of endcapping can also affect the retention of basic compounds. For information about Shim-pack PFPP columns, refer to Table 4.

One example of using a PFPP column for analysis of basic compounds is described below.

- **Example of Using a Shim-pack Scepter PFPP-120 Column for Simultaneous Analysis of Functionally Beneficial Components in Coffee that Contains Basic Compounds (from Application News 01-00280-EN)**

Chromatograms from the simultaneous analysis of a standard mixture of basic compounds determined to be functionally beneficial components in coffee are shown in Figure 28, and Figure 29 shows the structures of these 5 compounds. The results show that the PFPP column was able to retain trigonelline and other polar basic compounds that are difficult to retain with a C18 column. Also, as shown previously, since the Shim-pack Scepter PFPP-120 column tends to retain cationic compounds more strongly than other Shim-pack PFPP columns, Sceper columns are recommended for achieving increased retention of cationic compounds.



1. Trigonelline 2. Pyrocatechol 3. Chlorogenic acid
4. Caffeine 5. Caffeic acid

Figure 28: Chromatograms of a Sample Containing a Mixture of Each Compound (10 mg/L each)

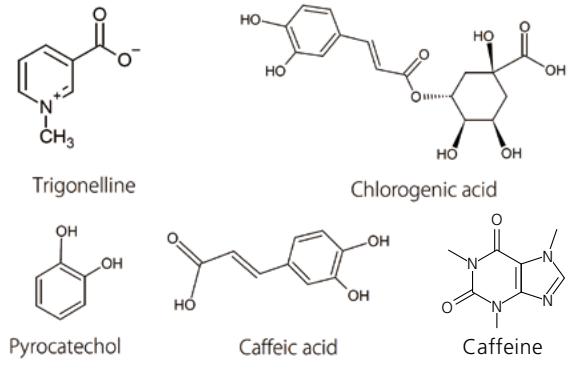


Figure 29: Structural Formulas of Functionally Beneficial Components in Coffee

Table 12: Analysis Conditions

System	: Nexera lite
Column (C18)	: Shim-pack Scepter C18-120 (150 mm x 4.6 mm I.D., 3 µm), P/N : 227-31016-05
Column (PFPP)	: Shim-pack Scepter PFPP-120 (150 mm×4.6 mm I.D., 3 µm), P/N : 227-31057-05
Mobile Phase	: A) 20 mmol/L (Sodium) phosphate buffer (pH 2.6) B) Acetonitrile
Time Program	: B conc. 0% (0.00-1.00min) → 10% (4.00min) → 20% (10.00-12.00min) → 70% (12.01-13.00 min) → 0% (13.01-18.00min)
Flow Rate	: 1.0 mL/min
Column Temp.	: 25 °C
Injection Vol.	: 5 µL
Vial	: SHIMADZU LabTotal Vial for LC/LCMS, P/N : 227-34001-01
Detection	: PDA Ch1 : 270 nm, Ch2 : 325 nm (SPD-M40)

To view Application News 01-00280-EN, click here.

5. Conclusion

This article addressed the advantages of using Shim-pack reversed phase columns with phenyl and PFPP functional groups as alternatives to alkyl phases. If you are unsure of which column to use for your application, refer to the selection chart for Shim-pack reversed phase columns shown in Figure 30 for guidance.

In terms of actual analysis, column selection can be quite simple, particularly when the column for use is specified, like in a USP method. When unspecified, column selection for method development can be challenging. Even columns with the same functional group modifications can have retention and selectivity differences due to variations in surface area,carbon load, and endcapping. For this reason, using multiple columns for screening is recommended, with consideration for alkyl, phenyl, and PFPP phases, as well as methanol and acetonitrile as the organic solvent.

The Shim-pack reversed phase column lineup provides a variety of separation selectivities for efficient method development. For automated screening and optimization of separation conditions including columns, consider LabSolutions™ MD and Method Scouting Solution software provided by Shimadzu to support LC robust is method development.

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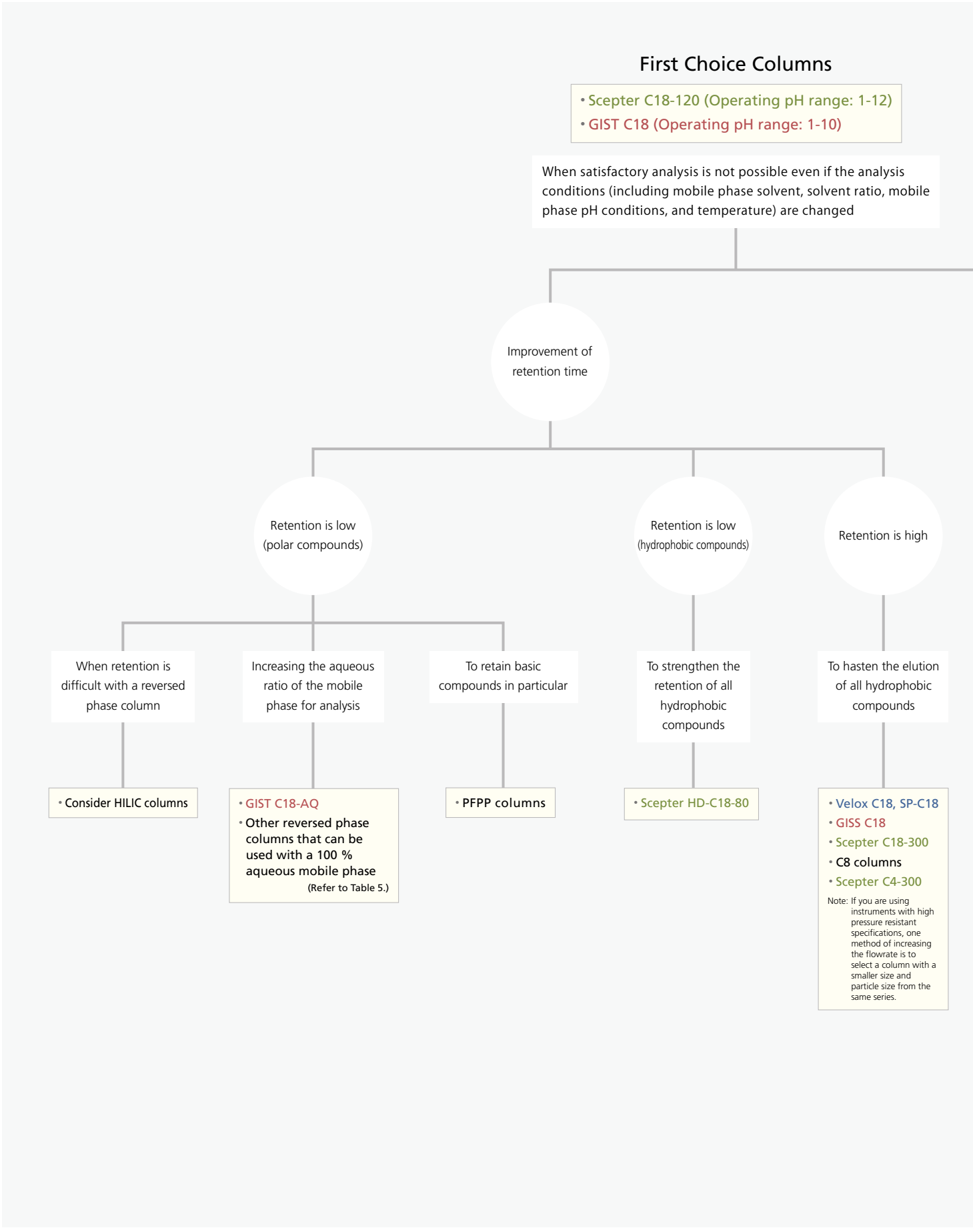
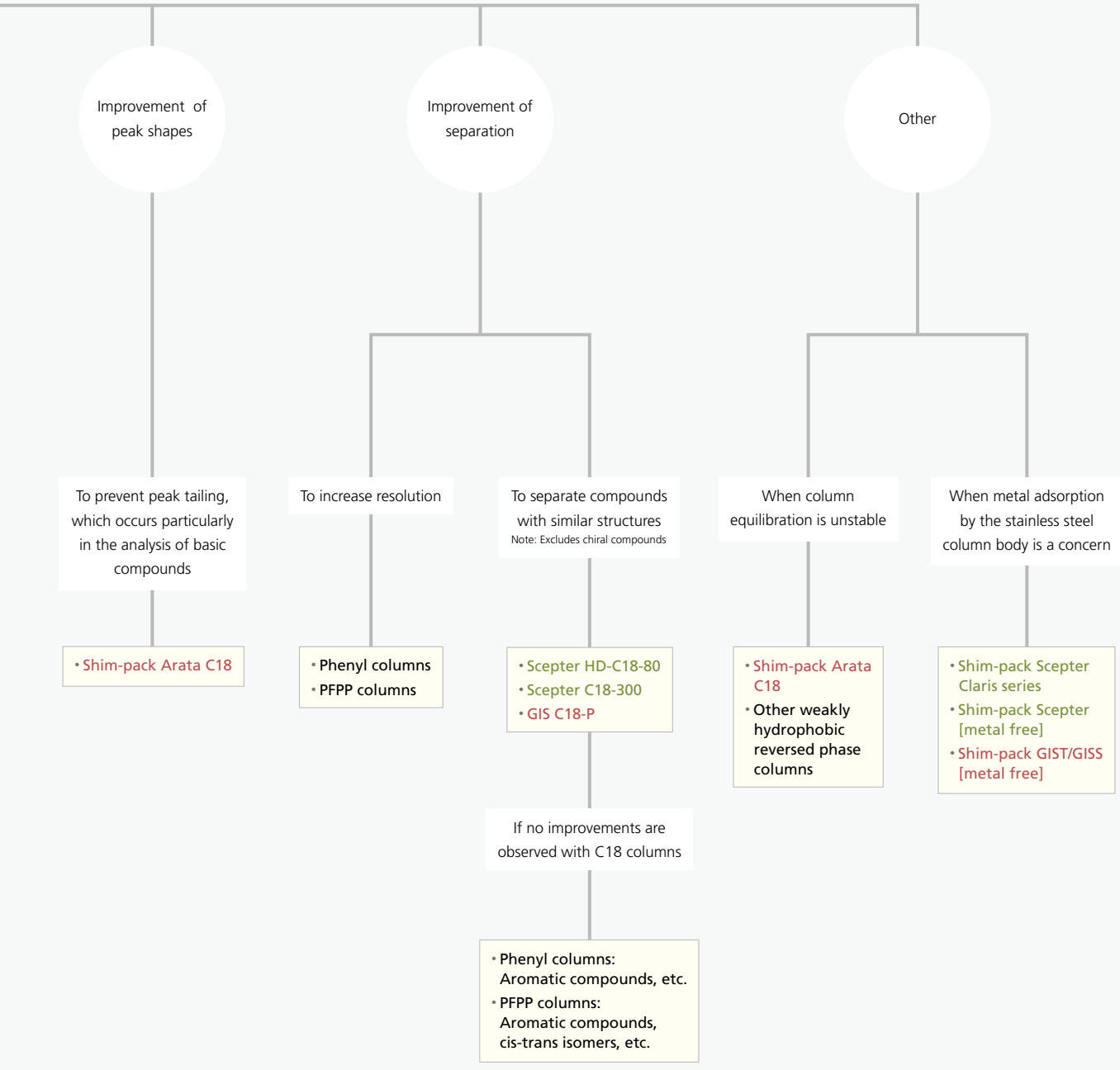


Figure 30: Chart for the Selection of Recommended General-Purpose Reversed Phase Columns from the Shim-pack Column Lineup

Red: Fully porous silica columns
Green: Fully porous organic silica hybrid columns
Blue: Superficially porous silica columns (Core shell silica columns)



*1 This is not a description of all of the Shim-pack columns.
*2 The separation selectivity differs significantly even for columns in the same group. Actual column selection may be more complicated than shown in this chart depending on the analytes and conditions.
The analysis results in this column selection chart are not guaranteed, so use this only as a reference.

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