

Wakosil-II 5C18 RS HPLC Columns

A BETTER SOLUTION

Most chromatographers will be familiar with the various types of tailing shown in **Figure 1**. Such tailing will occur when HPLC columns are used in a manner beyond their capabilities. The tailing peak 1 in these chromatograms is procainamide HCl, which has basic functional groups typical of many drugs. Procainamide HCl doesn't exhibit tailing on Wakosil II 5C18 RS columns as shown in **Figure 2** even though the chromatogram was generated under the same conditions as the chromatograms in Figure 1. The reason for this superior performance is that Wakosil II 5C18 RS is a highly deactivated stationary phase based on a high purity support. Many chromatographers expect that chromatographic supports are made of "high purity" silica but this is not always the case.

PRODUCT DATA

Figure 1. Common commercially available packings

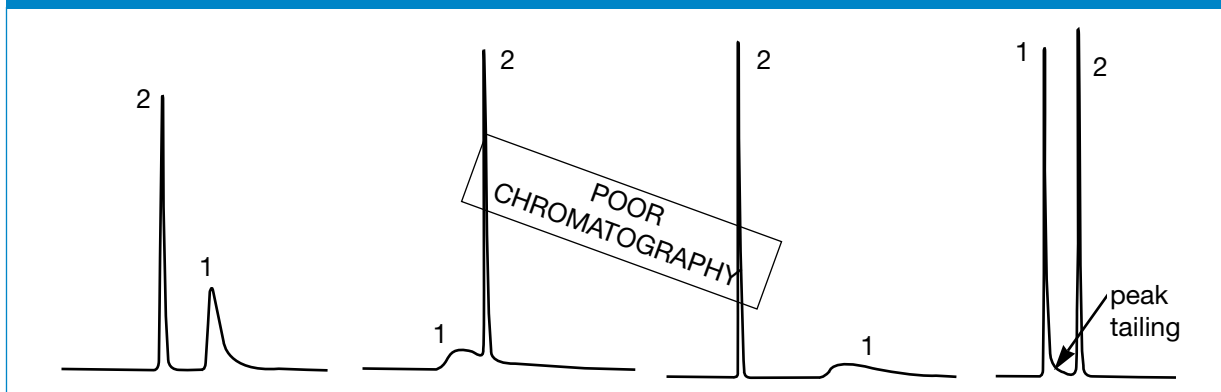
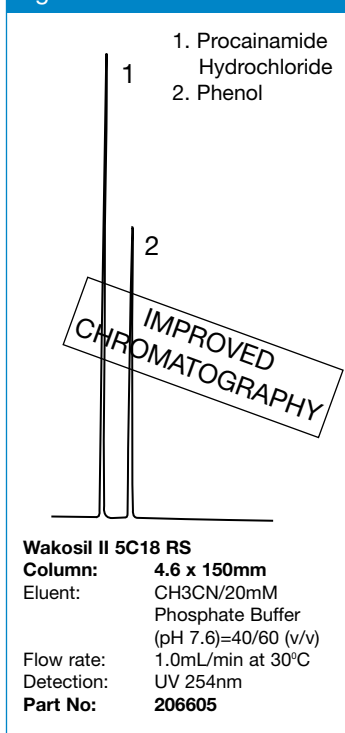


Figure 2. Wakosil-II 5C18 RS



Manufacturers of HPLC stationary phases began to focus on the purity of silica supports, ever since it became evident that the impurities incorporated in silica surfaces can contribute to peak tailing. In the past, HPLC packing manufacturers attempted to solve this problem by sterically shielding the silica surface with the small alkyl groups. This improved the chromatography of moderately polar compounds, but basic compounds would still exhibit excessive tailing on these *endcapped* columns. In recent times sophisticated washing procedures have been employed to remove surface impurities and the resultant HPLC columns have shown improved chromatographic performance, particularly with basic compounds that are prone to tailing. The performance of these *base deactivated* materials was not ideal and left considerable room for improvement. The next step in the evolution of HPLC packing materials has been the development of high purity silicas.

The primary impurities in silica based packing materials are metal ions. These are the main causes of peak tailing in reversed phase chromatography. Chelating analytes can bind directly to metal ions on the silica surface, causing poor peak shape which affects resolution from other compounds and also impairs the sensitivity of the analysis. Metal ions also increase the activity of residual silanols making them more acidic. This induces tailing in basic compounds, making them virtually undetectable in many cases. The presence of metal ions also influences the bonding of stationary phases such as C18 to the silica surface. This can effect the uniformity of the surface coverage with alkyl groups, thereby leaving more residual silanols and metal ions exposed so that they are able to influence analytes susceptible to their effects. A high purity silica like that used for Wakosil columns can minimize these effects, eliminating any visible tailing of difficult analytes.

An excellent test for the presence of active silanols is to examine the chromatography of pyridine in an unbuffered solution. Pyridine is quite strongly basic, and has a high affinity for silanols, as the nitrogen in pyridine is able to undergo hydrogen bonding with any silanols on the silica surface. Therefore tailing of the pyridine peak is an indication of available silanols present on reverse phase columns. Furthermore, any silanols will retard the elution of the pyridine. Thus, a C18 column should give a sharp pyridine peak that shows no evidence of tailing, and pyridine should elute before phenol. Wakosil II 5C18 RS illustrates the type of performance (**Figure 3**) that should be expected of a C18 column that has a high purity silica support.

The presence of metal ions on the silica surface is best indicated by analyzing the chromatography of a strong chelate. Phthalic acid is an excellent test sample as it readily binds with available metal ions as shown in **Figure 5**, causing peaks to tail. Phthalic acid shows no sign of tailing on Wakosil II 5C18 RS in **Figure 4**. This is another indication of the high purity silica used for the manufacture of Wakosil II 5C18 RS.

Figure 3. Pyridine derivative

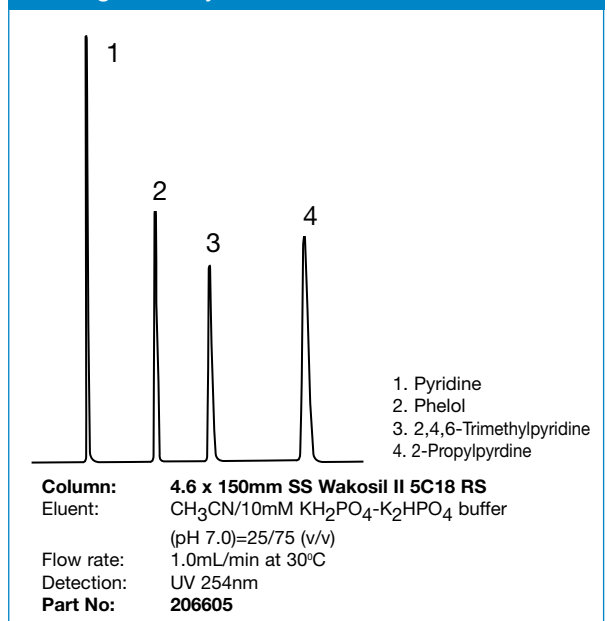


Figure 4. Carboxylic Acids

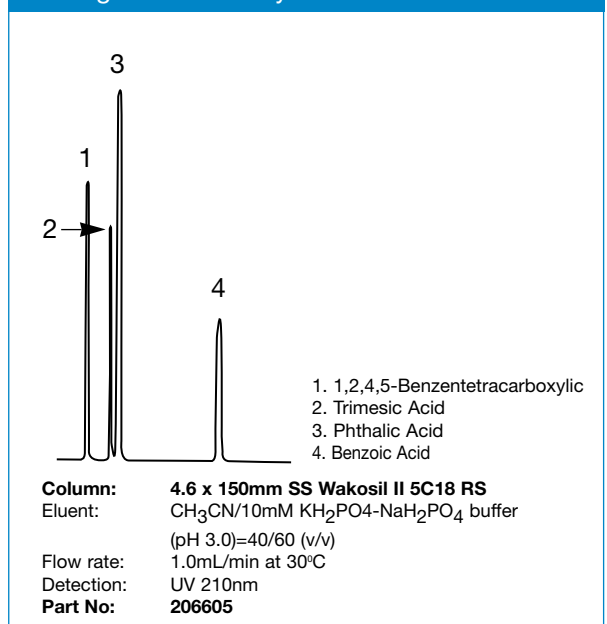
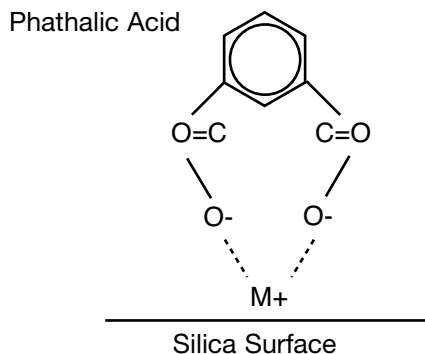
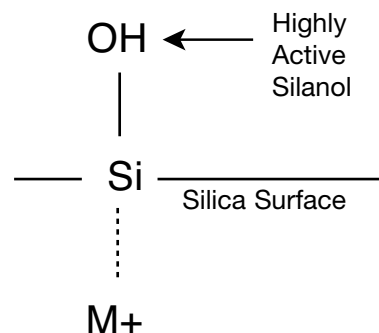


Figure 5. 5A. Metal on Silica Surface



5B. Internal Metals



Benzylamine should not be retained significantly on a C18 column in a buffered system. **Figure 6** shows a chromatogram comparing benzylamine with uracil, which is largely unretained. As can be seen, benzylamine is retained slightly. This is probably due to electrostatic interaction with some surface silanols. The sharp peak indicates that this influence is negligible and that Wakosil II 5C18 RS has a high hydrophobicity due to its uniform surface coverage. This illustrates the efficient phase bonding technology used to produce Wakosil II 5C18 RS.

Wakosil II 5C18 RS shows superior chromatographic performance due to the low levels of silanols and metal ions on its high purity base silica, as well as the use of state of the art phase bonding technology. The acceptable level for metallic impurities in a chromatographic silica support is 10ppm or lower for each of the respective metals. From **Table 1** it can be seen that most popular silica supports have much higher metal content. Consequently, many popular HPLC columns show evidence of silanol interaction despite the use of sophisticated endcapping procedures.

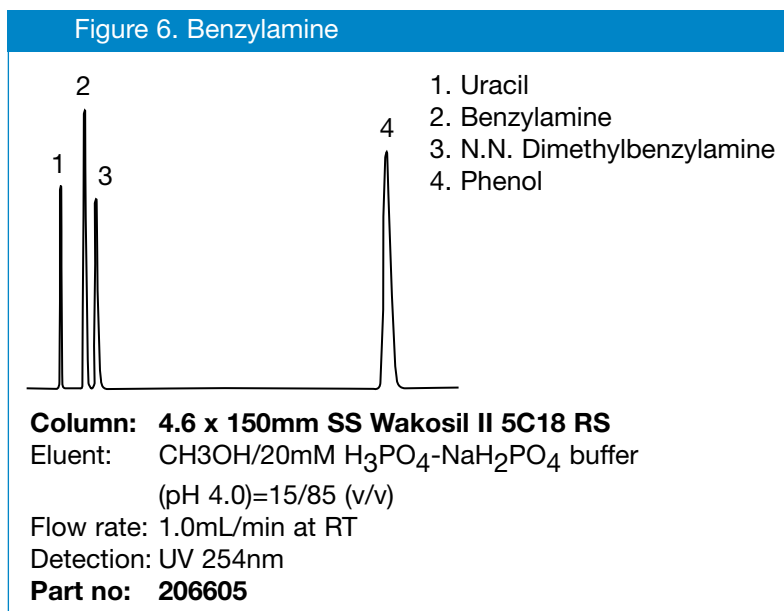


Table 1

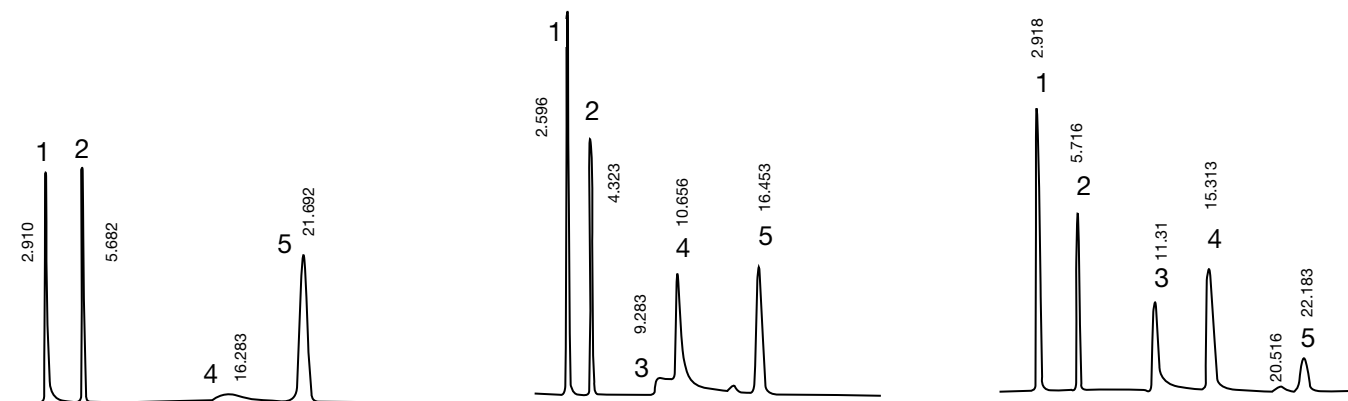
Metal Content of Wakosil II versus Common Silica								
Silica	Na	K	Mg	Al	Fe	Ca	Ti	Zn
Wakosil II	5ppm	1ppm	3ppm	7ppm	6ppm	4ppm	1ppm	1ppm
Common	37-4220ppm	4-64ppm	6-64ppm	20-150ppm	20-303ppm	6-444ppm	ND	ND

ND = Not Determined

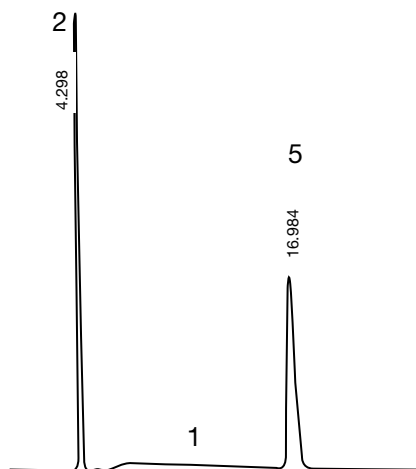
Wakosil II 5C18 RS has obvious advantages over standard HPLC columns and this is borne out by its chromatographic performance. It can be shown that Wakosil II 5C18 RS also has superior performance to other high purity silica columns. Acetylacetone, 8-quinolinol, and *N*-benzoyl-*N*-phenylhydroxylamine are very prone to tailing. The chromatograms in **Figure 7** compare how well Wakosil II 5C18 RS and other high purity silica based C18 columns perform this separation as opposed to a popular C18 column.

Wakosil II 5C18 RS shows some tailing of the 8-quinolinol peak. The other high purity silica based C18 columns exhibit varying degrees of tailing of the 8-quinolinol as well as varying amounts of tailing of the acetylacetone and *N*-benzoyl-*N*-phenylhydroxylamine peaks. The popular C18 column did not chromatograph the target compounds at all due to silanol interactions. This comparison illustrates the importance of high purity silica, and is also an indication of the importance of endcapping. The superior performance of Wakosil II 5C18 RS in comparison to that of other high purity silica C18 columns, is directly attributable to its superior endcapping technology.

Figure 7. 8-Quinolinol Analysis

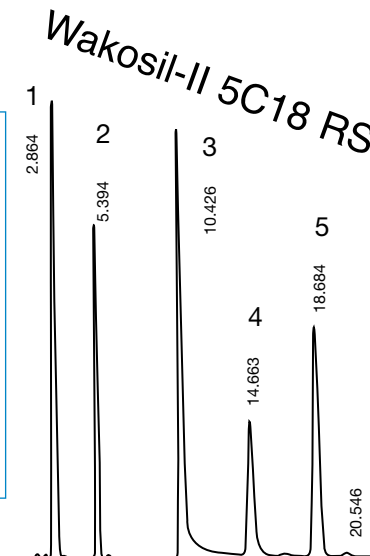


These high purity silica packings show varying degrees of tailing of 8-Quinolinol & other problem compounds



This common C18 cannot analyse 8-Quinolinol & other difficult compounds.

Eluent: MeOH/20mM K2HPO4 (pH 7.6) = 40/60 (v/v)
 Flow Rate: 1.0mL/min at 30°C
 Detector: UV 254nm 0.16 AUFS
 Instrument: Shimadzu LC-6A
 Column Size: 4.6mm ID x 150mm
 Sample: 1) Acetylacetone (2,4-Pentadione)
 2) Phenol
 3) 8-Quinolinol (Oxide)
 4) N-Benzoyl-N-Phenylhydroxylamine
 5) Benzene
 Sample Size: 15µL



Wakosil II 5C18 gives the best performance for 8-Quinolinol analysis.

Wakosil II 5C18 RS are highly endcapped C18 HPLC columns based on a high purity spherical silica support. These columns feature a highly uniform surface coverage of C18 for better chromatography. Wakosil II 5C18 RS comes in SGE's unique column format to ensure optimum column efficiencies. This means that Wakosil II 5C18 RS is now available in glass lined tubing for the true metal-free analysis. SGE also supplies Wakosil II 5C18 RS in stainless steel for routine HPLC work. The advantages of SGE's Wakosil II 5C18 RS columns make them the first choice for reversed phase chromatography.

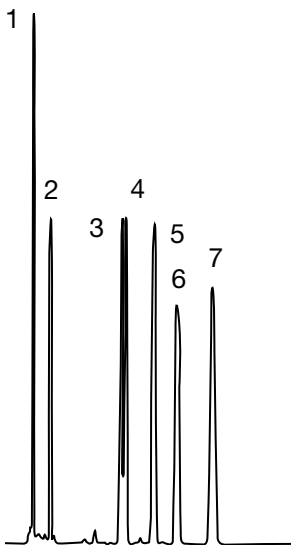
Wakosil II C18 RS Specifications

- Particle Size: 5µm and 3µm
- Pore Size: 120Å (by BET)
- Pore Volume: 1.1mL/g (by BET)
- Specific Surface Area: 350m²/g (by BET)
- Carbon Coverage: 17% (by Elemental Analysis)
- Mechanical Stability: 600Kg/cm²

NEW

Wakosil II 3C18 RS is now available with the same chromatographic characteristics as its 5 micron counterpart. This means higher efficiency 3 micron Wakosil II C18 columns are available for LC-MS and other demanding analyses.

Vitamins - Fat Soluble

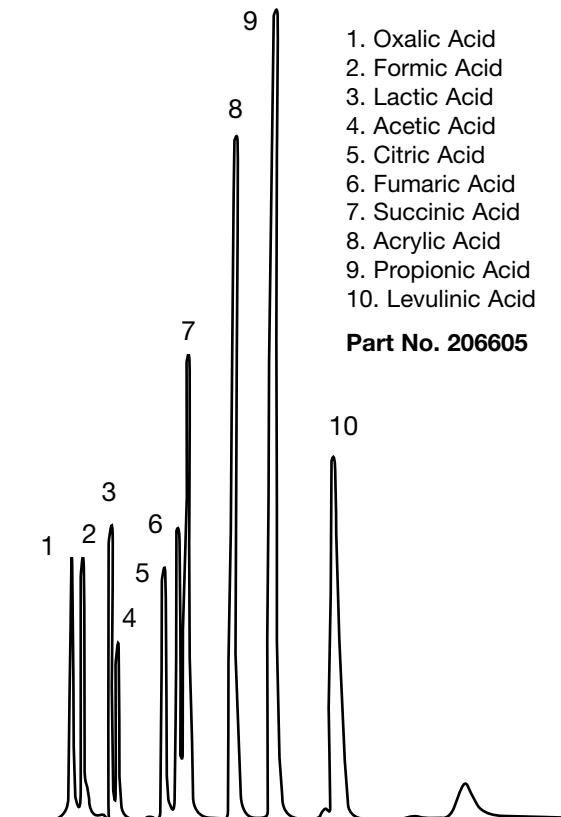


1. Vitamin K3
2. Vitamin A
3. Vitamin D2
4. Vitamin D3
5. Vitamin E
6. Vitamin E Acetate
7. Vitamin K1

Part No. 206605

Column Size: 4.6 x 150mm
Eluent: 25% Methanol, 75% Acetonitrile (v/v)
Flow Rate: 1.0mL/min at 40°C
Detector: UV 280nm

Organic Acids

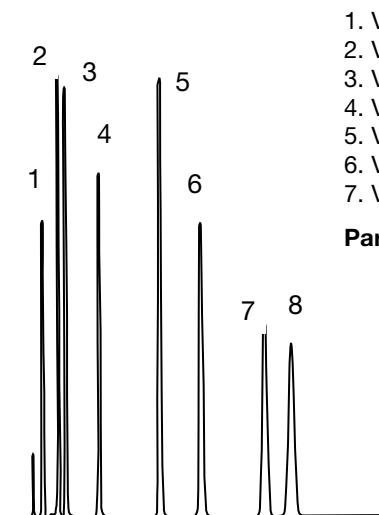


1. Oxalic Acid
2. Formic Acid
3. Lactic Acid
4. Acetic Acid
5. Citric Acid
6. Fumaric Acid
7. Succinic Acid
8. Acrylic Acid
9. Propionic Acid
10. Levulinic Acid

Part No. 206605

Column Size: 4.6 x 150mm
Eluent: 20mM H₃PO₄-NaH₂PO₄ buffer (pH2.5)
Flow Rate: 1.0mL/min at 30°C
Detector: UV 210nm

Vitamins - Water Soluble

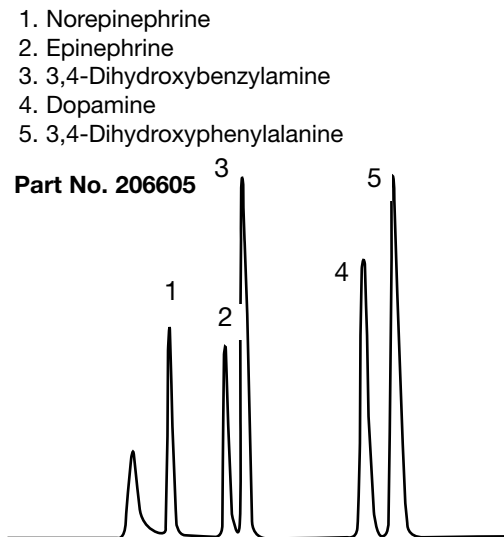


1. Vitamin K3
2. Vitamin A
3. Vitamin D2
4. Vitamin D3
5. Vitamin E
6. Vitamin E Acetate
7. Vitamin K1

Part No. 206605

Column Size: 4.6 x 150mm
Eluent: CH₃CN/0.1% H₃PO₄/5mM Hexane-sulfonic acid
Na salt=10/90 (v/v)
Flow Rate: 1.0mL/min at 40°C
Detector: UV 210nm

Chatecholamine



1. Norepinephrine
2. Epinephrine
3. 3,4-Dihydroxybenzylamine
4. Dopamine
5. 3,4-Dihydroxyphenylalanine

Part No. 206605

Column Size: 4.6 x 150mm
Eluent: 50nM H₃PO₄-NaH₂PO₄ buffer (pH2.5)
Flow Rate: 1.0mL/min at 40°C
Detector: UV 235nm

Wakosil-II 5C18 RS HPLC Columns

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Ordering Information

Wakosil II™ 5C18 RS - 5 micron columns

Column Length	Stainless Steel	Glass Lined Columns			
	4.6mm ID	4.6mm ID	4.0mm ID	2.0mm ID	1.0mm ID
10mm Guard	205018	<i>use 205019</i>	205019	209205	209105
50mm	208605	208505	208405	208205	208105
100mm	206205	206905	207032	207027	207082
150mm	206605	206805	207025	207028	207081
250mm	206505	206705	207026	207029	207080

Wakosil II™ 3C18 RS - 3 micron columns

Column Length	Stainless Steel	Glass Lined Columns			
	4.6mm ID	4.6mm ID	4.0mm ID	2.0mm ID	1.0mm ID
10mm Guard	<i>use 2050192</i>	<i>use 2050192</i>	2050192	2092052	2091052
50mm	2086052	2085052	2084052	2082052	2081052
100mm	2062052	2069052	2070322	2070272	2070822
150mm	2066052	2068052	2070252	2070282	2070812
250mm	2065052	2067052	2070262	2070292	2070802



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