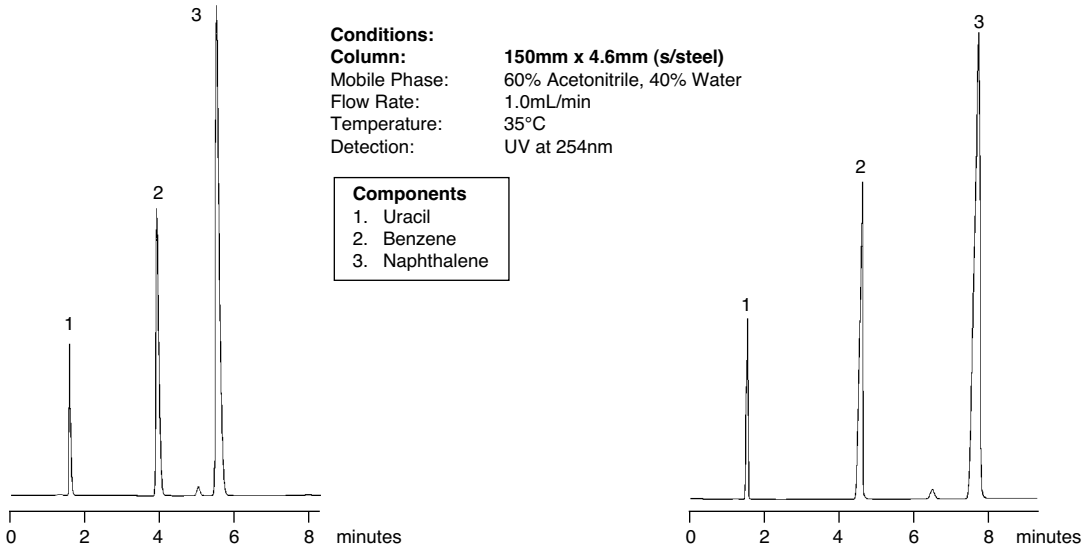
Wakosil II™ 5C8 RS

FAST REVERSE PHASE HPLC COLUMNS

C8 VERSUS C18

It is common knowledge that C8 columns exhibit lower retention of molecules with hydrophobic character, than their C18 counterparts. This is illustrated by the simple comparison in **Figure 1.** This phenomenon can be very useful to chromatographers, as the use of C8 columns can lead to significant time savings.

Figure 1: Retention comparison



HIGH PURITY SILICA WAKOSIL II 5C8 RS

Basic compounds have a tendency to exhibit extreme tailing on reverse phase columns due to strong secondary interactions with the underlying silica support. Wakosil II 5C8 RS columns avoid these secondary interactions, thereby ensuring excellent peak shapes for basic compounds as shown by the sharp pyridine peak in **Figure 2.** Pyridine has a strong affinity for active sites found on silica and will exhibit severe tailing on many reversed phase columns unless strong buffers are used. Wakosil II 5C8 RS is able to give a sharp pyridine peak even though no buffer is used. This excellent chromatography is made possible by Wakosil's high purity silica support that has minimal surface impurities. Wakosil II 5C8 RS is highly deactivated and is also thoroughly endcapped by proprietary processes. This makes Wakosil II 5C8 RS columns ideal for LC/MS applications as strong buffering of mobile phases is not required.

Figure 2: Pyridine Test

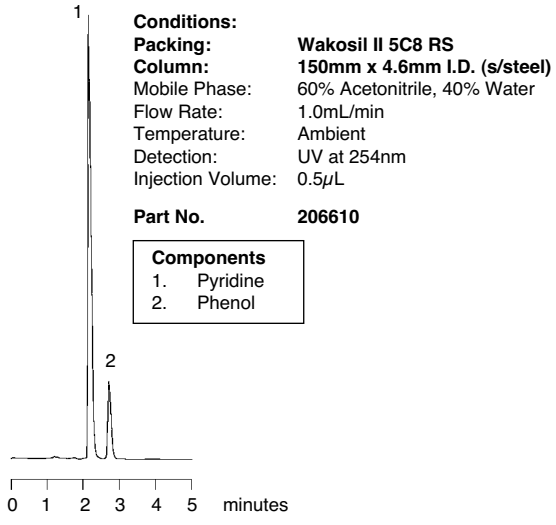
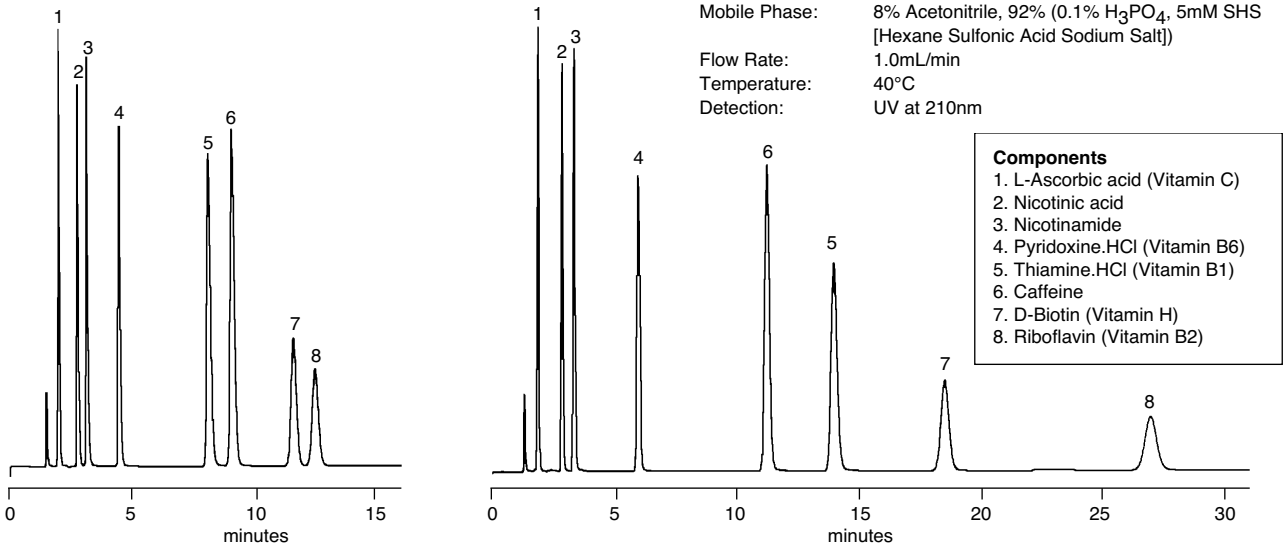


Figure 3: Water Soluble Vitamins



Wakosil II 5C8 RS Part No. 206610

Wakosil II 5C18 RS Part No. 206605

METHOD DEVELOPMENT

Any chemist who does not have access to a HPLC-MS system, will be familiar with the time consuming task of injecting individual standards and adjusting conditions to determine the elution order. This process can be streamlined considerably by the use of a C8 column. **Figure 3** compares the analysis of Water Soluble Vitamins on Wakosil II 5C8 RS and Wakosil II 5C18 RS columns.

On the C8 column, the retention times of Thiamine, Biotin, and Riboflavin are about half that of the C18 column. This translates to a time saving of many hours during method development and validation. Of course the greatest time saving is the shorter analysis time using the C8 column for the routine analysis.

Figure 4: Nicotinic Acid Derivatives

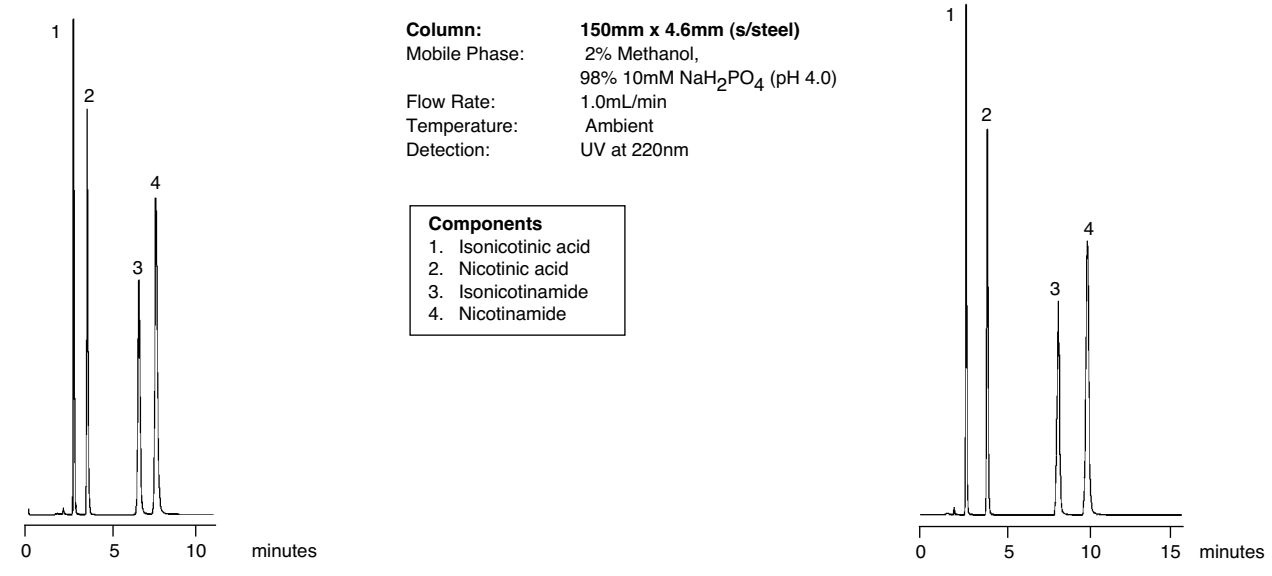


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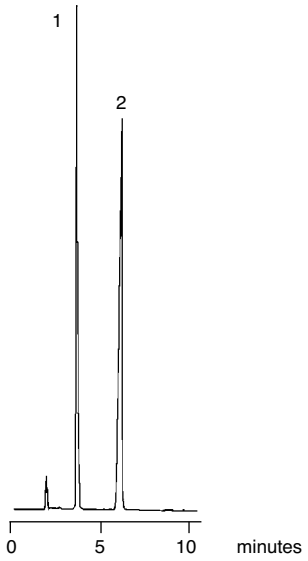
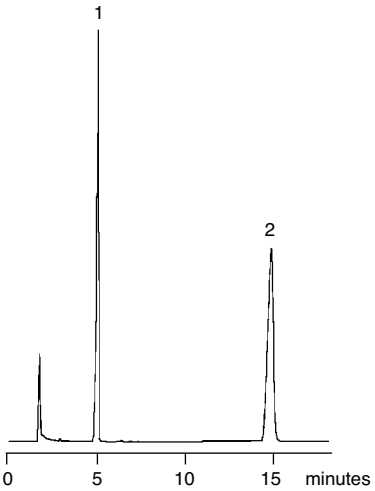
Figure 5: Glutathiones



Column: **150mm x 4.6mm (s/steel)**
Mobile Phase: 50mM $NaClO_4$, 0.1% H_3PO_4
Flow Rate: 1.0mL/min
Temperature: 40°C
Detection: UV at 215nm

Components
1. Glutathione, Reduced Form
2. Glutathione, Oxidized Form



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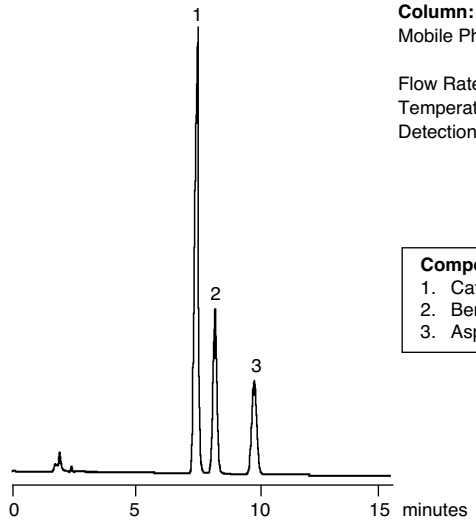
C8 SELECTIVITY

Chromatographers generally expect the same elution order for C8 and C18 columns. In most cases this holds true (**see Figures 4 to 7**) but there are also cases where the selectivity is quite different as illustrated in **Figure 3**. In this case the thiamine and caffeine peaks elute in reverse order on the C8 column. The difference in selectivity can also be an advantage as shown in **Figure 8**. Here, the Salicylic Acid and Vanillic Acid peaks are separated by the C8 column but not by the C18 column. The C8 column also has the advantage of a reduced run time. For some analyses C8 columns have the potential to act as confirmation columns without changing the mobile phases.

Figure 6: Food Additives in a Diet Drink



Column: **150mm x 4.6mm (s/steel)**
Mobile Phase: 10% Acetonitrile, 90% 0.1M KH_2PO_4
Flow Rate: 1.0mL/min
Temperature: 35°C
Detection: UV at 210nm

Components
1. Caffeine
2. Benzoic Acid
3. Aspartame

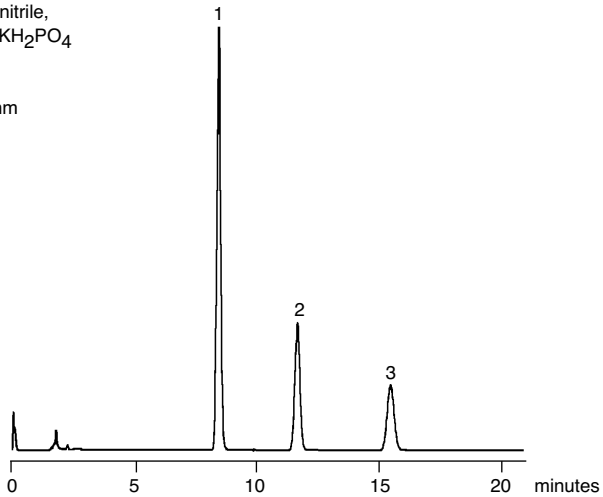


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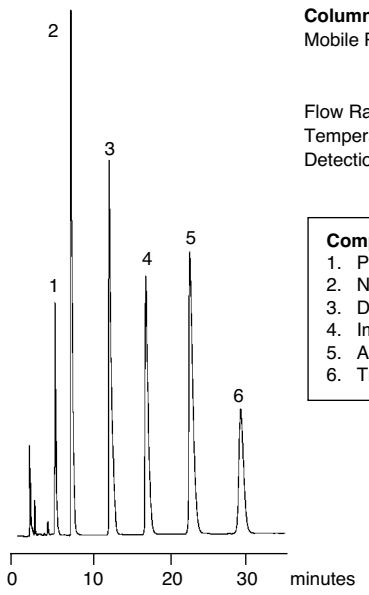
Figure 7: Tricyclic Antidepressants

Column: **150mm x 4.6mm (s/steel)**
Mobile Phase: 62% Methanol, 38% 20mM K_2HPO_4, KH_2PO_4 (pH 6.5)
Flow Rate: 1.0mL/min
Temperature: 40°C
Detection: UV at 254nm

Components
1. Protriptyline HCl
2. Nortriptyline HCl
3. Doxepin HCl
4. Imipramine HCl
5. Amitriptyline HCl
6. Trimipramine Maleate



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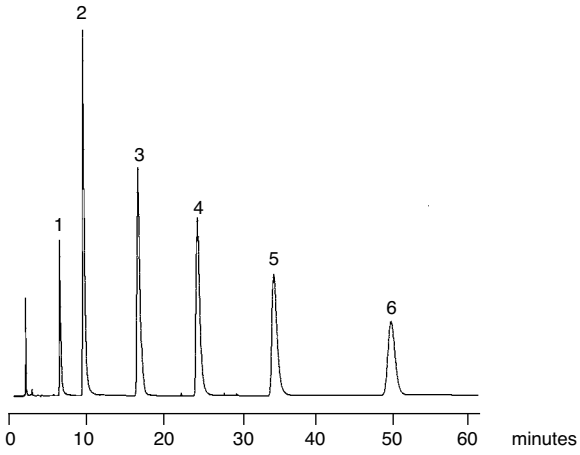

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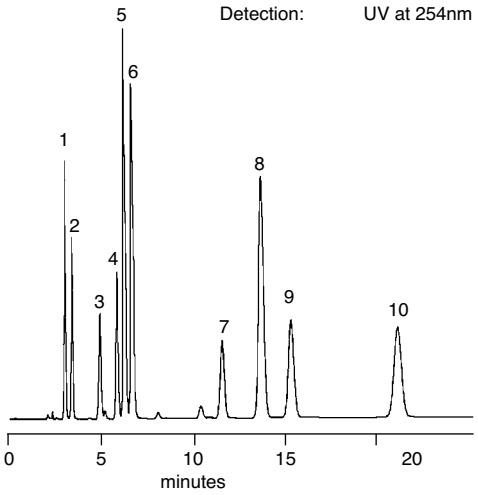
WAKOSIL II™ 5C8 RS - FAST REVERSE PHASE HPLC

The faster separations that are possible with C8 columns are illustrated by **Figures 3-8.** Wakosil II 5C8 RS columns enable chromatographers to increase their productivity by shortening run times during method development and routine analysis. Analysis of samples that are difficult to chromatograph, such as basic compounds, is made easier by the highly deactivated nature of Wakosil II 5C8 RS columns. This allows sharp peaks to be achieved without the use of strong buffers, further streamlining the method development process. The high deactivation of Wakosil II 5C8 RS also makes these columns ideal for LC-MS analysis.

Figure 8: Aromatic Organic Acids

Column: **150mm x 4.6mm (s/steel)**
Mobile Phase: 10% Acetonitrile, 90% 50mM KH_2PO_4, H_3PO_4 (pH 4.0)
Flow Rate: 1.0mL/min
Temperature: 40°C
Detection: UV at 254nm

Components
1. 3,4-Dihydroxyphenylacetic acid
2. Gentisic acid
3. p-Hydroxyphenylacetic acid
4. Salicylic acid
5. Vanillic acid
6. Caffeic acid
7. p-Coumaric acid
8. m-Coumaric acid
9. Ferulic acid
10. o-Coumaric acid



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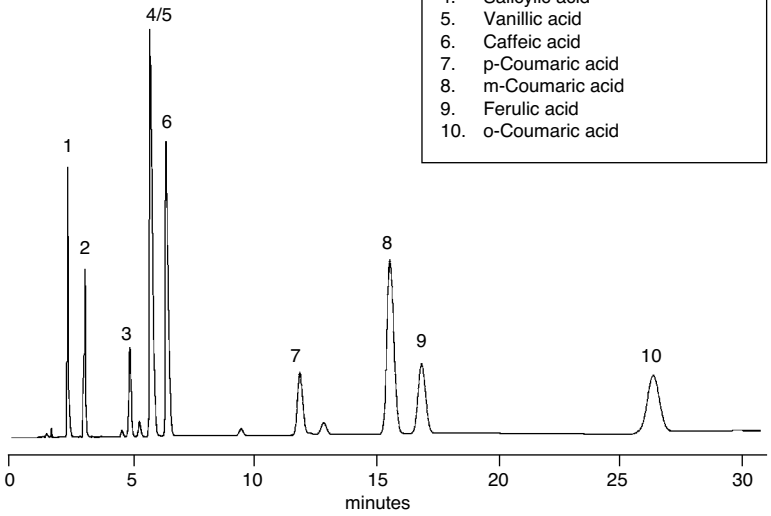

Wakosil II 5C18 RS Part No. 206605

Wakosil II™ 5C8 RS

FAST REVERSE PHASE HPLC COLUMNS

ORDERING INFORMATION -WAKOSIL II 5C8 RS

Column Length	Stainless Steel 4.6mm ID	Glass Lined Columns 4.6mm ID	4.0mm ID	2.0mm ID	1.0mm ID
10mm Guard	use 205016	use 205016	205016	209210	209110
50mm	208610	208510	208410	208210	208110
100mm	206210	206910	207132	207137	207182
150mm	206610	206810	207131	207136	207181
250mm	206510	206710	207130	207135	207180

WAKOSIL II 5C8 RS - VITAL STATISITCS

Particle Size:	5 microns
Pore Size:	120Å
Pore Volume:	1.1 mL/g
Specific Surface Area:	350 m^2/g
Carbon Coverage:	10%
Phase Bonding:	Monomeric
pH range:	2 - 7.5
Mechanical Stability:	600 kg/cm^2
Endcapping:	ultrar endcapped

For further information on Wakosil II 5C18 HPLC columns please request publications: PD-0168-H and PD-0154-H or see web site.

www.sge.com

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