

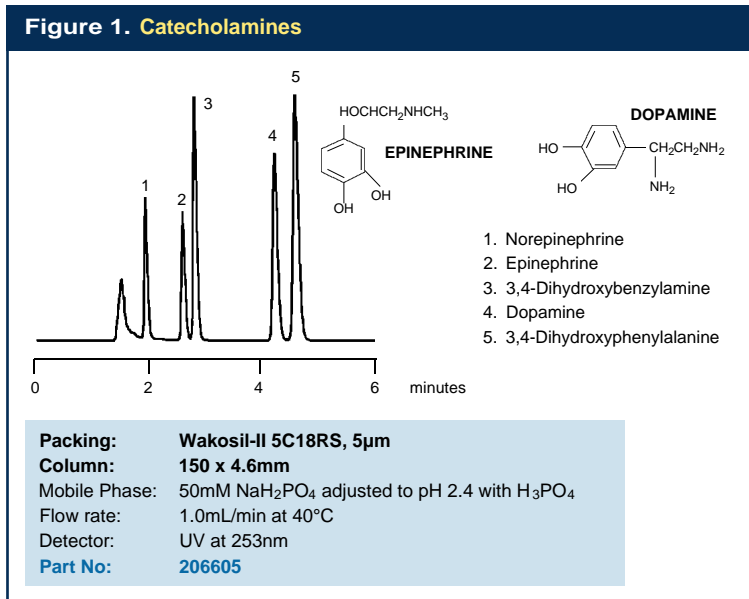
Wakosil II™ 5C18RS

Pharmaceutical Analysis by HPLC

The right HPLC column is a key component in quality control procedures for pharmaceutical products. The columns must be able to reproducibly and reliably separate the components of the typically difficult samples (Figure 1).

SGE Wakosil II 5C18 RS columns have all the features to make it the ideal packing for many pharmaceutical analyses as it provides better resolution and superior longevity to common HPLC packings.

Peak shape is another vital factor to enable accurate quantification of products and more importantly, impurities. This is especially essential for drug development work where extensive testing is carried out to analyse metabolites, side products and other impurities. Most HPLC columns would be expected to achieve this kind of performance, but unfortunately many



pharmaceutical compounds exhibit signs of tailing on common HPLC columns. In some cases the analytes cannot even be seen!

In theory the surfaces of the silica supports used for HPLC packings are totally covered with the bonded phase, usually

octadecylsilane. In practice the silica surface is littered with metal impurities and active silanols. These active groups interact strongly with the amino, carboxylic acid, hydroxy and other polar groups within analytes to cause tailing as in Figure 2 where Procainamide Hydrochloride, a cardiac depressant, shows excessive tailing and adsorption in the column. These same functional groups are also integral parts of molecules with biological activity, and as such are of greatest interest to the pharmaceutical industry. The manufacturers of most HPLC packings attempt to screen these active groups with short chain alkanes to prevent any interaction. The example in Figure 2 shows that this approach doesn't always work. Combining the use of a high purity silica, a surface treatment to remove excess silanols, and the latest endcapping technology,

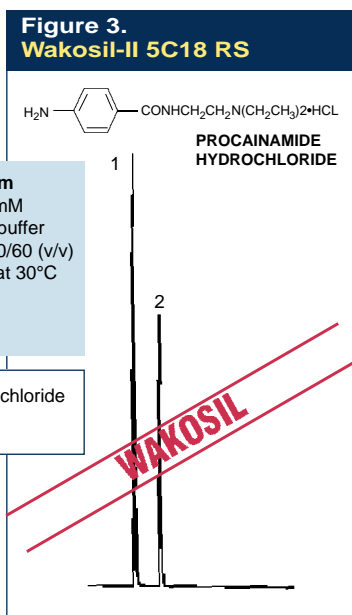
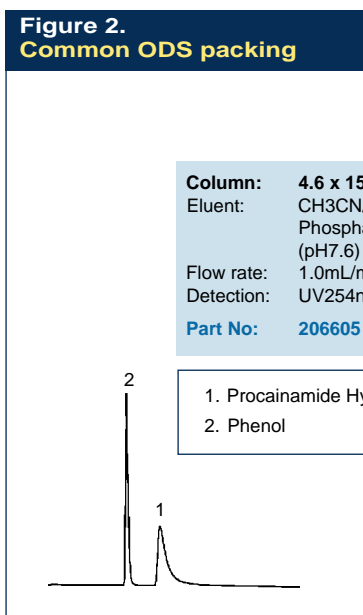
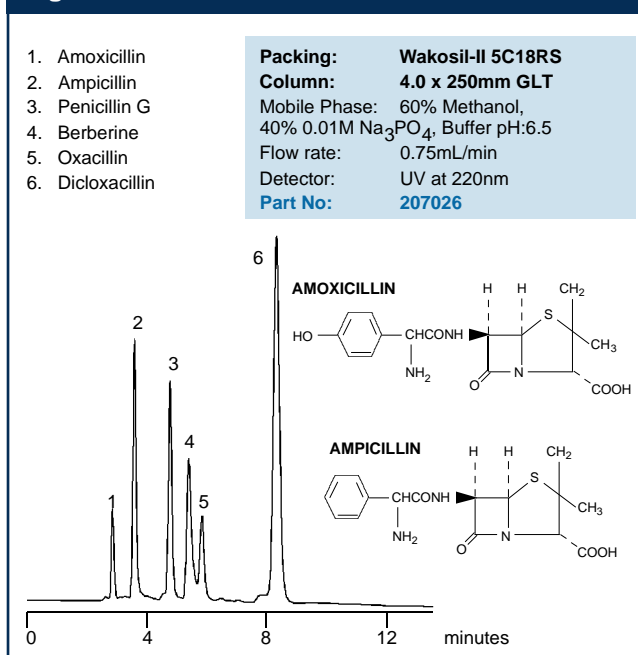


Figure 4. Anti-bacterials Determination



WAKOSIL II™ 5C18-RS COLUMNS ORDERING INFORMATION

Column ID:	4.6mm ID S/Steel		4mm Glass Lined Tubing	
Length:	150mm	250mm	150mm	250mm
Part No.	206605	206505	207025	207026

WAKOSIL II™ 5C18 RS PACKING

PROPERTY	EFFECT	RESULT
High purity base silica	- fewer active silanols - fewer surface impurities	- Less tailing of basic compounds - Less tailing of chelates - Larger pH operating range - Longer column life
Super-endcapping	- very few available silanols - very few available impurities	- Less tailing of basic compounds - Less tailing of chelates - Larger pH operating range
Uniform C18 surface coverage	- High hydrophobicity - Less interaction with base silica	- Greater reproducibility - Sharper peaks
Carbon Coverage: 17%	- High hydrophobicity - Less interaction with base silica	- Higher retention of non-polar analytes - Greater selectivity
Uniform spherical silica surface	- Even packing bed	- Higher efficiencies - Better peak symmetry - Longer column life
Pore size: 120 Å	- Enables larger molecules to enter Wakosil particles	- Suitable for the analysis of a large range of compounds
Specific surface area: 350 m ² /g	- Allows high carbon coverage	- See "Carbon Coverage"
Mechanical stability: to 600Kg/cm ²	- Withstands high backpressures	- Longer column life - High flow rates possible

Most importantly SGE is an ISO 9002 accredited company so all packing and testing procedures are documented to guarantee the reproducibility of all SGE Wakosil II 5C18 RS columns making them a perfect choice for method development and validation.

minimises the number of available active groups on the silica surface. This results in the chromatographic performance illustrated in **Figure 3** where Procainamide Hydrochloride and Phenol shows no sign of tailing on an SGE Wakosil II 5C18 RS column.

In general, pharmaceutical compounds have complex structures which can make them easy to separate, but within a group of compounds the differences can be very subtle, making separation difficult. For a HPLC column to efficiently separate similar compounds, it must have a high efficiency with respect to the target analytes. To achieve this the packing must have certain features. It must be spherical with a small particle size distribution so that when packed a uniform packing bed is formed to allow an even flow of mobile phase through the column. The HPLC packing must have a uniform surface coverage with its

bonded phase. Ideally the packing will have a pore size that is suitable to allow easy penetration by the analyte molecules to maximise the interaction between them and the bonded phase. For most pharmaceutical compounds a pore size between 100 and 150Å is optimal. **Figure 4** illustrates the separation of a series of penicillins by a SGE Wakosil II 5C18 RS column. In this case there is good separation between each penicillin despite their similar structures. Berberine, another anti-bacterial, is the only compound to elute close to another peak (Oxacillin) but its structure is unrelated and there are other coincidental chromatographic factors that cause Berberine to elute close to Oxacillin.

The nature of most pharmaceuticals necessitates the use of buffers to resolve the analytes. This can lower column lifetimes as the silica support is slowly dissolved. There are several factors that improve column lifetimes. A stable packing bed is necessary as any settling will cause a void. As mentioned previously, spherical packing with a small particle size distribution will give a stable bed. As buffers attack the silica support at its active sites, a high purity silica with thorough endcapping is far more stable than conventional silicas. Uniform phase loading also contributes to the longevity of a HPLC packing as the bonded phase helps screen the silica support from the influence of any buffer. Thus, all the characteristics that contribute to better chromatography of pharmaceutical compounds also act to increase column durability.