TECHNICAL NOTE

Liquid Chromatography

A Comparison of Quasar C18 and Inertsil ODS-3 HPLC Columns

Introduction

The most commonly used LC reversed-phase alkyl bonded stationary phase is octadecyl carbon chain (C18)-bonded silica, which is denoted as USP classification L1. C18 columns have a broad applicability from pharmaceuticals to food and environmental analyses. However, not all C18 columns are alike. Simply swapping a C18 column from one manufacturer to another can result in differences in retention time, resolution and even selectivity. Differences can arise due to variations in hydrophobicity, silanol activity, packing quality, particle size distribution, and silica purity, to name a few.[1]

This technical note provides details of a comparative study between PerkinElmer Quasar™ C18 and GL Sciences Inertsil ODS-3 silica-based columns (150 x 4.6 mm, 5 µm), covering the following areas:

- Relative Hydrophobicity
- Efficiencies for a Neutral Compound
- Evaluation of Silanol Activity
- Application example – analysis of clotrimazole

LC columns always have several parameters quoted including pore size, carbon load, and surface area. Table 1 shows the LC column stationary phase specifications for the two columns evaluated. It is important to note that, without chromatographic evaluation, these parameters will not provide enough information alone to accurately predict differences in selectivity and retention.

Table 1. LC column stationary phase specifications for Quasar C18 and Inertsil ODS-3 (150 x 4.6 mm, 5 µm) columns.

Column	Carbon Load (%)	Pore Size (Å)	Surface Area (m^2/g)	End Capping	Silica Type (A/B)
Quasar C18	17.0	100	380	Yes	B
Inertsil ODS-3	15.0	100	450	Yes	B

Relative Hydrophobicity

Hydrophobicity is a measure of the bonded stationary phase's ability to hydrophobically interact with analyte carbon groups. The retention factor (k) is a method of evaluating how much interaction the analyte has had with the alkyl chain. k is calculated using the retention time of the analyte and the time at which an unretained compound elutes (e.g. uracil in reversed-phase HPLC) (Equation 1). Calculating the k value for hydrophobic/neutral compounds is a good marker of hydrophobicity.

Low k values indicate little to no retention has taken place, which can be problematic. In cases such as these, potential impurities can easily co-elute, resulting in poor selectivity. Higher k values indicate that the analyte is more highly retained. A stationary phase with greater hydrophobicity will experience greater analyte retention (thus greater k) for a hydrophobic compound.[2,3] Excessive retention reduces sample throughput, with increased analysis times, and the peak height will also decrease as peak bandwidth increases.[3]

Some benefits of increased retention include:

- Greater opportunity to resolve analytes without increasing column length.
- Water content of the mobile phase does not need to be increased to improve retention, yielding greater MS sensitivity.

$$k = \frac{(t_r - t_0)}{t_0}$$

Equation 1. Retention factor (k) equation, where t_r is the retention time of the analyte, and t_0 is the time at which the unretained compound elutes.[2]

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Figure 1 shows the chromatograms of a neutral compound test mix obtained on the Quasar C18 and Inertsil ODS-3 columns. Table 2 details the k values obtained for each of the compounds. Quasar has a highly retentive stationary phase due to the high surface area of the silica, combined with the optimized ligand bonding process applied. Both columns demonstrated similar relative hydrophobicity, with the Inertsil having slightly higher k values. However, high retention needs to be accompanied by good peak shape and it is, therefore, important to also evaluate column efficiencies.

Table 2. Retention factor (k) of neutral compounds using the Quasar C18 and Inertsil ODS-3 columns.

Column	Retention Factor, k	
	Quasar C18	Inertsil ODS-3
Uracil	0.0	0.0
DMP	0.5	0.4
Toluene	1.9	2.1
Biphenyl	4.0	4.3
Phenanthrene	5.8	6.0

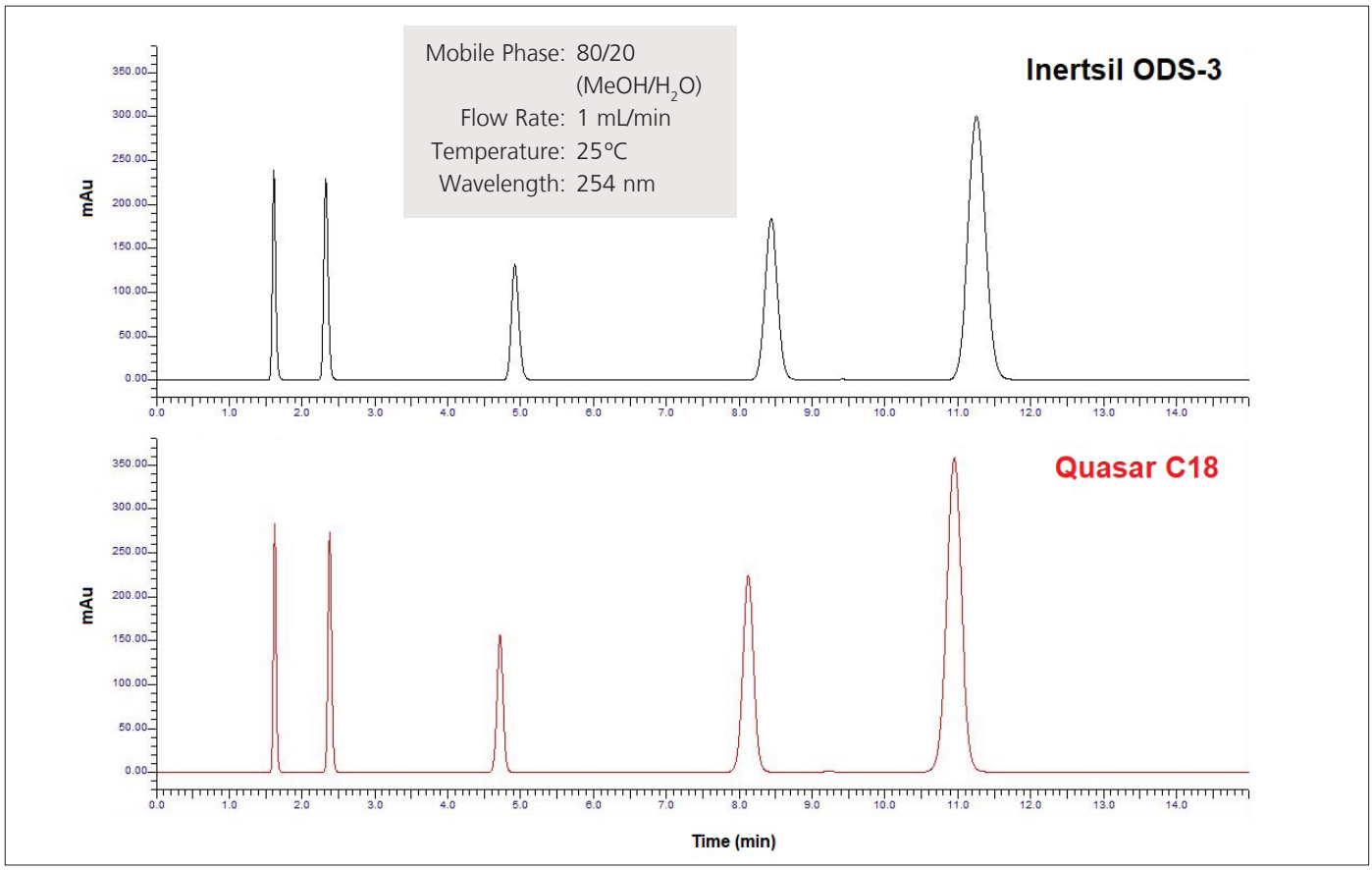

Figure 1. Chromatograms of a neutral compound test mix on Quasar C18 and Inertsil ODS-3 columns.

Column Efficiency for Neutral Compounds

High retention needs to be accompanied by good peak shape for the most effective separations. Therefore, it is important to calculate column efficiencies to gain insight into overall column performance.

Column efficiency is a measure of the peak dispersion on the column, which reflects the column performance. It is typically reported as plates (N) or plates per meter (N/m). For a given column length, a greater number of plates implies less dispersion of chromatographic bands, narrower peaks, and a more efficient separation.[4] It is important to note that later eluting peaks, which look broad in comparison with early eluting peaks, may have a higher column efficiency.[3]

Using neutral compounds for efficiency measurements allows retention from unwanted secondary interactions to be minimized. This ensures the primary interaction is the hydrophobic interaction between the alkyl chain and the neutral analyte; that in turn enables the data generated to provide a greater indication on factors such as particle size, packing efficiency, and particle size distribution. Smaller average particle sizes, higher quality packing and narrow particle size distributions all tend to result in higher efficiencies.[4]

In the hydrophobicity study, Inertsil showed to be slightly more retentive than Quasar. However, high retention needs to be accompanied by good peak shape, and the Inertsil showed much lower efficiencies when compared with Quasar (Table 3). When investigating the efficiency of toluene, Quasar demonstrated a 35 % higher efficiency in comparison with Inertsil. Figure 2 shows an example of the peak shapes obtained on the two columns for toluene. Higher efficiencies generally indicate narrower peaks, and thus allow greater scope to resolve critical pairs.

Table 3. Retention factor (k) of neutral compounds using the Quasar C18 and Inertsil ODS-3 columns.

Column	Column Efficiency (N/m)	
	Quasar C18	Inertsil ODS-3
Uracil	52674	38614
DMP	66854	47668
Toluene	99102	73250
Biphenyl	92400	69887
Phenanthrene	85369	65077

Evaluation of Silanol Activity

Using neutral compounds is useful for determining packing efficiency and efficiency of a column. However, it does not indicate the activity of the silica surface or how the column might perform when separating basic/cationic compounds. Basic compounds can frequently interact with weakly acidic underivatized residual silanols (Si-OH) on the surface of the silica particles. This unwanted secondary retention causes the analytes to be retained longer than the 'bulk' of the analyte molecules, which results in peak tailing and impacts on the separation efficiency of the column.[5]

Efficiencies and peak shapes of basic compounds are affected by silanol interactions, thus comparison of efficiency and tailing factors for a basic compound, such as pyridine (used in this study), can be used as an indicator of silanol activity. A tailing factor of one indicates a perfectly symmetrical peak, whilst peak tailing occurs when the tailing factor is >1. Phases with 'very low' levels of silanol activity tend to also have higher column efficiencies. Silica purity is also an important factor for LC columns in order to obtain peaks with little tailing and maintain high efficiency separations.[6]

Table 4 demonstrates the system suitability results of pyridine using the Quasar C18 and Inertsil ODS-3 columns. Figure 3 shows the peak shapes obtained on the two columns for pyridine. The mobile phase conditions were at near neutral pH to promote interaction between pyridine and silanols. No buffer was used in this study as its presence in the mobile phase can mask potential interactions of the silica surface and the analyte.

Table 4. System suitability results of pyridine using the Quasar C18 and Inertsil ODS-3 columns.

Column	Pyridine	
	Quasar C18	Inertsil ODS-3
Column Efficiency (N/m)*	44185	33637
Tailing Factor (5 %)	1.29	1.27
Tailing Factor (10 %)	1.21	1.21
Peak Width (50 %) (s)	3.57	4.23
Peak Height (µV)	144402	123191

*Column efficiency calculated using Foley-Dorsey method.

Both Quasar and Inertsil showed similar tailing factors at 5 % and 10 % peak height. However, Inertsil demonstrated a 24 % drop in efficiency when compared with Quasar. Additionally, the peak obtained using the Quasar column was less broad, with a smaller peak width (taken at 50 % peak height) and greater peak height, resulting in greater sensitivity. The Quasar C18 phase demonstrated excellent peak shape due to its ultra-high purity silica base and low residual silanol activity.

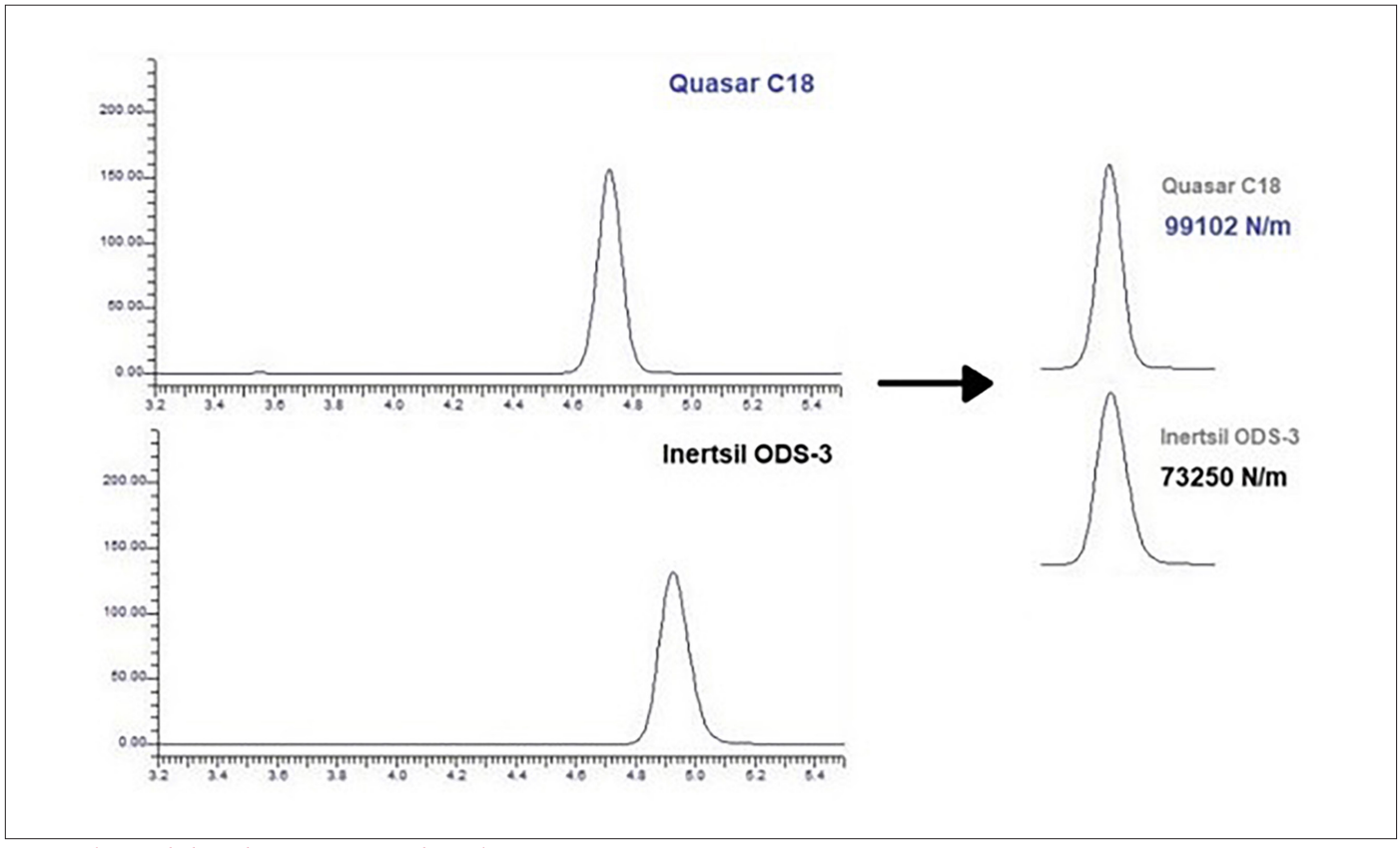

Figure 2. Toluene peak obtained using Quasar C18 and Inertsil ODS-3.

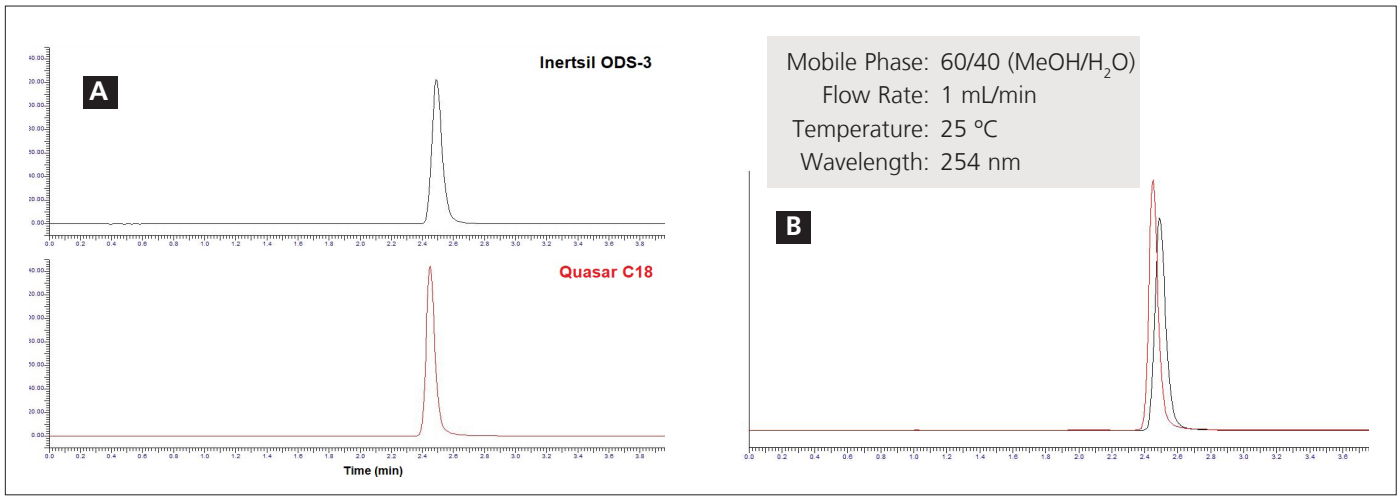

Figure 3. A) pyridine peak obtained using Quasar C18 (red) and Inertsil ODS-3 (black) columns. B) Overlay of pyridine peaks on Quasar C18 (red) and Inertsil ODS-3 (black).

Application: Analysis of Clotrimazole using Quasar C18 and Inertsil ODS-3 columns

The following application, conducted by a Pharmaceutical CRO in Chile, describes the use of a Quasar C18 (150 x 4.6 mm, 5 µm) column in comparison with an Inertsil ODS-3 (150 x 4.6 mm, 5 µm) column for the analysis of clotrimazole (Figure 4). Clotrimazole is an azole anti-fungal medication used primarily in the treatment of a wide range of fungal dermal infections.[7] All HPLC method parameters are shown in Table 5.

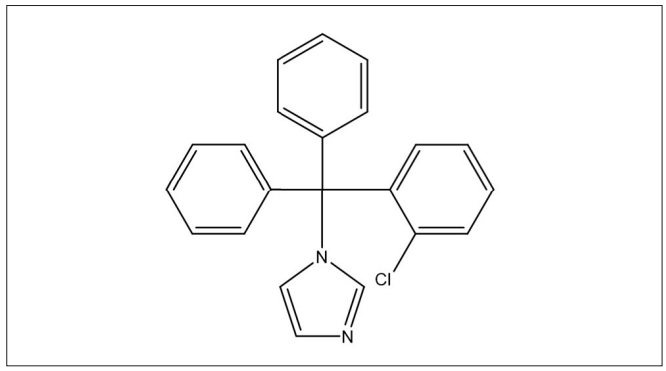

Figure 4. Chemical structure of clotrimazole.

Table 5. HPLC method parameters for analysis of clotrimazole using Quasar C18 and Inertsil ODS-3 columns.

Instrument	LC with PDA Detector			
Columns	150 mm	4.6 mm	5 µm	Quasar C18 (N9308802)
	150 mm	4.6 mm	5 µm	Inertsil ODS-3
Mobile Phase	**A:** Methanol **B:** Buffer (4.35 g/L K_2HPO_4) 78 % **A**, 22 % **B**			
Flow rate	1.5 mL/min			
Column Temperature	30 °C			
Sample Temperature	25 °C			
Wavelength	220 nm			
Injection Volume	20 µL			
Analyte	Clotrimazole (0.13 mg/mL in Mobile Phase)			

Results and Discussion

The analysis of clotrimazole using the Quasar and Inertsil columns is shown in Figure 5. Table 6 details the retention times, and system suitability parameters for clotrimazole using the two different columns. The Quasar C18 phase is ideally suited to the analysis of small molecules, providing higher efficiency (calculated using the USP tangential method) and better peak shape than the Inertsil ODS-3 column for the analysis of clotrimazole. This is due to Quasar's optimized ligand bonding technology and ultra-high purity silica base, which minimizes unwanted silanol interactions. Although Quasar demonstrated a slightly lower retention factor (k) in comparison with the Inertsil ODS-3 column, this does not impact overall performance. Quasar also provided greater resolution between the analyte and solvent peaks. This was a parameter calculated as part of the company's internal protocol.

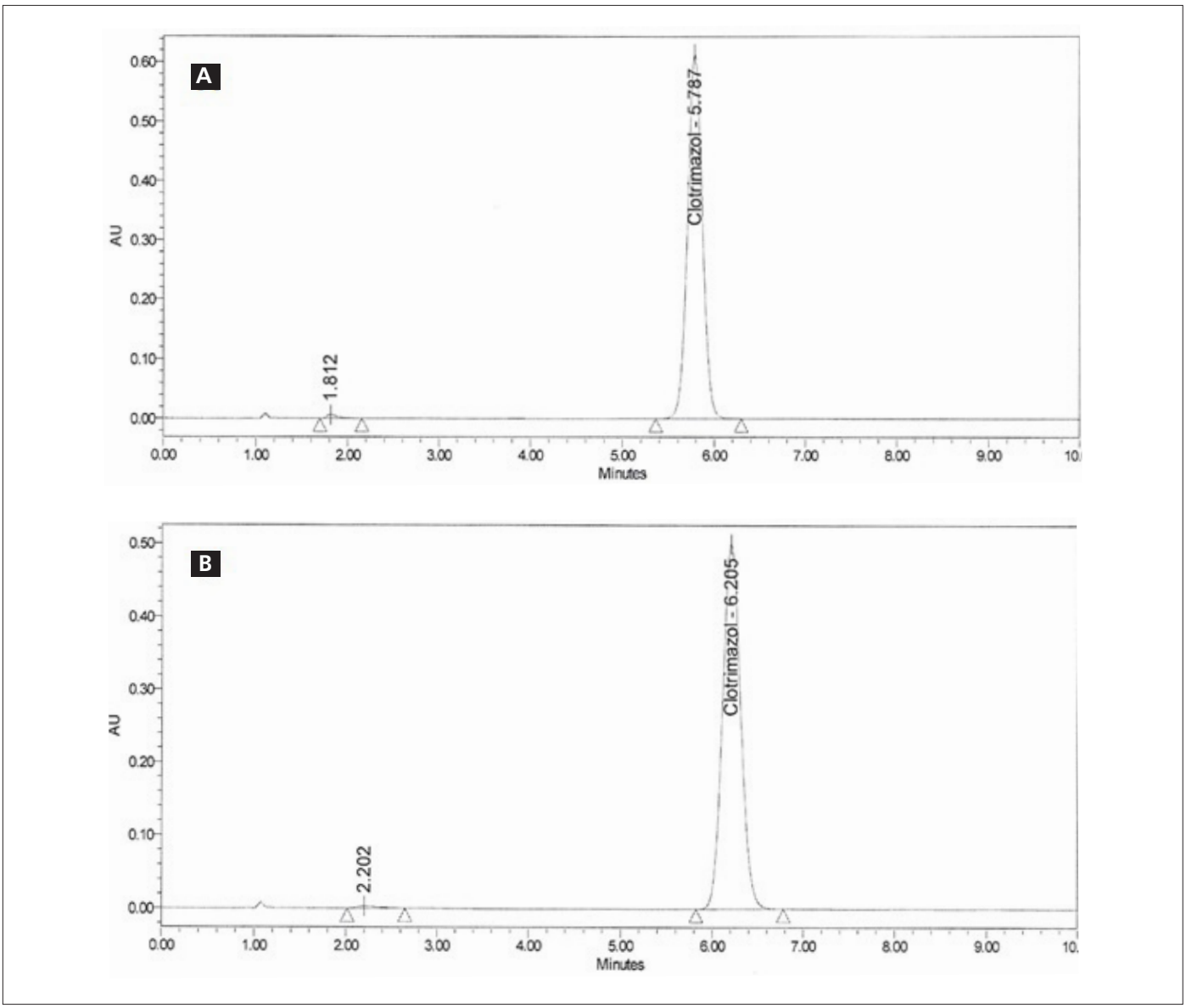

Figure 5. Analysis of clotrimazole using Quasar C18 150 x 4.6 mm, 5 µm (A) and Inertsil ODS-3 150 x 4.6 mm, 5 µm (B).

Table 6. System suitability results for the analysis of clotrimazole* using the Quasar C18 and Inertsil ODS-3 columns (150 x 4.6 mm, 5 µm).

Column	Retention Time (min)	k	Resolution	Peak Area (µV*sec)	Peak Area RSD (%)	Peak Efficiency (N)	Tailing Factor (USP)
Quasar C18	5.78	4.46	16.57	6920431	0.02	6121	1.02
Inertsil ODS-3	6.20	4.86	10.80	6926178	0.06	4591	1.10

*Taken from an average of three injections.

Conclusion

In this in-depth study, evaluation of relative hydrophobicity, efficiencies for a neutral compound and silanol activity have been investigated using PerkinElmer Quasar C18 and GL Sciences Inertsil ODS-3 columns. It has been shown that, despite being the same bonded phase, there are differences between these columns. Further information, for a variety of columns, can be found in the technical note entitled 'A Comparison of Fully Porous C18 Reversed Phase HPLC Columns'. https://www.perkinelmer.com/libraries/TCH-Comparison-of-FullyPorousC18ReversedPhase-HPLCColumns.

Both columns have shown to have similar levels of relative hydrophobicity, with Inertsil having slightly greater retention. High retention does, however, need to be accompanied by good peak shape, which Quasar has shown. Quasar demonstrated much higher column efficiencies than Inertsil for neutral compounds. Additionally, Quasar has demonstrated very high efficiency and very low peak tailing for a basic compound, pyridine, due to its ultra-high purity silica base and low residual silanol activity.

Finally, using the analysis of clotrimazole as an example application, Quasar C18 demonstrated a slightly lower retention factor but provided much higher efficiency and better peak shape than the Inertsil ODS-3.

References

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