

## Improving Peak Capacity while Maintaining Selectivity using CORTECS Columns on an Agilent 1290 LC System

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### Abstract

Columns packed with superficially porous particles have been used in many LC assays to improve peak shape and increase sample throughput. These benefits can be achieved regardless of the LC system so long as proper pairing of particle size and column dimension is taken into consideration. The work presented herein shows the benefits of a superficially porous CORTECS C<sub>18</sub> Column compared to a fully porous column with similar bonded technology on an Agilent 1290 LC System. Additionally, a competitive superficially porous column was tested. Comparisons of peak capacity demonstrate the ability of CORTECS C<sub>18</sub> Columns to provide improved resolution while maintaining selectivity versus similarly bonded superficially porous and fully porous particle technologies.

### Benefits

- Over a 10% increase in peak capacity using the CORTECS C<sub>18</sub>+ Column compared to the fully porous CSH C<sub>18</sub>
- Comparable selectivity between superficially and fully porous materials, allowing for minimal re-development of methods
- Increased peak capacity of CORTECS C<sub>18</sub>+ Column compared to a competitive superficially porous C<sub>18</sub>

## Introduction

Superficially porous particles (SPP) have become a popular technology used in liquid chromatography and consist of an impermeable center with a fully porous outer layer. This particle design was first introduced in the mid-1960s by Horvath for use in ion chromatography.<sup>1</sup> Since then, vast improvements have been made to the manufacture of these materials, including tight control over the porous layer thickness, the method of adhering the porous layer to the solid core, and the chemical nature of both the porous and non-porous layers. This particle technology provides benefits to column efficiency compared to their fully porous particle counterparts due to improvements in the A term (eddy diffusion) and B term (longitudinal diffusion) of the van Deemter equation. The A term is directly affected by the uniform particle size of a SPP compared to a fully porous particle. Uniform particle size leads to tighter packed beds with similar flow channels around and through the particles. The B term is affected by the solid core, which limits the overall permeability of the particle and reduces the volume of the column accessible to the analytes for diffusion.

The increased efficiency of these materials can help resolve critical pairs in troublesome assays.<sup>2</sup> In addition, using columns packed with superficially porous particles to modernize out-of-date monograph methods can grant more versatility in method transfer. Transferring USP monograph methods is strictly controlled by the USP general chapter <621>, wherein the new column must maintain either a  $L/d_p$  (length to particle size ratio) or  $N$  (theoretical plates) within a certain range of the original reference column. This can limit the length and size of the new column for method transfer. However, with superficially porous particles, transferring methods by  $N$  can often be employed to enhance separations by reducing run times, thereby increasing throughput and minimizing solvent consumption.<sup>3-5</sup>

In this work, we investigate a separation of six sulfa drugs using a superficially porous CORTECS C<sub>18</sub> Column. Here, we evaluate this column against a competitive superficially porous particle column and a column packed with fully porous particles, each functionalized with a C<sub>18</sub> ligand. The comparison was performed using an Agilent 1290 LC System, where the CORTECS C<sub>18</sub> Column demonstrates improved peak capacity, even over the competitive superficially porous column. Moreover, the CORTECS C<sub>18</sub> Column gave similar selectivity to the alternative columns tested, which could potentially simplify method transfer between columns.

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## Experimental

### Sample Description

Neat standards of sulfadiazine, sulfathiazole, sulfamethazine, sulfamethoxypyridazine, sulfachloropyridazine, and sulfisoxazole were made at 13 µg/mL concentrations using water as the sample diluent.

### LC Conditions

LC system:	Agilent 1290 Infinity II
Detection:	UV detection at 254 nm using a DAD
Vials:	TruView LCMS Certified Clear Glass vial (p/n:186005668CV)
Column(s):	CORTECS C <sub>18</sub> +, 2.1 x 50 mm, 2.7 µm (p/n: 186007395)  XSelect CSH C <sub>18</sub> , 2.1 x 50 mm, 2.5 µm (p/n: 186006101)  Competitor Superficially Porous C <sub>18</sub> , 2.1 x 50 mm, 2.7 µm
Column temp.:	30 °C
Sample temp.:	Ambient
Injection volume:	1 µL
Flow rate:	0.33 mL/min
Mobile phase A:	0.1% formic acid in water

Mobile phase B:

0.1% formic acid in acetonitrile

Gradient:

*See Table*

## Gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
0.00	0.33	95	5	6
6.30	0.33	40	60	6
6.90	0.33	40	60	6
7.05	0.33	95	5	6
9.00	0.33	95	5	6

## Data Management

Chromatography software:

Empower 3 Feature Release 5

Informatics:

Empower 3 Feature Release 5

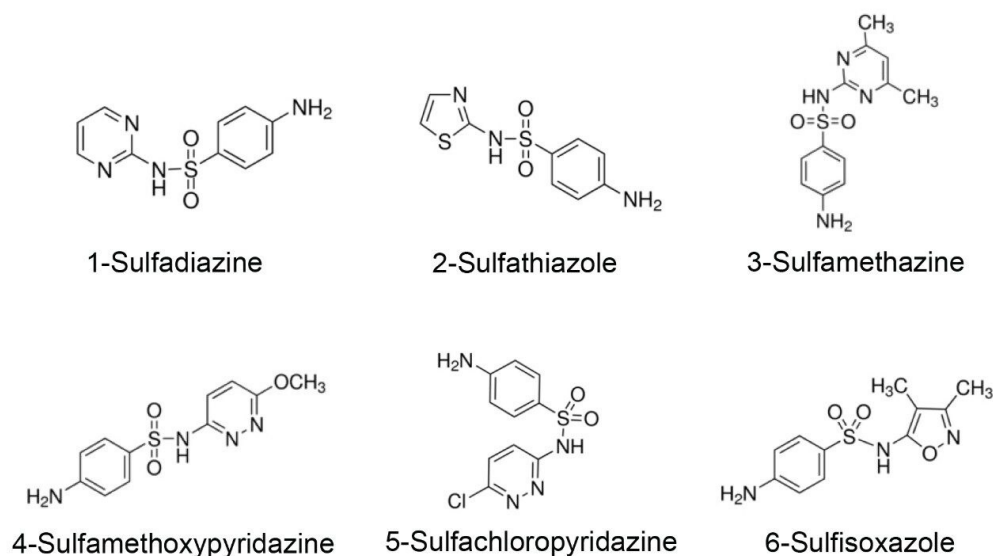


Figure 1. Chemical structures of the six sulfa drugs.

## Results and Discussion

Superficially porous particles provide many benefits for UHPLC separations, including higher efficiencies with comparable system backpressure versus fully porous particles of equivalent size. One benefit of superficially porous particles is that these particles are more uniform in size due to the nature of their manufacturing. This allows them to be packed more efficiently into a column.<sup>1</sup> Optimized packing has a significant impact on the efficiency of the columns as described by the van Deemter equation and plots for superficially porous vs fully porous particles.<sup>6-8</sup> This is due to the increased homogeneity of the superficially porous particles packed beds, which has a direct effect on column efficiency.

The van Deemter equation is used to measure the empirical additive sources of dispersion in a column. The equation is composed of three terms, A: eddy diffusion, B: longitudinal diffusion, and C: mass transfer.

Advancements in packing superficially porous particles reduce these contributions to dispersion, as associated with the A term, by creating uniform flow channels within the column. At optimal linear velocity the B term is also significantly reduced when using superficially porous particles as the impervious solid core reduces the volume available for dispersion. For small molecules, the C term, or mass transfer, is not a significant contributor, however

for the analysis of large molecules, the C term does have a more significant impact.<sup>9</sup>

An example of these benefits can be seen in the analysis of six sulfa drugs on two different superficially porous particle columns, and a fully porous particle column. All columns tested were 2.1 x 50 mm with 2.x  $\mu\text{m}$  particles. The superficially porous particles are 2.7  $\mu\text{m}$  while the fully porous particles are 2.5  $\mu\text{m}$ . The mobile phases used were water and acetonitrile each containing 0.1% formic acid. A flow rate of 0.33 mL/min and a column temperature of 30 °C was used. Gradient details are outlined in the methods section.

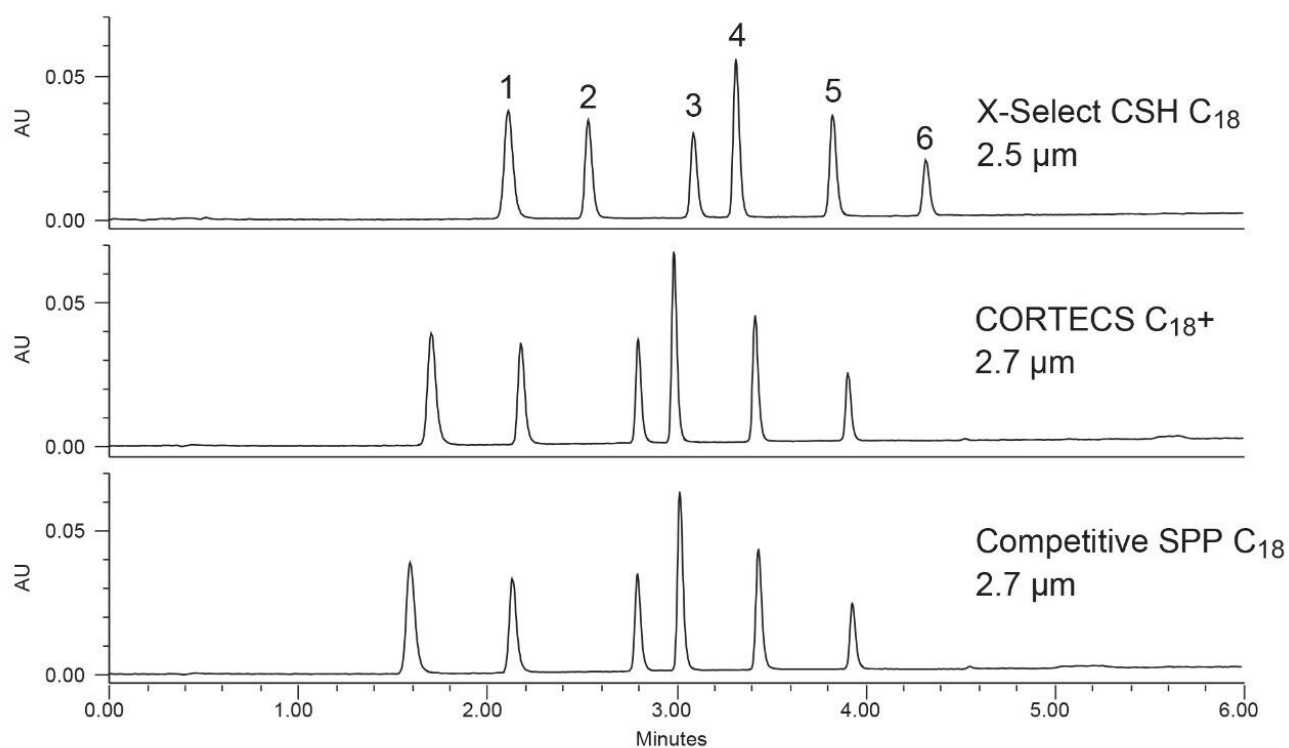


Figure 2. Separation of six sulfa drugs on three columns: XSelect CSH C<sub>18</sub>, a CORTECS C<sub>18</sub>+ and a competitive superficially porous (SPP) C<sub>18</sub>. Peak identities: 1) sulfadiazine, 2) sulfathiazole, 3) sulfamethazine, 4) sulfamethoxyipyridazine, 5) sulfachloropyridazine, and 6) sulfisoxazole.

At first glance, the separation appears comparable between the three columns. Relative retention times compared to sulfadiazine, shown in Table 1, confirm that the three materials have very similar selectivity. Both superficially porous particle columns have higher relative retention times compared to the fully porous particle column, however the values from the CORTECS C<sub>18</sub> and XSelect CSH C<sub>18</sub> Columns are closer to each other than

the competitor SPP Column. This could be due to differences in column chemistry between the three phases. However, even though differences in relative retention are seen, similar overall selectivity is seen in the chromatography.

	CORTECS C <sub>18</sub> +	Competitor SPP	CSH C <sub>18</sub>
Sulfadiazine	-	-	-
Sulfathiazole	1.28	1.34	1.20
Sulfamethazine	1.64	1.76	1.46
Sulfamethoxypyridazine	1.75	1.90	1.57
Sulfachloropyridazine	2.01	2.16	1.81
Sulfisoxazole	2.29	2.47	2.05

Table 1. Relative retention times (alpha values) using sulfadiazine as the reference peak.

The most pronounced difference in this separation can be seen by examining the peak widths of the six analytes at 4.4% peak height and using these values to determine peak capacity. Peak capacity was calculated as described in Equation 1. Peak capacity results are shown in Table 2.

$$P_c = 1 + \left( \frac{t_g}{W_{avg}} \right)$$

Equation 1. Peak capacity calculations where  $P_c$  is peak capacity,  $W_{avg}$  is average peak width at 4.4%, and  $t_g$  is gradient time.

	Avg. peak widths at 4.4% peak height (min)		
	CORTECS C <sub>18</sub> +	Competitor SPP	CSH C <sub>18</sub>
Sulfadiazine	0.111	0.114	0.114
Sulfathiazole	0.088	0.097	0.091
Sulfamethazine	0.072	0.078	0.084
Sulfamethoxypyridazine	0.068	0.074	0.079
Sulfachloropyridazine	0.073	0.078	0.086
Sulfisoxazole	0.071	0.076	0.084
Average peak width	0.0805	0.0862	0.0897
Peak capacity	79	74	71

Table 2. Average peak widths (n=3) for each analyte on each column, and calculated peak capacity using a gradient time of 6.30 minutes.

The superficially porous particle columns achieve higher peak capacity compared to the fully porous particle column. The peak capacity of the competitor SPP Column was 3% higher than that of the XSelect CSH C<sub>18</sub> Columns, while the CORTECS C<sub>18</sub> Column was approximately 11% higher. It should be noted that the CORTECS Column was able to improve the peak capacity of the separation by almost 7% compared to the other superficially porous particle column. The differences seen between these columns may be attributed to the differences in packing procedures for the two columns or the differences in particle chemistry. While not indicative of all assays, the analysis of sulfa drugs serves as an example of the benefits an analyst can obtain by using CORTECS Columns.

## Conclusion



CORTECS LC Columns, built using superficially porous silica base particles, are an ideal column choice for any LC system. Improved separation performance and increased column efficiency of superficially porous particle columns are achieved by the reduction in additive sources of dispersion from the A and B terms of the van Deemter equation. Notably, superficially porous particles can be packed more uniformly in a column, thus creating ideal conditions for reducing band-broadening and improving efficiency. All of these benefits can be examined more thoroughly by looking at van Deemter plots for superficially porous vs fully porous particles. One example of separation efficiency improvements with superficially porous particles is seen with the gradient separation of sulfa drugs. A CORTECS C<sub>18</sub> Column improved the peak capacity of the separation compared to a fully porous particle column while retaining the selectivity of the separation. Even when compared to the competitive SPP column, the CORTECS C<sub>18</sub> Column demonstrated enhanced peak capacity.

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