Application Note

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Superior Sample Capacity for Basic Compounds with a Formic Acid Mobile Phase

A comparison of the Agilent InfinityLab Poroshell 120 CS-C18 to traditional C18 columns

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Abstract

Six basic pharmaceutical compounds were separated on a formic acid and acetonitrile gradient. Two superficially porous particle C18 columns were compared: the charged surface Agilent InfinityLab Poroshell 120 CS-C18 and a traditional C18 bonded phase column. The charged surface column demonstrated better peak shape than the traditional C18 column for these basic analytes with a simple, universally compatible formic acid mobile phase. The performance of the traditional C18 column requires the use of a less desirable trifluoroacetic acid mobile phase for improvement of peak shape. Further investigation demonstrated that sample loading capacity for these basic analytes is also enhanced with the charged surface column, allowing lower limits of detection for impurity analyses.

Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. These columns are more efficient at lower pressure compared to their totally porous particle column counterparts.[1] This efficiency is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution in the column.[2]

The most popular particle size for superficially porous particle columns is 2.5 to 3 µm. These particles produce similar efficiency to traditional sub-2 µm columns, while generating approximately 50% of the backpressure. High efficiency can contribute to resolving closely eluting peaks, while low backpressure allows flexibility with LC instrumentation.

Agilent recently developed a new bonded phase chemistry on the 2.7 µm InfinityLab Poroshell 120 particles. This phase was created by applying a positive charge to the silica surface, then functionalizing the particle with a C18 bonded phase. The Agilent InfinityLab Poroshell 120 CS-C18 column provides enhanced loadability and peak shape for basic analytes under weak ionic strength mobile phase conditions, such as formic acid. Formic acid is an ideal mobile phase modifier, creating simple and highly reproducible conditions for LC analysis. Formic acid allows highly transferable methods across LC detectors, including its exceptional compatibility with LC/MS detection. The remarkably flexible 2.7 µm InfinityLab Poroshell 120 CS-C18 column can be used across many instrument and detector platforms, allowing effortless transfer between laboratories with varying instrumentation.

This study demonstrates the performance of a charged surface superficially porous particle column, the InfinityLab Poroshell 120 CS-C18 column. This column improved peak shape and loadability for basic pharmaceutical compounds compared to a traditional C18 column.

Experimental

An Agilent 1290 Infinity II LC system with an Agilent Ultivo triple quadrupole LC/MS (LC/TQ) was used in this experiment. The system was modified from its standard configuration to have lower system volume and dispersion. Table 1 shows the configuration details. Five LC columns were used in this experiment and are listed in Table 1. Tables 2 to 4 show the LC and TQ method parameters.

The six pharmaceutical compounds analyzed in this work were purchased from Sigma-Aldrich (St. Louis, MO, USA). Figure 1 shows their structures, and Table 5 displays the concentrations at which they were analyzed. Trifluoroacetic acid was also purchased from Sigma-Aldrich. Formic acid (p/n G2453-85060) and LC/MS-grade acetonitrile (p/n G2453-85050) were obtained from Agilent. Water was 0.2 µm filtered 18 MW from a Milli-Q system (Millipore, Burlington, MA, USA).

Table 1. System configuration.

Agilent 1290 Infinity II LC System Configuration	
Agilent 1290 Infinity II flexible pump (G7104A)	• Degasser • Seal wash pump • 35 µL solvent mixer: Agilent Jet Weaver, 35 µL/100 µL (p/n G4220-60006) • Firmware: B.07.23 [0009]
Agilent 1290 Infinity II vialsampler (G7129B)	• Sample thermostat (p/n G7167-60101) • Metering parameter: seat assembly PEEK 0.12 mm, sample loop 20 µL, analytical head 20 µL • Autosampler → heater: capillary, stainless steel, 0.12 × 105 mm, SL/SL (p/n 5500-1238) • Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716) • Cap, screw, blue, PTFE/red silicone septa, 100/pk (p/n 5182-0717) • Vial insert, 250 µL, glass with polymer feet, 100/pk (p/n 5181-1270) • Firmware: D.07.23 [0009]
Agilent InfinityLab LC Series integrated column compartment (G7130A)	• Integral type: G7129B • 3.0 µL heat exchanger • Heater → column: A-Line quick-connect assembly, 105 mm, 0.075 mm (p/n 5067-5961) • Column → flow cell: capillary, stainless steel, 0.075 × 220 mm, SV/SLV (p/n 5067-4784) • Firmware: B.07.23 [0009]
Agilent Ultivo LC/TQ (G6465A)	• Agilent Jet Stream ESI Source
Agilent 1290 Infinity II diode array detector (G7117B)	• Ultralow dispersion Max-Light cartridge flow cell, 10 mm, 0.60 µL (p/n G4212-60038) • UV lamp (5190-0917) • Firmware: D.07.23 [0009]
LC columns	• Agilent InfinityLab Poroshell 120 CS-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-942) • Traditional C18 on superficially porous particles, 2.1 × 100 mm, 2.7 µm • Traditional endcapped C18 on superficially porous particles, 2.1 × 100 mm, 2.7 µm • Traditional nonendcappped C18 on superficially porous particles, 2.1 × 100 mm, 2.7 µm • Traditional C18 on totally porous particles, 2.1 × 100 mm, 1.7 µm

Table 2. UHPLC method parameters.

Method	Column	Mobile Phases	Elution Conditions	Injection Volumes	Column Temperature	Detection
1	Agilent InfinityLab Poroshell 120 CS-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-942)	A: water B: acetonitrile C: 2% formic acid in water	0.4 mL/min, 21% B, 5% C, isocratic	Peak shape comparison: 1 µL of 5 µg/mL six-compound standard	30 °C	UV at 254 nm, 80 Hz ---and--- Ultivo/TQ ESI+ dMRM
2	Traditional C18 on superficially porous particles, 2.1 × 100 mm, 2.7 µm	A: water B: acetonitrile C: 2% formic acid in water	0.4 mL/min, 35% B, 5% C, isocratic			
3	Traditional C18 on superficially porous particles, 2.1 × 100 mm, 2.7 µm	A: water B: acetonitrile C: 2% trifluoroacetic acid in water	0.4 mL/min, 36% B, 10% C, isocratic			
4	Agilent InfinityLab Poroshell 120 CS-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-942)	A: water B: acetonitrile C: 2% formic acid in water	0.4 mL/min, 20% B, 5% C, isocratic	Sample loading capacity: 0.5 µL of 1 mg/mL to 0.1 ng/mL amitriptyline in water Impurity analysis: injection volume varies		
5	Traditional endcapped C18 on superficially porous particles, 2.1 × 100 mm, 2.7 µm	A: water B: acetonitrile C: 2% formic acid in water	0.4 mL/min, 32% B, 5% C, isocratic			
6	Traditional nonendcappped C18 on superficially porous particles, 2.1 × 100 mm, 2.7 µm	A: water B: acetonitrile C: 2% formic acid in water	0.4 mL/min, 32% B, 5% C, isocratic			
7	Traditional C18 on totally porous particles, 2.1 × 100 mm, 1.7 µm	A: water B: acetonitrile C: 2% formic acid in water	0.4 mL/min, 32% B, 5% C, isocratic			

Table 3. LC/TQ sources method parameters.

MS Source	Set Point
Gas Temperature	150 °C
Gas Flow	12 L/min
Nebulizer	20 psi
Sheath Gas Temperature	250 °C
Sheath Gas Flow	5 L/min
Capillary Voltage	2000 V

Table 4. LC/TQ acquisition method parameters.

Compound Name	Precursor (*m/z*)	Product (*m/z*)	Fragmentor (V)	CE (V)	Polarity
Amitriptyline	278.2	117.1	115	21	Positive
Amitriptyline	278.2	91	115	25	Positive
Desipramine	267.2	72.1	90	13	Positive
Desipramine	267.2	44.1	90	50	Positive
Doxepin	280.2	115	115	50	Positive
Doxepin	280.2	107.1	115	21	Positive
Imipramine	281.2	86.1	75	13	Positive
Imipramine	281.2	58.1	75	45	Positive
Nortriptyline	264.2	233.2	100	13	Positive
Nortriptyline	264.2	91.1	100	25	Positive
Trimipramine	295.2	100.2	90	25	Positive
Trimipramine	295.2	58.2	90	25	Positive

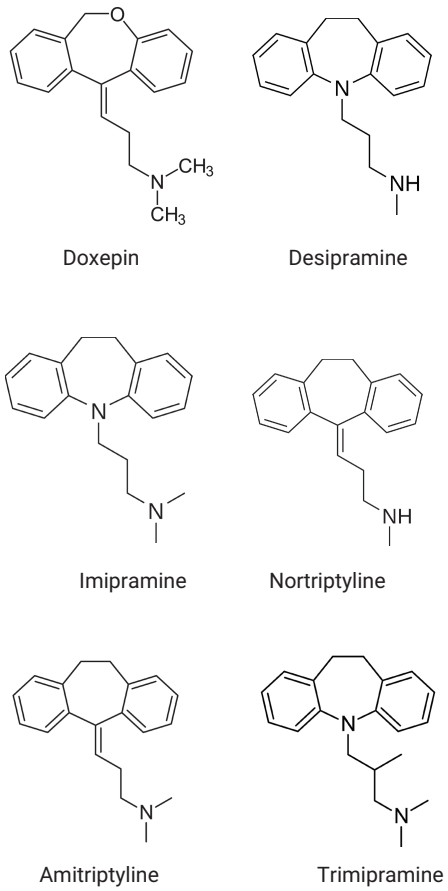

Figure 1. Compounds of interest.

Results and discussion

Figure 2 compares the performance of the first three analysis scenarios outlined in Table 2, using the six compound standard listed in Table 5. The top chromatogram shows the separation of six basic pharmaceutical compounds on the InfinityLab Poroshell 120 CS-C18 column with a simple formic acid mobile phase. The middle chromatogram illustrates the performance of a traditional C18 column with the same mobile phase. The traditional C18 column produces wider peaks than the charged surface C18. The bottom chromatogram shows that peak widths of the traditional C18 column can be improved with the use of a trifluoroacetic acid mobile phase, as shown in the chart of peak widths. Trifluoroacetic acid is often considered an undesirable mobile phase because it has an extremely low pH. The low pH can be damaging and difficult to completely clean from columns and LC systems. For LC/MS users, trifluoroacetic acid also reduces signal intensity significantly due to ion suppression.

Table 5. Standard concentrations.

Analytes (In Elution Order)	Six Compound Standard (Prepared in Water)	Impurity Standard (Prepared in Water)
Doxepin	5 µg/mL	n/a
Desipramine	5 µg/mL	n/a
Imipramine	5 µg/mL	2 µg/mL
Nortriptyline	5 µg/mL	n/a
Amitriptyline	5 µg/mL	0.8 mg/mL
Trimipramine	5 µg/mL	2 µg/mL

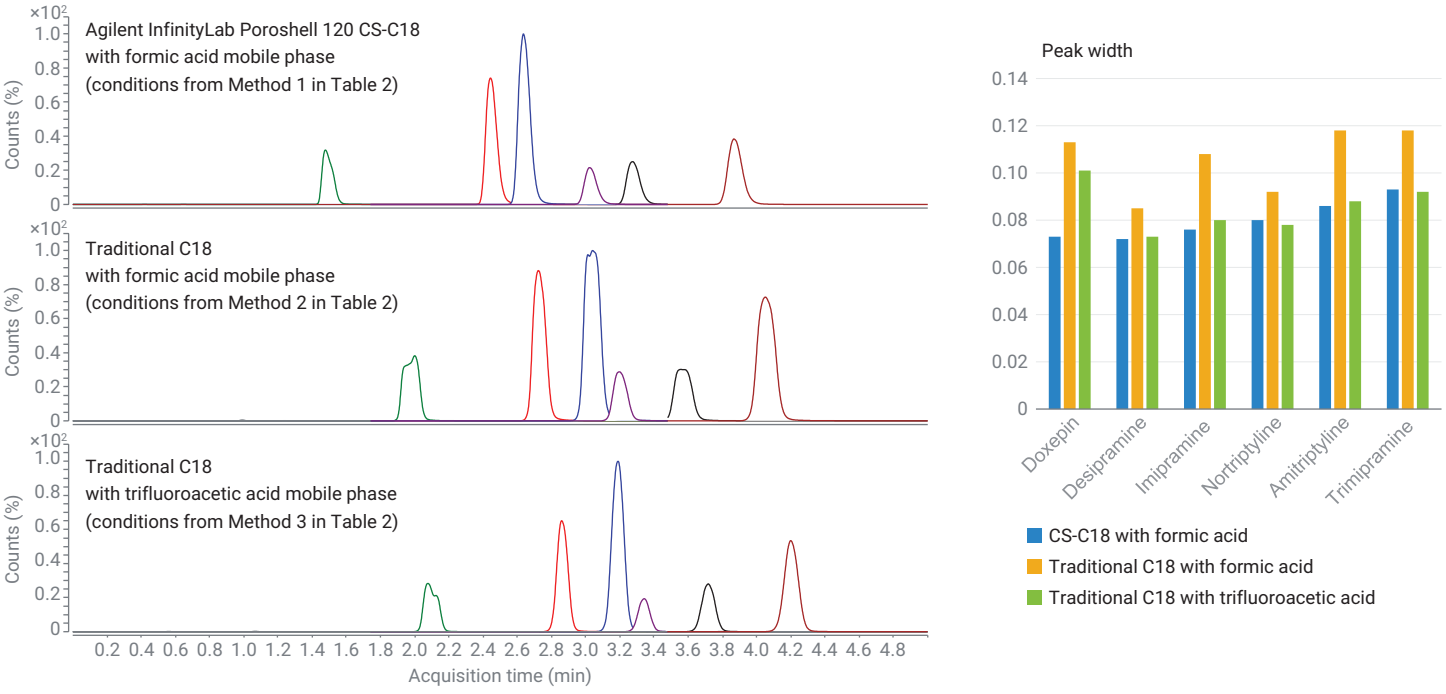

Figure 2. Comparison of chromatographic performance of basic pharmaceutical compounds.

Additional investigation into basic analyte loading capacity is shown in Figure 3. For this study, serial dilutions of amitriptyline were prepared and injected onto the four C18 columns. Peak width at half height was tracked as a measure of column performance. For each of the C18 columns used, the acetonitrile content was slightly modified to ensure similar overall retention for amitriptyline, k' = 7. Overload is assessed when the peak shape degrades as a function of the amount of sample injected onto the column. As more sample is injected onto the column, each of these C18 columns has a degree of column overload. This overload is noted by the increase in peak width at higher sample loads in Figure 3, and as shown in the corresponding chromatograms in Figure 4. However, Figures 3 and 4 both clearly demonstrate how the charged surface CS-C18 maintains a consistently narrower peak for amitriptyline. The narrow peak width maintains even when the column is overloaded, compared to the three different traditional C18 columns.

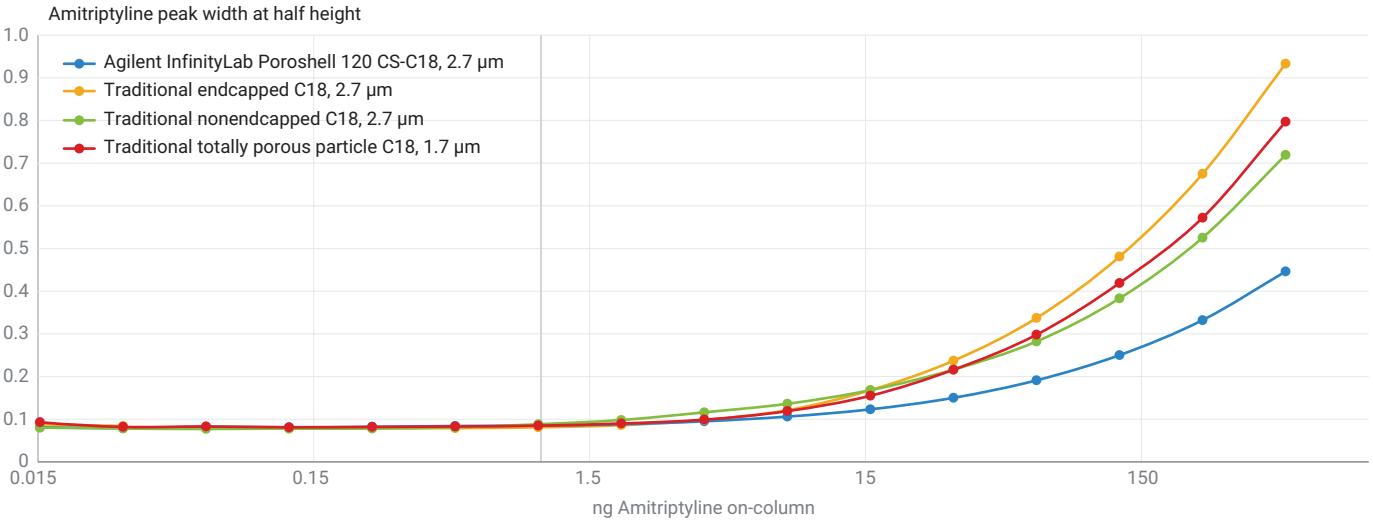

Figure 3. Sample loading capacity curves for CS-C18 and traditional C18 columns; methods 4 to 7 as described in Table 2.

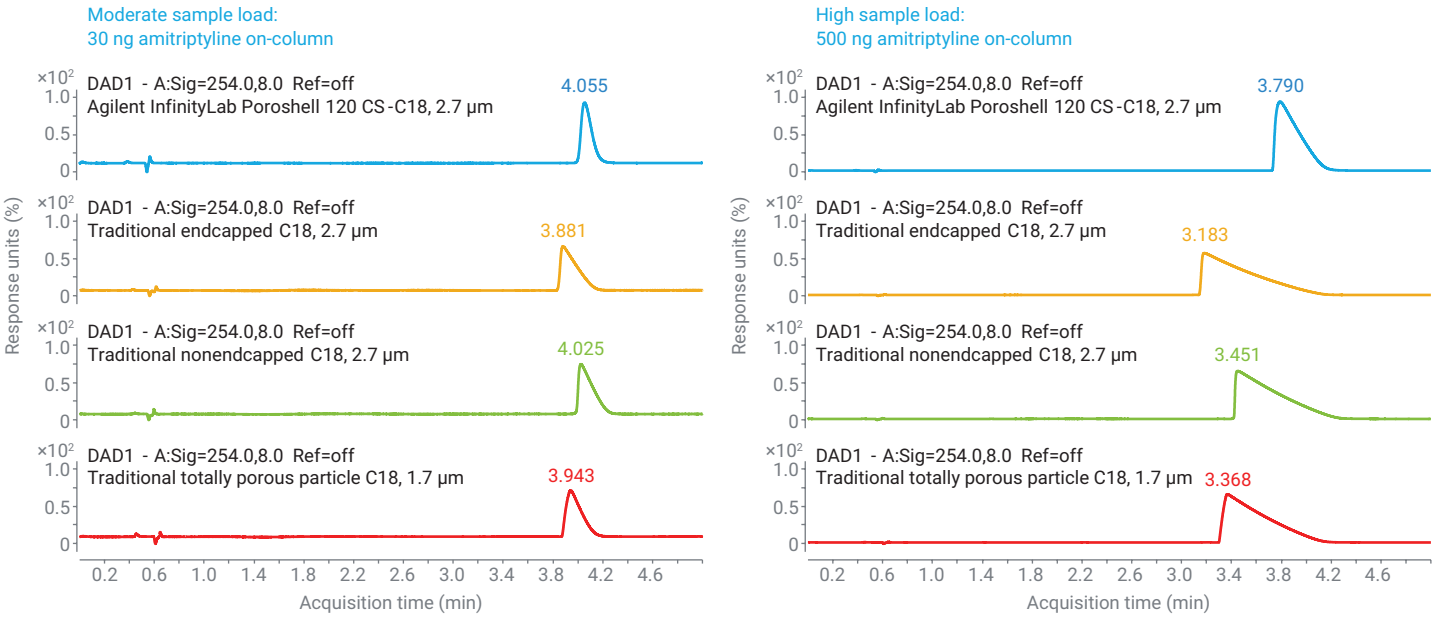

Figure 4. Performance differentiation between CS-C18 and traditional C18's at moderate and high sample load.

Reducing this overload effect for the InfinityLab Poroshell 120 CS-C18 column means that it can excel in impurity analyses for basic compounds. During impurity analyses, there is typically a concentrated main compound, as well as additional analytes of interest at low concentration levels. The impurity analysis in Figure 5 shows a concentrated sample of amitriptyline with impurities at 0.25% by weight. The injection volume is increased incrementally to allow sufficient detection and quantitation of the low concentration impurities. However, as the injection volume increases, the amount of amitriptyline on column passes the point of overload and results in peak broadening and tailing. With the InfinityLab Poroshell 120 CS-C18 column, the main peaks stay narrow enough to provide adequate resolution of the impurities before and after the main peak. However, for the traditional C18, the extreme main peak broadening consumes the earlier eluting impurity, and the significant peak tailing distorts the peak shape of the later eluting impurity. The charged surface CS-C18 can accommodate larger injections of the main compound, without compromising the chromatography of neighboring peaks. The larger injections increase the sensitivity of basic compound impurity analyses and allow detection and quantitation of ultra-low level impurities, compared to traditional C18 columns.

The InfinityLab Poroshell 120 CS-C18 column with a formic acid mobile phase delivers improved results for these basic pharmaceutical compounds at high- and low-level concentrations with UV and MS detection.

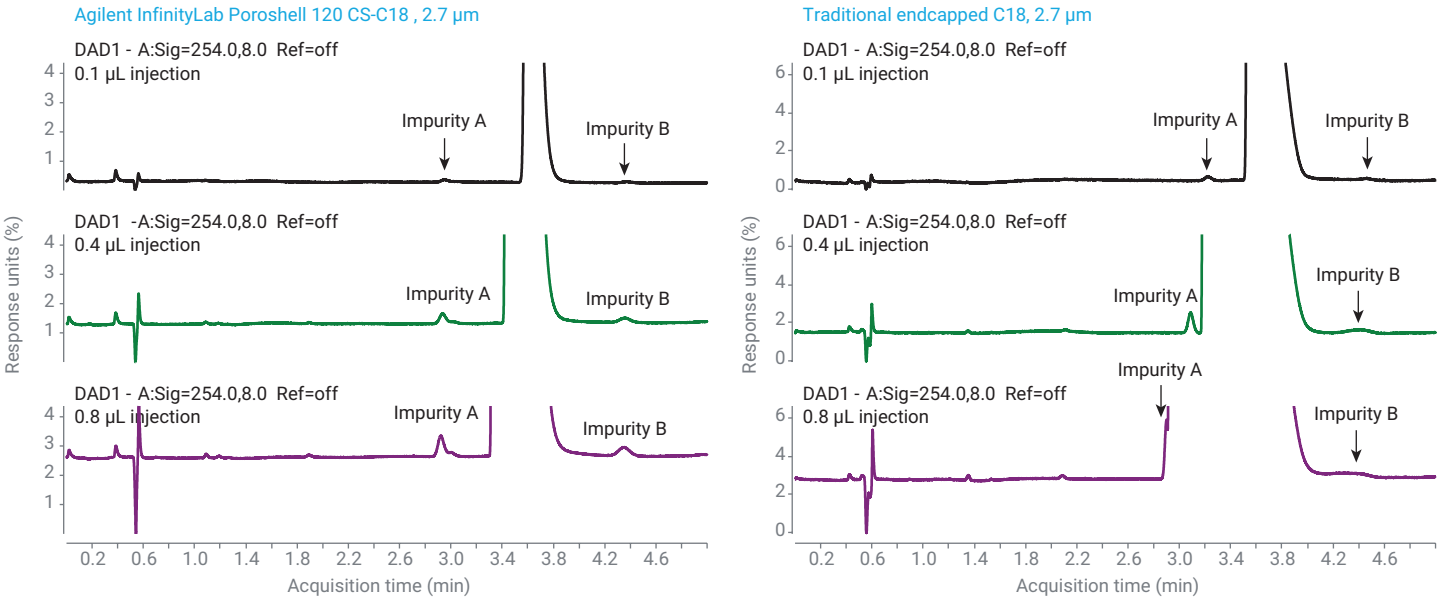

Figure 5. Impurity analysis with amitriptyline + 0.25% impurities on CS-C18 and a traditional C18; methods 4 to 5 as described in Table 2.

Conclusion

The charged surface Agilent InfinityLab Poroshell 120 CS-C18 column offers improved performance for basic analytes under simple formic acid mobile phase conditions compared to a traditional C18 bonded phase with the same mobile phase. Superior peak shape results in taller and sharper peaks, which provide better resolution and sensitivity. CS-C18 also offers improved sample loading capacity, maintaining excellent basic compound peak shape, and allowing more sensitive impurity analyses. Traditional C18 columns require the use of trifluoroacetic acid mobile phase to produce similar peak shapes. Trifluoroacetic acid is a less desirable mobile phase modifier since it is known to contaminate systems and columns, as well as cause significant ion suppression in LC/MS.

References

1. Gratzfield-Hugsen, A.; Naegele, E. Maximizing Efficiency Using Agilent InfinityLab Poroshell 120 Columns. *Agilent Technologies application note*, publication number 5990-5602EN, **2016**.
2. Meyer, V. R. Practical High-Performance Liquid Chromatography. Fourth Edition, Wiley, 2004; p. 34.

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