Application Note

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Improved Peak Shapes for Basic Analytes Using Agilent InfinityLab Poroshell 120 CS-C18 Columns

A comparison to traditional C18 columns with formic acid mobile phase for improved LC/MS sensitivity

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Abstract

Six basic pharmaceutical compounds are separated on a formic acid and acetonitrile gradient. Two superficially porous particle C18 columns are compared: a 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 formic acid mobile phase. While the performance of the traditional C18 column can be improved using a more complex buffered mobile phase, LC/MS sensitivity decreases as a result of ion suppression by the buffer salt.

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 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. The formic acid modifier 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 improves peak shape and sensitivity 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. Two LC columns were used in this experiment and are listed in Table 1. Tables 2 to 4 show the LC and TQ method parameters.

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]
Agilent 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

Table 2. UHPLC method parameters.

Method	Column	Mobile Phases	Elution Conditions	Injection Volumes	Column Temperature
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, 2 to 35% B in 5 min, with 5% C held constant throughout the analysis	5 µL of 5 µg/mL standard ---or--- 0.5 µL of 50 ng/mL standard	30 °C
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, 10 to 50% B in 5 min, with 5% C held constant throughout the analysis		
3	Traditional C18 on superficially porous particles, 2.1 × 100 mm, 2.7 µm	A: water B: acetonitrile C: 200 mM ammonium formate in water, pH 3.0	0.4 mL/min, 11 to 51% B in 5 min, with 10% C held constant throughout the analysis		

The six pharmaceutical compounds analyzed in this work were purchased from Sigma-Aldrich (St. Louis, MO, USA). Figure 1 shows compound structures, and Table 5 displays the concentrations at which they were analyzed. Ammonium formate 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).

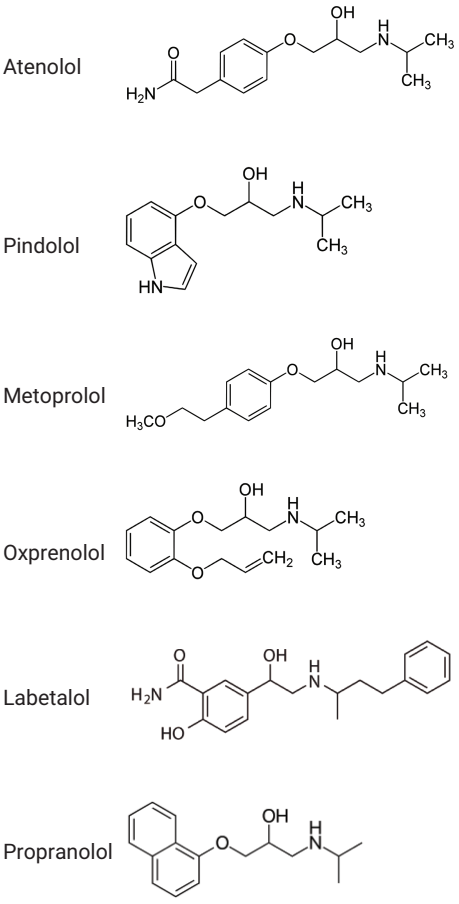

Figure 1. Compounds of interest.

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
Atenolol	267	145	110	28	Positive
Atenolol	267	56	110	28	Positive
Labetalol	329	161.9	110	35	Positive
Labetalol	329	91	110	60	Positive
Metoprolol	268.2	133	110	20	Positive
Metoprolol	268.2	77	110	69	Positive
Oxprenolol	266	116	113	12	Positive
Oxprenolol	266	72	113	16	Positive
Pindolol	249	116	110	16	Positive
Pindolol	249	56	110	28	Positive
Propranolol	260	116	95	16	Positive
Propranolol	260	56	95	28	Positive

Table 5. Standard concentrations.

Analytes (In Elution Order)	High Concentration Standard (Prepared in Water)	Low Concentration Standard (Prepared in Water)
Atenolol	5 µg/mL	50 ng/mL
Pindolol	5 µg/mL	50 ng/mL
Metoprolol	5 µg/mL	50 ng/mL
Oxprenolol	5 µg/mL	50 ng/mL
Labetalol	5 µg/mL	50 ng/mL
Propranolol	5 µg/mL	50 ng/mL

Results and discussion

Figure 2 compares the performance of the three analysis scenarios outlined in Table 2, using the high concentration 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, which resulted in 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 an ammonium formate buffered mobile phase. The improvement is shown in the chart of peak widths. For each of these separations, the acetonitrile gradient was slightly modified to ensure similar overall retention for all analytes within this chromatographic comparison.

The charged surface and traditional C18 columns were compared again in Figure 3, with the same analytes at low concentration, as described in Table 5. The best performing conditions for each column were chosen: CS-C18 with formic acid, and traditional C18 with ammonium formate. While peak shapes were acceptable for all analytes on both columns, sensitivity was reduced for the traditional C18 due to ion suppression from the mobile phase salt, ammonium formate.

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

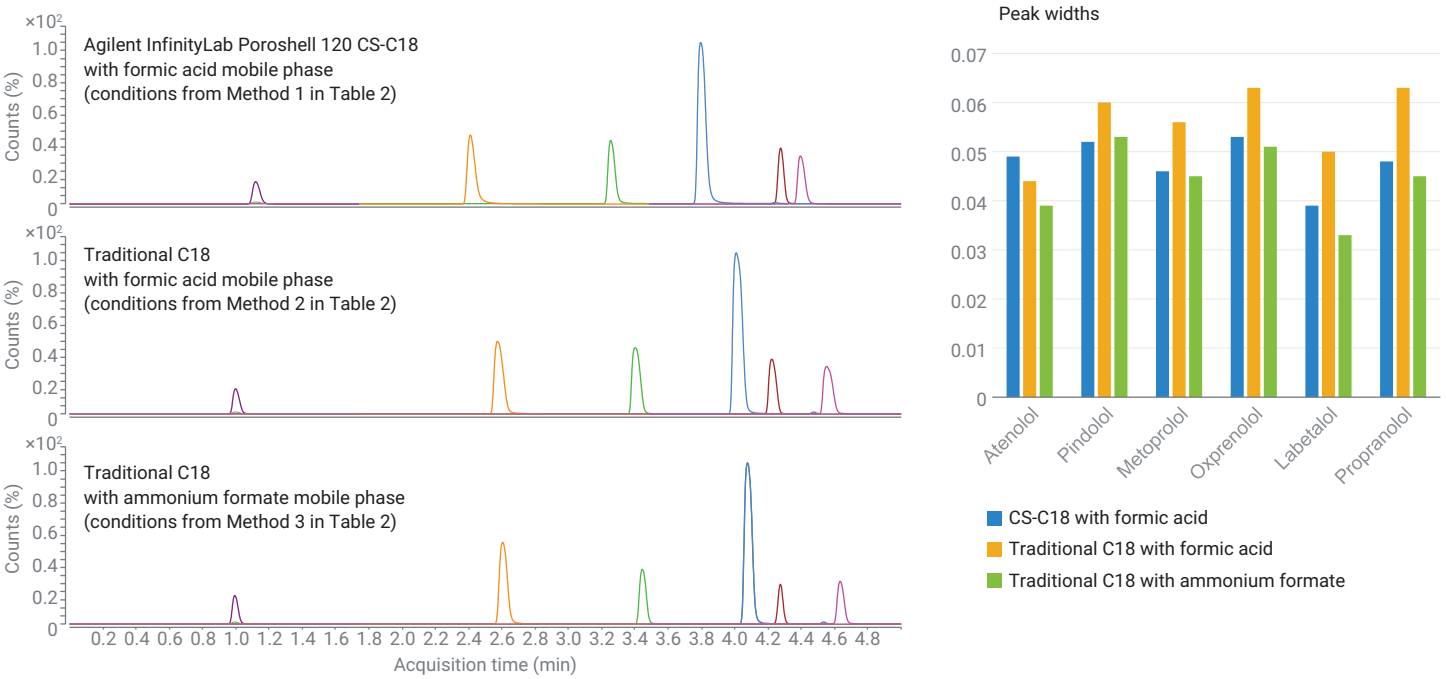

Figure 2. Comparison of chromatographic performance of basic pharmaceutical compounds.

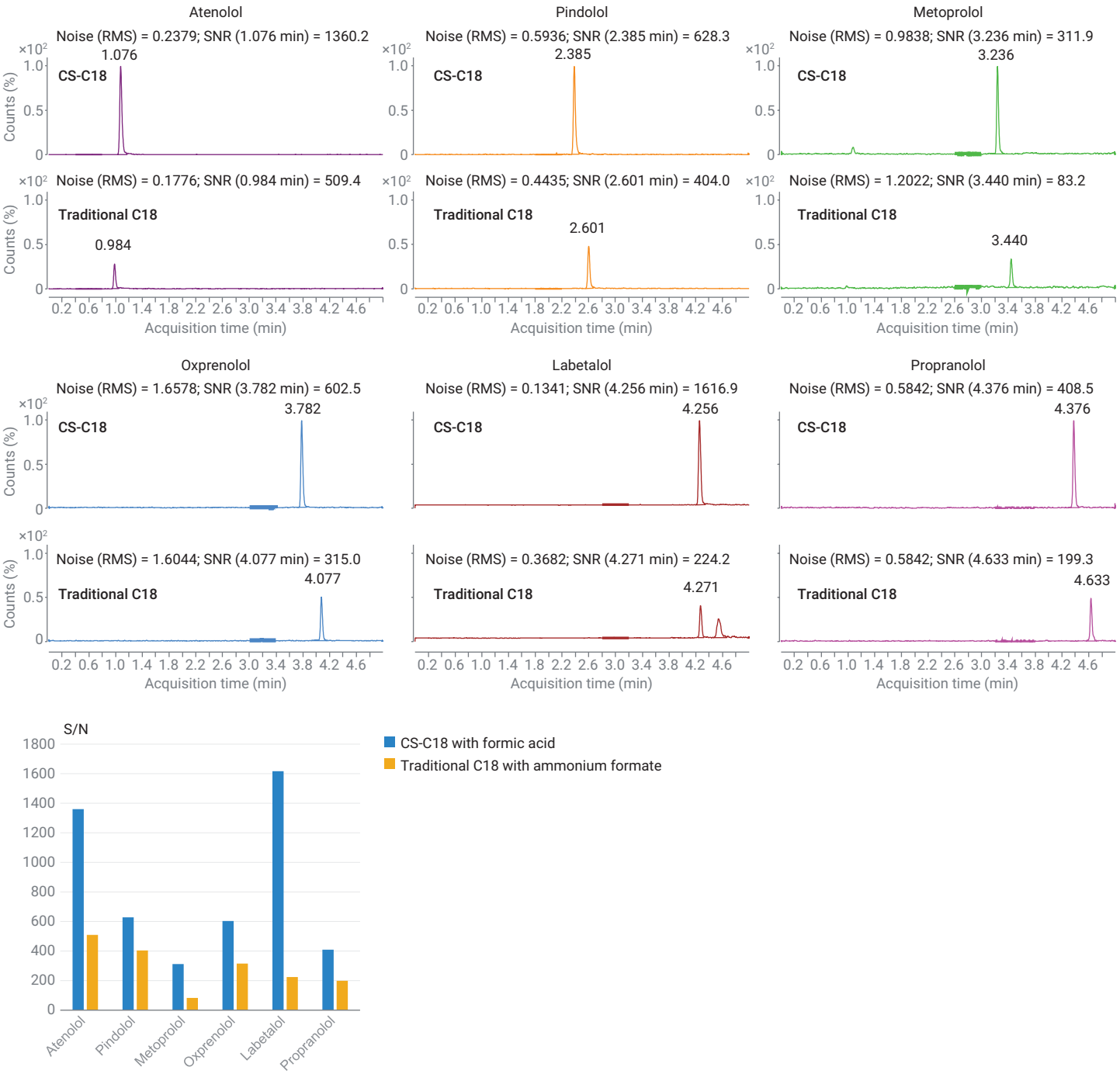

Figure 3. LC/MS sensitivity comparison with low concentration standard.

Conclusion

The charged surface of the InfinityLab Poroshell 120 CS-C18 column offered improved performance for basic analytes. The improvement was under simple formic acid mobile phase conditions compared to a traditional C18 bonded phase with the same mobile phase. Superior peak shape resulted in taller and sharper peaks, which provided better resolution and sensitivity. The traditional C18 column can produce similar peak shapes with the use of a more complex buffered mobile phase; however, LC/MS sensitivity was reduced due to ion suppression from the buffer salt.

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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