Separation of Eight Benzodiazepines on an Agilent Poroshell 120 EC-C18, 1.9 µm Column

Application Note

Small Molecule Pharmaceutical

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Abstract

Eight benzodiazepines are analyzed with an Agilent Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm column using a formic acid and acetonitrile gradient. The eight structurally similar compounds were baseline-resolved in 5 minutes, with a minimum resolution of 2.2.

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Introduction

Superficially porous particle LC columns are a popular tool in liquid chromatography. These columns generate high efficiency at lower pressure compared to their totally porous particle column counterparts [1]. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column [2]. The current trend with superficially porous particles is to reduce particle size for further efficiency improvements. The higher efficiency can be used to speed up analyses or improve results by increasing resolution and sensitivity.

This application note demonstrates the UHPLC performance of an Agilent Poroshell 120 EC-C18, 1.9 µm column, and its ability to baseline resolve eight closely related compounds.

Experimental

An Agilent 1290 Infinity LC System was used in this experiment. The system was modified from its standard configuration to have very low system volume and dispersion. Table 1 shows the configuration details, and lists the Agilent LC column that was used in this experiment. Table 2 shows the LC method parameters.

The eight benzodiazepines that were analyzed in this work were purchased as a mixed component solution from Cerilliant; their structures are shown in Figure 1. Formic acid was purchased from Sigma-Aldrich. Acetonitrile was purchased from Honeywell (Burdick and Jackson). Water was 0.2 µm filtered 18 MW from a Milli-Q system (Millipore).

Table 1. UHPLC System Configuration

Agilent 1290 Infinity LC system configuration	
Agilent 1290 Infinity Binary Pump (G4220A)	35 µL Solvent mixer: Jet weaver, 35 µL/100 µL (G4220-60006)
Agilent 1290 Infinity High Performance Autosampler (G4226A)	Seat assembly, ultra low dispersion, for Agilent 1290 Infinity Autosampler (G4226-87030) Autosampler → Heater: Capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784) Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (5182-0716) Cap, screw, blue, PTFE/red silicone septa, 100/pk (5182-0717) Vial insert, 250 µL, glass with polymer feet, 100/pk (5181-1270)
Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)	Heat exchanger, low dispersion, 1.6 µL, double (G1316-60005) Heater → Column: A-Line quick-connect assembly, 105 mm, 0.075 mm (5067-5961) Column → Flow cell: Capillary, stainless steel, 0.075 × 220 mm, SV/SLV (5067-4784)
Agilent 1290 Infinity Diode Array Detector (G4212A)	Ultra-Low Dispersion Max-Light Cartridge Flow Cell, 10 mm (G4212-60038)
Agilent OpenLAB CDS ChemStation Edition Revision C.01.05 [35]	G4220A: B.06.53 [0013] G4226A: A.06.50 [003] G1316C: A.06.53 [002] G4212A: B.06.53 [0013]
Agilent LC Column	Agilent Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm (693675-902)

Table 2. UHPLC Method Parameters

Column	Mobile phase	Flow rate (mL/min)	Mobile phase composition	Injection volume (µL)	Sample	Thermostated column compartment (°C)	Diode array detector
Agilent Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm	A) 0.4 % formic acid in water B) acetonitrile	0.5	36 % B isocratic	0.5	Cerilliant B-033 Benzodiazepine Multicomponent Mixture-8; 250 µg/mL of each component in acetonitrile; see Figure 1 for structures	20	254 nm, 80 Hz

Results and Discussion

Figure 2 shows the separation of eight bezodiazepines on a Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm column. All compounds were baseline-resolved, with a minimum resolution of 2.2, in 5 minutes. The benzodiazepines were difficult to separate because they are structurally very similar (Figure 1). However, the Poroshell 1.9 µm column had sufficient efficiency and resolving power to successfully separate this sample.

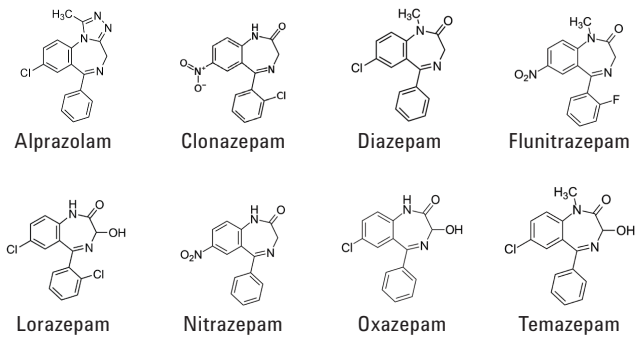

Figure 1. Compounds of interest.

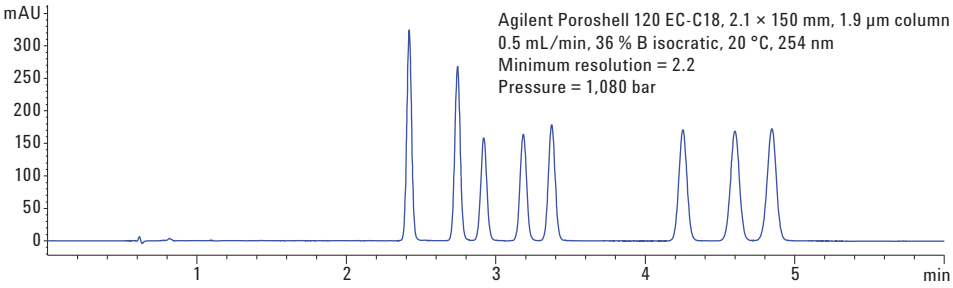

Figure 2. Separation of benzodiazepines on an Agilent Poroshell 120 EC-C18, 1.9 µm column.

Conclusions

The Agilent Poroshell 120 EC-C18, 1.9 µm column was used to accomplish a challenging separation of eight benzodiazepines. The very high efficiency of this small superficially porous particle column provided sufficient resolution to baseline-resolve the closely related compounds on a 2.1 × 150 mm column.

References

1. A. Gratzfield-Hugsen, E. Naegele. Maximizing efficiency using Agilent Poroshell 120 Columns. *Agilent Technologies Application Note*, publication number 5990-5602EN, **2016**.
2. V. R. Meyer. Practical High Performance Liquid Chromatography. Fourth Edition, p. 34. Wiley (2004).

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