Application Note

Environmental

Modernizing HPLC Methods with Agilent InfinityLab Poroshell 120 Columns

Tripling throughput in the analysis of carbonyl-DNPH derivatives using Poroshell 120 SB-C18 columns

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Abstract

An original method for carbonyl-dinitrophenylhydrazine (DNPH) derivatives analysis, developed on a traditional 5 µm column, was transferred and scaled to different particle sizes of Agilent InfinityLab Poroshell 120 SB-C18 columns, including 4, 2.7, and 1.9 µm. Significant reduction in run time and solvent consumption could be achieved when scaling the method from a larger, totally porous particle column to smaller, superficially porous columns.

Introduction

Carbonyl compounds are hazardous substances that usually exist in air and soil. Many regulations from the USA, EU, China, and Japan are set to control levels of carbonyl compounds in various environmental samples.[1,2] Because of their highly volatile and reactive nature, carbonyl compounds are often converted to DNPH derivatives prior to LC analysis.

In this application note, an original method for analysis of 16 carbonyl-DNPH derivatives using an Agilent ZORBAX StableBond C18, 4.6 × 250 mm, 5 µm was transferred to a 4 µm Poroshell 120 SB-C18 with the same dimensions. The method was then scaled to different particle size columns, including 2.7 and 1.9 µm, while maintaining resolution. Run time and solvent consumption was significantly reduced.

Experimental

Reagents and chemicals

All reagents were HPLC grade or higher. HPLC-grade acetonitrile was bought from J. T. Baker (Center Valley, PA, USA). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). Standards stock solution was from Anpel Laboratory Technologies (Shanghai, China). The standard mixture solution was made by diluting stock solution with acetonitrile to a concentration of 10 µg/mL.

Equipment and materials

- **Column inlet:** Agilent InfinityLab Quick Connect LC fitting (p/n 5067-5965)
- **Column outlet:** Agilent InfinityLab Quick Turn LC fitting (p/n 5067-5966)
- Agilent vial, screw top, amber, write-on spot, certified, 2 mL (p/n 5182-0716)
- Agilent bonded screw cap, bonded blue, PTFE/red silicone septa (p/n 5190-7024)
- Agilent InfinityLab solvent bottle, amber, 1,000 mL (p/n 9301-6526)
- Agilent InfinityLab Stay Safe cap, GL45, three-port, one-vent valve (p/n 5043-1219)
- Eppendorf pipettes and repeater

Instrumentation

- Agilent 1290 Infinity II high speed pump (G7120A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 1290 Infinity II DAD (G7117B)
- Agilent OpenLab CDS, Version C.01.07

Table 1. The target carbonyl-DNPH compounds analyzed in this application note.

No.	Name	CAS	Stock Concentration (µg/mL)
1	Acetaldehyde-DNPH	1019-57-4	100
2	Acetone-DNPH	1567-89-1	100
3	Acrolein-DNPH	888-54-0	100
4	Benzaldehyde-DNPH	1157-84-2	100
5	2-Butanone-DNPH	958-60-1	100
6	Butyraldehyde-DNPH	1527-98-6	100
7	Crotonaldehyde-DNPH	1527-96-4	100
8	Cyclohexanone-DNPH	1589-62-4	100
9	Formaldehyde-DNPH	1081-15-8	100
10	Hexaldehyde-DNPH	1527-97-5	100
11	Methacrolein-DNPH	5077-73-6	100
12	Propionaldehyde-DNPH	725-00-8	100
13	*o*-Tolualdehyde-DNPH *m*-Tolualdehyde-DNPH	1773-44-0 2880-05-9	100 100
14	*p*-Tolualdehyde-DNPH	2571-00-8	100
15	Valeraldehyde-DNPH	2057-84-3	100

Table 2. HPLC conditions.

Column	Mobile Phase Composition	Flow Rate (mL/min)	Injection Volume (µL)	Thermostatted Column Compartment (°C)	Diode Array Detector
Agilent ZORBAX StableBond C18, 4.6 × 250 mm, 5 µm (p/n 880975-902) Agilent InfinityLab Poroshell 120 SB-C18, 4.6 × 250 mm, 4 µm (p/n 680970-902)	Water/ACN: 0 to 8 min, 57% ACN, 8 to 10 min 57 to 75% ACN, 10 to 17 min 75% ACN	1.5	5	50	360 nm, 5 Hz
Agilent InfinityLab Poroshell 120 SB-C18, 4.6 × 150 mm, 2.7 µm (p/n 683975-902)	Water/ACN: 0 to 5 min, 57% ACN, 5 to 6 min 57 to 75% ACN, 6 to 10 min 75% ACN	1.5	3	50	360 nm, 40 Hz
Agilent InfinityLab Poroshell 120 SB-C18, 3.0 × 100 mm, 1.9 µm (p/n 685675-302)	Water/ACN: 0 to 3.3 min, 57% ACN, 3.3 to 4 min 57 to 75% ACN, 4 to 6.7 min 75% ACN	0.64	0.85	50	360 nm, 80 Hz

Results and discussion

The original separation of 16 carbonyl-DNPH derivatives on a totally porous particle ZORBAX StableBond C18, 4.6 × 250 mm, 5 µm column was accomplished in 14 minutes and is shown in Figure 1B. Figure 1A shows the same sample and LC method on an InfinityLab Poroshell 120 SB-C18, 4.6 × 250 mm, 4 µm column. The 4 µm superficially porous particle column is designed to generate doubled efficiency compared to a 5 µm totally porous particle column with the same phase. As a result, increased resolution can be achieved when using a 4 µm superficially porous particle column without any method changes.

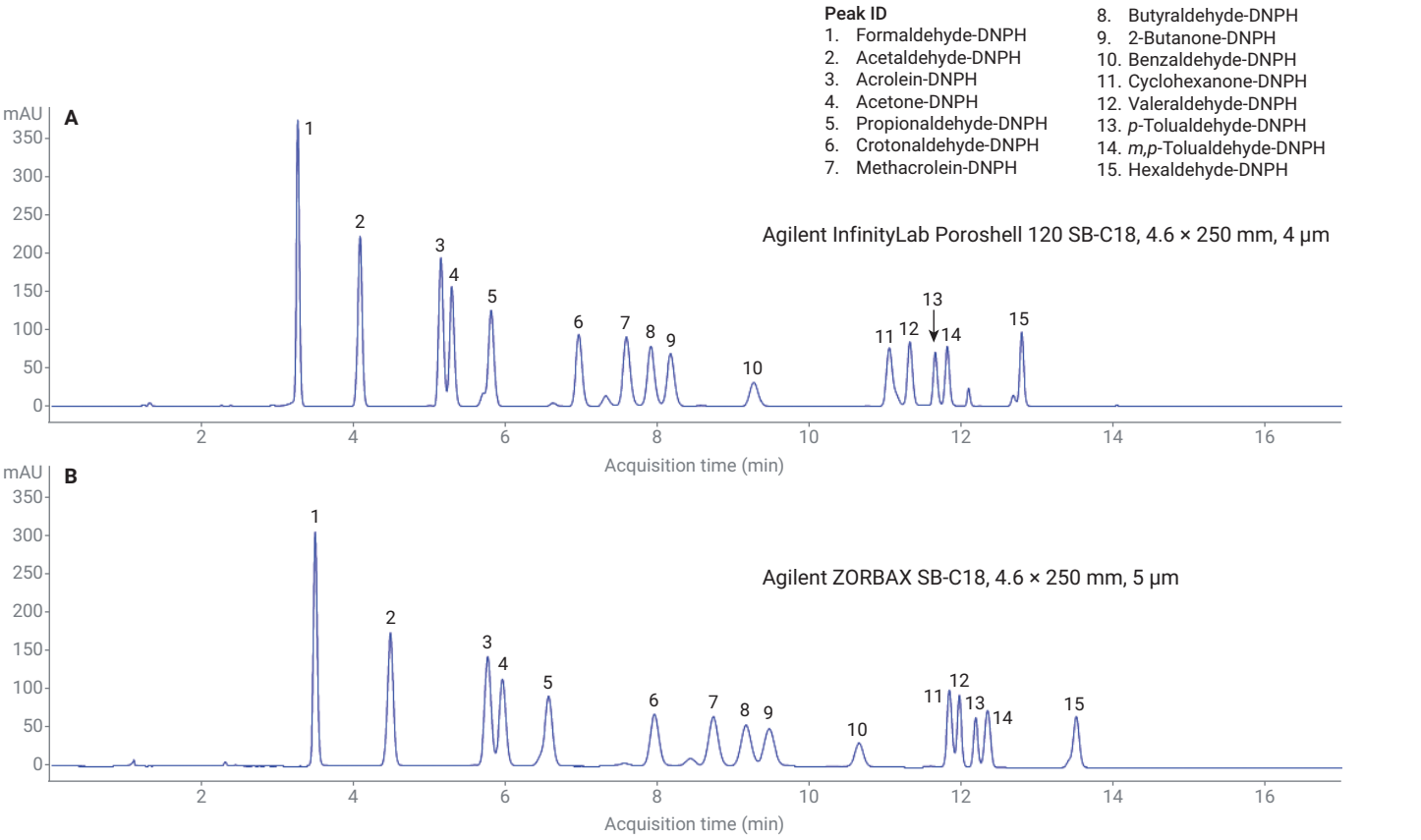

Figure 1. Comparison of chromatograms generated using the Agilent InfinityLab Poroshell 120 SB-C18 column and ZORBAX StableBond C18 column.

The method scalability between 4, 2.7, and 1.9 µm superficially porous particle columns is shown in Figure 2. All three columns have similar length-to-particle-diameter ratios (L/dp). This allows the separation to be transferable between columns with different particle sizes. The advantages of using a smaller particle size and a shorter column include run time and solvent consumption saving. Compared to the SB-C18 250 mm, 4 µm method, the analysis on the 2.7 µm column was 40% faster, and used 41% less mobile phase while maintaining the same resolution. The analysis on the 1.9 µm column was 61% faster and used 83% less mobile phase compare to the 250 mm column. The results are summarized in Table 3.

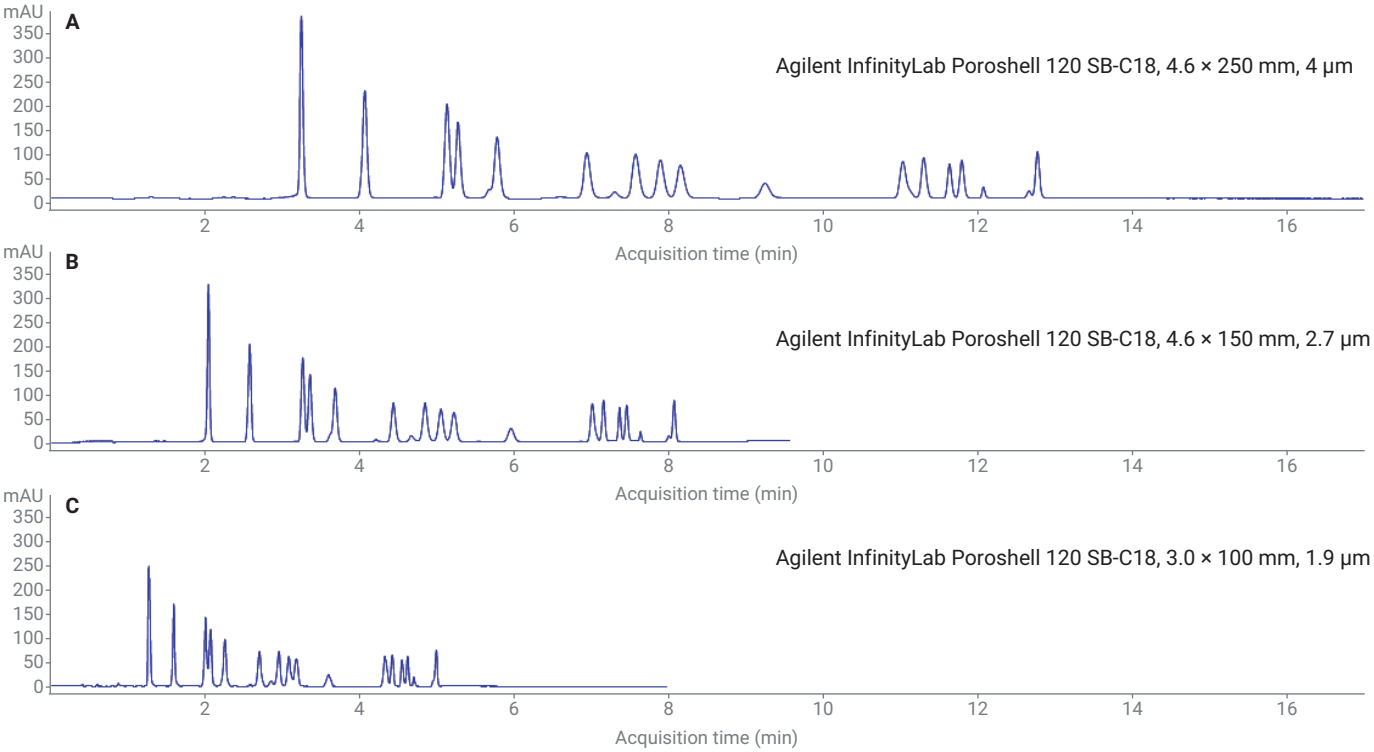

Figure 2. Chromatograms of method scaling between 4, 2.7, and 1.9 µm Agilent InfinityLab Poroshell 120 SB-C18.

Table 3. Results of using columns with different dimensions.

Column Dimensions	L/dp	Run Time	Solvent Consumption
4.6 × 250 mm, 4 µm	62.5	17 min	25.5 mL
4.6 × 150 mm, 2.7 µm	55.6	10 min (–41%)	15 mL (–41%)
3 × 100 mm, 1.9 µm	52.6	6.7 min (–61%)	4.3 mL (–83%)

Conclusion

Separation using traditional columns, such as a ZORBAX StableBond C18, 5 µm column, can be improved using Poroshell 120 SB-C18, 4 µm with the same column dimensions. Due to the matching selectivity, the method can be easily scaled for different particles families (ZORBAX and Poroshell) and particle sizes. Columns with smaller particles enable the use of shorter columns, which can significantly reduce run time and solvent consumption.

References

1. Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC). EPA Method 8315a, *Environmental Protection Agency*, Washington, DC, USA, **1996**.
2. Soil and Sediment — Determination of Carbonyl Compounds — High Performance Liquid Chromatography. HJ 997-2018, Standards of Environmental Protection, China, **2018**.

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