Application Note

Consumer Product Testing

Analysis of Fluorescent Brighteners in Masks using an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl Column

Authors

Rongjie Fu
Agilent Technologies (Shanghai) Co. Ltd.

Xia Wu
Agilent Technologies (China) Co. Ltd.

Abstract

Nine fluorescent brightener compounds were analyzed with an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column (4.6 × 150 mm, 4 µm) using a gradient HPLC method and FLD detection. The method was compared to a method using a totally porous particle column, the Agilent ZORBAX Eclipse Plus Phenyl-Hexyl. The method was also scaled to a smaller particle, shorter column, the Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 1.9 µm. All the fluorescent brightener compounds analyzed were well resolved on all of the columns.

Introduction

Fluorescent brightening agents (FBAs), also known as optical brightening agents (OBAs), are colorless to weakly colored organic compounds. They are designed to brighten colors or mask yellowing in plastics, lacquers, paints, inks, photo-processing solutions, and fibers. Fluorescent brighteners are forbidden additives in consumer products like food and cosmetics, however they may be added to the textiles used to make face masks. A Chinese regulation[1] prohibits the use of these compounds in masks for children and requires proof of the absence of mobile fluorescent brighteners.

In this application note, both an Agilent ZORBAX Eclipse Plus Phenyl-Hexyl (4.6 × 250 mm, 5 µm) column and an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column (4.6 × 150 mm, 4 µm) were used for the analysis of nine fluorescent brighteners with a gradient method[2] using LC/FLD. The method was then scaled to a UHPLC column, the Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column (3.0 × 100 mm, 1.9 µm).

Experimental

Reagents and chemicals

All reagents were HPLC grade or higher. HPLC-grade methanol and acetonitrile were bought from Merk (Billerica, MA, USA). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). Ammonium acetate was purchased from Sigma-Aldrich. Standards were purchased from Anpel Laboratory Technologies (Shanghai, China). The standard mixture solution was made by dissolving the fluorescent brightener compounds in acetonitrile. The separate concentrations of the individual compounds are in Table 1.

Equipment and materials

- **Column inlet:** Agilent InfinityLab Quick Connect LC fitting (part number 5067-5965)
- **Column outlet:** Agilent InfinityLab Quick Turn LC fitting (part number 5067-5966)
- Agilent vial, screw top, amber, write on spot, certified, 2 mL (part number 5182-0716)
- Agilent bonded screw cap, bonded blue, PTFE/red silicone septa (part number 5190-7024)
- Agilent InfinityLab solvent bottle, amber, 1 L (part number 9301-6526)
- Agilent InfinityLab Stay Safe cap, GL45, three-port, one-vent valve (part number 5043-1219)
- Agilent Captiva Econofilter, PTFE membrane, 13 mm diameter, 0.2 µm pore size (part number 5190-5265)

Instrumentation

An Agilent 1290 Infinity II High Speed Configuration LC system installed with ultralow dispersion kit (5067-5189), consisting of the following modules:

- Agilent 1290 Infinity II high speed pump (G7120A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 1260 Infinity II FLD Spectra (G1321B)

An Agilent 1290 Infinity II flexible configuration LC system, consisting of the following modules:

- Agilent 1290 Infinity II flexible pump (G7104A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 1260 Infinity II FLD (G7121A)

Table 1. The fluorescent brightener standard compounds analyzed in this application note.

No. (Elution Order)	Name	CAS	Concentration (µg/mL)
1	Fluorescent brightener 220	16470-24-9	5.24
2	Fluorescent brightener 85	12224-06-5	0.912
3	Fluorescent brightener 113	12768-92-2	5.12
4	Fluorescent brightener 351	38775-22-3	0.332
5	Fluorescent brightener 71	16090-02-1	0.945
6	Fluorescent brightener 162	3271-05-4	0.937
7	Fluorescent brightener 140	61968-71-6	0.995
8	Fluorescent brightener 135	1041-00-5	0.0968
9	Fluorescent brightener 199	13001-40-6	0.931

Table 2. LC conditions.

Column	Mobile Phase Composition	Optimized Gradient	Flow Rate (mL/min)	Injection Volume (µL)	Thermostatted Column Compartment (°C)	FLD
Agilent ZORBAX Eclipse Plus Phenyl-Hexyl, 4.6 × 250 mm, 5 µm	A) 20 mM ammonium acetate in water B) acetonitrile: methanol (4:1)	Time (min) A% B% 0 95 5 1 95 5 5 70 30 15 50 50 16.7 10 90 21.7 20 90 21.8 95 5 26.7 90 5	1.0	8	25	Ex 365 nm, Em 430 nm @4.63 Hz
Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 4.6 × 150 mm, 4 µm		Time (min) A% B% 0 95 5 0.6 95 5 3 70 30 9 50 50 10 10 90 13 10 90 13.1 95 5 16 95 5	1.0	5	25	Ex 365 nm, Em 430 nm @4.63 Hz
Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 1.9 µm		Time (min) A% B% 0 95 5 0.2 95 5 1 70 30 3 50 50 4 10 90 5 10 90 5.01 95 5 6.0 95 5	0.8	1.4	25	Ex 365 nm, Em 430 nm @74 Hz

Results and discussion

The method developed on an Agilent ZORBAX Eclipse Plus Phenyl-Hexyl, 4.6 × 250 mm, 5 µm column shows baseline separation for all fluorescent brighteners. To improve efficiency and reduce solvent consumption and run time, the method was transferred to a superficially porous particle (SPP) column with the same phase, as shown in Figure 1. The shorter 150 mm InfinityLab Poroshell 120 Phenyl-Hexyl column provided almost the same resolution as a 250 mm ZORBAX Eclipse Plus Phenyl-Hexyl column, while allowing an analysis time reduction of 40%. This time saving is possible because a 4 µm superficially porous particle column provides twice the efficiency of a 5 µm totally porous particle (TPP) column. The gain in resolution can also be used differently: 4 µm SPP columns can replace 5 µm TPP columns to achieve higher efficiency and better resolution of existing methods, e.g. when the resolution of a critical peak pair is not sufficient. In this case, there was no need to change the existing method or change column dimensions.

The method was also scaled to a smaller SPP column, the Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 1.9 µm. Compared to the InfinityLab Poroshell 120 Phenyl-Hexyl, 4.6 × 150 mm, 4 µm, a 56% of time saving was achieved in the analysis using the InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 1.9 µm. The resolution was also improved due to the larger column length to particle diameter ratio (Figure 2). The 1.9 µm superficially porous particle columns provide an extra 20% efficiency above that of 1.8 µm superficially particle columns. However, instruments should be configured with low-volume capillaries and flow cells to ensure that the full resolving power is achieved.[3]

Overlaying the chromatograms from six consecutive injections demonstrates good reproducibility with the modified gradient method on the InfinityLab Poroshell 120 Phenyl-Hexyl, 4.6 × 150 mm, 4 µm column, as shown in Figure 3. A real sample was prepared as follows: weigh 1.0 g of cut mask pieces (5 × 5 mm) into a 50 mL volumetric flask with 20 mL of 70% DMF; extract using a sonicator at 50 °C for 40 minutes. After cooling, take the upper level of the liquid and filter it with an Agilent Captiva Econofilter PTFE membrane into sample vials, then analyze by HPLC (Figure 4). There were no target compounds detected in the sample.

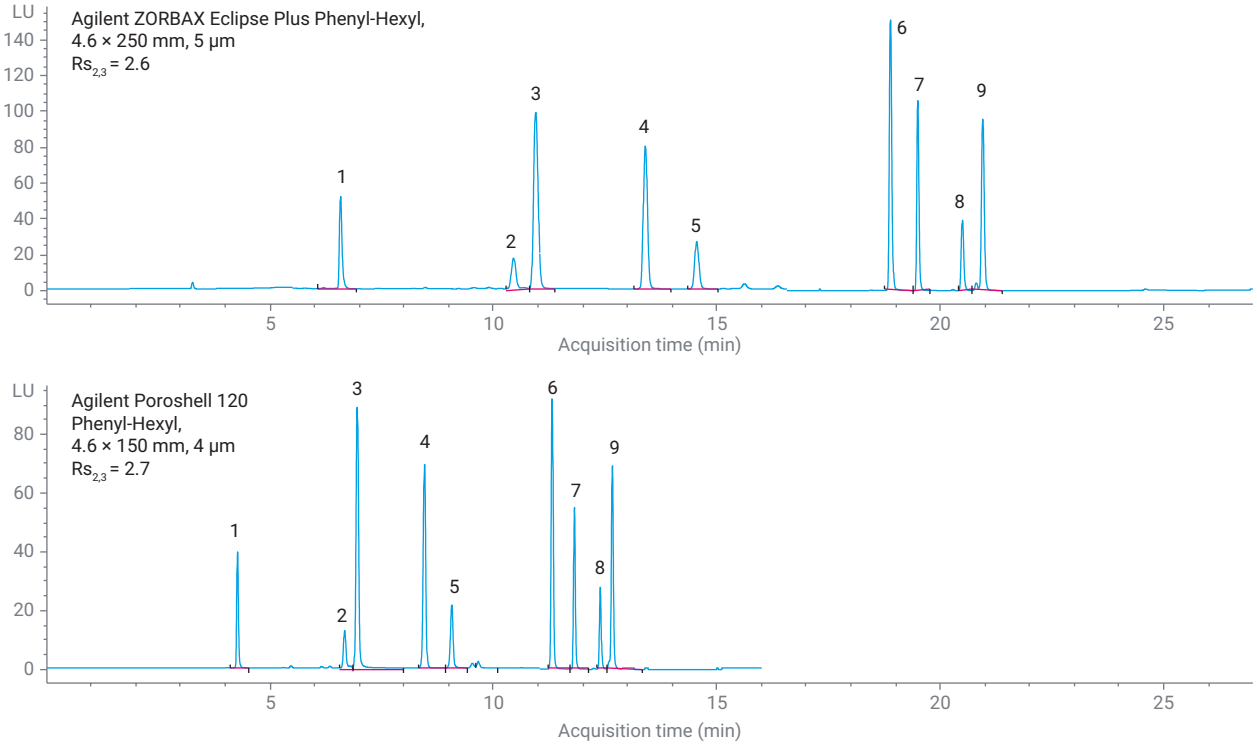

Figure 1. Comparison of standards chromatograms with an Agilent ZORBAX Eclipse Plus Phenyl-Hexyl, 4.6 × 250 mm, 5 µm column and an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 4.6 × 150 mm, 4 µm column.

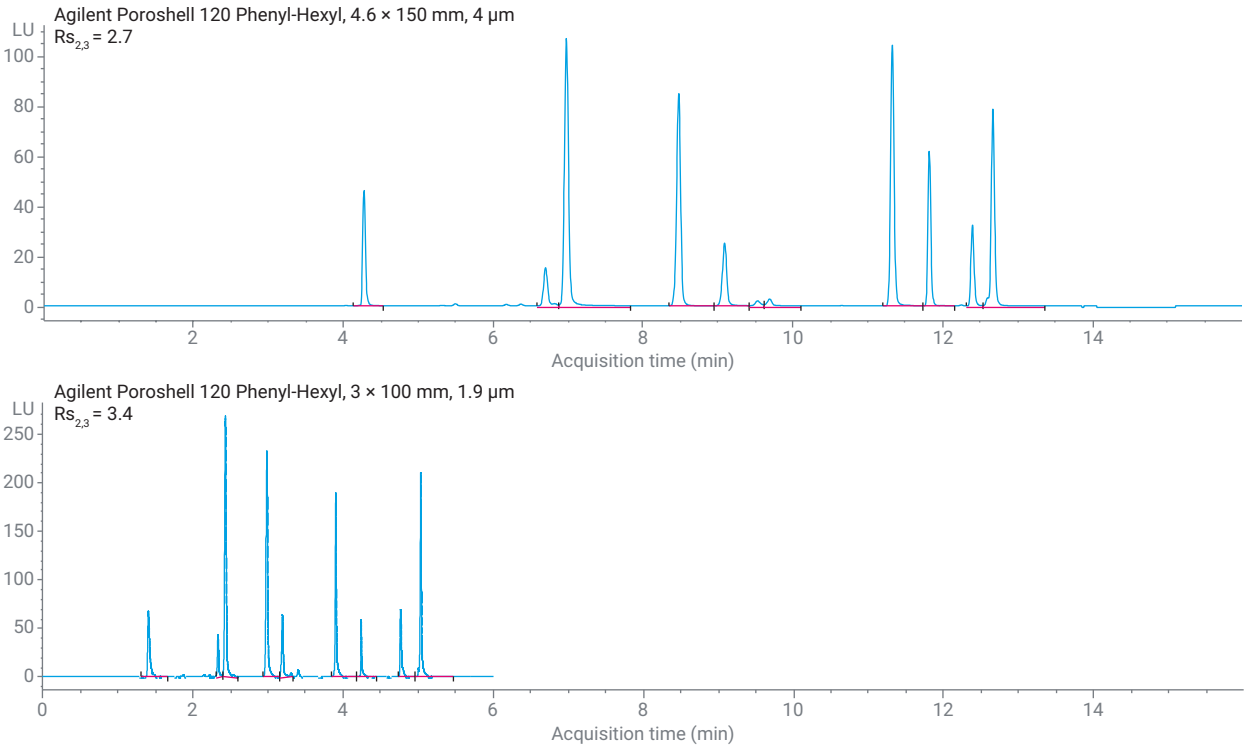

Figure 2. Chromatograms of the method scaled from an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 4.6 × 150 mm, 4 µm column to an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 1.9 µm.

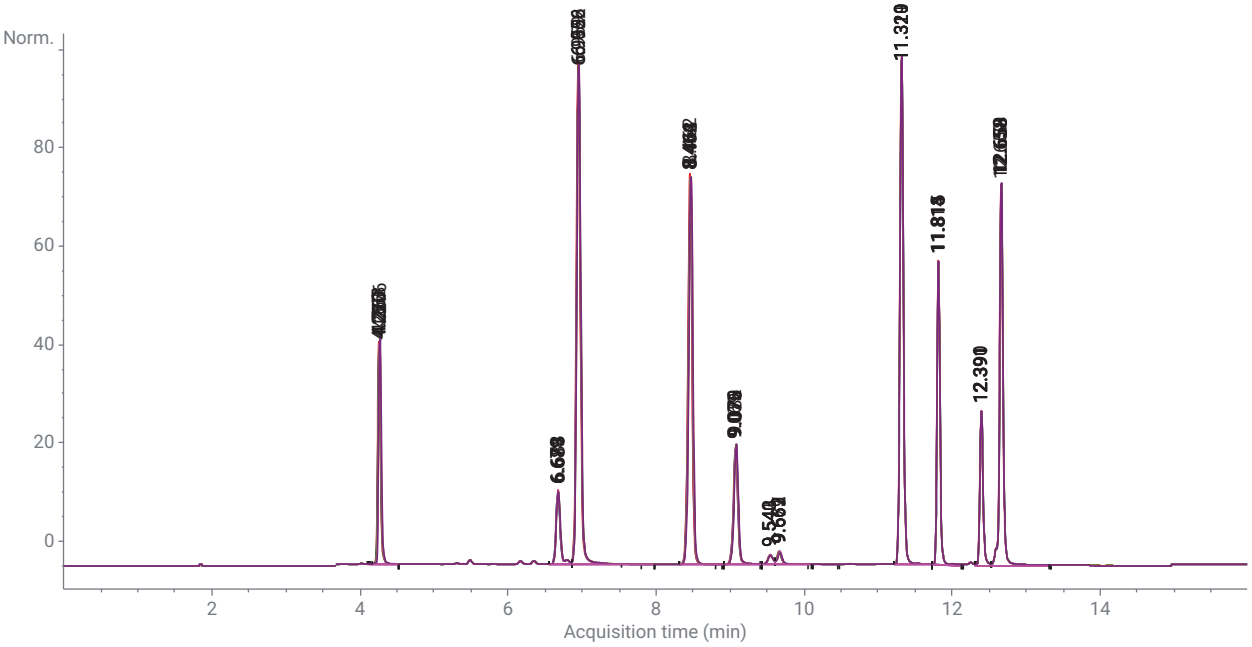

Figure 3. An overlay of chromatograms of six consecutive injections of fluorescent brightener standards with an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 4.6 × 150 mm, 4 µm column.

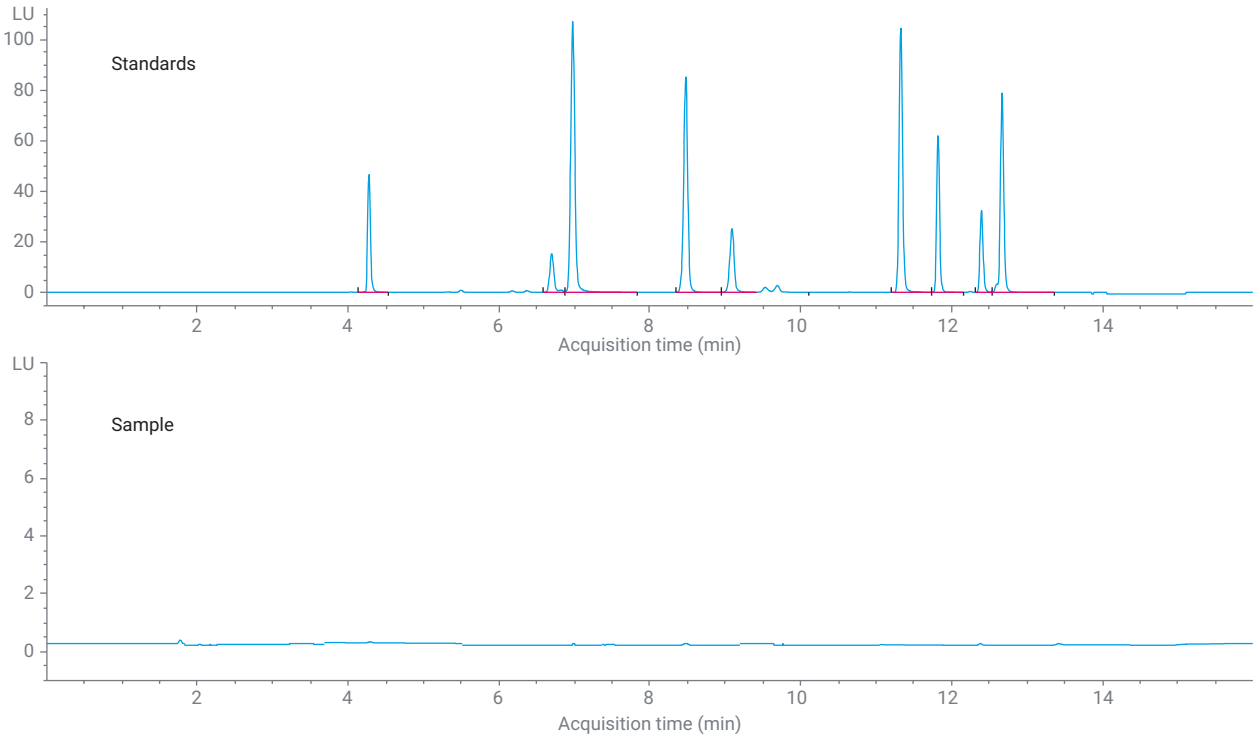

Figure 4. Analysis of mask sample with an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 4.6 × 150 mm, 4 µm column.

Conclusion

The Agilent InfinityLab Poroshell 120 Phenyl-Hexyl 4 µm column can easily replace the totally porous Agilent ZORBAX column, saving time while maintaining resolution. The method optimized on the 4 µm column can be used for routine analysis of fluorescent brightener in face masks. The highly efficient Agilent InfinityLab Poroshell 120 1.9 µm column is a powerful chromatographic separation tool that can provide higher resolution within a shorter analysis time.

References

1. Technical Specification of Children Mask, China Standard, GB-T 38880-**2020**.
2. Hu Yiguang; Zhang Xianchen; and Wang Jingli. Detection of Several Common Fluorescent Whitening Agentin Cotton Textiles. Cotton Textile Technology, *44(4)*, 39.
3. Anne Mack. Instrument, Method, and Sample Optimizations to Get the Most from Agilent InfinityLab Poroshell 120, 1.9 µm Columns. *Agilent Technologies application note*, publication number 5991-7560EN (**2017**).

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