High resolution separations by supercritical fluid chromatography using a coupled column approach with the Agilent 1260 Infinity Analytical SFC System

Application Note

Pharmaceutical and Chemical Industry

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Abstract

In Supercritical Fluid Chromatography (SFC), pressure drop is substantially smaller than in liquid chromatography because of the lower viscosity of the mobile phase. Consequently, longer (or coupled) columns packed with small particles can be used at relatively low pressure, resulting in high plate numbers and high resolution separations.

This Application Note demonstrates the high resolution separation of a pharmaceutical test mixture, a pesticide standard and a cosmetic sample on a 1 m column (4 × 25 cm) using the Agilent 1260 Infinity Analytical SFC system.

Introduction

The potential for using serially coupled columns in Supercritical Fluid Chromatography (SFC) to obtain high efficiency and high resolution separations has already been recognized for many years [1,2]. Due to the low pressure drop in SFC as a result of the lower viscosity of supercritical carbon dioxide compared to classical mobile phases in HPLC, columns can be coupled in series and operated at relatively low pressure (< 300 bar). For example, if four 25 cm columns packed with 5 µm particles are coupled in series to give a 1 m long column, the resulting plate number approaches 100,000. This high efficiency chromatographic set-up offers interesting possibilities for the separation of complex mixtures or can be used as a generic high resolution tool.

The Agilent 1260 Infinity Analytical SFC system was evaluated for high efficiency analysis of a pharmaceutical mixture, a pesticide sample, and a perfume sample. The performance of the system is demonstrated.

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Experimental

Solutions

Stock solutions of pharmaceutical test solutes were prepared in methanol at concentrations between 1 and 5 mg/mL, depending on solubility. These stock solutions were mixed to obtain a 15 component pharmaceutical test mixture at 100 µg/mL. For ibuprofen and fenoprofen, a higher concentration (800 µg/mL) was used to have a similar UV response for all compounds at 254 nm. A pesticide sample containing 27 different pesticides at 100 µg/mL was obtained from Dr. Ehrenstorfer GmbH (Pesticide-Mix 34, Dr. Ehrenstorfer, Augsburg, Germany). The perfume sample was obtained from a commercial source.

Instrumentation

An Agilent 1260 Infinity Analytical SFC system (G4309A), consisting of an Aurora SFC Fusion A5 and a modified Agilent RRLC binary system, was used.

All analysis were performed on a set of four serial coupled Agilent ZORBAX RX-SIL 25 cm × 4.6 mm, 5 µm columns. For comparison, the pharmaceutical test mixture was also analysed on a single (25 cm) column.

The modifier was 20 mM ammonium acetate in methanol. The experimental conditions are summarized in Table 1.

Conditions	
Columns	Agilent ZORBAX Rx-SIL, 4.6 mm id × 25 cm, 5 µm (4 ×)
Supercritical fluid	CO_2
Modifier	MeOH with 20 mM CH_3COONH_4
Outlet pressure	150 bar
Flow rate	2.0 mL/min
Gradient A (Pharma)	0–80 min: 5–40% (4 columns) 0–20 min: 5–40% (1 column)
Gradient B (Pesticide)	0–60 min: 5–15% (4 columns)
Gradient C (Perfume)	0–40 min: 5–22% (4 columns)
Temperature	40 °C
Injection volume	5 µL
Detection	DAD, 254 nm for Pharmaceutical mix DAD, 254 nm for Pesticide mix DAD, 315 nm for Perfume sample

Table 1
Experimental conditions.

Results and Discussion

1. Pharmaceutical analysis: Comparison of 1 versus 4 columns

A gradient method was developed for the separation of the 15 component pharmaceutical mixture using one single column of 25 cm. The gradient was from 5% to 40% modifier in 20 min.

For comparison the sample was analyzed on a series of four coupled columns (effective column length: 1 m) using a scaled gradient (gradient time × 4, 5% to 40% modifier in 80 min). The resulting chromatograms are compared in Figure 1.

Significantly better resolution is obtained with the high efficiency coupled column approach. This is clearly demonstrated for peaks 8, 9, 1, and 3 and for peaks 5, 4, 7, 10, and 11. The separation of late eluting solutes 13 and 14 is hardly increased. This is prob-

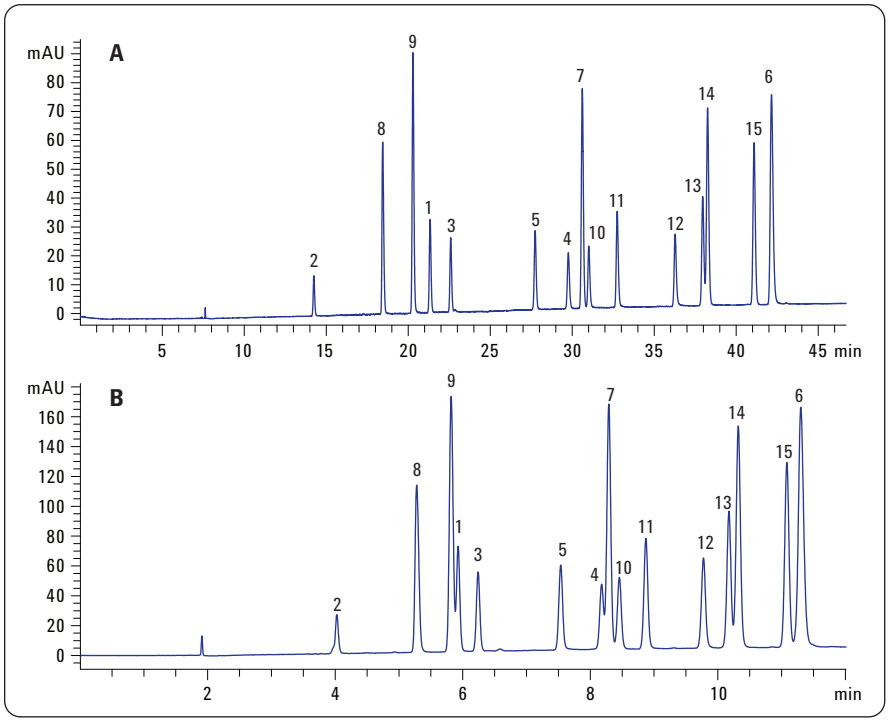

Figure 1
Gradient separation of the 15 component pharmaceutical mixture on A) 4 coupled columns (5% to 40% modifier in 80 min) and B) single column (5% to 40% modifier in 20 min). Peak identity: 1. Caffeine; 2. Ibuprofen; 3. Theophylline; 4. Theobromine; 5. Thymine; 6. Adenine; 7. Uracil; 8. Fenoprofen; 9. Flurbiprofen; 10. Cortisone; 11. Prednisone; 12. Hypoxanthine; 13. Hydrocortisone; 14. Prednisolone; 15. Sulfamethoxazole.

ably due to the influence of mobile phase density at higher percentages of organic solvent. Changes in solvent density can alter and influence selectivity in SFC significantly; and should be considered when methods are translated.

The plate number, calculated using isocratic runs, was 23,000 for caffeine and 18,000 for prednisone on the single 25 cm column. The plate numbers, measured on the serial coupled column set, were 94,000 and 76,000, respectively, reflecting the linear increase of plate number with column length. Consequently, the peak capacity increased. The calculated peak capacity for a 25 cm column and the 20 min gradient of 188, is increased to 347 on the 1 m column using the 80 min gradient (peak capacity is proportional to $\sqrt{N}$).

The analysis of the pharmaceutical mixture was repeated 10 times. Retention time repeatability was better than 0.1% (0.09% for caffeine, 0.06% for cortisone, 0.06% for prednisolone) and peak area repeatability was better than 0.5% (0.38% for caffeine, 0.34% for cortisone, 0.33% for prednisone).

2. Pesticide analysis

It has been demonstrated that packed column SFC is suitable for the analysis of different classes of thermolabile and/or polar pesticides that are not amenable to GC, including carbamates, phenylurea, sulfonylurea, and triazines [2,4]. A pesticide mixture containing triazines and phenylurea was analyzed on the four serially coupled column set using a 60 min gradient. The obtained chromatogram demonstrates excellent separation of all pesticides with baseline separation of almost all analytes (Figure 2). The separation mechanism resembles normal phase type separation since more polar pesticides (for example, the atrazine metabolites) elute after the more hydrophobic compounds (atrazine). Good sensitivity, comparable with HPLC-DAD is obtained. The SFC conditions applied here are compatible with MS detection and the method can be coupled to APCI-MS detection as well.

3. Perfume analysis

The analysis of natural products, including essential oils, and plant extracts, is an interesting application area for analytical SFC since high resolution is required. A perfume sample was analyzed by high resolution SFC using the serial coupled silica column approach. The chromatogram displayed in Figure 3 demonstrates excellent separation of all major components. The peak capacity was approximately 300 with the conditions applied (5% to 22% modifier in 40 min gradient). This is comparable to GC (using a 30 m × 0.25 mm 7column, 40 min analysis time) and can be considered as orthogonal separation and analytical technology.

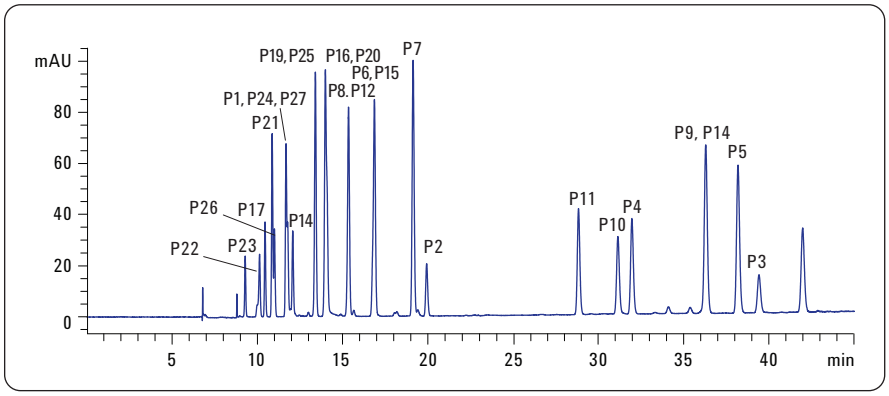

Figure 2
Separation of pesticide mixture.
Peak identity: P1. Atrazine; P2. Desethyl-atrazine; P3. Desethyl-desisopropyl-atrazine; P4. Chlorotoluron; P5. Chloroxuron; P6. Chlorpropham; P7. Crimidine; P8. Cyanazine; P9. Diuron; P10. Fenuron; P11. Isoproturon; P12. Linuron; P13. Metamitron; P14. Metazachlor; P15. Methabenzthiazuron; P16. Metobromuron; P17. Metolachlor; P18. Metoxuron; P19. Metribuzin; P20. Monolinuron; P21. Prometryn; P22. Propazine; P23. Propham; P24. Sebuthylazine; P25. Simazine; P26. Terbuthylazine; P27. Terbutryn.

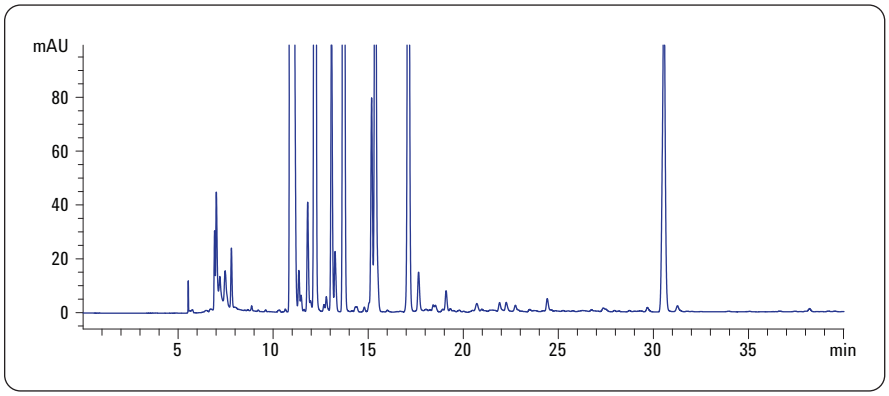

Figure 3
Separation of perfume sample.

Conclusions

This Application Note demonstrates that high resolution separations can be obtained by packed column SFC using the Agilent 1260 Infinity Analytical SFC system and serial coupled columns. Efficiencies in the order of 100,000 plates and peak capacities above 300 can be obtained, while excellent retention time and peak area repeatability are maintained, as demonstrated by the analysis of a pharmaceutical sample, a pesticide mixture, and a perfume sample.

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