DIONEX

Technical Note 701

Sub One-Minute, Nine-Component Gradient HPLC Separation for Increased Productivity Using an Acclaim® 120 3-µm C18 Column

INTRODUCTION

In recent years the need for increased productivity has led to shorter chromatographic runs. For this purpose the column length is usually shortened to 5–10 cm (from 15–25 cm) in addition to optimizing other parameters like flow rate, gradient settings, and column temperature. When shortening the column length, resolution is compromised. Therefore, to maintain adequate resolution and peak capacity, the particle diameter is reduced. While 5- and 3-µm particles are routinely used, sub 2-µm particle columns are also available. The main disadvantage with smaller particles is a significant increase in backpressure, which results in a higher instrument work load and, subsequently, more frequent maintenance intervals. In addition, reducing the particle diameter also increases the requirements for preventing column clogging. To avoid these drawbacks it is necessary to find out how much run times can be shortened with routinely used particle diameters and standard HPLC equipment without compromising chromatographic performance.

This article describes the use of a 3-µm Acclaim 120 C18 4.6 × 50 mm reversed-phase column for the gradient separation of a nine-component mixture within 39 s using standard HPLC equipment.

EQUIPMENT AND METHODS

The separation was performed with a Dionex Summit® HPLC System consisting of a P680A HPG High-Pressure Gradient Pump with Micro-Flow Kit, an ASI-100T™ Thermostatted Autosampler with 100-µL syringe and loop, a TCC-100 Thermostatted Column Compartment with 10-µL eluent preconditioner, a UVD 340U Photodiode Array Detector with a 395-nL flow cell (P/N 6065.1800N) and Chromeleon® Chromatography Management Software (version 6). The nine-component test mixture was separated by gradient elution at 60 °C on an Acclaim 120 C18, 3-µm reversed-phase column (4.6 x 50 mm, P/N 059131) with a flow rate of 5.2 mL/min. The eluents were (A) water and (B) acetonitrile. Gradient: Concave gradient (curve 7) from 40 to 90% B in 0.65 min, hold for 0.2 min. One microliter of a nine-component test mixture (alkylphenones and acetanilide, each 100 mg/L) was injected. UV detection was performed at 240 nm with a 2-nm bandwidth and a 10-Hz data collection rate. For reduced gradient delay and reduced extra column volume, 0.18 mm i.d. capillaries were used, and the sample loop was switched out of the fluidic path shortly after injection.

RESULTS AND DISCUSSION

Figure 1 shows an overlay of five consecutive injections of a nine-component mix. Excellent separation of all peaks is achieved within 39 s (RT between last and first eluting peak), with the use of a standard 3-µm particle separation column and standard HPLC equipment. Peak width at half height ranged from only 0.4 to 0.7 s. Application of a curved gradient optimized the resolution between peaks 4 and 5 to a value of 2.2. The reproducibility was 0.13% retention time RSD and 0.69% area RSD. With overlapped sample injection (the next injection is prepared during the current run) the cycle time was only 63 s. The column temperature of 60 °C decreased the eluent viscosity and allowed the use of higher flow rates for reduced chromatographic run times. The generated backpressure was 230 bar (3336 psi) which is approximately 50% of the backpressure expected with the use of an equivalent sub 2-µm column. The Acclaim column chemistry provided excellent peak shapes with-out the need to add modifiers (like formic acid) to the mobile phases.

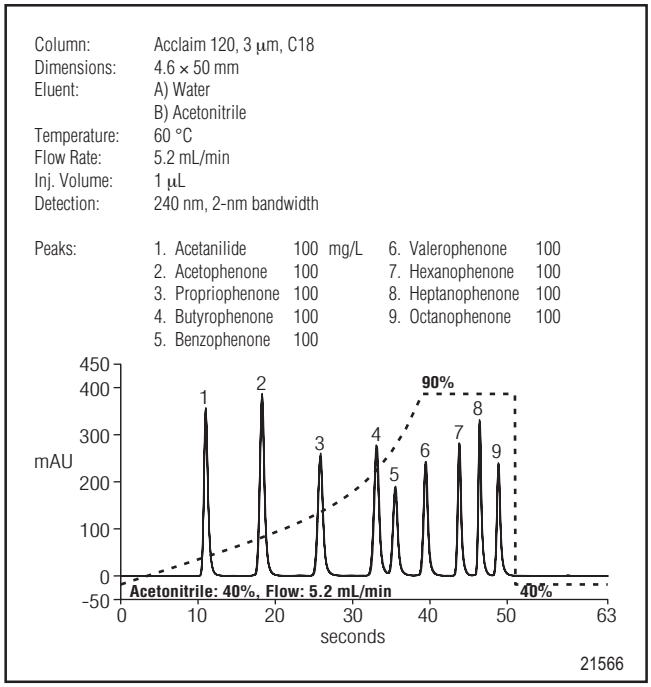

Figure 1. *Overlay of five consecutive UV chromatograms of a nine-component test mixture (alkylphenones and acetanilide).*

CONCLUSION

The use of an Acclaim 3-µm particle column in combination with standard HPLC equipment for high-throughput applications was demonstrated. Separation of a nine-component test mixture within 39 s and with cycle times of only 63 s was achieved while maintaining good chromatographic performance. System backpressure was kept moderate, resulting in a lower workload on the analytical equipment. This moderate backpressure prolongs the life of parts subject to wear—including seals—and, therefore, increases instrument uptime.

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* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.

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