Screening Impurities in Fine Chemicals Using the Agilent 1290 Infinity LC System

Application Note

Fine Chemical

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Abstract

The Agilent 1290 Infinity LC System with ultra violet/visible (UV/VIS) Diode Array detection (DAD) is used to analyze octyl-dimethyl-p-aminobenzoic acid for the presence of impurities. The system is used for the chromatographic separation of the compound from its impurities on 3.0 and 2.1 mm id C18 columns, of various lengths, with 1.8-um packing materials prepared in 600-bar (9000 psi) or special 1200-bar (18,000 psi) configurations. The ability of the 1290 Infinity LC System to operate with long, high resolution columns under conditions of rapid analysis is demonstrated with low viscosity acetonitrile (ACN) and higher viscosity methanol (MeOH) solvent conditions.

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Introduction

The analysis of impurities in starting materials, intermediates and finished products intended for a wide range of final uses is essential for ensuring product quality, performance, and consumer safety. The general conditions for successful analysis of impurities by high-performance liquid chromatography (HPLC) include gradient elution and multi-wavelength monitoring of the overall separation and may benefit from other detectors including evaporative light scattering (ELSD) and mass spectrometers (MS). Because impurity determination is the primary goal, one needs to ensure that mobile phase, vials, and HPLC components are free of minor impurities that might lead to confusing results during the analysis. Careful preparation of diluent blanks and blanks that might represent contamination sources due to additional sample preparation, such as filtration, are also appropriate. The analysis sequence is likely to include runs of the production material, solvent or diluent blank runs. It is also typical to include limit standards prepared by diluting the primary component to the lowest level where detection of impurities might be required. Finally, it is generally essential to include an authentic high purity reference standard.

Para-aminobenzoic acid (PABA) has historically been used as an ultra-violet filter ingredient in sunscreen formulations. As its use can increase the risk of skin cancer a derivative in the form of octyl-dimethyl-p-aminobenzoic acid (OD-PABA), is currently and more commonly used. However, PABA may be formed as a degradate of OD-PABA, so it is important to monitor its potential presence in samples of OD-PABA. As a commercial product, the purity of OD-PABA is important to manufacturers, for the purposes of safety and economics. In this work we investigate the capability of the Agilent 1290 Infinity LC system (UHPLC system with 1200 bar pressure limit) to detect impurities in OD-PABA samples with UV/VIS Diode Array detection.

The structure of the OD-PABA compound analyzed in this work is shown in Figure 1.

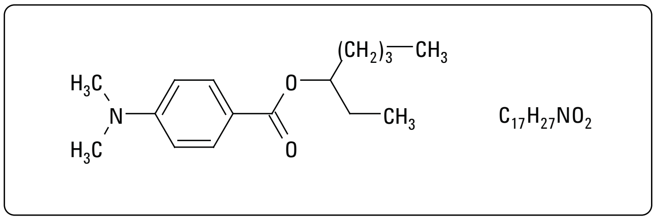


Figure 1.   Octyl-dimethyl-p-aminobenzoic acid (OD-PABA).

Experimental

Sample Preparation

The primary OD-PABA solution was prepared at a concentration of 1 mg/mL in 2-propanol and subsequently diluted to lower concentrations as needed. Injection volumes of 0.2–2 µL were made into the LC/DAD system.

LC Method Details

LC Conditions

Agilent 1290 Infinity LC system binary pump G4220A,
Agilent 1290 Infinity LC system autosampler G4226A
Agilent Thermostatted Column Compartment G1316C with switching valve
Agilent 1290 Infinity system diode array UV/VIS detector G4212A with 10 mm path fiber optic flow cell

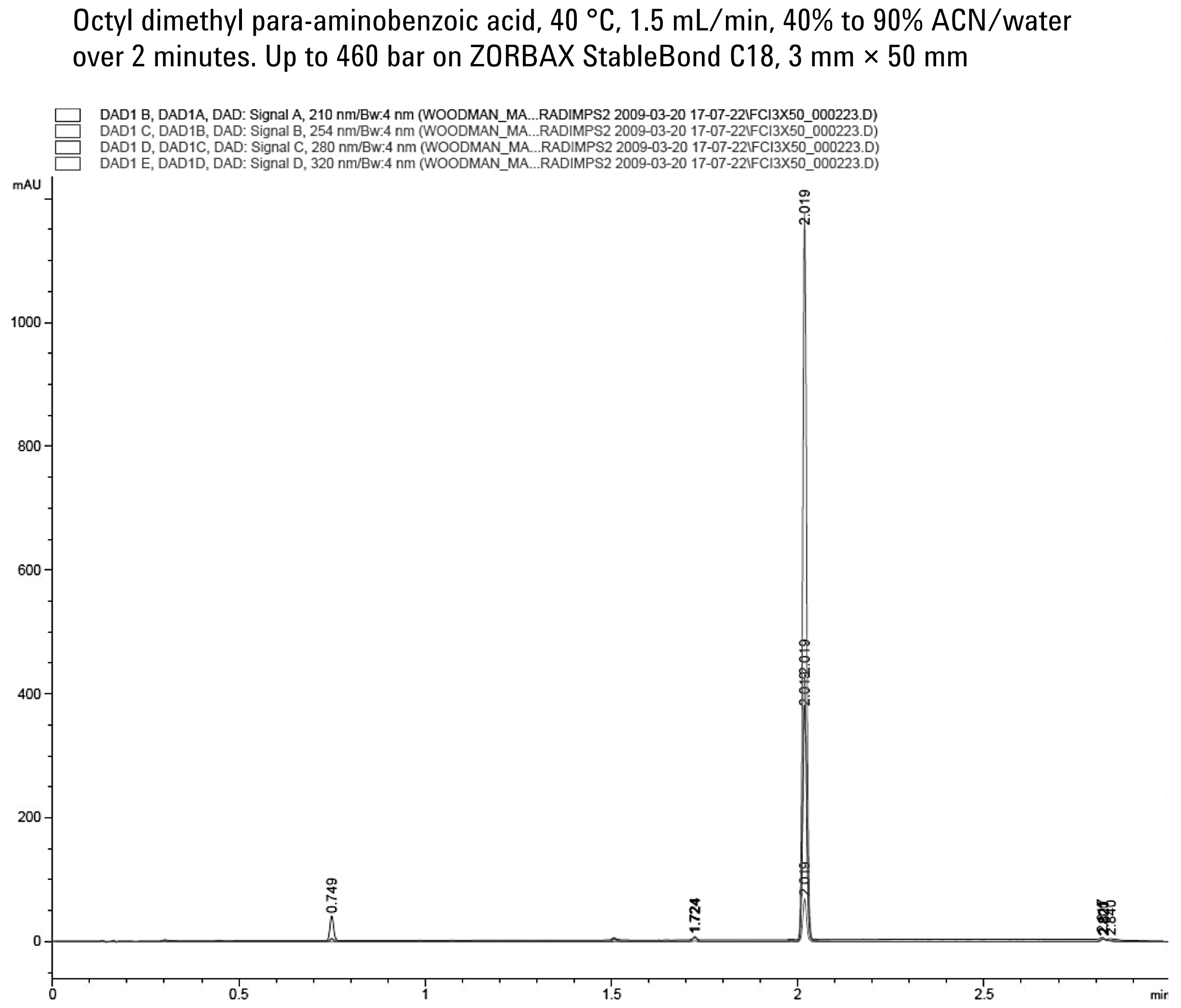
Columns:	(See individual figures for specific usage) Agilent ZORBAX SB-C18 RRHT, 3 mm × 50 mm, 1.8 µm 600 bar p/n 827975-302 Agilent ZORBAX SB-C18 RRHD, 2.1 mm × 100 mm, 1.8 µm 1200 bar, p/n 858700-902 Agilent ZORBAX SB-C18 RRHD, 2.1 mm × 150 mm, 1.8 µm 1200 bar, p/n 859700-902
Column temp:	40 °C
Mobile phase:	A = HPLC grade water B = Acetonitrile (ACN) or methanol (MeOH) (See individual figures)
Flow rate:	See individual figures
Gradient:	Gradient: the gradient conditions were either 40% to 90% ACN or 50% to 100% MeOH. The gradient slope was maintained at 3.5% organic phase increase per column volume, altering gradient time and flow rate accordingly. This is based on calculations using a modification of the Agilent Method Translator. [1]

UV Conditions

Monitoring 210, 254, 280 and 320 nm, bandwidth 4 nm, reference wavelength off

Results and Discussion

The UV response of OD-PABA, with four wavelengths monitored, is shown at a retention time of 2 min in Figure 2. Multi-wavelength monitoring of the separation provides a simple way to account for multiple impurities and assist in the selection of a final wavelength condition that can maximize sensitivity for all detected analytes.



Octyl dimethyl para-aminobenzoic acid, 40 °C, 1.5 mL/min, 40% to 90% ACN/water over 2 minutes. Up to 460 bar on ZORBAX StableBond C18, 3 mm × 50 mm

Figure 2. *Multi-wavelength UV chromatogram of OD-PABA production material on a 3 mm × 50 mm ZORBAX Rapid Resolution High Throughput (RRHT) column. The chromatogram demonstrates the typical difficulties encountered with this type of separation, which are a need for wide dynamic range detection and sensitive impurity measurement. The peak at 0.75 minutes is confirmed by retention time matching and UV spectra to be PABA, the primary impurity in the mixture.*

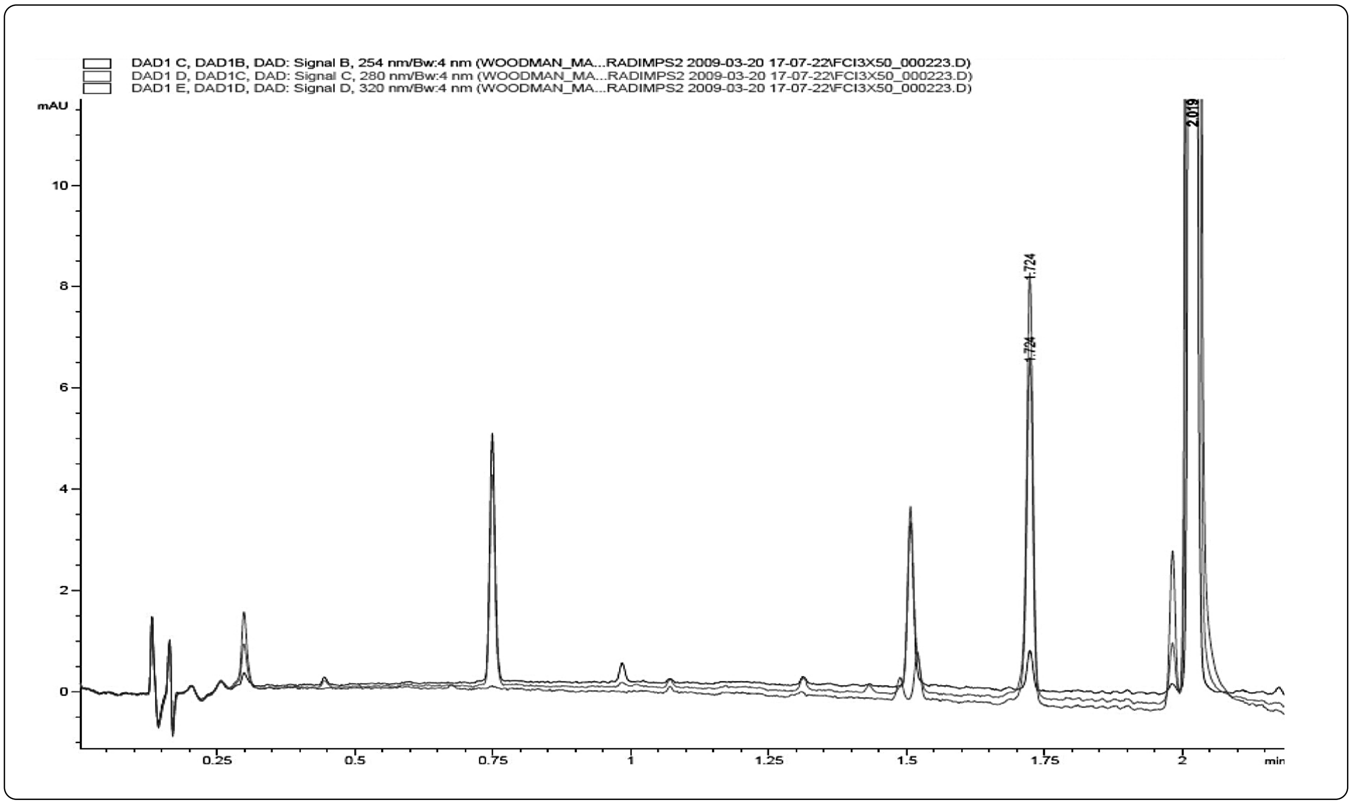


Figure 3. An expanded presentation of the chromatogram shown in Figure 2 based on the 3 mm × 50 mm gradient separation.

In Figure 3 the expanded multi-wavelength chromatogram allows us to see close detail and shows the number of impurities, as well as several areas where chromatographic resolution is clearly inadequate for individual component measurement. Despite the small particle size used in this column, the relatively short length limits the total resolution. As we move to longer column dimensions we will often reduce column diameter to reduce overall solvent consumption at the same time.

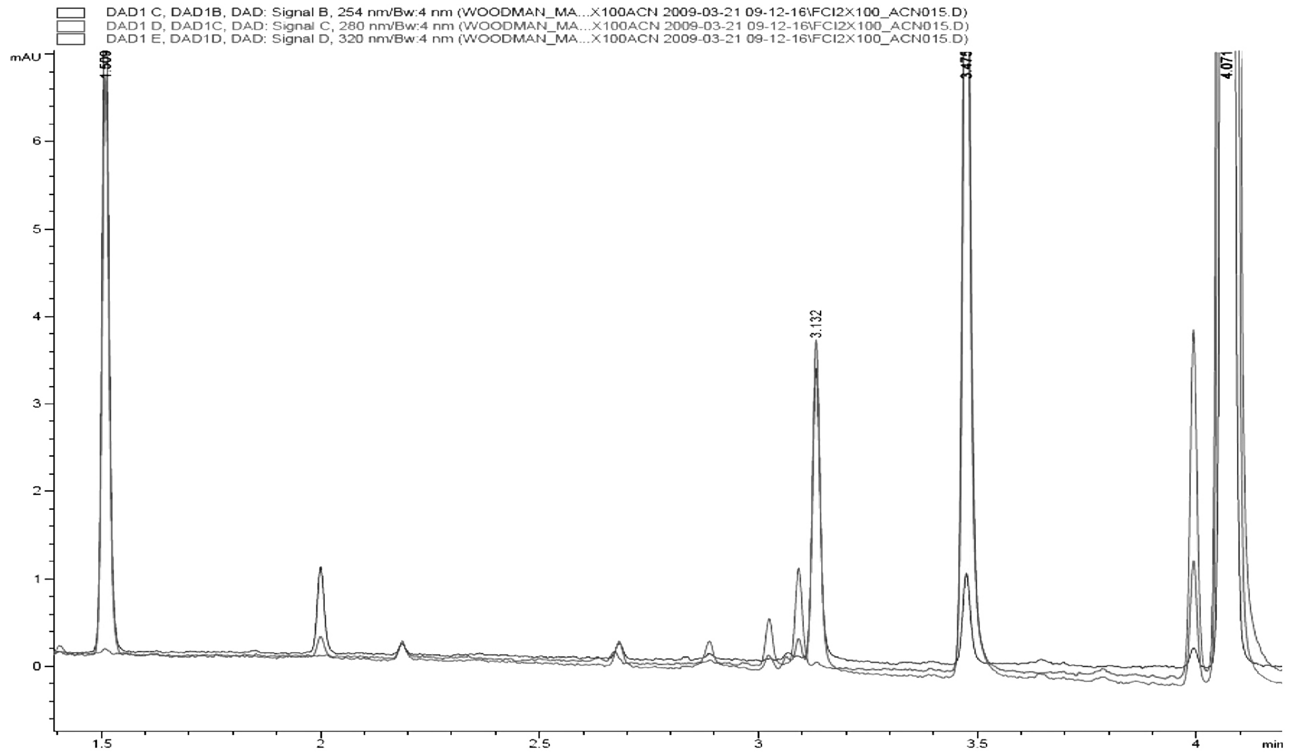


Figure 4. Analysis of the standard material on a 2.1 mm × 100 mm Agilent ZORBAX StableBond C18 column prepared for operation at 1200 bar pressure limit. Acetonitrile water gradient, 0.74 mL per minute, gradient time 4.0 minutes.

In Figure 4, we see that increasing the length of the column has resulted in a significant increase in the resolution of some of the observed components. To further increase resolution it would be practical to explore longer columns or explore alternative mobile phase or column chemistries.

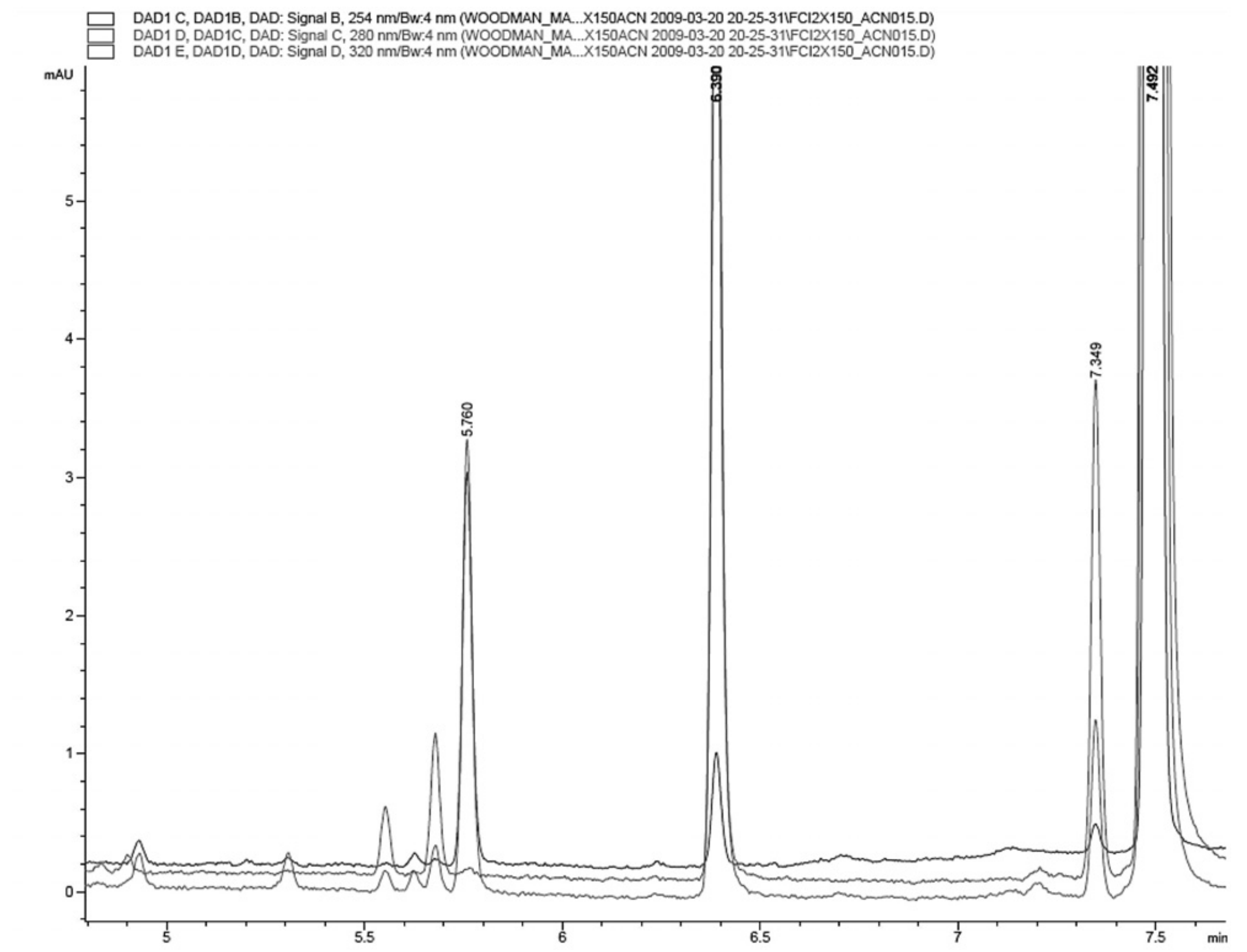
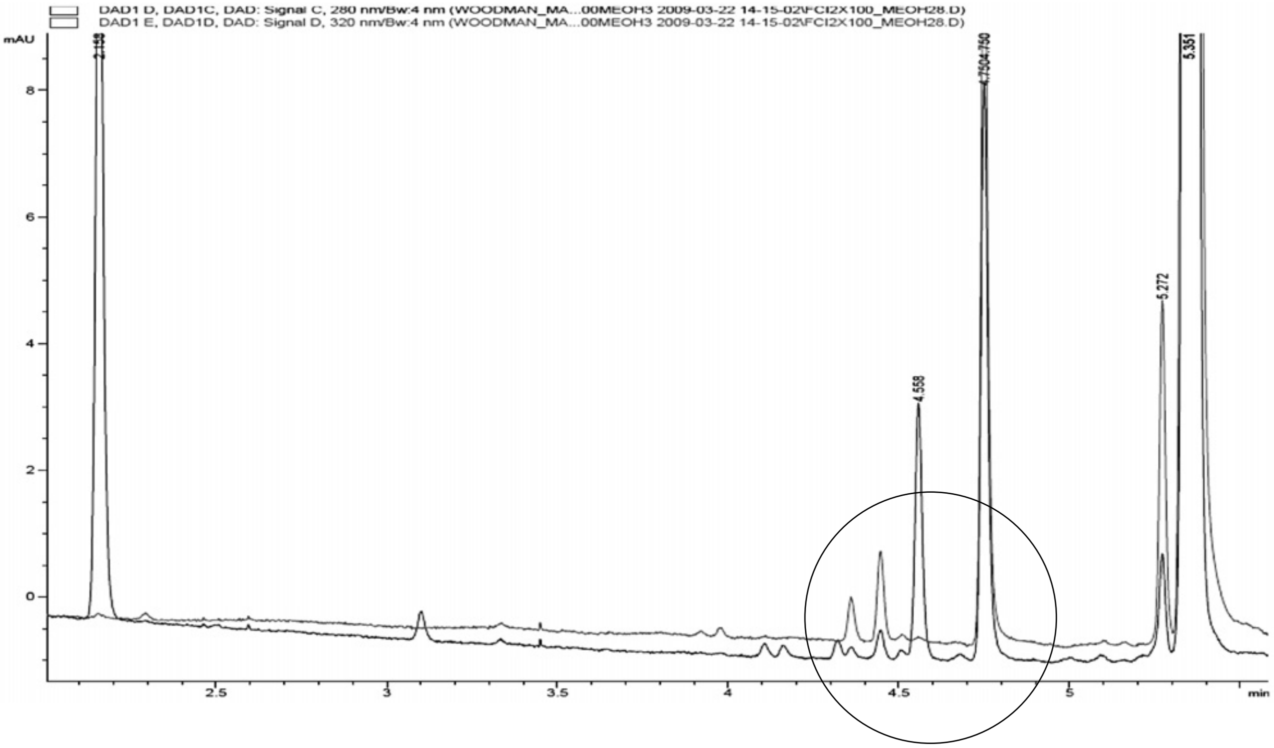


Figure 5. An expanded view of the acetonitrile separation using the same gradient slope on a 2.1 mm × 150 mm column rated for 1200 bar operating pressure. Agilent ZORBAX StableBond C18, 1.8 µm.

The increased column length clearly gives more resolution, however the increased back pressure also limits the flow rate if one is to operate in a conservative range of operating pressure. The Agilent 1290 Infinity LC system and associated ZORBAX Rapid Resolution High Definition (RRHD) chemistries are capable of operating pressures up to 1200 bar, approximately 18,000 psi. To ensure robust and rugged system operation many users typically specify the upper pressure limit for a method at a value less than 80% of the rated operating pressure.



Octyl dimethyl para-aminobenzoic acid, 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes.
Up to 845 bar on Agilent ZORBAX StableBond C18, 2.1 mm × 100 mm, 1.8 µm.

Figure 6. Separation of the sample mixture on a 2.1 mm × 100 mm Agilent ZORBAX StableBond C18, using methanol as the organic phase. Flow rate 0.52 mL/min gradient time 5.7 min, for a gradient of 5% to 100% methanol

When considering the fundamental components of the resolution equation we are all quite familiar with the concepts of capacity, selectivity, and efficiency. Increasing the column length, like decreasing the particle size of the packing material, will increase the efficiency of the overall separation. Because the increase in efficiency yields a relatively low return in terms of resolution, users often need to ensure that the capacity factor is optimized by exploring alternative chemical variables that could promote increased selectivity in the separation.

In Figure 6 we see the dramatic results achieved by changing the separation conditions from using acetonitrile as the organic phase to methanol. If this separation was highly dependent on monitoring the separation at very low wavelengths one might find the UV cutoff of the methanol, 205 nm, to be problematic. In this example, however, the highly conjugated structures of the parents and related impurity structures allow sensitive detection at wavelengths well above the UV cutoff of common organic solvents used in reversed phase chromatography. In about the same amount of analysis time as the example in Figure 5, we achieve significantly higher selectivity leading to more resolved impurities while reducing overall solvent consumption and eliminating the need for expensive acetonitrile as the organic phase.

Octyl dimethyl para-aminobenzoic acid, 0.52 mL/min, 50% to 100% MeOH/water over 5.7 minutes.
Up to 845 bar on Agilent ZORBAX StableBond C18, 2.1 mm × 100 mm, 1.8 µm, 40 °C.

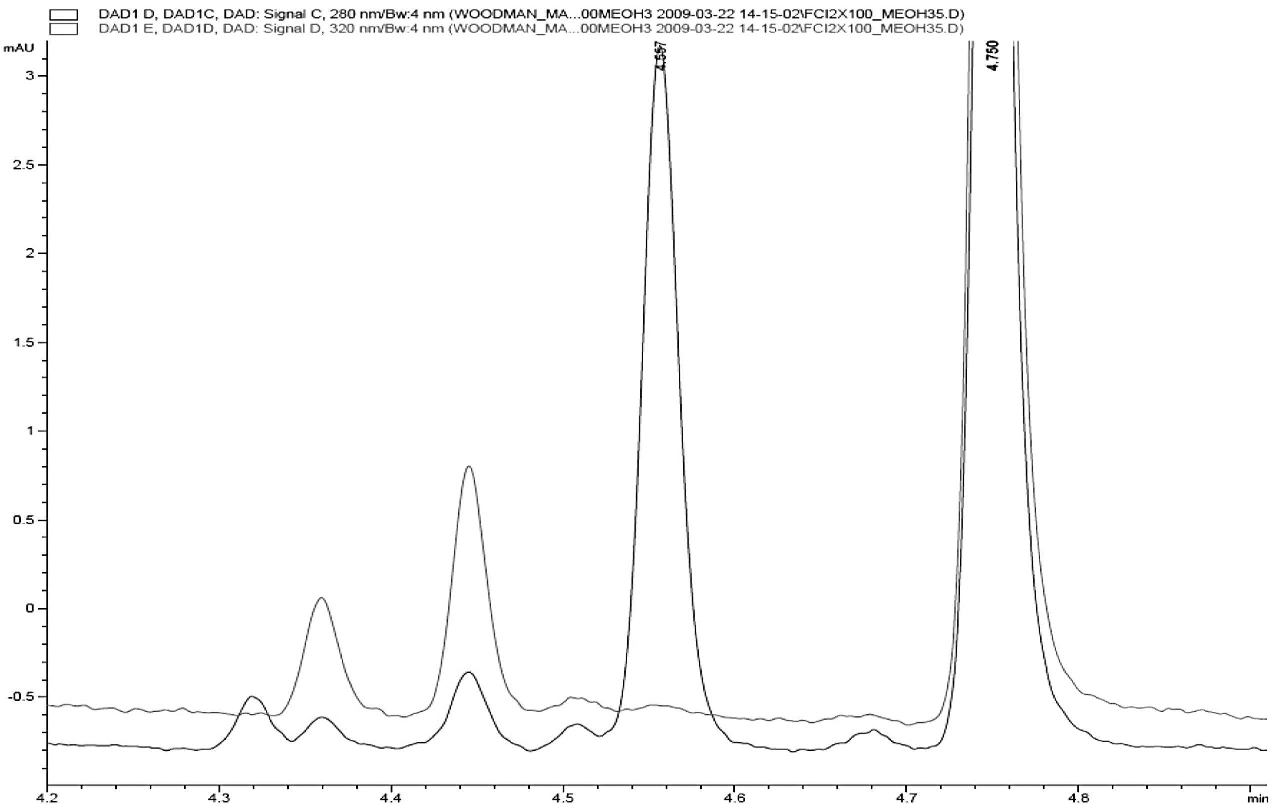


Figure 7. An expanded view of the small region of the chromatogram in Figure 6 showing a significant number of low concentration impurities. Conditions as in Figure 6. Estimated impurity concentrations for the smallest peaks in this figure are less than 0.02%.

Conclusions

The detection of low-level impurities in synthetic materials and highly refined natural products is of critical importance to the ultimate utility of these substances. Rapid analysis by HPLC using high-resolution columns and appropriately chosen organic phases ensures consistent results with rapid analysis turnaround time. Using the Agilent 1290 Infinity LC system, we were able to easily demonstrate UHPLC capabilities well within the operating range of the designed system. Higher throughput could still be achieved with this system by increasing flow rate and simultaneously reducing the gradient segment time to reproduce the gradient slope in a shorter total analysis time.

For More Information

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References

1. http://www.chem.agilent.com/en-US/products/instruments/lc/pages/gp60931.aspx)

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