



Optimizing PAH LC/PDA Separation Using Fully-Automated Spectral Processing and Qualitative Spectral Analysis Tools

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PAH analysis for environmental quality control has always been a challenging separation in LC, requiring application of binary gradient chromatography. The use of the PDA due to the nature of the PAH compounds spectra has always been a valuable information source for component identification, peak purity and quantitation. The goal of this work was to explore the opportunity to develop an isocratic separation of a PAH standard mix and be able to perform accurate identification/quantitation through the use of an automated spectral processing software. IRIS™, the new spectral processing software developed by PerkinElmer, provides all the conventional tools useful for spectral evaluation and a full suite of automated functions to seamlessly plug into the workflow the relevant spectral information.

Instrumentation/Equipment

Analysis was performed with a PerkinElmer® Series 200 PDA system with Quaternary pump, High Throughput Autosampler with Peltier tray, all controlled under the integrated TotalChrom®/IRIS software. The HPLC column used for the analysis was a PerkinElmer PAH column 150 × 3.3 mm, 5 μm. All solvents (CH₃CN, H₂O) were HPLC grade. PAH standards were obtained from Restek (Bellefonte, PA).

Experimental

Initial gradient separation was performed using 40% CH₃CN in H₂O to 95% in 20 minutes in a 25 minutes run at flow rate of 1.5 mL/min. A real-time chromatogram was monitored at 254 nm. Spectra were acquired from 200 to 700 nm. Results are shown in Figure 1a. Isocratic separation was performed at 80% CH₃CN in H₂O at 1.5 mL/min using similar detection parameters (Figure 1b).

Results

Figure 1a shows a complete separation of compounds and excellent library matching when compared to pure standards. Applying an isocratic method and a subsequent automated spectral investigation in this method development phase, a single coelution was observed (Figure 1b) as a failure in the automated peak purity report (Figure 1c). Extracting selected wavelengths allowed reliable quantitation of the coeluted compounds. A new method for routine analysis was then optimized to automatically extract/quantify/report analytical data (Figure 1d).

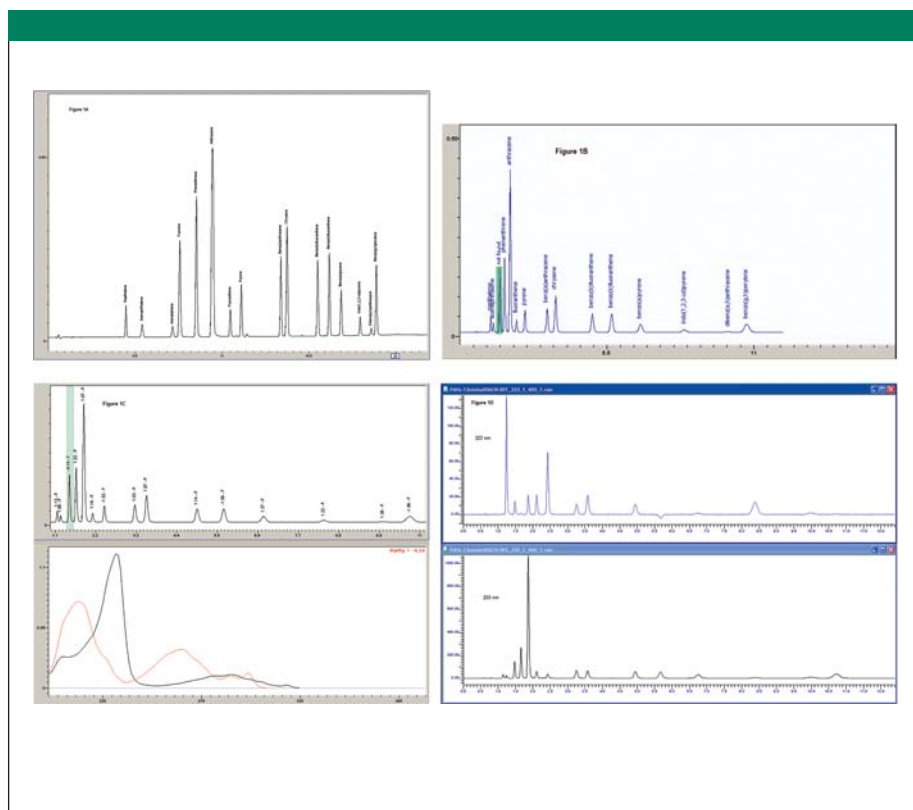


Figure 1.

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