Performance PerSPECtives

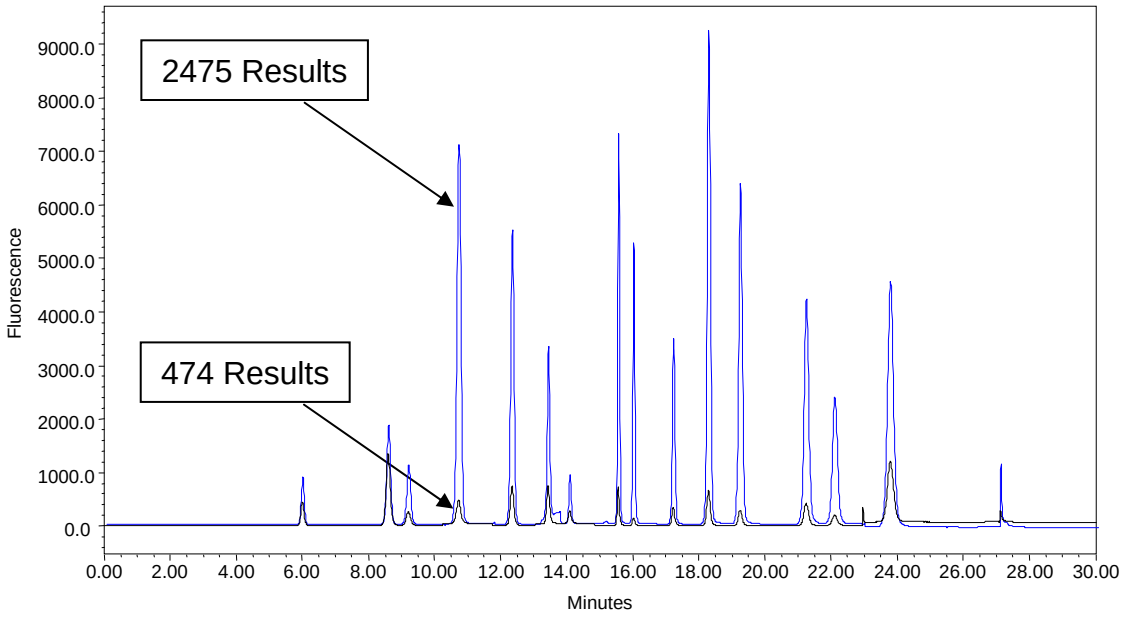
Waters® 2475 Multi-λ Fluorescence Detector: **Enhanced Sensitivity (1 of 2)**

The Waters® 2475 Multi-λ Fluorescence Detector offers unsurpassed sensitivity for quantitating low levels of target compounds, whether naturally fluorescent or derivatized with a fluorescent tag (See Waters Performance PerSPECtive 720000448EN that describes the technology that provides improved performance). This Performance PerSPECtive reveals the superior application results obtained on the 2475 Multi-λ Fluorescence Detector compared to previous generation products.

Enhanced Detector Sensitivity: Fluorescence detection is a highly sensitive technique for HPLC. Typically, femtogram or parts per trillion (ppt) sample levels can be detected. Because detector noise is influenced by many factors, (mobile phase, sample composition, smoothness of eluent flow, stability of eluent temperature, etc.) evaluation of chromatographic sensitivity is best achieved by comparing the signal-to-noise ratio (S/N) of the resolved peaks. This method provides a better representation of detector and total system performance.

Figure 1 and Table 1 compare results obtained from the analysis of very low concentrations (200-400 ppt) of sixteen Polynuclear Aromatic Hydrocarbons (PAHs) on the Waters 2475 Multi-λ Fluorescence Detector vs. Waters 474 Fluorescence detector. The superior performance of Waters 2475 detector is in part a result of a unique axially illuminated flowcell design resulting in detection sensitivity exceeding that of conventional fluorescence detectors that use cuvette-shaped flowcells (See Waters Performance PerSPECtive 720000448EN).

Figure 1. Enhanced Fluorescence Detection of Waters 2475 (Top) vs. Waters 474 (Bottom) Fluorescence Detectors.



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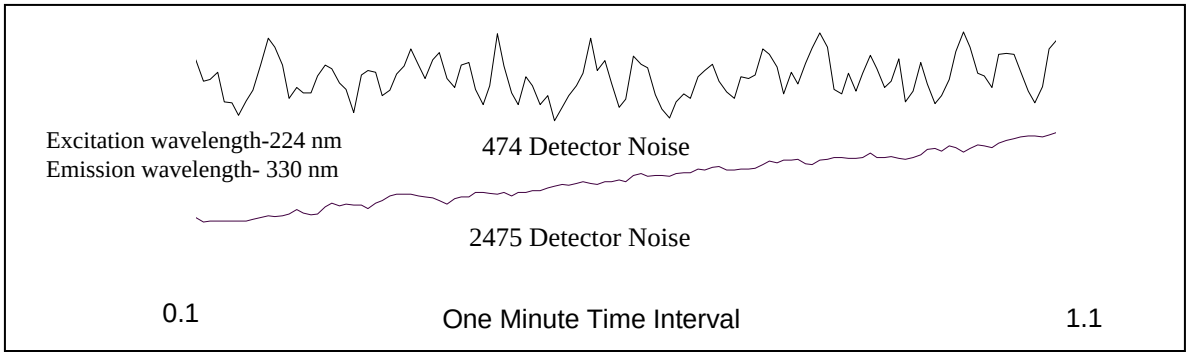
Increased Signal with Reduced Baseline Noise: Table 1 compares peak height and signal-to-noise data obtained on Waters 474 vs. 2475 for a PAH analysis. Note the increased performance of Waters 2475 Multi-λ Fluorescence Detector by more than two orders of magnitude. This is a result of both an increase in signal (i.e., peak height) with a substantial decrease in detector noise.

Table 1. Comparison of Peak Height & Signal-to-Noise:
Waters 474 vs. Waters 2475 Multi-λ Fluorescence Detector

	474 Peak Ht	2475 Peak Ht	474 S/N	2475 S/N	**S/N Increase**
Benzo(b) fluoranthene	3,837	227,980	827	276,005	**334**
Benzo(k) fluoranthene	17,029	451,949	3,672	547,153	**149**
Benzo(a) pyrene	7,844	322,654	1,691	390,621	**231**
Dibenzo(a,h) anthracene	8,088	38,248	1,744	462,769	**265**
Benzo(g,h,i) perylene	2,421	151,466	522	183,373	**351**
Indeno (1,2,3-cd) perylene	208	19.864	45	24,048	**534**

Note: Baseline noise was calculated using a 30-second time segment
474 average peak to peak noise = 4.637
2475 average peak to peak noise = 0.826



Reduce Fluorescence Response Variability: In traditional fluorescence detectors, emission response is directly proportional to the intensity of the excitation energy that illuminates the sample. Therefore, fluorescence response decreases as the light source ages. Waters 2475 detector has a unique feature that normalizes its photomultiplier tube response using Raman band of water, ensuring that each 2475 detector produces consistent data throughout the life of the lamp.

Summary:

- Technology contained in Waters 2475 Multi-λ Fluorescence Detector improves sensitivity for the analysis of fluorescent compounds, such as PAHs, by increasing detector signal while reducing baseline noise.
- Innovative features contained in Waters 2475 detector help ensure that high quality results are obtained throughout the useful life of the detector lamp. In addition, these same features minimize detector-to-detector response variability that is commonly experienced using traditional fluorescent detectors.

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