Performance PerSPECtives

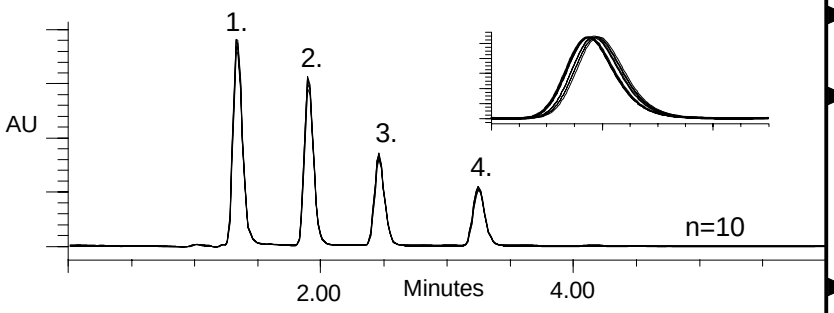
Retention Time Vs Peak Area

What is the relationship?

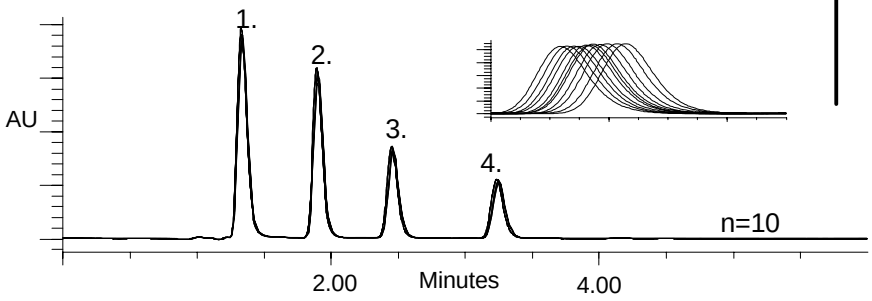
Peak retention time reproducibility has been redefined with the introduction of the Waters 2690 Separations Module. Smoothness of flow, constant pressure, as well as accurate and precise solvent proportioning are a few of the enhancements that allow the 2690 to perform beyond traditional expectations. Peak retention time is the first level of peak identification used by chromatographers. Retention time reproducibility is important to identify compounds confidently. Other benefits can be realized by improving retention time reproducibility. When performing quantitative evaluations, retention time reproducibility can be directly linked to peak area reproducibility. What relationship does the reproducibility of retention time have with the reproducibility of peak area?

Below are two sets of data generated with a Waters 2690 Separation Module utilizing automated solvent blending (WPP214). The top set of data (Set A) are 10 injections with a constant flow rate of 1 ml/min. The bottom set (Set B) was generated with the same instrument by varying the flow rate 0.001 ml/min between each of the 10 injections to span a flow rate range from 0.995 - 1.005 ml/min in order to imitate poor retention time reproducibility. This experiment was used to calculate the effects of retention time reproducibility on peak area reproducibility.

Set A



Set B



Conditions:

- Waters 2690 Separation Module
- Symmetry® C18 3.9 x 150mm 1 mL/min, 60:40 methanol:water, 30°C, 254nm
- PQ standard mixture acetone, C2, C3, C4 alkylphenones

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Retention Time versus Area

From this data, one can deduce that as retention time reproducibility gets worse, so does peak area reproducibility. The figure below is a graph of retention time (x-axis) versus peak area (y-axis) for peak 3 over 10 injections of the PQ separation. The data Set A and Set B correspond to the earlier data.

As retention time reproducibility worsens, peak areas have more variability suggesting that good retention time % RSD produces better area % RSD and larger retention time % RSD generates poor area reproducibility . Also, the peak area counts trend up with the increase in retention time. This information supports the need for high performance HPLC equipment to assure reproducible results, both retention time for identification and peak area for quantitation.

Why should a user be looking to achieve the highest level of performance? Because their results (quantitation and calibration) are directly linked to retention time reproducibility.