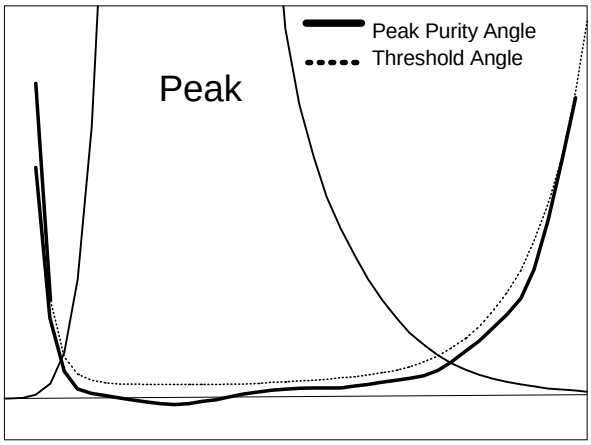
Performance PerSPECtives

Waters 996 Photodiode Detector: Peak Purity III

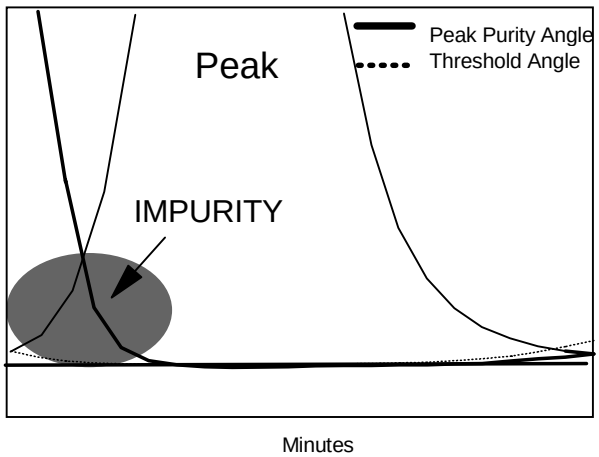
Interpretation of peak purity plots

This is the third in a series of Performance Perspectives which discuss peak purity. The others are WPP16 and WPP17. The graphics of the Waters Millennium® software are very useful for interpreting peak purity analysis. The Purity Angle plot (heavy line) measures spectral differences at each data point across a peak compared to the peak apex spectrum. When the Purity Angle is less than the Threshold Angle (dashed line), all spectra differences are due to random events caused by spectral noise. When the spectral differences are greater than the threshold, there is a coeluting impurity or impurities.



This figure is the typical appearance of a peak purity plot for a chromatographic peak consisting of only one compound.

The increase in the spectral differences at front and back of the peak are caused by increasing noise relative to the peak absorbance.



This figure is typical of a coeluting impurity on the leading edge of a peak, where the purity angle increases above the threshold. The reason you may not see a shoulder on the peak is the impurity may not have absorbance at the monitoring wavelength or it may be present in a fairly low concentration. The Waters 996 with Millennium software not only identifies coeluting compounds, but shows where these coelutions are within the chromatographic peak.

Knowing the location of the impurity can help to optimize a chromatographic separation.

Waters

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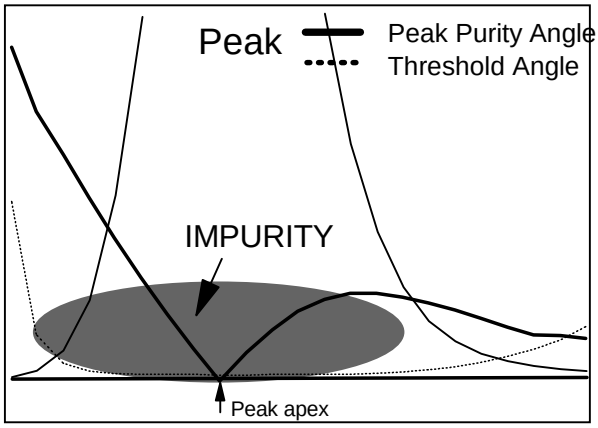
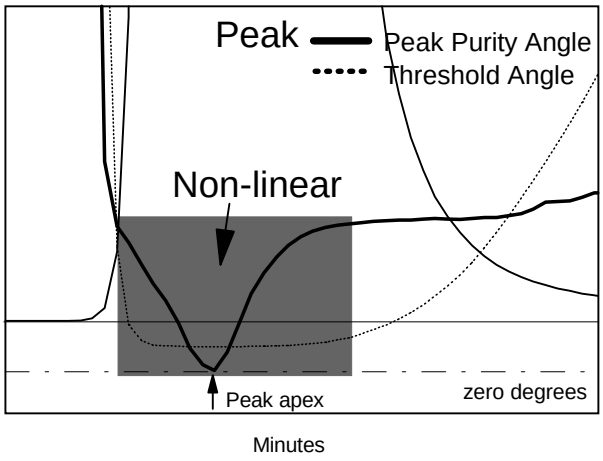
Interpretation of peak purity plots



This is an example of a peak purity plot where the coelution is poorly resolved from the analyte peak. As a result, the apex, reference spectrum is a mixture both compounds. Therefore, the purity plot changes across the entire peak.



There are situations where peak purity plots will indicate spectral differences but not the presence of a coeluting impurity. The most common is the shown here. When the concentration of the analyte is high enough that detector linearity is exceeded at some wavelengths, the apex spectrum is different than the others across the peak. A clue to this is the flat line in the purity plot above the zero degrees. If linearity is the problem, diluting the sample will produce a purity plot like the first figure.

Peak purity can be a very helpful tool. However, when using this analytical technique the analyst must be cautious in the interpretation of the results. By working with known standards, you can learn what the peak purity plot will look like and the changes which can be expected in the peak purity values with changes in factors like analyte concentration. After that, when the purity plot changes or the purity angle value increases, you will have confidence to interpret it as the presence of a coeluting impurity. The absence of a coelution when using a photodiode array detector should not be interpreted as chemical purity. (see WPP16)