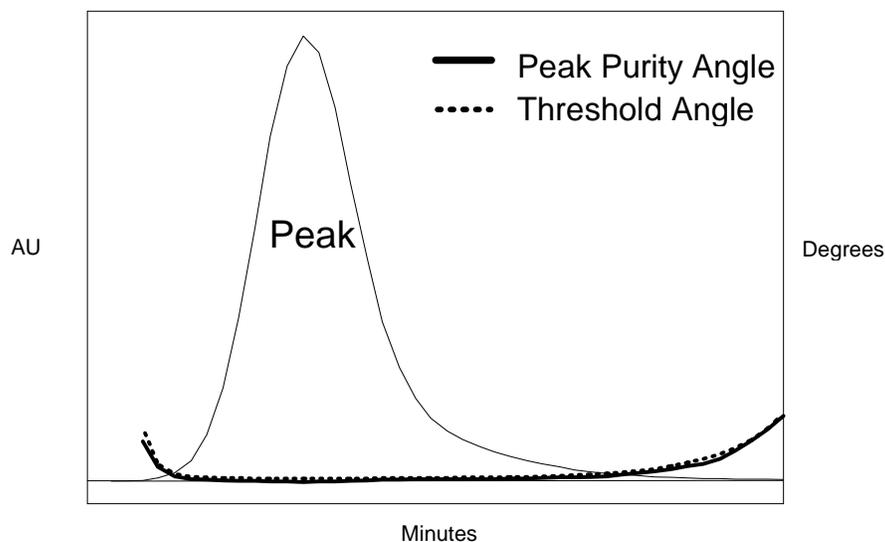


Waters 996 Photodiode Detector: Peak Purity II

Peak purity plot

Peak purity was defined in Performance Perspective WPP16. To simplify interpretation, Waters Millennium® software presents both graphical representations and numerical results for peak purity.

Peak purity is an analysis of spectral differences, assuming the impurities are spectrally different from the analyte. The spectral analysis is achieved using vector analysis algorithms (Gorenstein, et al., LC-GC 12, no.10, 1994, pp.768-772). The more similar the spectra are, the closer the value is to 0.0 degrees; the more spectrally different, the larger the value (90 degrees is the maximum). When no significant spectral differences are found within a peak, one can conclude that the peak is spectrally homogeneous and probably contains a single compound. Millennium analyzes all the spectra (data points) across the peak. The data is converted into vectors, compared and graphically plotted so that the results can be visualized as shown in the figures.

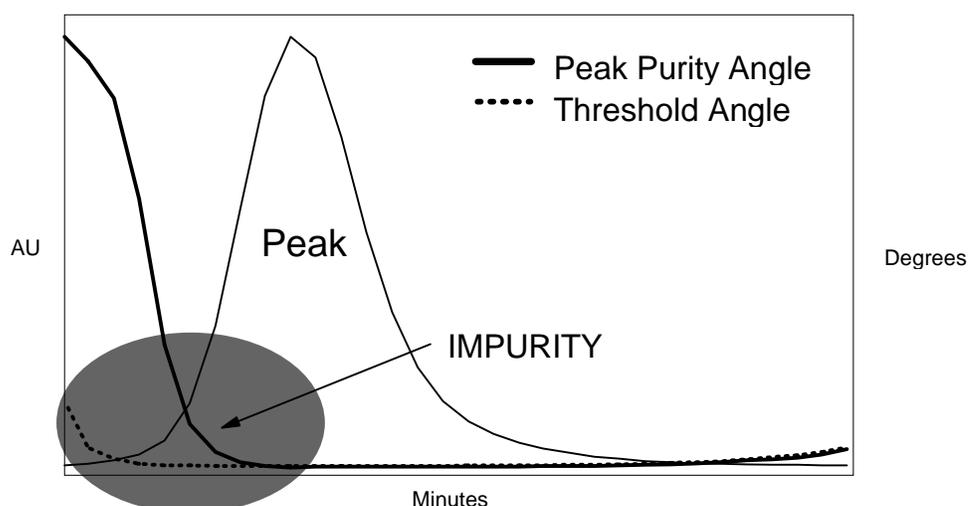


The above figure is the peak purity plot for a chemically pure chromatographic peak. Notice there are no valleys or shoulders on the peak. The thick solid line represents the spectral differences across the peak. The reference point is the apex spectrum. Most of the spectra in the peak are close to zero difference, which is characteristic with a pure compound. At low absorbances the baseline noise contributes to spectral differences.

Peak purity plot - coelution

Millennium estimates the spectral noise and the random events which cause small changes in spectra, from a user chosen segment of baseline. This is called the threshold. When the purity plot is above this noise threshold, the spectral changes are recognized to be greater than random noise. This provides a level of confidence in that a spectral impurity has been found.

When an impurity is detected, the purity plot rises above the threshold line. This indicates spectral differences beyond noise contributions and implies the presence of more than one compound in the chromatographic peak. In the example below, the coeluting impurity is in the leading edge of the peak. To determine if there is one or more impurity present, another feature of the Millennium software called Multicomponent Peak Purity can be used. This feature, will analyze for the number of spectrally distinct compounds in the coelution from one to four or more.



To automate the peak purity analysis, numerical values for Purity Angle and Threshold Angle are calculated by the Millennium software. They are averages of all the measured angles weighed by the absorbance in the peak. In this way, the greater the absorbance the more importance is given to the peak and threshold values. The lower the purity angle, the more similar the spectra across the peak, the more closely the Purity Angle will approximate zero.

Peak purity analysis is very useful during methods development to confirm all components have been chromatographically separated; and in quality control to warn the analyst that an unexpected coeluting impurity has appeared. Customized Millennium reports can be generated which automatically flag purity values that are higher than expected which indicate the presence of an impurity.