Enhanced Diode Array Detector Sensitivity and Automated Peak Purity Control

Technical Note

Introduction

The most widely used detection technique for HPLC analysis is UV absorption. Over the decades, single and multi-wavelength detectors have been optimized for use as highly sensitive devices for quantitative analysis at specific wavelengths, however one drawback of these detectors is that they only provide limited information for compound identification.

The introduction of the diode array detector helped overcome this problem by providing access to quantitative as well as qualitative spectral information for a wide wavelength range. Agilent Technologie introduced the first diode array detector for liquid chromatography in 1982. Since that time significant improvements in the design of both the hardware and software have been made, resulting in the introduction of the Agilent 1100 Series diode array detector (DAD). A schematic view of the optical design of this detector is given in figure 1.

In order to obtain optimum lamp energy in the UV and visible ranges, a tungsten lamp is positioned in line with a deuterium lamp. By using a software-programmable slit combined with the 1024 element diode array, the optimum between wavelength resolution and sensitivity is easily achieved.

This Technical Note demonstrates the outstanding performance of the Agilent 1100 Series DAD as a very sensitive UV-Visible detector and the capabilities of the instrument for peak-purity analysis and peak confirmation of trace level.

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Results and discussion

High sensitivity UV-detection

The most important criterion for performance measurement in UV detection is a high and reproducible signal-to-noise ratio for the compound of interest. In addition, detector drift and wander over the whole wavelength range have to be monitored. Baseline stability results typical for the Agilent 1100 Series DAD are shown in Figure 2, where baseline noise in the UV range is below 1×10^{-5} AU. The lower part of the figure illustrates the decrease in baseline noise that is achieved by having a tungsten lamp to cover the visible wavelength range. This allows for high sensitivity measurement when combined with the 13 µl standard flow cell (10 mm cell path length.)

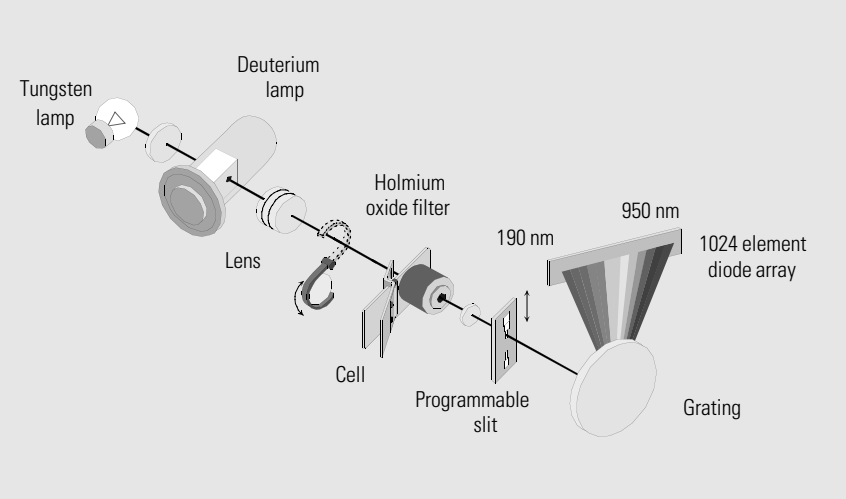

Figure 1
The optical design of the Agilent 1100 Series diode array detector

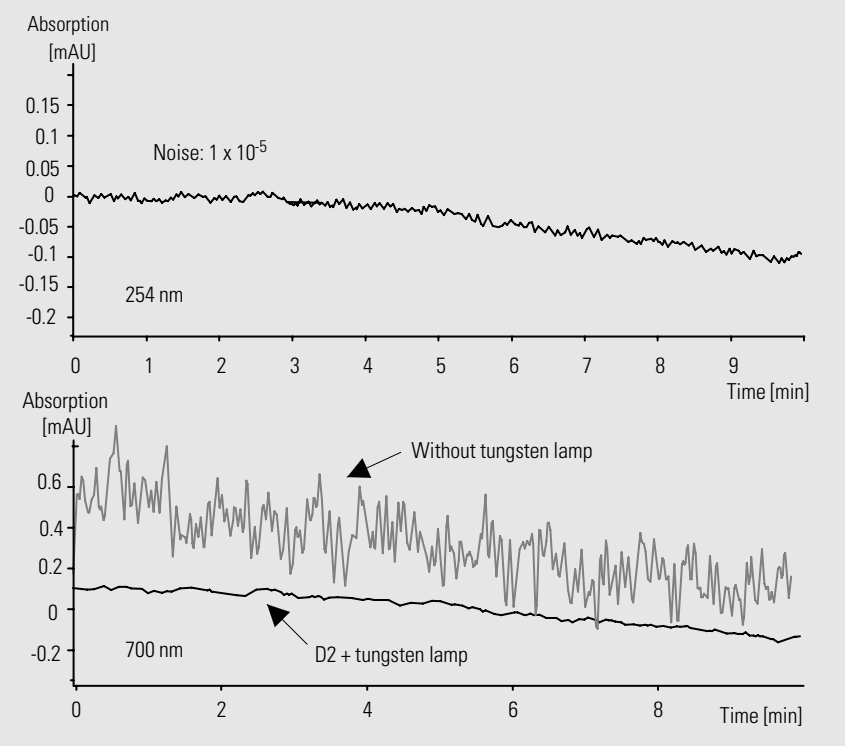

Figure 2
Baseline stability with the Agilent 1100 Series diode array detector

The upper part of this figure illustrates the typical noise in the UV range. The lower figure shows the difference in noise for operation with and without the tungsten lamp in the visible range.

Conditions	
Column	Hypersil BDS, 3 µm, 125 × 3 mm
Mobile phase	Water/Acetonitrile 2/8 isocratic
Flow rate	0.2 ml/min
Slit	1 nm

In order to optimize signal/noise each application requires a thorough optimization of DAD parameters at trace level. The graphical user interface of the Agilent 1100 Series DAD gives easy access for bandwidth, slit width, response time and reference wavelength. Figure 3 shows the results of analysis of anthracene at the 10 pg level at 251 nm with a signal/noise ratio of 23 after optimization.

Multiwavelength analysis

For selective quantification of known compounds multiwavelength detection is better. This requires that the substances show absorption at substantially separated wavelengths. Since diode array detectors can collect data over the whole range of wavelengths, they are ideally suited for use as multiwavelength detectors.

Figure 4 shows an example from food analysis. The green appearance of lemonade is created by adding a yellow and blue color. To ensure compliance with regulatory requirements the colors are identified by simultaneous monitoring at non-interfering wavelengths and quantified according to the appropriate directives.

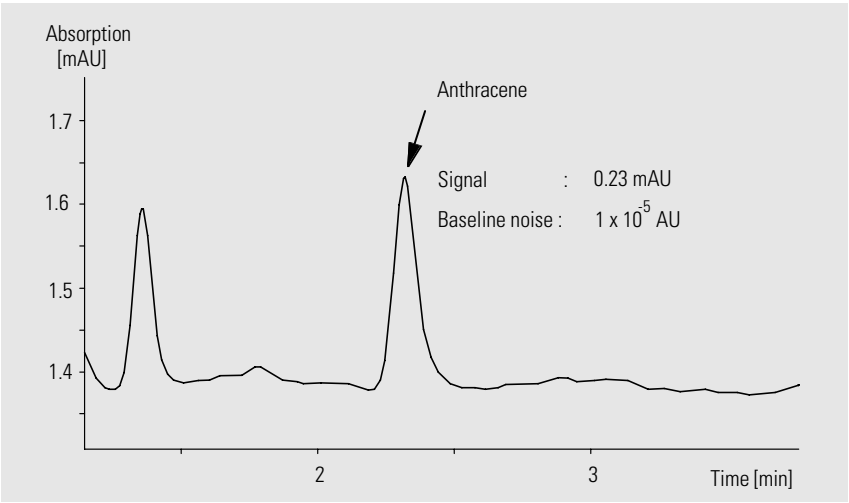

Figure 3
UV-signal sensitivity in environmental trace analysis

Conditions

Sample	Anthracene standard, 10 pg
Column	Hypersil ODS, 3 µm, 100 × 2.1 mm
Mobile phase	Water/Acetonitrile 3/7 isocratic
Flow rate	0.4 ml/min
Wavelength	251 nm

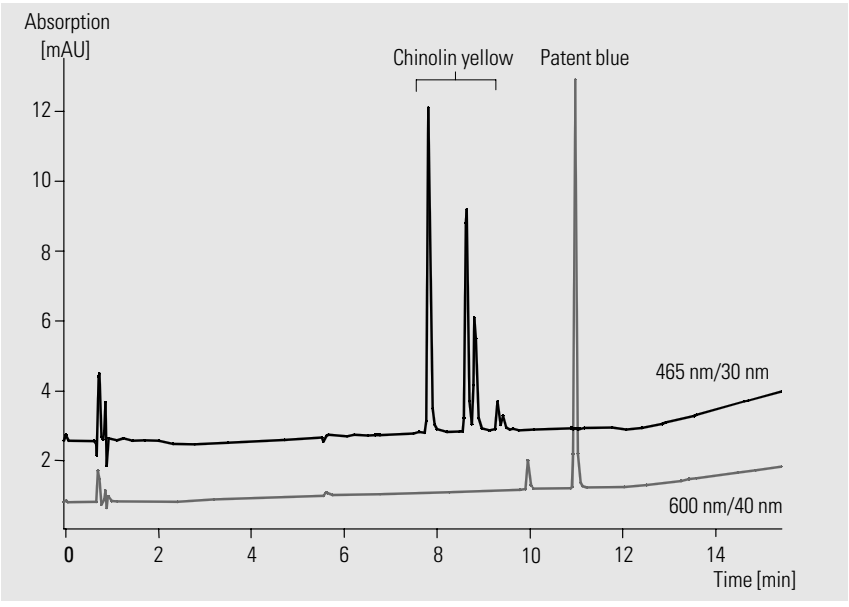

Figure 4
Analysis of colors from Woodruff lemonade by multi-wavelength detection

The peak for patent blue reflects a concentration of 7.45 ng dye

Conditions

Column	Hypersil BDS, 3 µm, 125 × 3 mm
	40 °C
Mobile phase	
A:	0.01 Disodiumhydrogenphosphate + Tetrabutylammonium–dihydrogenphosphate in water, pH 4.2,
B:	Acetonitrile
Flow rate	0.8 ml/min
Gradient	15%B to 40%B in 10 min, to 90%B in 19 min

Extended wavelength detection

A variety of chemicals and natural products are available for use as colors and dyes to address current interests in fashioned products. In order to prevent health damage arising from this practice an increasing range of colors are regulated with national directives. The Agilent 1100 Series DAD is well suited for monitoring in the visible wavelength range. The addition of a tungsten lamp leads to superior baseline stability (figure 2) and more complete spectra (figure 5).

Peak identification at trace level

Retention time and absorption at a single wavelength is not sufficient to prove the identity of compounds in environmental analysis. The complexity of real life samples are such that additional information is needed to confirm peak identity. With a diode array detector complete spectral information is available from any time and wavelength during the run. One common practice is to compare the obtained spectra from a peak with a library or to check for peak-purity by comparison of spectra taken throughout a single peak. With the Agilent 1100 Series DAD both techniques can be applied automatically as part of a method and therefore run routinely for a sequence of samples, for example overnight. Spectra can either be collected throughout the analytical run or peak controlled to save memory space.

Library search

Figure 6 shows an example for ultimate spectral sensitivity. A comparison of sample results with library spectra confirms the peak identity. At the limit of detection for pyrene, at pg–level, the spectrum taken during the run matches with the library spectrum recorded at high concentration. With the availability of pure standard compounds, customized libraries can be established by the individual user for a particular application. The Agilent ChemStation software calculates the correlation between the library and the experimental spectra and reports match factors. A match factor of 1000 (correlation factor = 1) describes identical spectra.

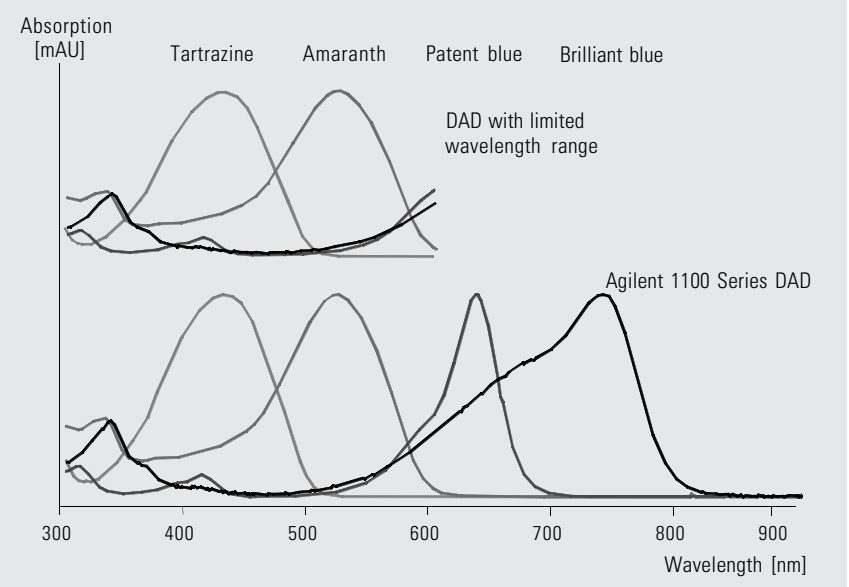

Figure 5
The Agilent 1100 Series DAD gives more complete spectra

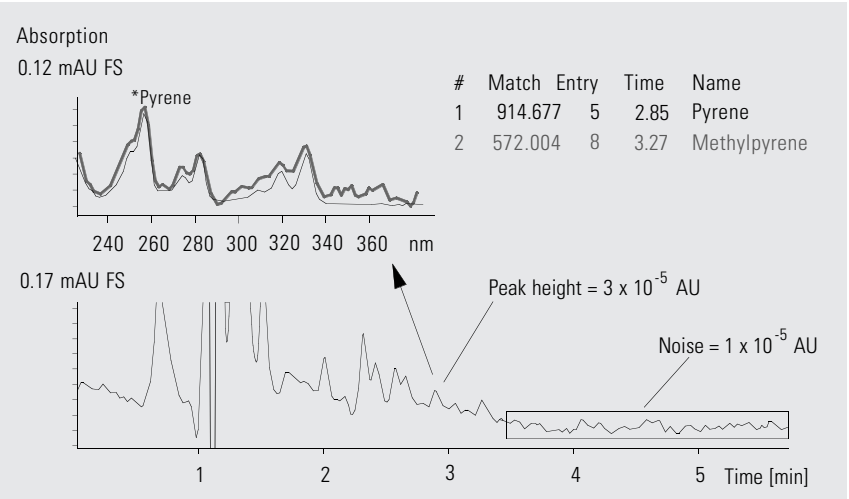

Figure 6
Spectral identification by library search at trace level concentrations

Peak-purity analysis

Normalized spectra from a pure peak will not differ whether they are recorded at the apex, at the beginning or at the end of a peak. Impurities with different spectra and comparable spectral absorption can be monitored as soon as some chromatographic resolution of the compounds is achieved.

The Agilent ChemStation provides several tools for the calculation and judgement of peak-purity. For notification purposes a threshold level can be set to inform the user about a certain level of spectral impurity. The similarity curve displays the match factors for all spectra in a peak, providing more detailed information and higher sensitivity. In addition, a threshold curve can be calculated to show the effect of noise on the spectral similarity. This gives the range for which spectral impurities are within the noise limit. Both curves can be combined into one graph—the peak-purity curve.

The following examples demonstrate how the Agilent 1100 Series DAD is able to identify the presence of even minor impurities within a chromatographic peak. These are different with respect to chromatographic resolution.

Two mixtures with common ingredients to analgesic formulations were analyzed under the same conditions. Concentrations of the analytes varied. The overlaid chromatograms in figure 7 highlight the limited resolution of phenacetin and salicylic acid (R = 0.9) and caffeine and paracetamol (R = 0.1).

The absorption bands of the two pairs show such overlay that there is no wavelength for sensitive quantification of the minor compound away from the major ingredient (figures 8 and 9).

Example 1: Phenacetin and salicylic acid

In the case of phenacetin and salicylic acid, chromatographic separation is nearly achieved. The identification of phenacetin as the impurity is done by analyzing peak-controlled spectra (upslope, apex and downslope.) Acquisition of peak-controlled spectra considerably reduces the requirements for data storage. With this mode, the threshold alarm is displayed if one match factor exceeds the threshold value.

Conditions	
Column	Hypersil BDS, 3 µm, 125 × 3 mm
Mobile phase	Water with sulfuric acid to pH 3/Acentonitrile 6/4 isocratic
Flow rate	0.25 ml/min

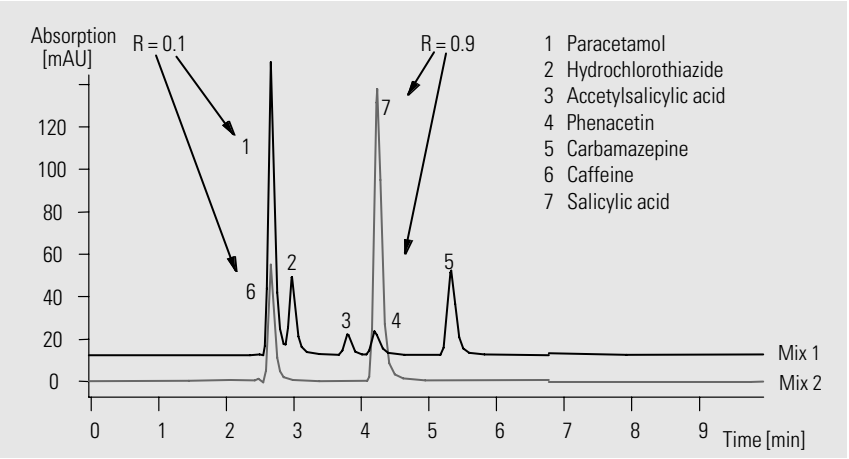

Figure 7
Chromatograms of two analgesics mix

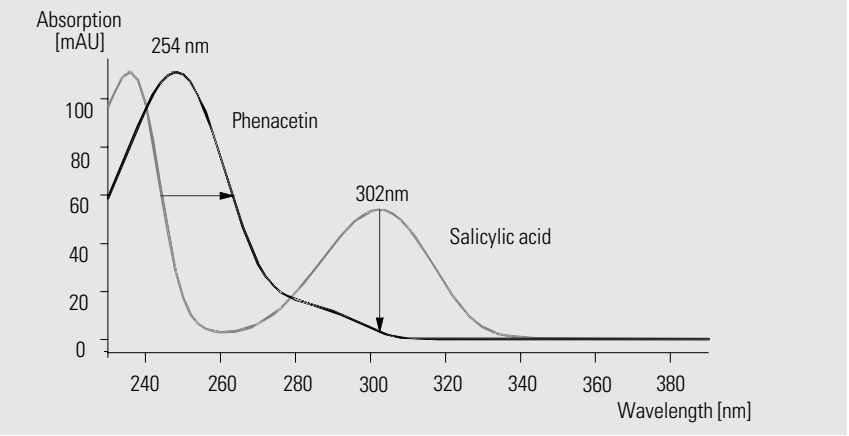

Figure 8
UV spectra for phenacetin and salicylic acid

Figure 10 shows the results for different levels of phenacetin as the impurity. It can be seen that phenacetin was detected as the spectral impurity down to the 0.5 % level. The peak spectra window gives the overlay of spectra taken. The peak-purity window offers a view as used in multi-wavelength detection.

Example 2: Caffeine and paracetamol

Figure 9 shows that caffeine and paracetamol should be monitored at 254 nm and 272 nm. However, at both wavelengths there is a UV absorption of the coeluting compound in the chromatogram (figure 7). In this case, analysis of peak-controlled spectra is only sufficient to detect an impurity level of 10 % paracetamol.

However with the full set of spectra available, calculation of the similarity and threshold curves can be done. Both are displayed as separate curves with the ratio of similarity and threshold values (figure 11). Thus the similarity of spectra is elucidated taking into account the influence of noise, especially at the start and end of a peak.

With the example of caffeine and paracetamol, as little of 0.1 % of paracetamol could be detected as spectral impurity.

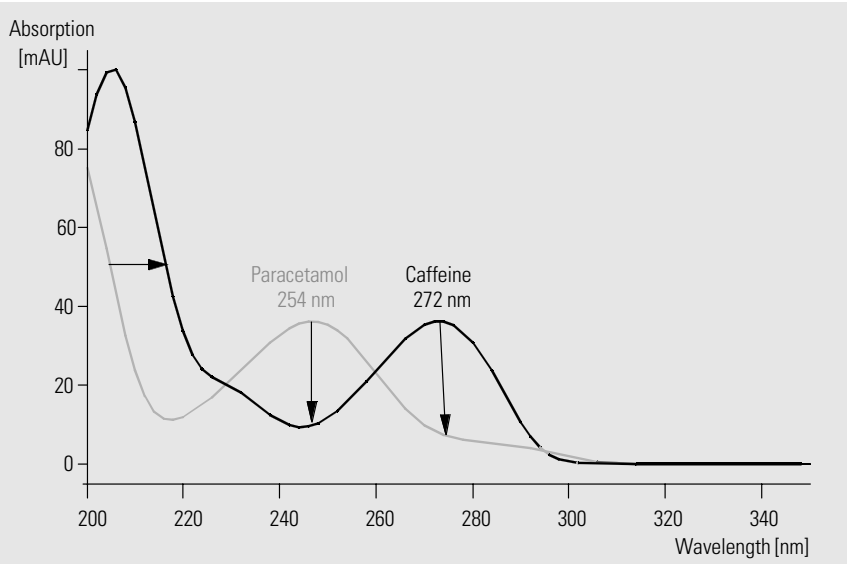

Figure 9
UV spectra for caffeine and paracetamol

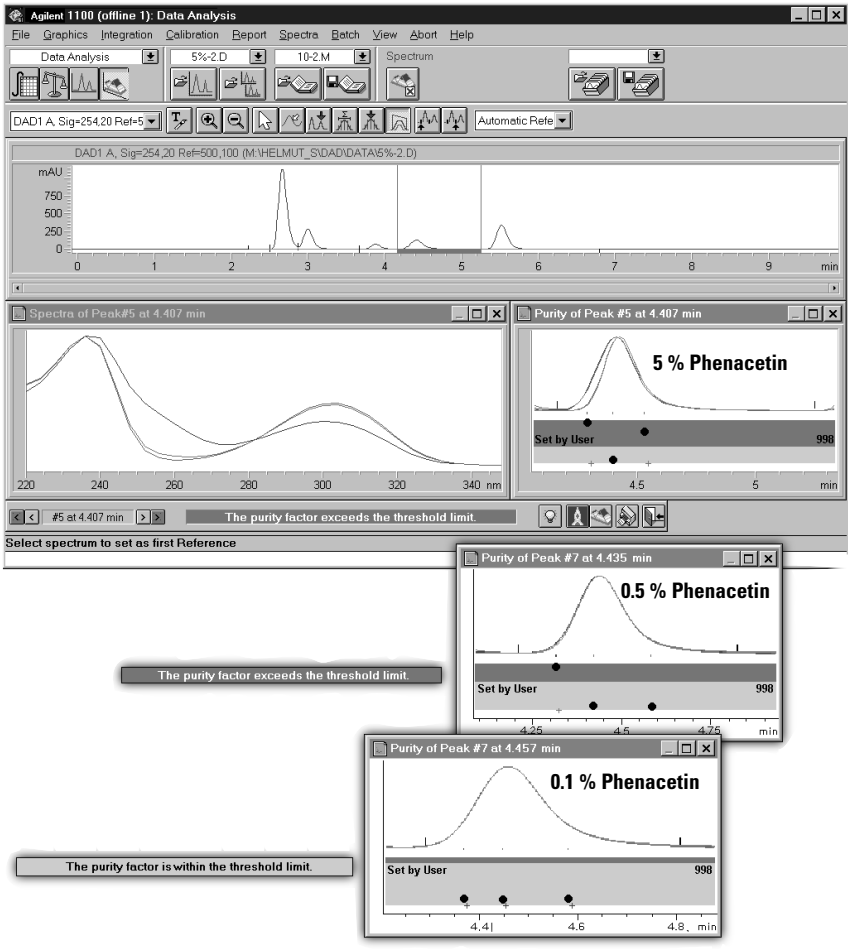

Figure 10
Peak-purity analysis with peak-controlled spectra, phenacetin as the impurity, coeluting with salicylic acid

Summary

The Agilent 1100 Series DAD gives excellent sensitivity for basic single and multiwavelength detection as well as for spectral identification of compounds. Even at trace levels the software on the Agilent ChemStation allows fast and accurate comparison of spectra with customer contributed libraries. Library searches as well as peak-purity analysis can easily be executed as part of a method. Additionally, the algorithms implemented in the peak-purity analysis can detect impurities down to the 0.1 % level.

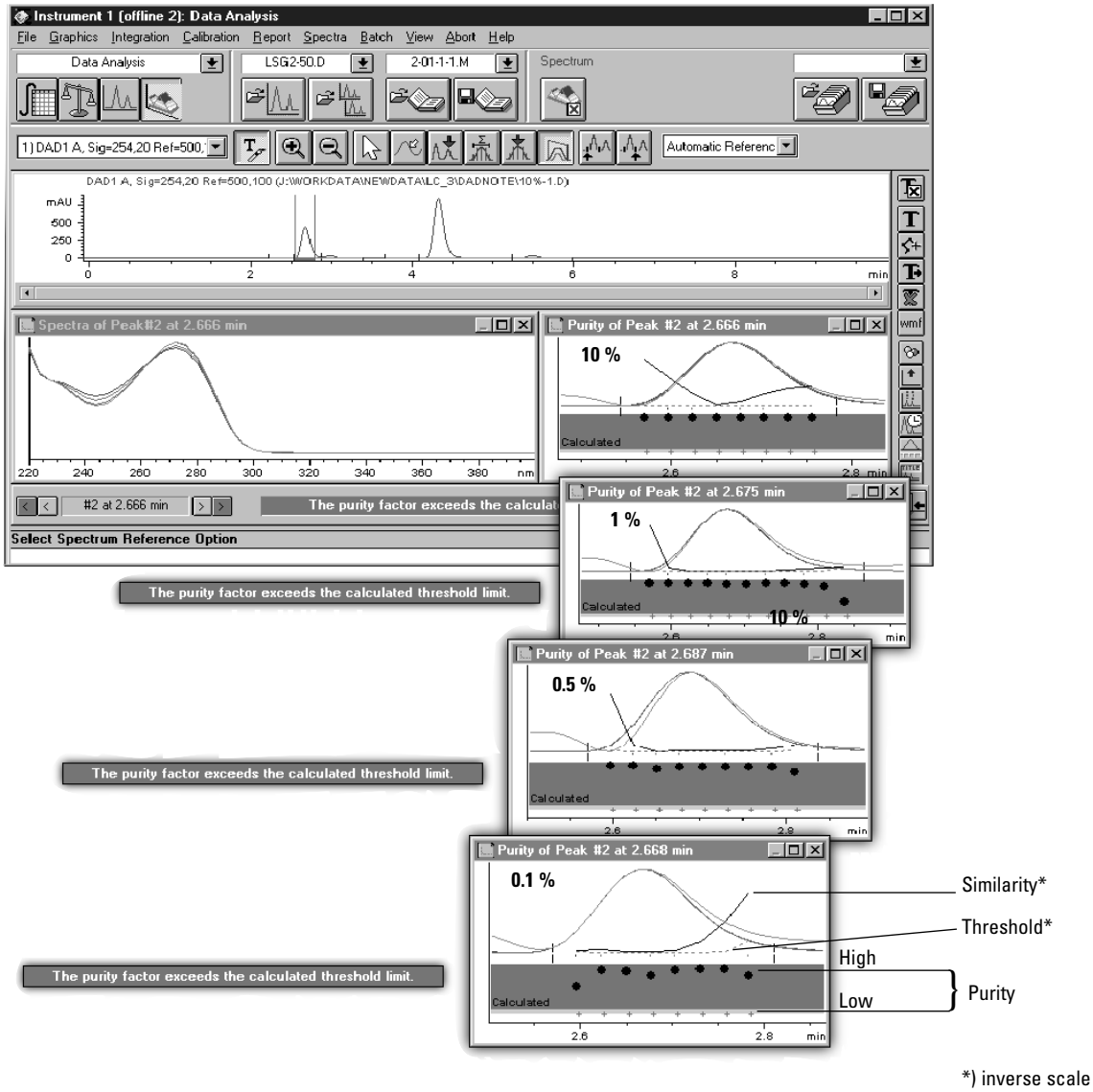

Figure 11
Peak-purity analysis for caffeine with different concentrations of paracetamol. From full spectral information detection of impurities at the 0.1 % level is possible

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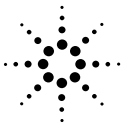

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