Flash chromatography with diode array scanning rapidly confirms fraction purity

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Isolera Spektra provides definitive fraction purity assessment prior to any post flash analysis. With this information chemists quickly know if a fraction contains a pure product suitable for further mass and structure confirmation or whether the impure fractions must be re-purified.

Chemists routinely analyze fractions gathered during flash purification with the same TLC technique used to determine the best separation conditions prior to purification. Once a fraction's purity is determined it is sent for mass and structure determination using LC-MS and NMR, respectively.

Post-purification purity analysis by Thin-Layer Chromatography (TLC) can be eliminated with Isolera® Spektra™, a new flash chromatography system from Biotage that features advanced photo-diode array (PDA) technology with post-purification fraction purity evaluation software.

Synthetic organic, medicinal, and natural product chemists are always in need of better and faster techniques to isolate their compound or compounds of interest. For most, flash chromatography with UV-triggered fraction collection is that technique. Most flash purification systems today allow the user to select one or two specific wavelengths to increase the probability that the compounds in the sample will be detected and collected.

Reaction mixtures and natural product extracts can contain multiple compounds and often the TLC analysis separates some, but not all of the components and chemists will not learn about this until after the collected fractions have been analyzed for purity by HPLC. If the fraction or fractions of interest are not pure, the fractions must be re-purified wasting significant time.

To eliminate this issue, Isolera Spektra, and its new detection technology called λ-All, provide compound detection at all available wavelengths, not just one or two. And to provide real-time purity analysis, the Isolera Spektra also measures, records, and displays the UV absorbance of each compound as it elutes from the cartridge. This data can be reviewed and analyzed during purification and in the final results.

In this application note we demonstrate how the advances in Isolera Spektra are used to determine the purity of each peak in a 5-component sample.

Results

For chemists synthesizing compounds or isolating compounds from natural resources, the number of components in the sample is not always known so co-elution using TLC and flash does occur. For this sample, the Isolera Spektra with the SNAP Ultra cartridge quickly separates four of the five sample components as predicted by TLC, Figure 1.

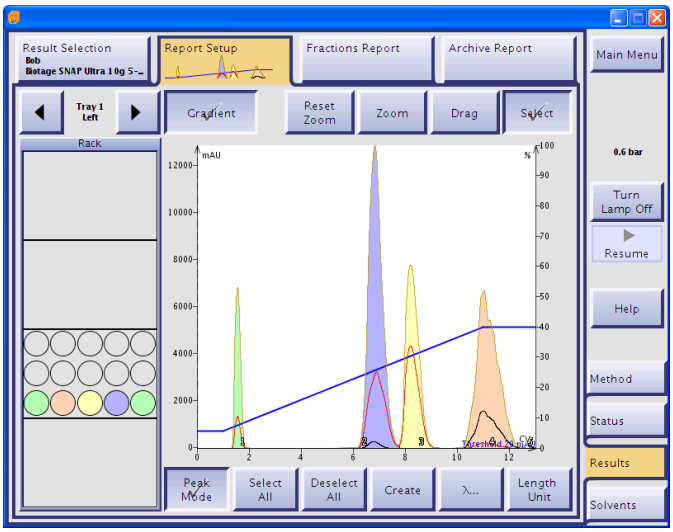

Figure 1: Flash chromatogram showing the separation of four of the five compounds in the test mixture.

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The Isolera Spektra PDA data can be used real-time or post-purification to determine fraction purity. Simply touching a peak displays the spectra for the peak or fraction. Moving the cursor through the peak displays any changes to the peak's spectra. In Figure 2, moving the cursor through peak 3 (yellow) shows spectral consistency indicating this peak is a single compound.

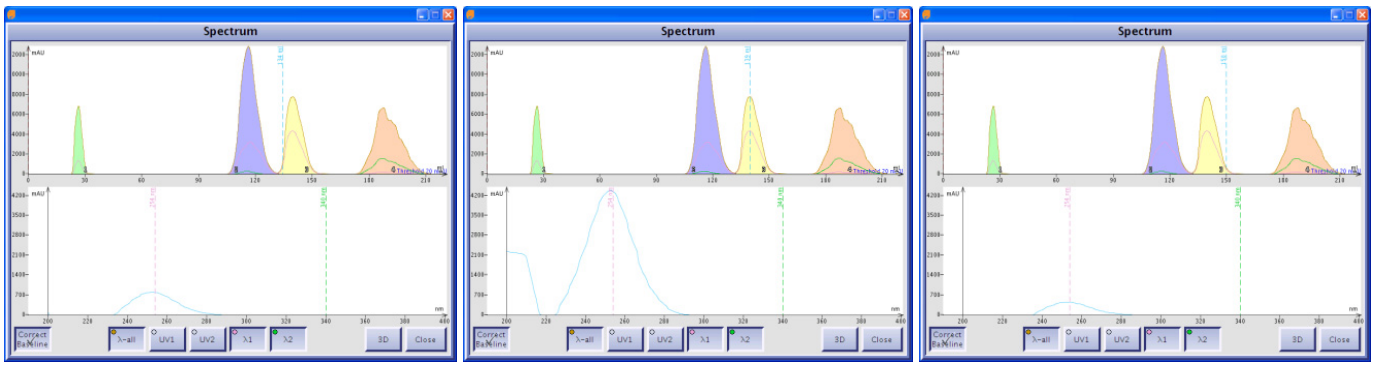

Figure 2: Fraction 3 (yellow) purity is ascertained by viewing the fractions spectra at the beginning, middle, and end of the peak. The spectra shape did not change indicating fraction 3 to be pure.

Fraction four (pink) has a slightly irregular shape. This may be the presence of multiple non-resolved impurities or compound solubility changes during purification. The Isolera Spektra 2-D analysis tool quickly determines if the fraction is pure, Figure 3.

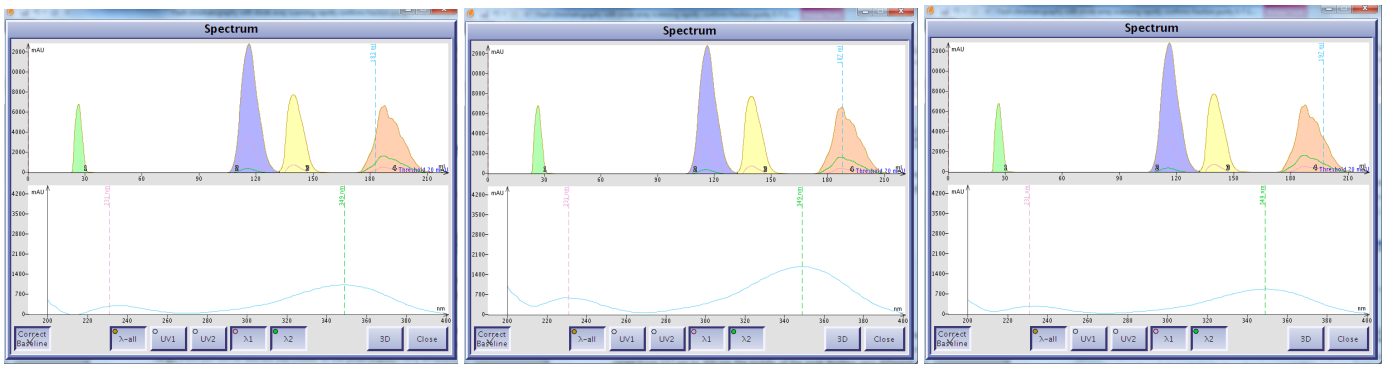

Figure 3: The Isolera Spektra 2-D analysis tool quickly shows if a misshapen peak is pure. Spectra of the peak's leading edge, apex, and trailing edge display identical UV maxima indicating this fraction is pure.

Following the same procedure in Figure 3 for fraction 2 (blue) it is quickly determined that the peak is a mixture of two compounds. The leading and trailing edges of the peak show a similar broad absorbance range from 227 nm to 300 nm but the middle of the peak displays very different, bi-modal spectra indicating an impure fraction, Figure 4.

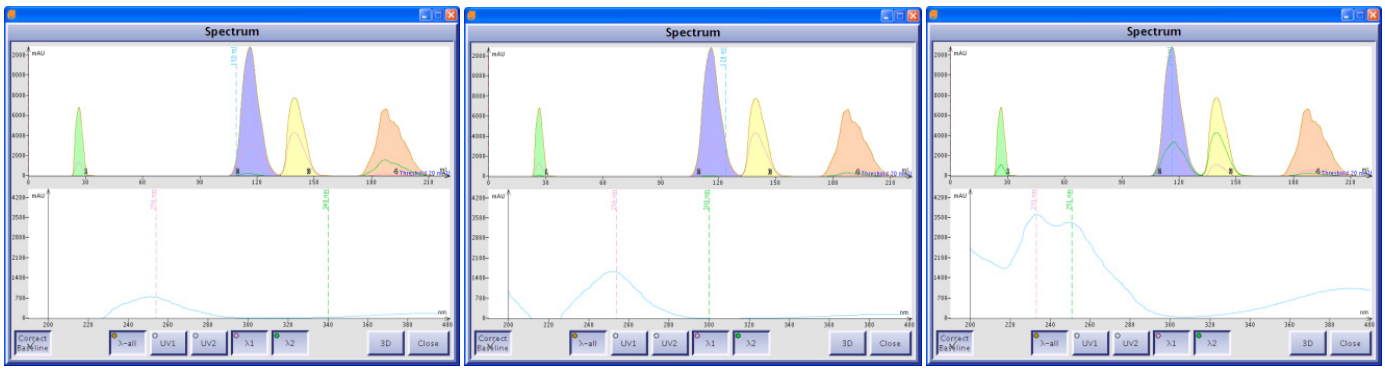

Figure 4: Analysis of the spectra for fraction 2 shows very different results depending on which part of the peak is evaluated. Moving the cursor through the peak clearly shows the presence of different spectra for the same fraction, proving fraction 2 is impure.

When viewed in 3-D the blue fraction's impurity is also noticeable as a slight shoulder on the right side of the peak (the green line at 251 nm) while the shape of the 3-D peak behind it at 233 nm (pink line) is symmetrical. These differences also indicate that the fraction is impure.

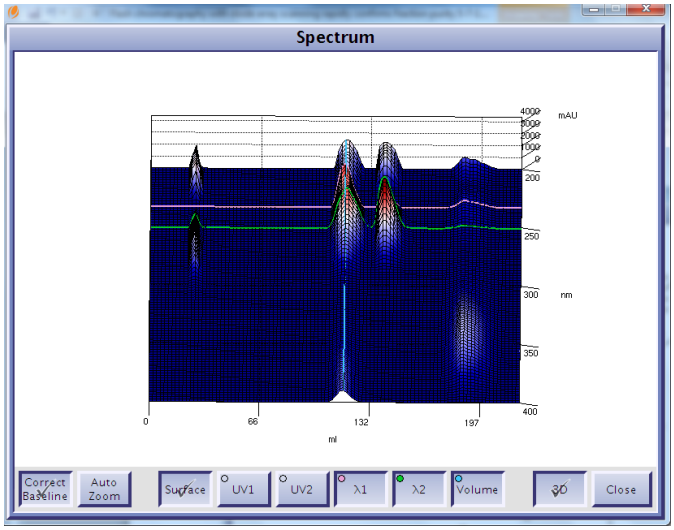

Figure 5: A 3-D graphical analysis provides supporting evidence of fraction purity or impurity. In the case of fraction 2, the peak shape at 251 nm (green line) is clearly asymmetric while other wavelengths at the same retention have symmetrical peaks.

Conclusion

Isolera Spektra provides definitive fraction purity assessment in both 2-D and 3-D prior to any post flash analysis. With this information chemists quickly know if a fraction contains a pure product suitable for further mass and structure confirmation or whether the impure fractions must be re-purified. This knowledge improves synthesis throughput and avoids any embarrassment of submitting impure fractions for analytical evaluation.

Experimental details

Components

- Naphthalene
- 2-Nitroaniline
- Butyl paraben
- Methyl paraben
- 4-Nitroaniline

Sample preparation

Dissolve each compound in acetone at a concentration of 0.1 g/mL.

Method development

TLC at 20% Ethyl acetate in Hexane

Compound	Rf
Naphthalene	0.84
2-Nitroaniline	0.32
Butyl paraben	0.32
Methyl paraben	0.24
4-Nitroaniline	0.11

Equipment used

Flash system:	Isolera Spektra Four equipped with a 200–400 nm UV detector
Flash cartridge:	Biotage® SNAP Ultra 10 g
Flow-rate:	12 mL/min
Solvents:	A: Hexanes B: Ethyl acetate
Equilibration:	5% B at 50 mL/min for 3 CV (51 mL)
Gradient:	5% B for 1 CV (17 mL) 5% to 40% B in 10 CV (170 mL) 40% B for 2 CV (34 mL)
Detection:	λ-All with baseline correction, 200–400 nm
Threshold:	20 mAU
TLC:	Biotage KP-SIL

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