# Real-time Fraction Purity Analysis in Reversed-phase Flash Chromatography

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Pure fractions are in high demand – impurities mean more work after purification. With new technology, fraction purity can be digitally analyzed directly during chromatography to reveal any problems on the fly.

In this application we will show how the Isolera Spektra is used to determine fraction purity, eliminating the need for other post-purification analysis.

Reversed-phase flash chromatography is growing as an alternative to expensive reversed-phase HPLC. Today's improved reversed-phase flash cartridges separate reaction mixtures and natural product extracts effectively, providing higher loading capacity and high fraction purity.

Unlike normal-phase flash chromatography that uses simple and easy to perform normal-phase TLC to analyze fraction purity, the use of reversed-phase TLC is not as easy or wide-spread. To analyze reversed-phase flash fraction purity chemists currently rely on reversed-phase HPLC, an expensive technique that is requires extra time to perform. With chemists needing to be as productive as possible, the extra time needed to complete post-purification analysis of a sample reduces the chemist's throughput.

## No more post purification analysis

Recently, Biotage launched a new flash purification system, Isolera™ Spektra, which includes real-time photo-diode array scanning and analysis. This advanced technology is new to flash chromatography and can eliminate the need for post-purification fraction analysis by recording, displaying, and saving the UV spectrum for each eluting compound. Analysis of the spectra can determine if a fraction is pure.

## Experimental

A mixture of four compounds was purified using reversed-phase flash chromatography with a MeOH/$H_2O$ on an Isolera Spektra Four.

| **Sample** | |
|---|---|
| **Caffeine** | 0.020 g |
| **Methyl paraben** | 0.025 g |
| **Propyl paraben** | 0.020 g |
| **Curcumin** | 0.050 g |
| **Dissolution solvent** | 6:4 Acetone/$H_2O$ |

| **Equipment** | |
|---|---|
| **Flash system:** | Isolera Spektra Four with variable UV detector |
| **Flash cartridge:** | Biotage® SNAP KP-C18-HS 12 g |
| **Solvents:** | A. $H_2O$<br>B. Methanol |
| **Equilibration:** | 30% B at 100 mL/min for 3 CV |
| **Gradient:** | 30% B for 2 CV<br>30% B to 100% B in 9 CV<br>100% B for 2 CV |
| **Flow-rate:** | 12 mL/min |
| **Detection:** | λ-All, monitor 254 nm and 280 nm |
| **Baseline correction:** | On |
| **Wavelength range:** | 200 nm–400 nm |
| **Threshold:** | 20 mAU |
| **Sample load:** | 23 mg |



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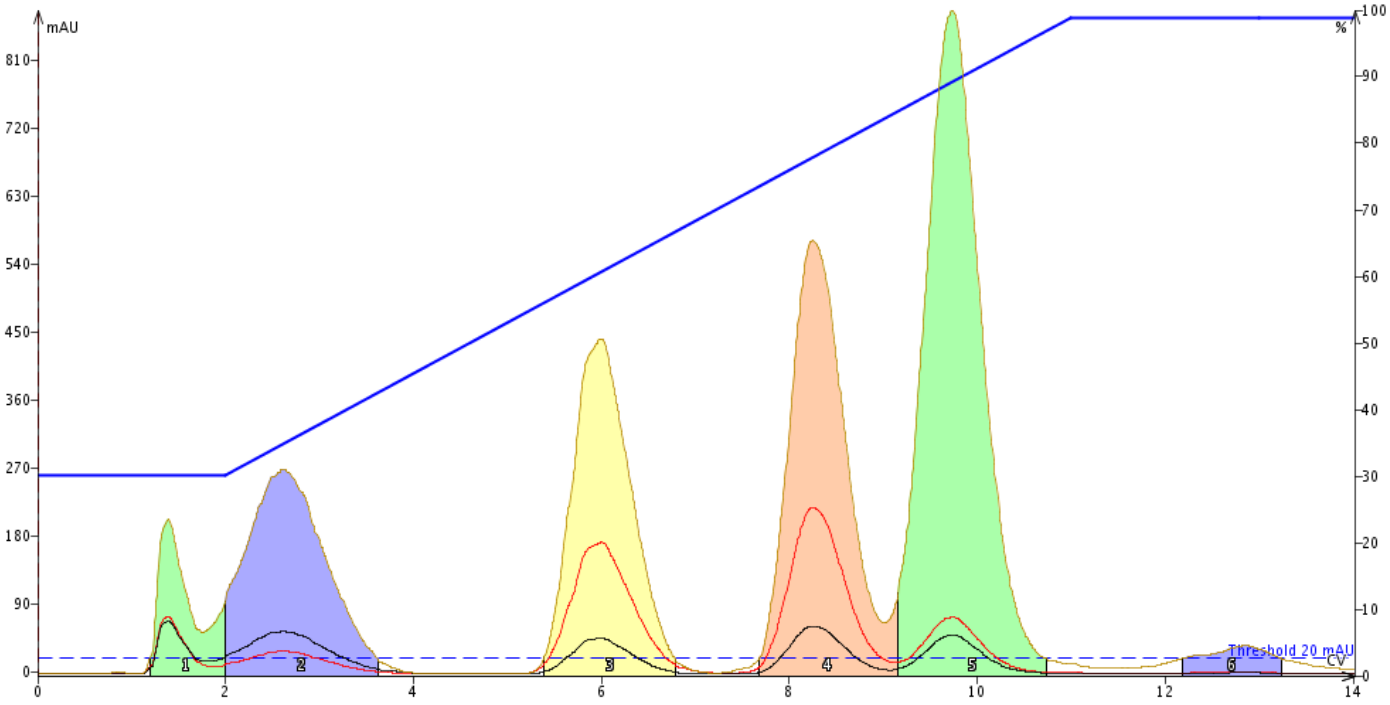


**Figure 1:** The Biotage® Isolera™ Spektra flash system used with a Biotage® SNAP KP-C18-HS cartridge fully separates the 4-component mixture as well as the injection solvent acetone (1st peak) and a lipophilic impurity (last peak).

## Results

The Isolera Spektra and Biotage SNAP KP-C18-HS cartridge fully separated the four compounds as well as acetone (from the injection) and a late eluting impurity. Each compound was collected into an individual test tube, Figure 1.

During the sample's purification the UV spectra of each peak was recorded and saved. This data helps determine both peak purity and compound ID. In Figure 2, each fraction has been highlighted (vertical dotted line above the peak on the chromatogram) displaying its spectrum in the lower half of the screen. The vertical dotted line (cursor) can be moved from peak to peak and within a peak. What is quickly determined is each peak's UV maximum. In Figure 2, the first eluting peak has a UV maximum of 266 nm which matches that of acetone. The second peak has multiple maxima with the two most prominent at 206 nm and 273 nm, characteristic of caffeine. Peak 5 has maximum at 200 nm, 262 nm and above 400 nm. This fraction's UV spectrum and yellow color identify the fraction as curcumin.

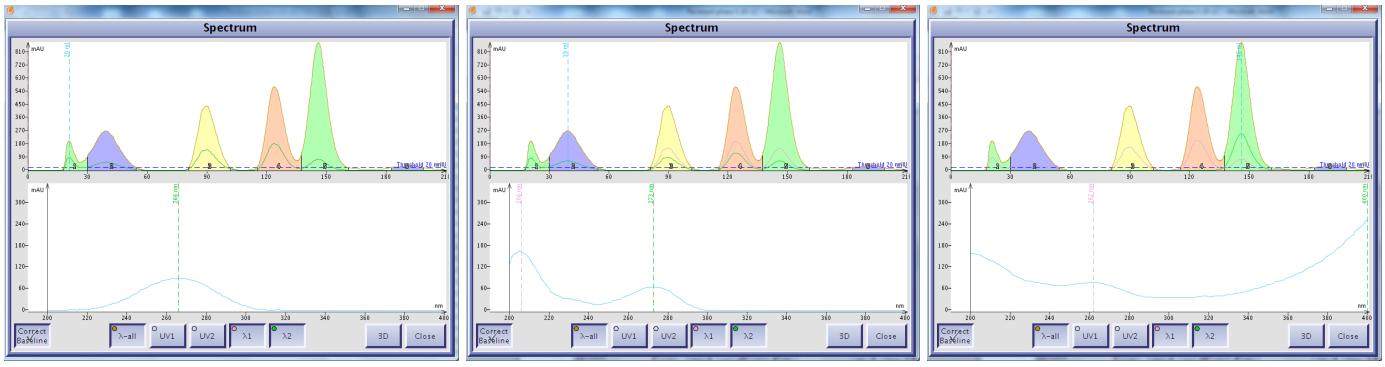


**Figure 2:** The Isolera Spektra records, displays, and saves the spectra for each eluting peak. This data displays the spectra of acetone (left), caffeine (middle), and curcumin (right).

The third and fourth peaks have very similar spectra with a primary UV maximum at 256 nm and a secondary maximum at 211 nm identifying them as parabens. There is a difference between peaks 3 and 4 at 200 nm where the slope from 200 nm to the valley at 225 nm is steeper for peak 4 (propyl paraben) than for peak 3, methyl paraben.

If a fraction is impure moving the cursor through the peak and watching the spectra pattern will show this. A spectrum that moves up and down in unison indicates a fraction or peak is pure; changes in spectra shape indicate an impurity.

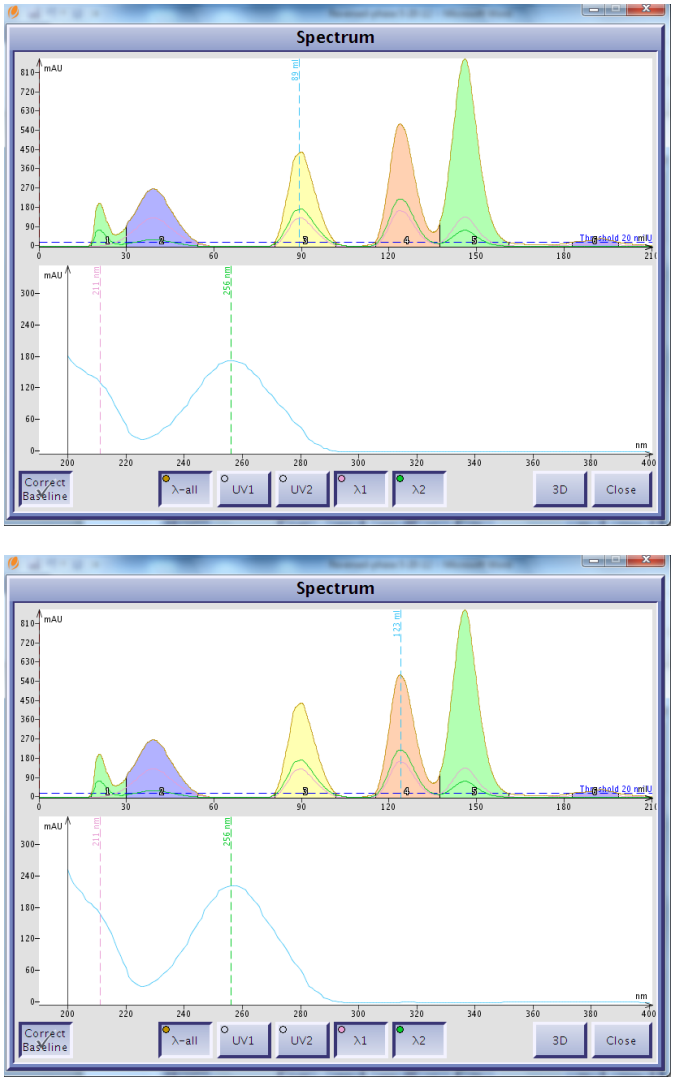


**Figure 3:** Methyl paraben (top) and propyl paraben (below) share similar UV absorption properties but can be differentiated by the different spectra between 200 nm and 225 nm.

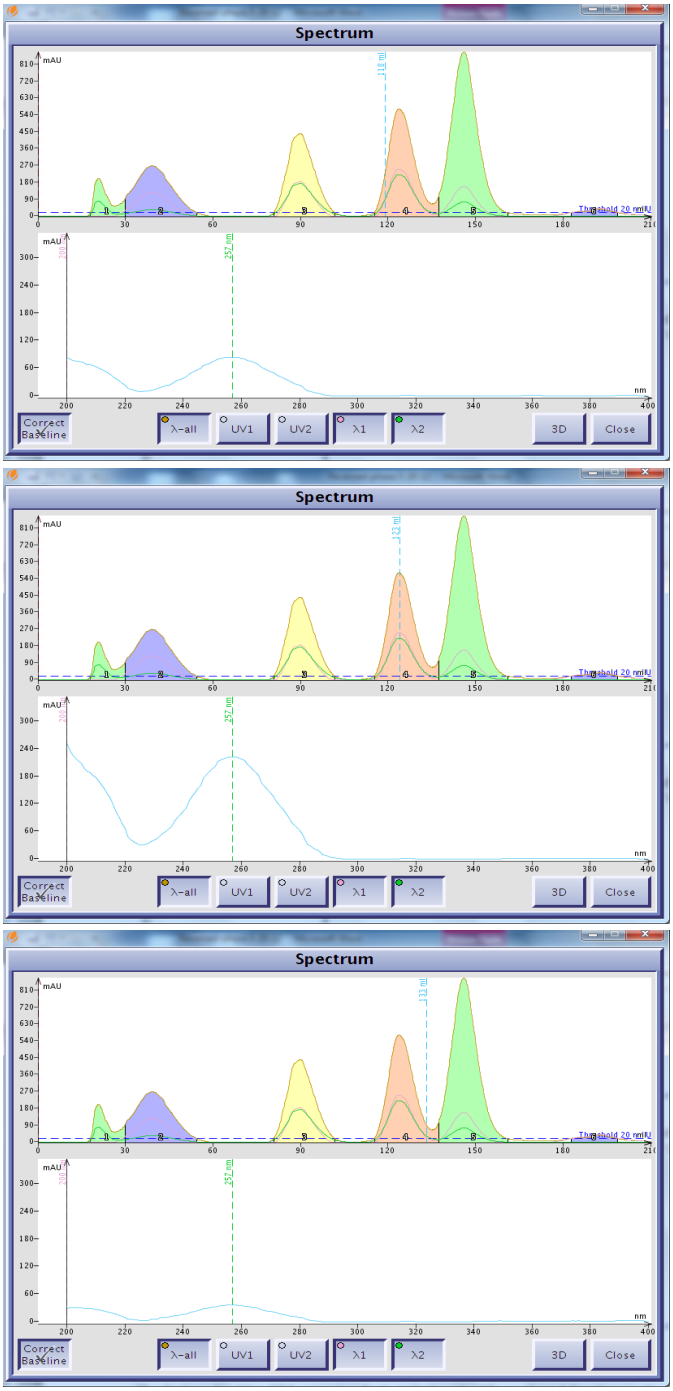


**Figure 4:** Fraction purity can quickly be determined by scanning a peak with the cursor and monitor the shape of the spectrum. Rise and fall of the curve is only natural, but any shift to the right or left would indicate an impure fraction. Scanning peak 4 (propyl paraben) shows very high purity.

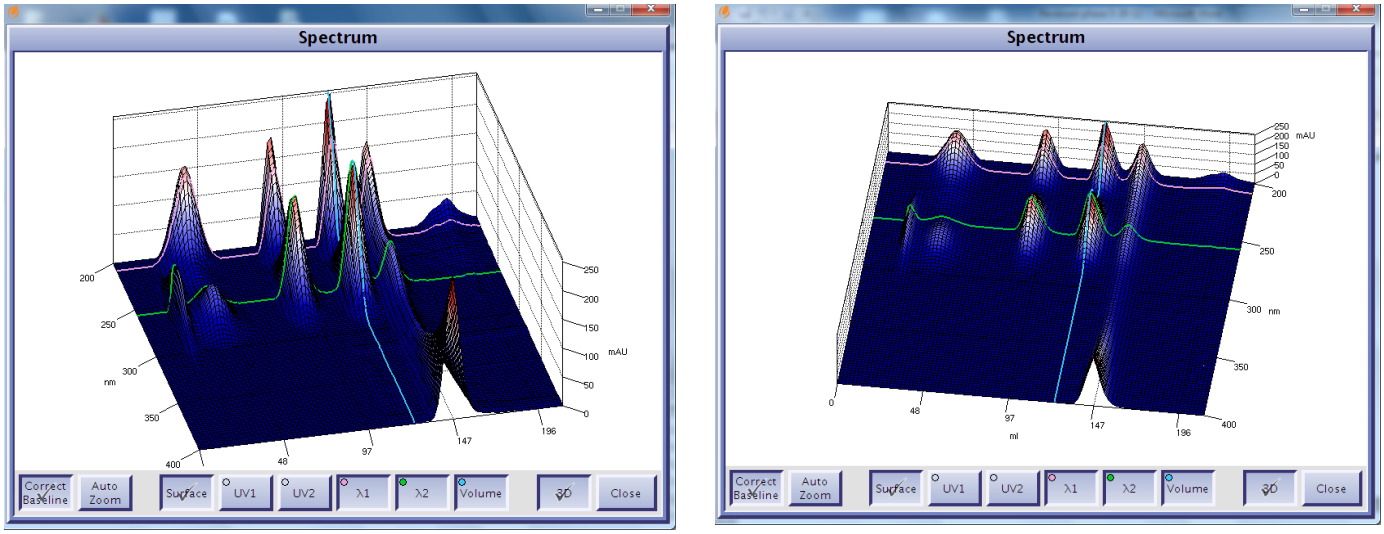


**Figure 5:** The Isolera Spektra displays chromatographic and spectral data in a rotatable 3D format providing additional purity analysis information.

With the Isolera Spektra the chromatographic and spectral data is reviewable in 3-D as well to provide an overview of each compound's purity relative to elution volume and wavelength.

## Conclusion

The Isolera Spektra PDA scanning feature helps identify peak and fraction purity in real-time saving chemists the time required to have flash purification fractions analyzed for purity by analytical methods such as HPLC.

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