# Ensuring Higher Levels of Purity in Target Submissions in Drug Discovery by Employing a Multi-detector Approach in Flash Chromatography

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# Abstract

During synthetic transformations, chemists continuously monitor their reactions using TLC spotting and manual UV detection as indicators of reaction completion. Complementary detection represents mass balance better than UV alone, giving the chemist more confidence about the relative amounts of the target compound being synthesized, unreacting starting materials, and reaction by-products being collected. RevealX™ detection technology employs multiple detection signals in flash chromatography, whereby allowing the chemist to estimate the relative masses of reaction components by comparing the relative peak size. The technology can be used to reassure confidence in target compound purities and gives more accurate understanding of component masses compared to UV alone.

# Objective

The multiple detectors of the Reveleris® system can be used to better estimate the relative amounts of mixture components being collected than UV alone. By separating mixtures of known relative mass composition using the Reveleris® system, it can be shown that the ELSD mass detector gives a better indication of the composition than UV.  Here we demonstrate the relative mass balance of 2 purifications and compare the response of ELSD to UV.  By using ELSD to complement UV, a greater picture of sample composition can be observed and more confidence of accurate reaction yields and sample amounts can be provided.

## *Flash Separation Conditions*

| | |
|---|---|
| **Column** | 12g Reveleris® silica |
| **Sample Size** | 10mL |
| **Injection Mode** | Liquid |
| **Flow Rate** | 36mL/min |
| **Equilibration** | 6 min |
| **Run Length**[1] | Varies |
| **Air Purge** | 1.5 min |
| **Slope Detection** | High Sensitivity |
| **ELSD Threshold Detection** | 3mV |
| **UV Threshold Detection** | 0.02au |
| **Collection Mode** | Collect Peaks |
| **Per Vial Volume Maximum** | 29mL |
| **Per Vial Volume Peaks** | 20mL |
| **Per Vial Volume Non-Peaks** | 20mL |
| **UV1 wavelength** | 254nm |
| **ELSD Carrier Solvent** | Isopropanol |
| **Solvent A** | Hexane |
| **Solvent B** | Ethyl acetate |
| **Color of ELSD Signal Trace** | Green |
| **Color of UV1 Signal Trace** | Blue |
| **Color of UV2 Signal Trace** | Red |
| **Color of Gradient Trace** | Gray |

[1]Determined by the gradient profile

# *Sample 1*

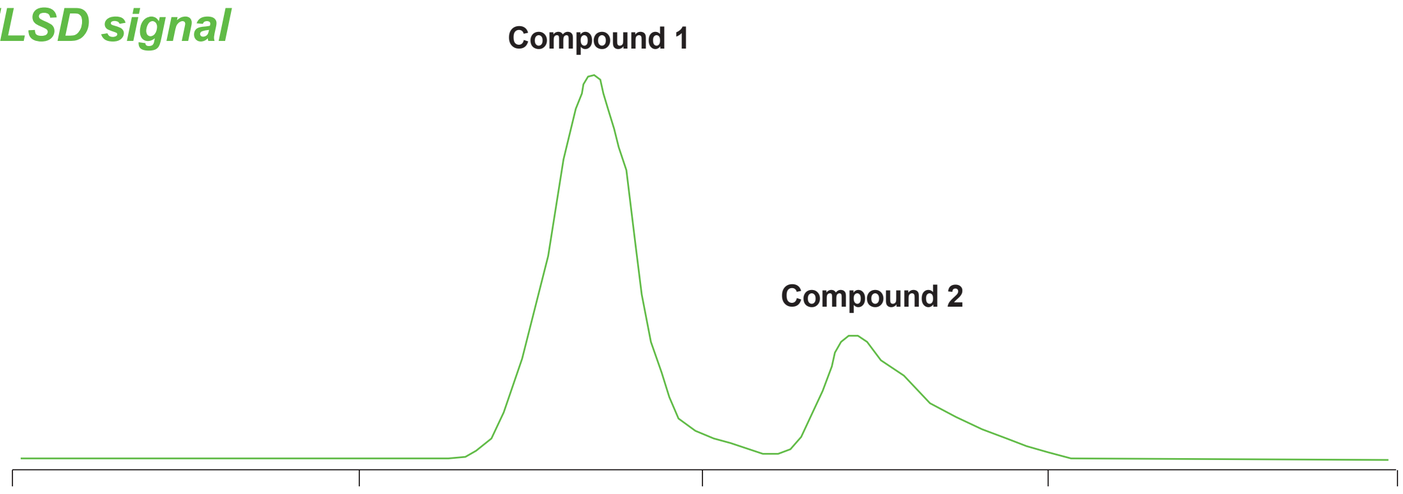
This challenge is the separation of a non-UV absorbing Compound 1 (cholesterol) from a UV absorbing Compound 2 (lidocaine) in a UV absorbing solvent (toluene). Under the conditions for this separation, Compound 2 tails badly, requiring a “Collect All” mode to achieve better recovery of Compound 2.

| Sample Relative Mass Composition | |
|---|---|
| **Component** | **Mass Ratio** |
| Compound 1 | 2.3 |
| Compound 2 | 1.0 |

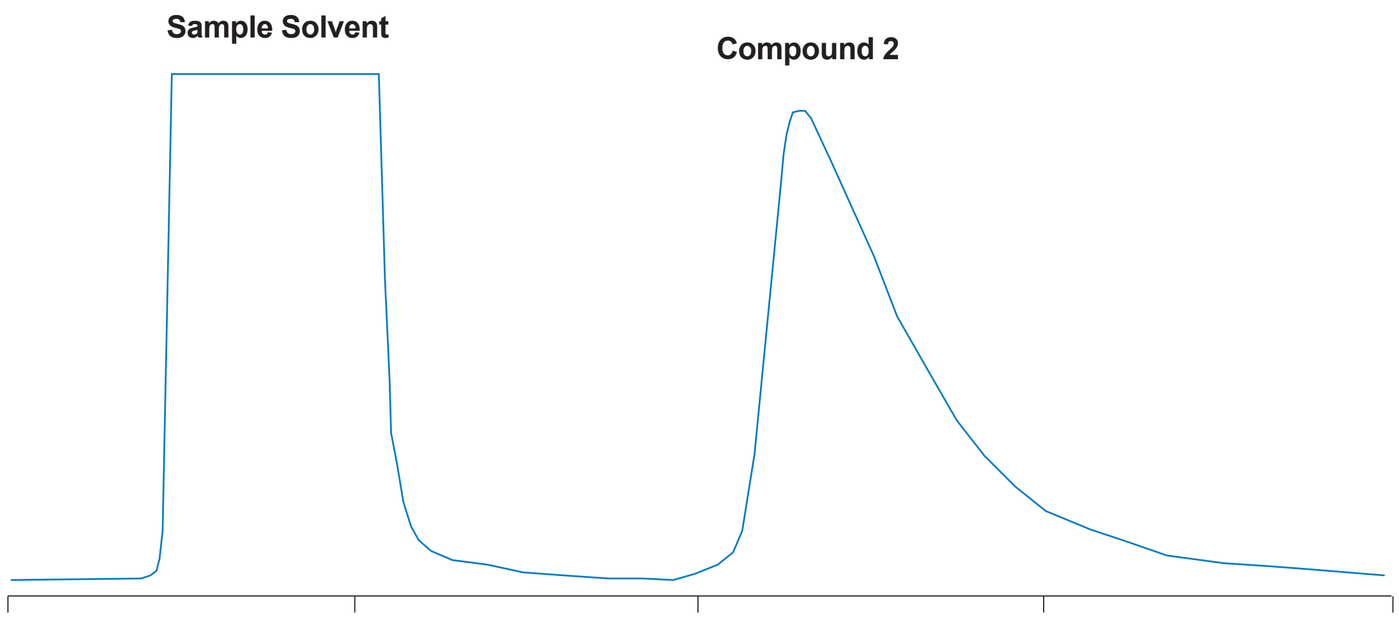
**Gradient:**

| **Time (min):** | 0 | 0.7 | 0.9 | 2.5 |
|---|---|---|---|---|
| **%B:** | 37 | 37 | 64 | 64 |

***ELSD signal***




***UV 254nm signal***




In this example, Compound 1 cannot be detected by UV. Without information provided by other methods of analysis, the UV signal could lead one to believe that the sample consisted only of Compound 2 and the sample solvent. The ELSD signal provides a more accurate picture of the actual sample composition.

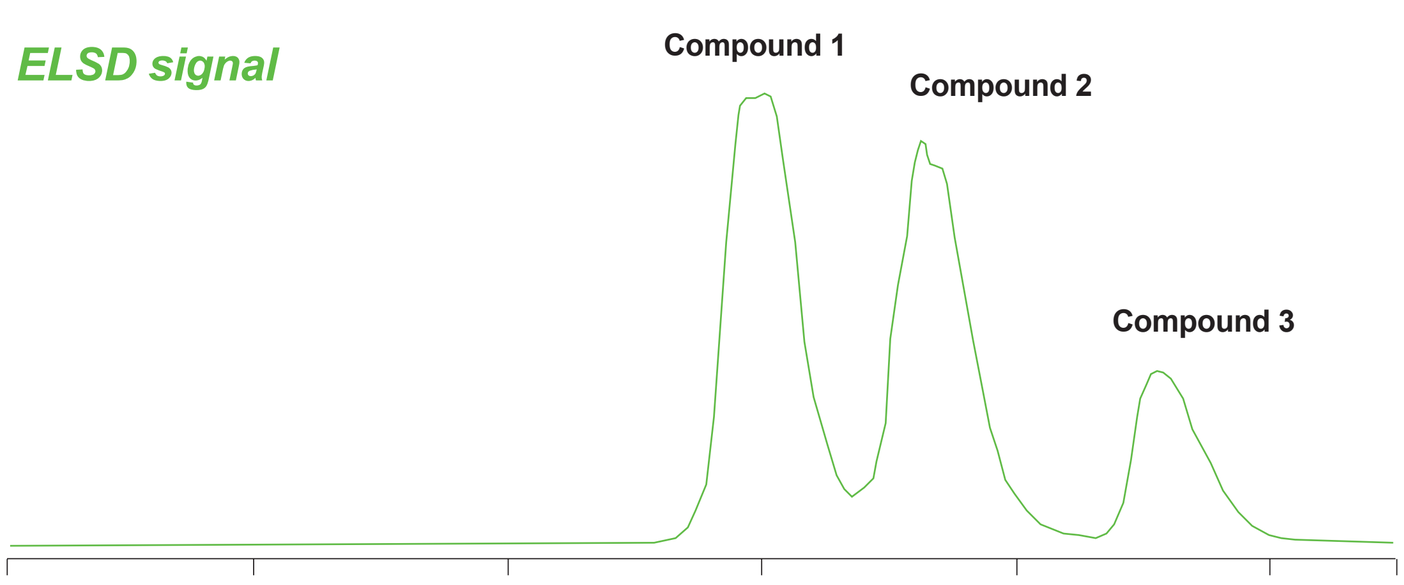
# *Sample 2*

| Sample Relative Mass Composition | |
|---|---|
| **Component** | **Mass Ratio** |
| Compound 1 | 2.3 |
| Compound 2 | 2.2 |
| Compound 3 | 1.0 |

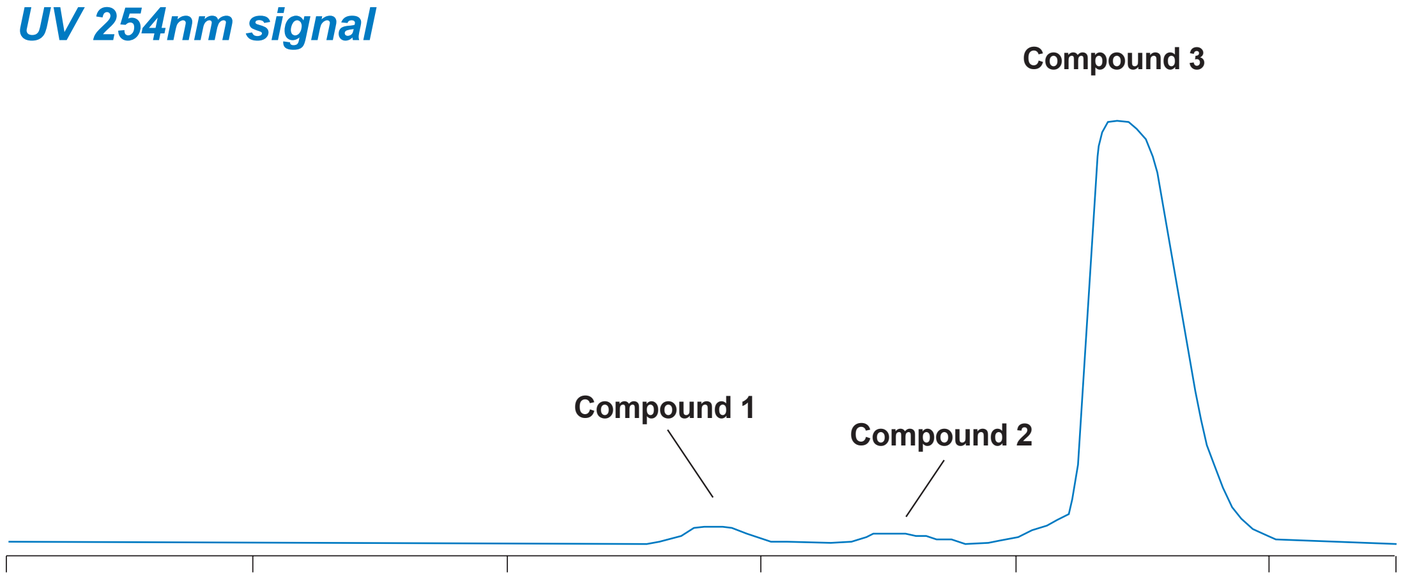
**Gradient:**

| **Time (min):** | 0 | 0.7 | 0.9 | 1.4 | 2.5 |
|---|---|---|---|---|---|
| **%B:** | 3 | 3 | 9 | 22 | 22 |

*ELSD signal*




*UV 254nm signal*




In this example, all three components are detectable by UV but the absorbance for Compound 3 is much larger than for the other two compounds. This can lead to the false conclusion that the sample consists mainly of Compound 3. The ELSD signal gives a much better representation of the true composition.

# Conclusion

The two examples show how relying on UV detection alone can lead to a false sense of the composition of the sample being separated. In both cases the UV 254nm signal would indicate that the last eluting component represents the majority of the sample, when it actually represents the smallest amount. By incorporating RevealX™ detection in the Revleris® flash system, a true picture of the composition of the sample is presented.

By using ELSD to complement UV, a greater picture of sample composition can be observed and more confidence of accurate reaction yields and sample amounts can be obtained. Combining these detection sources in a single flash system optimizes performance and purity without the need to continuously optimize and configure auxiliary detectors and fluidics.