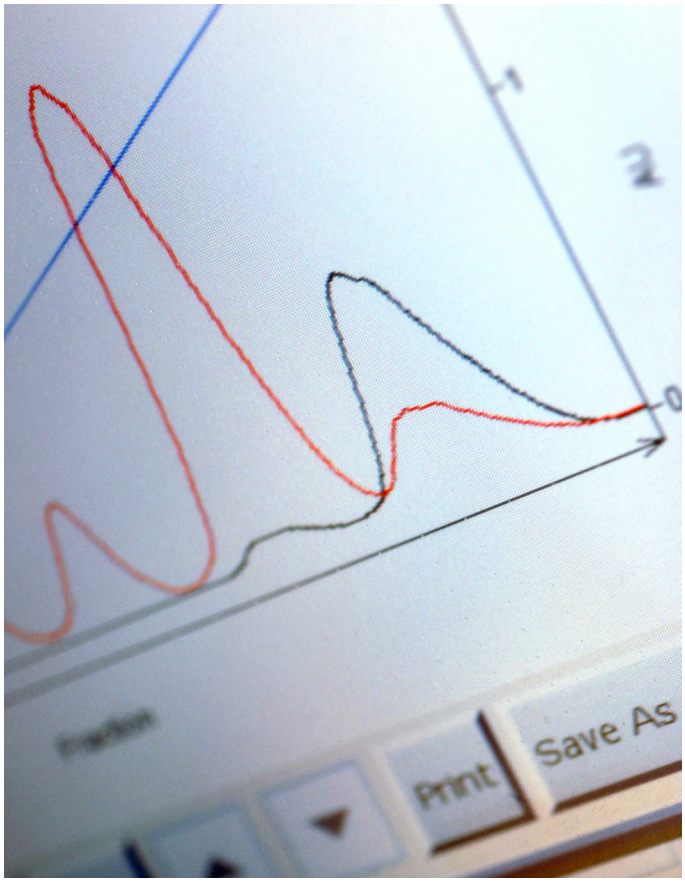
Flash Chromatography: Using UV Detection with UV Absorbing Mobile Phases

Sunil Rana and Panagiotis Ioannidis

The UV absorption spectrum of some solvents overlaps with the product they dissolve, meaning that fraction collection processes cannot distinguish between solvent and product. Luckily, there is technology that solves this problem.



Flash purification has proven to be the most effective technique for purification, especially at gram-scale and when speed is required. During the past ten years flash cartridges and instrumentation have evolved significantly. Some purification evolutionary paths that developed were based on and around technological barriers at the time. For example, even though there are much better solvents in normal phase chromatography (from the point of view of dissolution of products), some solvents evaded flash, due to their own intrinsic ability to absorb UV. Solvents such as acetone, toluene/ethylacetate are examples of these. Their own UV absorption overlaps with the product they dissolve, such that a fraction collection process cannot distinguish between solvent and product (Figure 1).

The Solution

Flash purification technology has improved vastly, and the situation described above is changing. The Biotage line of Isolera™ instrumentation has led the way in eliminating this small but very practical issue of coping with solvent UV absorption (Figure 2).

Baseline Correction

Isolera™ Spektra purification instruments provide an alternate automated in-system means of dealing with absorbing solvents. Isolera Spektra is an advanced software package, designed to work with Isolera family of purification instruments to further enhance the functionality. One of its key features is the elimination of baseline drift, based on solvent absorptions. The purification chemist only needs to load the purification column and run samples as normal. When selecting 'Baseline Correction' in the software, the system automatically takes care of the whole process. Isolera Spektra systems will first create a real baseline based on the actual solvent/gradient composition intended in the purification run, hold it in memory and then subtract it when the purification occurs (Figure 2b). This real baseline correction is far more accurate and reliable than other systems which may use calculations and algorithms, as it samples the actual solvent to be used for the purification, rather than arbitrarily applying a theoretical list to a real life comparison.

Conclusion

In conclusion, we have shown that it is possible to completely eliminate a drifting baseline in UV detected purification by the use of advanced baseline correction features. Unlike many other systems, Isolera™ Spektra automatically organizes real blank runs for the chemist to create true background spectra and ensure maximum fidelity of the resulting baseline data (Figure 1), enabling almost any UV absorbing solvent combination to be used for flash purification (Figure 2).

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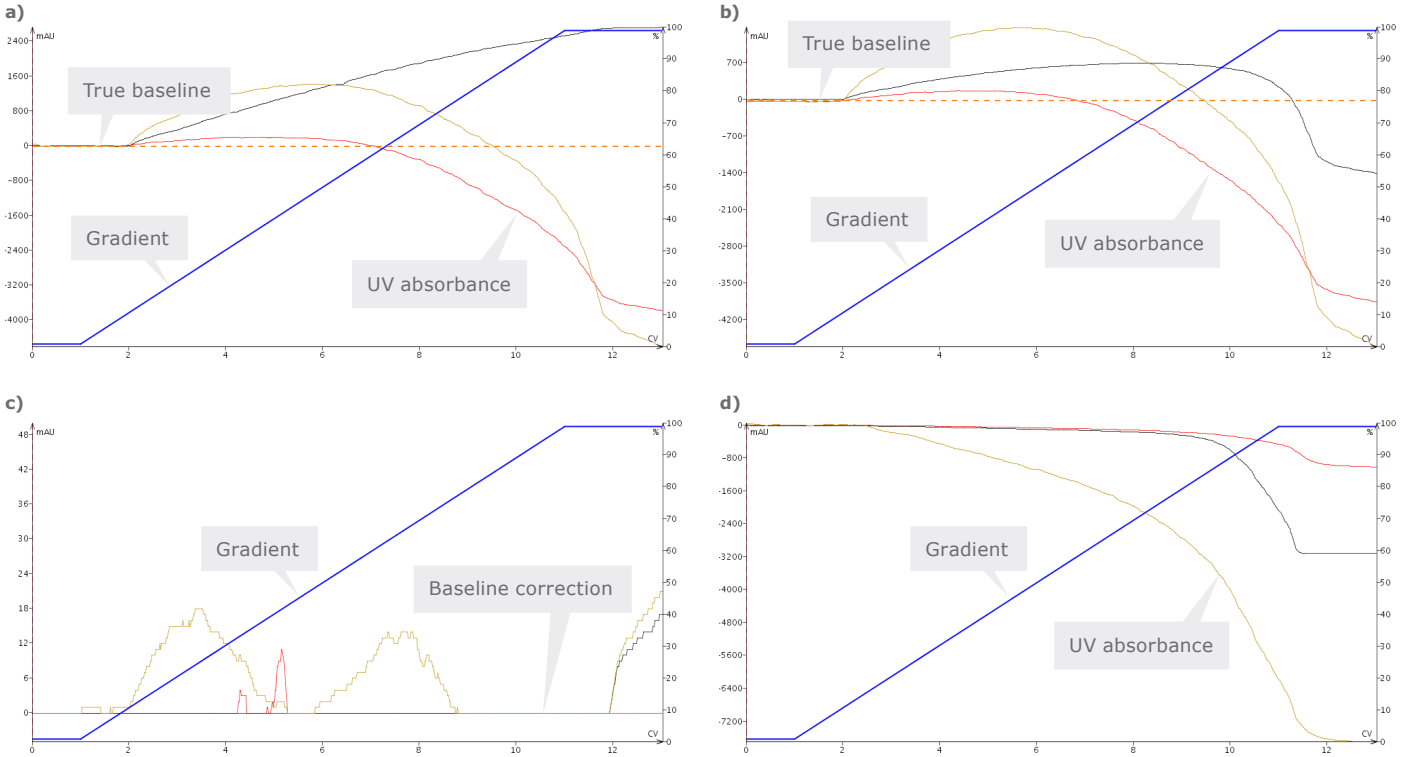

Figure 1. Results of blank experiments. UV-absorption curves for a 0–100% gradient. The results indicate that it is impossible to use these solvents with any collect parameters other than Collect All. System used: Isolera™ One running Spektra 2.0. UV-detector: 200–400 nm.

a) Blank experiment @ 220 nm (red line), 265 nm (black line) and λ-all (beige line) detection [no cartridge, baseline correction off]. Solvent A: toluene; Solvent B: acetone.

b) Blank experiment @ 220 nm (red line), 280 nm (black line) and λ-all (beige line) detection [no cartridge, baseline correction off]. Solvent A: toluene; Solvent B: acetone.

c) Baseline correction on @ 220 nm (red line) 280 nm (black line) and λ-all (beige line) detection. Solvent A: toluene; Solvent B: acetone.

d) Blank experiment @ 220 nm (red line), 262 nm (black line) and λ-all (beige line) [baseline correction off]. Solvent A: toluene; Solvent B: ethylacetate.

e) Baseline correction on. Solvent A: toluene; Solvent B: ethylacetate.

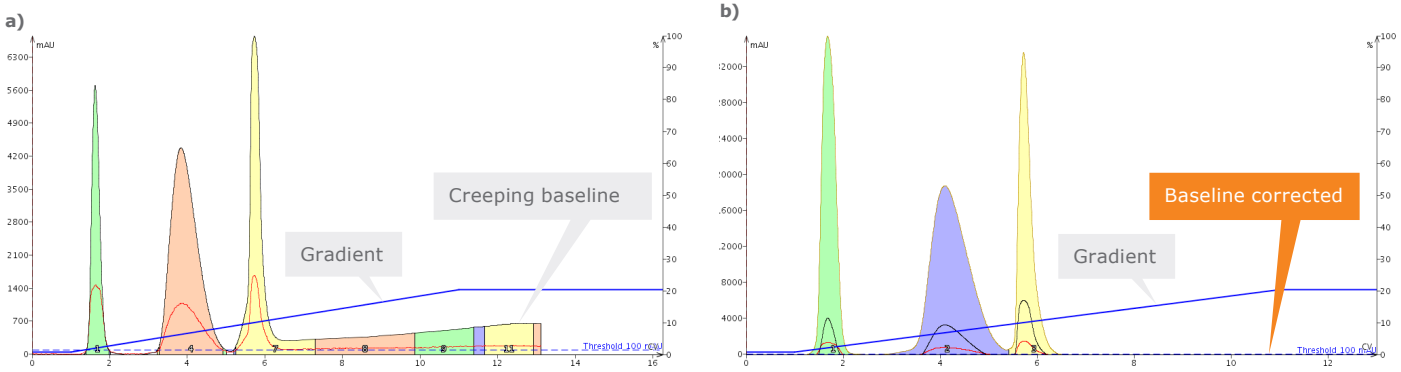

Figure 2. Results of purification runs. System used: Isolera™ One running Spektra 2.0. UV-detector: 200–400 nm. Solvent A: Toluene; Solvent B: Acetone. Wavelengths used: 220 nm (red line), 280 nm (black line) and λ-all (beige line). Cartridge: Biotage® SNAP KP-Sil, 25 g. Sample: Nitronaphthalene, 2-Nitroaniline, 3-Nitroaniline (1.1:1 in acetone, 0.6 g/mL) 1 ml sample gives 2.4 % loading.

a) Example purification @ 265 and 280 nm detection [baseline correction off].

b) Example purification [λ-all: ON, baseline correction on].

Part Number: AN092.v1

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