Isolating trace quantities of product

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Chromatography Application Note AN26

Overview

Isolating small quantities of products within a large sample is generally difficult and lengthy by flash chromatography. However, tailored automated flash chromatography instrumentation can assist scientists seeking a convenient and rapid resolution of this issue.

This application note describes the isolation by flash chromatography of products present in trace amounts within a sample.

Background

Some branches of the chemical industry running organic chemistry syntheses at a large scale level are seeking to isolate small quantities (0.1%–1.0%) of impurities contained in a large product batch. An example of this situation is the Active Pharmaceutical Ingredient (API) large-scale preparation by the custom synthesis manufacturing industry. The U.S. Food and Drug Administration requires isolation and full characterization of all small quantity impurities contained in synthesized APIs.

The isolation of such small quantities of organic compounds contained in an API batch can be inconvenient and time consuming. Often, the manufacturer must repeat several purifications using flash chromatography to gather each impurity in an exploitable quantity.

In this situation, a simple and reliable single-step purification technique would be beneficial. The use of a Combi*Flash*® Companion® equipped with a UV detector containing a 2.0 mm pathlength flow cell represents a viable solution to convenient small quantity impurity isolation and collection.

Results and Discussion

The Combi*Flash* Companion is factory-equipped with a UV detector with a standard 0.1 mm pathlength flow cell. An optional 2.0 mm pathlength flow cell is available for this UV detector.

In order to investigate whether a 0.1 mm pathlength flow cell could detect small quantities of impurities, a sample mixture containing 10 g methyl paraben, 10 mg acetophenone, and 10 mg p-aminobenzoic acid was prepared.

The sample mixture was next run on a Combi*Flash* Companion equipped with a 0.1 mm pathlength flow cell. The resulting chromatogram (Figure 1) showed detection of the large quantity of methyl paraben only.

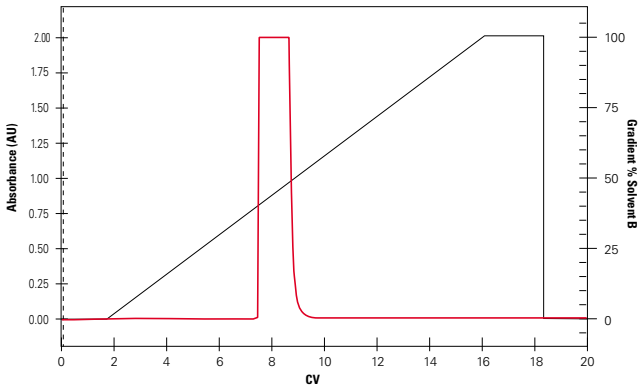

Figure 1: Combi*Flash* Companion equipped with 0.1 mm pathlength flow cell Chromatogram shows sample mixture purification attempt on 330 g normal phase Redi*Sep* column with hexane/ethyl acetate.

The next step of the investigation exchanged the 0.1 mm pathlength flow cell inside the Combi*Flash* Companion with the optional 2.0 mm pathlength flow cell. This larger pathlength makes the UV detector more sensitive. The same sample mixture was run with detection by the 2.0 mm pathlength flow cell (Figure 2).

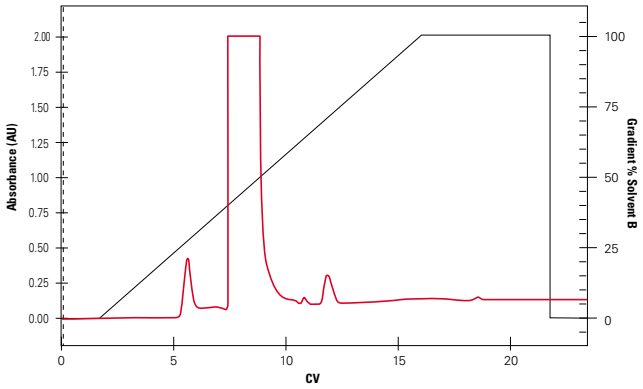

Figure 2: Combi*Flash* Companion equipped with 2.0 mm pathlength flow cell Chromatogram shows sample mixture purification on 330 g normal phase Redi*Sep* column with hexane/ethyl acetate.

The 2.0 mm pathlength flow cell allowed detection of the two small quantity (0.1%) impurities in the sample mixture. This is illustrated by the two peaks at 6 and 12 Column Volumes (CV). The 0.1 mm pathlength flow cell did not detect them.

Therefore, a Combi*Flash* Companion equipped with an optional 2.0 mm pathlength flow cell allows synthetic organic chemists to isolate and collect small quantities of secondary products within a large sample batch.

Combi*Flash* Companion flow cell information

A user can quickly exchange the flow cell inside the Combi*Flash* Companion. Therefore, for standard flash chromatography purifications, the user would operate the Combi*Flash* Companion with the standard 0.1 mm pathlength flow cell. For projects involving small quantity impurities isolation and collection, the user would install the optional 2.0 mm pathlength flow cell.

Experimental

Table 1: Method Parameters

Instrumentation:	Teledyne Isco Combi*Flash*® Companion® equipped with a UV detector containing a 2.0 mm pathlength flow cell	
Column	330 g Normal Phase Redi*Sep*	
Sample Loading Method	10 g methyl paraben, 10 mg acetophenone, and 10 mg 4-aminobenzoic acid pre-coated on normal phase silica gel	
Wavelength	254 nm	
Mobile phase:	Solvent A: Hexane	Solvent B: Ethyl acetate
Flow Rate:	100 mL/minute	
Equilibration Volume:	2 column volumes	
Gradient:	% Solvent B	CV
	0	Initial
	0	1.8
	100	14.2
	100	2.2
	0	0.0
	0	1.8

Conclusion

Increasing the UV detector's flow cell pathlength from 0.1 mm to 2.0 mm successfully achieved the isolation of small quantities of products within a large sample.

This application illustrated the use of the Combi*Flash* Companion equipped with a more-sensitive UV detector for the isolation of products present in trace amounts within a sample.

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