Why is Reversed-phase Flash Chromatography Use Increasing?

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Introduction

Flash chromatography is the primary tool used by medicinal chemists for reaction mixture purification. Over the past 10 or so years, the use of reversed-phase flash chromatography for reaction mixture purification has increased dramatically due to both chromatographic and non-chromatographic reasons.

Some reasons for this switch from normal-phase include...

- Synthesis of increasingly polar compounds
- Synthesis of increasingly complex reaction mixtures
- Conservation/environmental concerns/green chemistry
- Cost savings
- RxN mix analysis performed by HPLC-MS
- Column reusability

In this poster, we will show an example of why chemists are increasingly using reversed-phase flash chromatography for purification of a complex reaction mixture.

Experimental Protocol

Reagents and Materials

Solvents used in study included: house DI water, hexane, ethyl acetate, acetonitrile and methanol (Reagents, Inc., Charlotte, NC).

Reagents used isatoic anhydride, benzyl amine, benzaldehyde, and acetic acid, all from Aldrich Chemical, Milwaukee, WI.

A Biotage® Initiator+ performed the synthesis.

A Biotage TLC plate (5x10 cm was used for method development.

Flash chromatography was performed using a Biotage® Dalton 2000 system with an APCI ion source.

Normal-phase flash chromatography used a 5-gram, Biotage® Sfär HC column and Samplet® cartridge for dry loading.

Reversed-phase flash chromatography used a 6-gram Biotage® Sfär C18 flash column.

A Biotage® V-10 Touch dried the reaction mixture.

Organic Synthesis

Isatoic anhydride, benzyl amine, benzaldehyde, and acetic acid were mixed with acetonitrile and reacted to create 3-benzyl-2-phenyl-2,3-dihydro-4(1H)-quinazolinone, Figure 1. The reaction mixture concentration was 247 mg/mL.

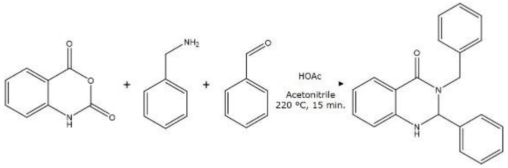

Figure 1. *Microwave-assisted organic synthesis reaction in acetonitrile at 220 °C.*

Thin-layer Chromatography

After synthesis, the reaction mixture was evaluated by TLC in 40% EtOAc in hexanes and separated a leading and trailing impurity away from the target product, Figure 2.

Figure 2. *Reaction mixture TLC using 40% EtOAc/hexanes shows the product (blue) with a leading and trailing impurity.*

Mass Analysis

The reaction mixture's detectable masses were determined using the Isolera Dalton 2000 with the solids analysis probe. Three +m/z were found, 314.8 (product), 226.8, and 235.4, Figure 3.

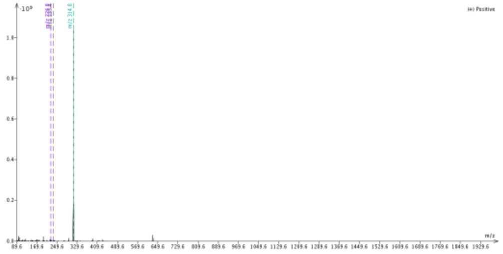

Figure 3. Positive ionization APCI mass analysis finds three masses, 314.8 (product), 226.8 and 235.5

Flash Chromatography

A normal-phase linear gradient based on the TLC data (10-80% ethyl acetate) provided a partial separation as expected by the TLC results.

The flash method separated the lead impurity (+m/z 235.4) from the other compounds but generated little resolution between the product and the trailing impurity (+m/z 226.8), Figure 4.

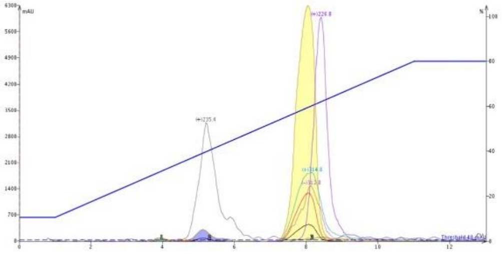

Figure 4. Reaction mixture normal-phase purification with mass identification. One impurity (+m/z 235.4) is fully separated from the product but the other impurity (+m/z 226.7) is not.

Reversed-phase

With normal-phase not providing an adequate purification result, I evaluated reversed-phase flash chromatography with a 50-80% acetonitrile in water gradient. The rationale being that reversed-phase's different separation mechanism (partitioning vs. adsorption/desorption) may provide an improved purification result.

Reversed-phase flash chromatography improved separation with the +m/z 226.8 impurity now eluting earlier than the product and the other impurity (+m/z 235.4) eluting last, Figure 5. In fact, reversed-phase flash even resolved a previously un-detected impurity with a +m/z 191.

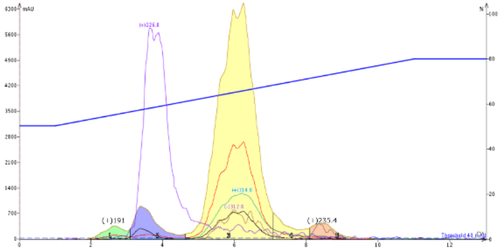

Figure 5. *Reaction mixture reversed-phase flash purification separates both major impurities away from product. One impurity (+m/z 226.8) elutes earlier than the product while the other impurity (+m/z 235.4) elutes well after with this solvent system. Another impurity with +m/z 191 is also separated.*

Conclusions

For reaction mixtures not well-purified using normal-phase methods, reversed-phase flash chromatography often provides better separations because of differing separation mechanisms. This is a major reason why reversed-phase flash chromatography use is increasing in medicinal chemistry research labs.

Besides improved purification results, reversed-phase is greener and, generally, less expensive than normal-phase.

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