Purifying Complex Reaction Mixtures via High-performance Flash Chromatography

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Introduction

Synthetic chemistry is hard enough; purifying the reaction product can be even more challenging. If your reaction mixture shows you made your target but also many by-products, what do you do? You can resynthesize using different reaction conditions, use advanced work-up procedures, or you can purify using high-performance flash chromatography.

For many chemists, purification is the logical option though it may be a challenge. In this poster, we show how using TLC and the right purification tools can overcome the purification challenge.

Experimental Protocol

Reagents and Materials

Solvents used in study included: hexanes, ethyl acetate (EtOAc), and acetone, all from 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.

TLC plates, silica, and the dry load system were also from Biotage.

Biotage® Sfär HC (5 g) flash cartridges and a Biotage® Selekt flash purification system were used for this study.

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

Organic Synthesis

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

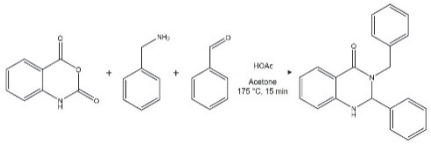

***Figure 1**. Microwave-assisted organic synthesis reaction in acetone at 175 °C.*

Thin-layer Chromatography

After synthesis, the reaction mixture was evaluated by TLC with both 20% and 30% EtOAc in hexanes, Figure 2.

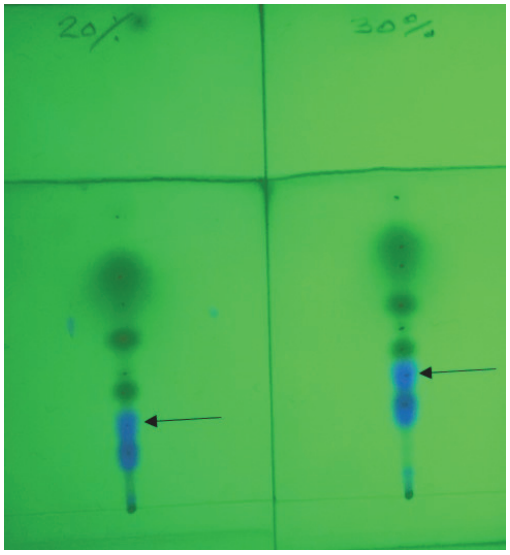

***Figure 2**. Reaction mixture TLC using 20% and 30% EtOAc/hexanes shows many compounds. The product is the top fluorescent spot. (arrow).*

Flash Chromatography

Biotage Selekt will created both linear and step gradients and estimate sample load based on the distance between the target compound and its two nearest eluting neighbors on a TLC plate. From the TLC results, gradients of 5-40% and 7-60% EtOAc were created from the 20% and 30% EtOAc TLC data, respectively. For both sets of Rf data, the Selekt software estimated a 140 mg sample load when the product (Rf 0.19 from the 20% plate and Rf 0.35 from the 30% plate) were targeted.

Dry loading with silica was used to ensure maximum separation efficiency. For the linear gradients, 0.4 mL of crude (100 mg) and 400 mg of silica were mixed, dried, and packed into a Biotage DLV (dry load vessel).

Linear Gradient Results

Both linear gradient purification results show a separation of the target compound (product) from its nearest eluting neighbors, Figure 3. However, the results do indicate there is room for improvement.

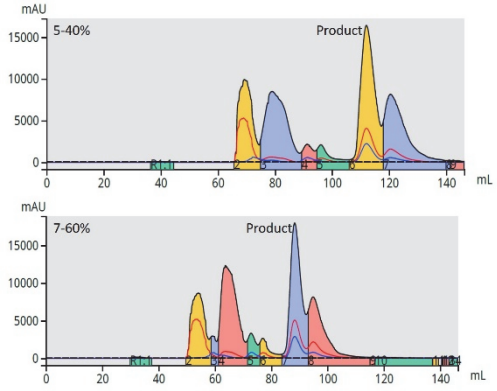

***Figure 3**. Linear gradient flash purification of the RxN mixture (100 mg load). Top - 5-40% EtOAc. Bottom - 7-60% EtOAc.*

Step Gradient Results

In an effort to improve the purification effectiveness, Selekt software was used to create a step gradient from the same TLC Rf values. Based on this data, a single step gradient (17% EtOAc for 4 column volumes (CV) followed by 20% EtOAc for 9.5 CV) was created and used. System estimated a load of 515 mg for the same 5-gram column.

To evaluate the step gradient, the remainder of the reaction mixture (200 mg) was purified, again using dry loading. The results clearly show that the purification effectiveness has improved with the target compound completely resolved from its impurities, Figure 4.

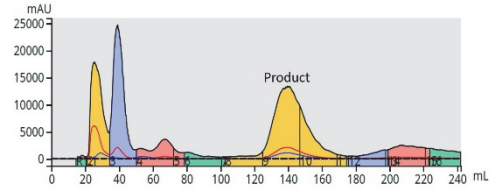

***Figure 4**. Step gradient purification of 200 mg crude reaction mixture on a 5-gram column provides a far superior purification.*

Conclusions

For complex reaction mixtures, a simple, automated step gradient based on two TLC plates' data will provide improved purification effectiveness. Such a properly designed step gradient increases column load capacity compared to a linear gradient.

Utilizing the Biotage Selekt system's ability to create gradients and estimate loading from TLC data eliminates guesswork of gradient design, appropriate column size, and crude mixture load.

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