Effective Chromatographic Scale-up Considerations

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Introduction

Moving Chemistry from Bench to Market, the theme for this ACS meeting, provides an opportunity to discuss the best ways to purify reaction mixtures at larger scale.

In this poster, we describe a simple methodology to maximize purification throughput while reducing organic solvent use.

Experimental Protocol

Reagents and Materials

Solvents used in study included: hexane, ethyl acetate, dichloromethane, dimethyl sulfoxide and dimethylformamide, all from (Reagents, Inc., Charlotte, NC).

Reagents used included: isatoic anhydride, benzylamine, benzaldehyde, and acetic acid all from Aldrich Chemical, Milwaukee, WI.

Synthesis was performed using a Biotage® Initiator+ microwave.

Biotage silica TLC plates (5x10 cm) were used for method development.

Flash chromatography was performed using a Biotage® Selekt system.

Normal-phase flash chromatography utilized 10-gram and 25-gram Biotage® Sfär HC columns.

Experimentation

Work performed for this poster compared the synthetic results for the same reaction with different reaction solvents.

TLC was used to determine the best reaction solvent and to create an optimal flash chromatography method.

Linear and step gradients were used for small scale flash (10-gram column). Scale-up was performed with the step gradient on the 25-g column.

Synthetic Results

As chemistry moves from discovery to production, method changes are typically made to improve workflow efficiencies. Reaction conditions that may be best in the lab (high temperature reactions with high boiling solvents (DMF, DMSO) may not translate to synthetic scale-up reactions due to solvent evaporation issues, as per this example, so alternative solvents are evaluated and incorporated.

In this reaction, four solvents were evaluated – DMF, DMSO DCM, and EtOAc, Figure 1.

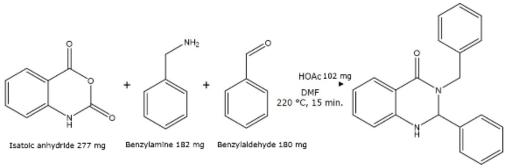

Figure 1. Reaction performed in each of the four solvents. The DCM reaction was performed at 150 °C.

Upon cooling, the EtOAc reaction product crystallized yielding 217 mg of pure compound. The other three reaction mixtures remained solubilized.

TLC analysis

Each reaction mix was evaluated for purity by silica TLC in 30% and 40% EtOAc in hexane, Figure 2.

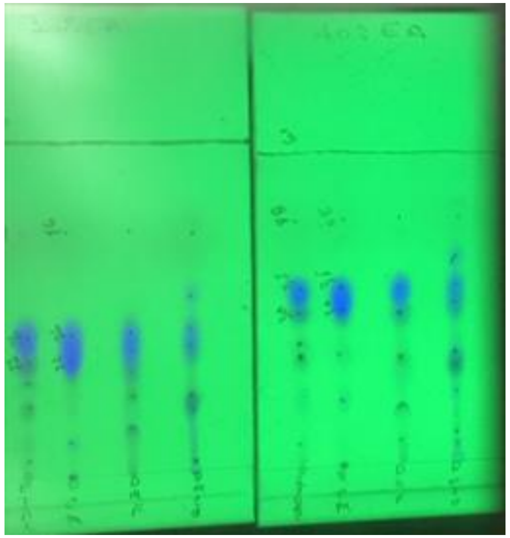

Figure 2. Reaction mixture TLC analysis shows DCM (second from left) is the most efficient synthesis solvent; the product is the blue spot.

The TLC data clearly show the product and some by-products to be fluorescent and that both DMF and DMSO reactions created more by-products indicating poorer product yields – not viable scale-up options. The EtOAc mother liquor and DCM reactions were cleaner but show a trailing impurity with the EtOAc impurity being more significant. That leaves the DCM reaction for chromatographic scale-up.

Linear Gradient

The TLC data was used to create a simple linear gradient, Figure 3. Linear gradients work well in discovery and even some smaller scale-up applications but not necessarily in a production mode.

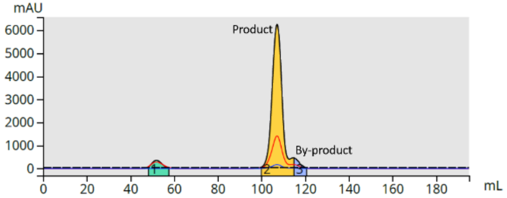

Figure 3. DCM RxN mix linear gradient partially purifies the product from the minor by-product (20 mg load) but consumed 195 mL

Step Gradient

While synthetic methods are not directly scalable, purification methods are. Linear gradient technology can be challenging in process scale flash chromatography but isocratic and step gradients are easily scaled.

Using the TLC data from both TLC runs (30% EtOAc and 40% EtOAc), the Selekt system created a short, 140-mL step gradient using the 10-gram column, Figure 4.

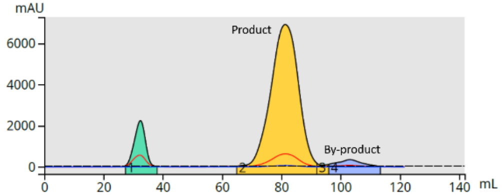

Figure 4. Step gradient purification of DCM RxN (20 mg) generates a superior separation and cuts solvent use 28% versus the linear gradient.

To further increase product purity for scale-up, the collection wavelength was changed from PDA 198-400 nm (inclusive), to 254 nm. While this reduced sensitivity, it increased the obtainable load (80 mg) while maximizing purity and yield, Figure 5.

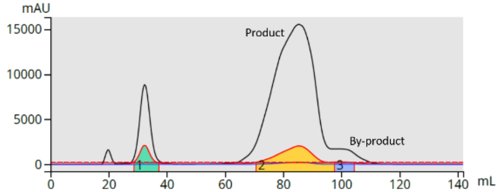

Figure 5. Step gradient with 80 mg load using the 10-gram column with detection at 254 nm.

Though some resolution was lost the method's scalability is shown using a 25-gram column at 5x the load, Figure 6.

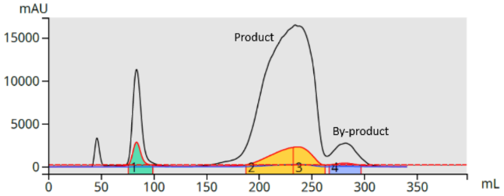

Figure 6. Five-fold scale-up purification of the DCM-solvated reaction using the 25-gram column shows reliable method scalability with minimal solvent consumption.

Mass spectral analysis showed the product to be 100% pure, Figure 7.

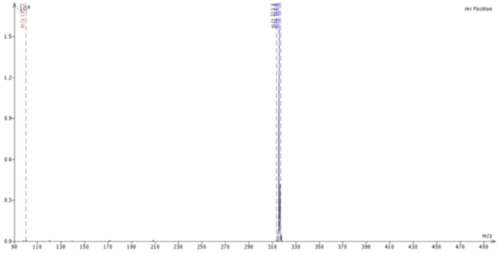

Figure 7. Mass spec analysis of the collected product peak showed no other masses.

Conclusions

Taking chemistry from the bench to the market efficiently requires optimized synthetic and purification routes. Choosing the right reaction solvent and purification method will help achieve this goal.

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