

Orthogonal Flash Chromatography Reduces Organic Solvent Use While Maximizing Workflow

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Introduction

In medicinal chemistry each intermediate and final product needs purification. Historically, the primary tool for reaction purification has been normal-phase flash chromatography sometimes followed by reversed-phase preparative HPLC to provide higher purity when needed. While this process works, it can consume large quantities of organic solvent and slow down the synthesis workflow.

In this poster we describe an orthogonal flash chromatography approach that uses a small silica normal-phase column at high sample load to provide a crude sample clean-up followed by a high load, small column, reversed-phase flash chromatography step to purify the synthetic product while minimizing solvent use.

Experimental Protocol

Reagents and Materials

Solvents: methanol, 2-propanol, ethyl acetate, hexanes, dichloromethane, and dimethyl sulfoxide (Reagents, Inc., Charlotte, NC), and in-house deionized water

Reagents: hippuric acid and α -methyl benzylamine (Sigma Aldrich, Milwaukee, WI).

Synthesis performed with a Biotage® Initiator+ with a 2-5 mL reaction vial.

Thin-layer chromatography was performed using a Biotage 1x3 inch TLC plate.

Flash chromatography was performed using a Biotage® Selekt system with a Biotage DLV, 10-gram Biotage® Sfär HC column, and a 12-gram Biotage® Sfär C18 flash column.

Evaporation was performed with a Biotage® V-10 Touch rapid solvent evaporator.

Goal

Highlight the benefits of using high-load normal-phase flash chromatography followed by reversed-phase flash purification to maximize product purity while minimizing solvent consumption.

Synthesis

The reaction was performed using the Biotage® Initiator+ microwave synthesizer at 150 °C for 15 min in dichloromethane, Figure 1.

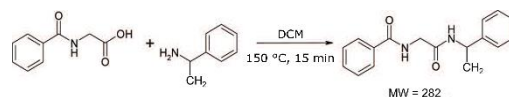


Figure 1. Microwave reaction of hippuric acid and α -methylbenzylamine.

The crude reaction yield measured at 1.7 grams after drying with the Biotage® V-10 Touch.

Thin-layer Chromatography (TLC)

Solvent A: hexanes

Solvent B: 3:1 ethyl acetate / 2-propanol

Ratio: 40% A, 60% B.

This solvent blend was used as a “green” dichloromethane/methanol replacement.

The TLC showed the presence of myriad, poorly resolved compounds, most of which were polar with low Rf values, Figure 2.

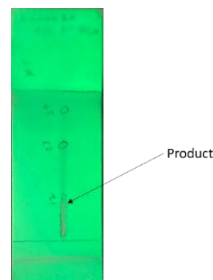


Figure 2. Reaction mixture TLC indicated the presence of many, mostly polar compounds.

Normal-phase Purification

System: Biotage® Selekt

Column: 10-gram Biotage® Sfär HC silica

Dry loading: 10-gram Biotage® Dry Load Vessel with ~7 grams of silica

Load: 1.7 grams

Solvent A: hexanes

Solvent B: (3:1) EtoAc/IPA

Gradient: 10%-80% B over 10 CV, hold for 80% for 15 CV, step to 100%, hold for 10.3 CV

Flow rate: 40 mL/min

This is a much higher loading percentage than what is typically used by chemists (1% load). For this reaction size, a 1% load would require a 200-gram column and >11-L of solvent.

The chromatogram revealed many partially separated compounds with the desired reaction product eluting relatively early (~100 mL) in a 550 mL long purification, Figure 3.

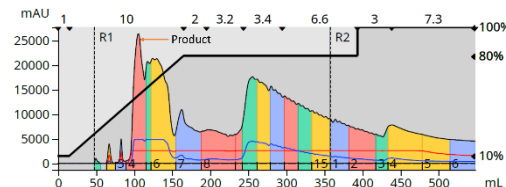


Figure 3. Normal-phase crude reaction mixture purification (17% load) provided a partial separation of the product (arrow) and by-products.

The partially purified product yield was 244 mg.

Reversed-phase Purification

The product was re-purified by reversed-phase flash chromatography because its different selectivity separates compounds normal-phase cannot.

System: Biotage® Selekt

Column: 12-gram Biotage® Sfär C18

Dissolution solvent: DMSO

Load: 0.244 grams

Solvent A: H₂O

Solvent B: MeOH

Gradient: 35-80% B over 10 CV

Flow rate 30 mL/min

Purified yield: of 210 mg

The purification removed several contaminating by-products, Figure 4.

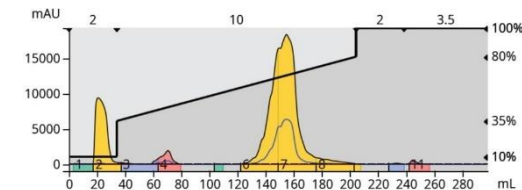


Figure 4. Reversed-phase purification of the normal-phase purified fraction (244 mg) provided a product yield of 210 mg.

Conclusions

Orthogonal flash purification reduced solvent consumption while maximizing product purity and workflow.

Overloading a small silica column partially purified the product using 20X less solvent (550 mL) than the normally used 200-gram column (>1 L) for a 1.7-gram purification.

Product re-purification by reversed-phase maximized purity and added only 300 mL to the total solvent consumption.