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## Introduction

Utilization of synthetic chemistry routes developed in late lead optimization is becoming more widespread as the desire to efficiently confirm pre-clinical compounds has increased. In some cases, chemical processes use reagents which are specific and cannot be substituted on scale up. Such reagents include transition metal catalysts.

These catalytic reactions using precious metals such as Pd, Pt, Ru and Rh, are becoming more popular in industrial synthesis due to atom economy and greener environmental credentials. However, limits for residual impurities or metal in final products/APIs, by FDA and EMEA (ICH Guidelines Q3D Step 5 version issued on 16.12.2014) are becoming increasingly challenging to meet.

Classical methods to remove these metals such as recrystallization, distillation or extraction are energy intensive or may be irreproducible. The use of carbon adsorption methods for example is also quite widespread but can have varying results, including significant loss of API.

## Overview

Grubbs catalysed olefin metathesis is one of the most important transition metal catalysed reactions outside of classical palladium chemistry. It is also empirically known that ruthenium can be more difficult to remove from chelated products, than palladium. As a popular vehicle for ruthenium we chose the second generation Grubbs catalyst in our studies due to its higher catalytic activity and stability towards moisture and air; in order to investigate the removal of ruthenium metal from the products of the diallyl malonate ring closing reaction.

This study presents the use and application of a convenient metal scavenging kit in a Ruthenium metal removal process. Our aim was to combine classic Grubbs chemistry with the concept of metal scavenging, to demonstrate how classical synthetic chemistry may be adapted to meet modern-day guidance on elemental impurities in the pharmaceutical industry. The strategy was threefold:

- » Screening
- » Application of screening to Grubbs reaction
- » Comparison of metal scavenger with carbon

## Experimental

**Reagents:** Dichloromethane (DCM) and Grubbs Catalyst™ (2<sup>nd</sup> Generation) were purchased from Sigma Chemical Co. (Dorset, UK) all reagents were analytical grade >99%+ except where specified.

### Experiment 1

#### Initial Colorimetric Screening

Since the colour of a model catalyst solution may be used as an indicator of metal content, a colorimetric and qualitative screening approach was first applied to a clean solution of Grubbs second generation catalyst. This enabled us to eliminate scavengers which had no noticeable effect under these conditions. (Figure 1).

#### Experimental Procedure

To a gravity rack manifold (Biotage p/n 123-2019) was added each of the 5 x 1 g/15 mL SPE style pre-packed metal scavengers in the Biotage kit (p/n K-MS-3) and also another commercially available Si-Thiol metal scavenger.

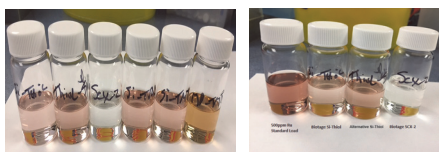
Each SPE column was conditioned with 10 mL of DCM under gravity and the filtrates discarded. 1 mL of a stock solution of Grubbs catalyst in DCM was (500 ppm) was diluted to 10 mL using DCM and added to each tube.

Each tube was further washed by adding DCM (2 mL) under gravity. Filtrates were collected, dried using a rotary evaporator at 40 °C and analysed for Ruthenium content by ICP-MS (Table 1).



**Figure 1.** Initial Colorimetric Screen using 500 ppm Grubbs catalyst solution in DCM, (left to right: ISOLUTE® Si-Thiol, ISOLUTE® SCX-2, ISOLUTE® Si-Trisamine, ISOLUTE® Si-TMT and Biotage® MP-TMT).

From our initial colorimetric screen (Figure 2a) it was clear to see that there was one scavenger which completely removed the colour from solution but interestingly we noted differences between some other metal scavengers, which by name were very similar (Figure 2b).



**Figure 2a (left):** Initial colorimetric screen of metal scavengers. Shows one clear winner, ISOLUTE® SCX-2 (third vial from the left). **Figure 2b (right):** comparison of two Si-Thiol metal scavengers. From left to right = 500 ppm control experiment (no scavenger), Biotage Si-Thiol metal scavenger, alternative Si-Thiol scavenger and ISOLUTE® SCX-2. When analysed by ICP, ISOLUTE® Si-Thiol was >10% more effective than an alternative under identical conditions.

Metal Scavenger	Residual Ru (ppm)	% Scavenged
SCX-2	<5	99
Biotage Si-Thiol	397	21
Comp Si-Thiol	449	10

**Table 1.** Residual ICP results for ISOLUTE® SCX-2, ISOLUTE® Si-Thiol and Competitor Si-Thiol.

**Table 1.** ICP results indicate more efficient Ru removal using ISOLUTE® Si-Thiol compared to an alternative so-called Silica Thiol in a clean solvent system. This increase in efficiency translates directly into reduced material usage (therefore costs) and also improved process workflow, with less solvent use and less evaporation, in scale-up.

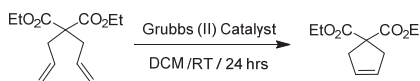
However, ISOLUTE® SCX-2 was shown to have the highest scavenging efficiency for Ru under these conditions. At that stage we suspected this was due to the additional ion-exchange interaction offered by ISOLUTE® SCX-2 to another handle (the basic ligand) in the Grubbs (II) catalyst. Regardless of mechanism of removal, we were pleased with the overall performance and continued the study.

## Experiment 2

### Focussed Screening Decision Making Process

An focussed scavenger screen was then applied to the product of the Grubbs ring closing metathesis reaction of diethyl diallylmalonate.

This simple reaction was used as a vehicle to furnish classical Grubbs reaction products along with an impurity profile that would be addressable by metal scavengers (Figure 3).

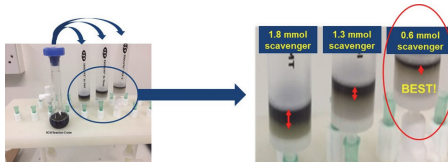


**Figure 3.** Ring closing metathesis of diethyl diallylmalonate.

### Experiment 2a. Crude Scavenger Screen

The reaction (Figure 3) was applied to our screening cartridges to investigate the effect of capacity on scavenging [ISOLUTE® Si-TMT and ISOLUTE® Si-Thiol have sulphur binding points but afford mono and bidentate binding stabilization to transition metal ions)].

An identical quantity and volume of crude reaction mixture was applied to 1 g/15 mL columns containing ISOLUTE® Si-TMT, ISOLUTE® Si-Thiol and ISOLUTE® SCX-2 metal scavengers and more fresh solvent added to ensure the reaction had washed down effectively (Figure 4).



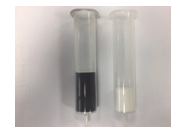
**Figure 4.** Comparative selectivity and binding of crude product of Grubbs ring closing metathesis, in terms of total Sulfur. Left: ISOLUTE® Si-TMT (1.8 mmol), Middle: ISOLUTE® Si-Thiol (1.3 mmol), Right: ISOLUTE® SCX-2 (0.6 mmol)

The metal scavenger ISOLUTE® SCX-2 (0.6 mmol capacity) was the most effective scavenger in screen. This was due to additional binding sites offered to the Grubbs catalyst ligand (ion exchange with ISOLUTE® SCX-2 and basic species). Comparison of ISOLUTE® Si-TMT and ISOLUTE® Si-Thiol suggests that increasing thiol scavenging capacity does not necessarily lead to increased Ru scavenging. This suggests an additional, possibly thermodynamic stability based mechanism of scavenging for the Ru.

### Experiment 2b. Focussed Scavenging Comparison

The above reaction was scaled up to gram scale and halted when product was obtained in reasonable yield (87%) and chemical purity. The crude reaction was evaporated and re-dissolved in DCM (10 mL). Larger scale scavenging cartridges (70 mL volume) consisting of 10 g

of ISOLUTE® SCX-2 and 10 g of activated carbon were prepared and equilibrated with 30 mL of fresh solvent (Figure 5). To each cartridge was added 10 mL of the reaction extract, followed by another 30 mL of DCM. The aliquots were collected, evaporated (Figure 6) and analysed for Ru by ICP (Table 2).



**Figure 5.** 10 g activated carbon (left in image), 10 g ISOLUTE® SCX-2 metal scavenger (right in image). Almost 50% more volume for the same mass of carbon compared to ISOLUTE® SCX-2 metal scavenger.

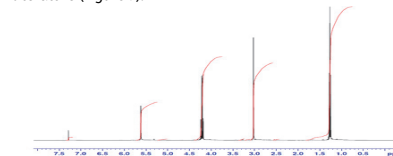


**Figure 6.** a) left crude reaction passed through activated carbon b) right - crude reaction passed through ISOLUTE® SCX-2 metal scavenger.

	Activated Carbon	SCX-2
PPM RU Before	500	500
PPM RU after	98	33
Mass Yield	84%	90%

**Table 2.** Ru removal and mass yield comparison Activated Carbon vs ISOLUTE® SCX-2

Although not the focus of our study, reaction product was also confirmed by <sup>1</sup>HNMR, and found to be consistent with the published literature (Figure 7).



**Figure 7.** <sup>1</sup>H NMR -Product from Ring Closing Metathesis of diethyl diallylmalonate

### Experiment 3- Comparison of Biotage metal scavenger with traditional carbon batch absorption

Experiment 1 (Figure 2a) consisted of some very powerful scavengers, but it was clear that only one (ISOLUTE® SCX-2) had immediate reactivity with the ruthenium catalyst based on the colour of the elution. Since it is very common to apply metal scavengers in 'batch-stir' work flow, we looked at the effect of an identical quantity of Biotage® MP-TMT but after simple overnight RT stirring. We used our standard crude product from experiment 2b for this.

10 mL of crude product in DCM was diluted to 20 mL and added to a 100 mL round bottom flask. 5 g of Biotage® MP-TMT dry resin was added and stirred at RT for 16 hrs. The mixture was filtered through a fritted SPE column and washed with DCM (2x20 mL). The combined extracts were evaporated to dryness. Final mass yield was excellent (88%), and the Ru content determined to be 60 ppm. Considering this was an unoptimized experiment based on a lab hypothesis, we were very pleased with the significant improvement to the Ruthenium scavenging using Biotage® MP-TMT metal scavenger.

## Conclusions

Using this methodology, Biotage Metal scavengers were easily applied to the removal of ruthenium from std solutions and also real reaction products.

Screening using the screening kit<sup>2</sup> provided a quick and efficient means of finding candidate metal scavengers to consider for future scale up.

We already know from previous work that metal scavengers are highly optimized for palladium but for more difficult scavenging applications (such as ruthenium), where excellent results are not possible in fast flow applications, we showed that traditional batch stir methods provide an excellent and powerful alternative. Although activated carbon can be used to remove metal, our previous studies and current work continues to show that modern metal scavengers are more effective and provide higher yield and purity API, whilst consuming far less reactor space. This results in a significant work flow and cost efficiency, from reactor efficiency and also solvent utilization and evaporation perspective.

## References

- [1 http://biotage.com/news/new-application-note--metal-mitigation-extractables-and-regulatory](http://biotage.com/news/new-application-note--metal-mitigation-extractables-and-regulatory)
- [2 http://biotage.com/product-page/metal-scavenging-screening-kits](http://biotage.com/product-page/metal-scavenging-screening-kits)