

# Manual vs Automated Flash Chromatography 2: Purification

## of Synthesised (2S-3S)-epoxygeraniol

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**Chromatography  
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### Abstract

The chiral epoxidation of the allylic alcohol geraniol allows for the enantioselective production of (2S,3S)-epoxygeraniol, by means of a Sharpless asymmetric synthesis. Purification of synthesised (2S,3S)-epoxygeraniol was accomplished by automated flash chromatography and manual glass-column chromatography. The success, efficiency, quality, and economics of each type of purification method were analysed and compared in order to determine which method of purification would be of greater benefit to chemists in professional and teaching settings. It was found that automated chromatography, using the Teledyne ISCO CombiFlash® NextGen 300+, was more successful, efficient, and economical, as compared to traditional manual-glass column chromatography.

### Background

Flash chromatography is commonly used as part of a laboratory experiment for undergraduate students. It is also in routine use during graduate research as synthesized compounds need to be purified. Flash chromatography is a simple, low-cost introduction to chromatography that is very effective in purifying compounds.

#### Advantages of open columns

Despite the advent of automated flash chromatography systems, open columns are still very popular in universities. They have a low initial capital cost, so many of them can be used at the same time. They also provide a sense of how flash chromatography is performed.

#### Disadvantages of open columns

Open columns are made of fragile glass that, when broken, requires cleanup of sharp shards and loose silica. The glass columns need to be packed and unpacked at the end of the experiment, exposing students to silica dust, solvents, and any retained compounds on the column. Only isocratic or step gradients are possible with open columns. The column requires more time to run and needs continual monitoring and management of solvent and fractions. A large number of TLC plates are required to identify the fractions of interest due to the lack of any detector.

#### Advantages of automated flash columns

Automated flash columns are self-contained, so there is no exposure to silica gel or any products or solvents

left on the column after the experiment is finished. The columns are optimally packed, giving improved resolution and reducing the possibility of co-eluting peaks. Although the columns are packaged in plastic, there is reduced solid waste because the detector shows which fractions should be combined, rather than using thin layer chromatography (TLC) plates to see when compounds elute. Automated systems allow experimentation with gradients and show the relationship between gradient steepness and resolution between peaks better than open columns. As there is no need to pack or clean columns, and the purifications are faster, more samples can be run in a given time, offsetting the parallel runs that can be done with open columns.

#### Disadvantages of automated flash columns

The major drawback of the automated system is the initial investment of the flash chromatography equipment, so fewer chromatography systems would be available than open glass columns. An ongoing investment in pre-packed columns is also required, alongside any maintenance costs associated with the equipment.

### Results and discussion

Experiment	Manual purification yield from crude product (%)*	Automatic purification from crude product (%)*
4	29.04	52.85
5	49.73	56.14

#### Yield and time analysis

(2S,3S)-epoxygeraniol was synthesised successfully and then purified using manual and automated flash chromatography. The success, efficiency, quality, and economics of each type of purification method were then analysed in order to determine which method of purification would be of greater benefit to a chemist, both in a professional chemistry environment and in an undergraduate teaching laboratory.

Firstly, the yield of pure product achieved from the synthesised crude was analysed in order to determine the efficiency of manual and automated purification.

The yield varied between each run of each manual and automated purification. This may be due to several

different possible factors.

It can be seen that a greater yield was indeed achieved from the automated purification, with 52.85 % and 56.14 % obtained respectively. In comparison, the manual purification achieved yields of 29.04 % and 49.73 % for experiments 4 and 5 respectively. This is likely due to the use of pre-packed columns in the automated purifications, which allowed for better efficiency as the silica is more tightly packed than in a manual column. This allows the crude product more interaction with the stationary phase allowing for better yield of the purified product. Another possibility for variable yield is the time the sample remains on the column. The epoxide ring may not be stable on silica and might open.<sup>1</sup>

The yields obtained for experiment 5, for manual and automated purifications, are relatively similar; however, the quality of purification, (which was considered to be more important than the yield,) was found to be significantly better in the automated purification.

NMR spectra of the crude product and the manual and automated purified product for experiment 5 were analysed. The results show that the product purified by the automated method contained fewer impurities and solvent residues. Moreover, the impurity peaks were significantly weaker when comparing the measured integral. This is in comparison to the product purified manually, which contained several peaks of trace impurities such as residual ethyl acetate, residual acetone, grease, and overlapping signals which can be attributed to hexane and ethyl acetate solvent. Both manual and automated purification techniques removed residual solvent and general impurities when comparing to the crude product NMR; however, the automated purification was more successful at purification than the manual technique.

Furthermore, it was determined that automated purification is more efficient regarding the time taken to complete one purification. The automated purification required 26.0 minutes for completion whereas the manual purification required a total of 135.0 minutes. This is a 417.24 % percent increase of time required for one purification, thereby confirming the greater efficiency of the automated purification. Additionally, when performing a manual purification, complete supervision of the procedure is required at all times. This is tedious and allows for an increase in the possibility of errors occurring during the manual purification procedure. Potential errors may include, for example, spillages; drying out of the silica rendering it unusable; disrupting of the silica in the glass column, and so on. These errors can make the separation less precise as the bands become broader which can cause a reduction of yield and purity. Conversely, an automated purification removes much of the human error involved

and does not require constant supervision whilst the column is running. For example, the running of the column can be paused at any time the user desires, such that mistakes are less likely to occur. Moreover, the *CombiFlash* instrumentation has sensors that notify the user when a solvent is low in quantity and prevents the system from running any purification column until the user replaces the solvent. This feature eliminates the production of incorrect chromatograms due to insufficient solvent.

Time in the lab can be optimized and errors decreased by removing the constant supervision and labour required by a manual procedure. An automated purification can take as little as 14.0 minutes total, when subtracting the column run time from the total time taken. This is a clear testament to the efficiency of an automated purification.

Additionally, it is much easier to learn and perfect the methodology of product purification using the automated system as compared to a manual one. This is because the manual glass column purification method is more complex and requires much more practice to become familiar with it than an automated purification, which is simpler.

Importantly, for an undergraduate teaching laboratory, the economics of experiments performed are significant. The total price of the materials used for a manual purification and two automatic purifications (using 4 and 12 g columns) were calculated. It was found that the automatic purifications came to a total of £19.81 and £44.70 for the 4 and 12 g columns, respectively. This was significantly less expensive than a manual purification which came to a total of £79.51. An automated system such as the Teledyne ISCO *CombiFlash* NextGen 300+ is an initially expensive investment, more so than manual column glassware materials. However, in industry, the majority of synthesised products are purified by automated means; thereby it should be essential to allow undergraduate chemistry students to have the experience of purifying products using automated instrumentation. However, it must be noted that not all undergraduate synthesis experiments can be purified with automated instrumentation models since not all contain an ELSD detector required for products that do not have a chromophore. The ELSD detector comes at an additional cost, and so may not be practical for a teaching laboratory. In a professional laboratory however, it would be essential, and is recommended when purchasing an automated flash chromatography system.

Therefore, although an initial investment into an automated instrumentation is required, it is more economical to run automated purifications and additionally can allow students to be better prepared for their careers as chemists after university.

While one notes the use of disposable flash columns, the silica gel for a manual column also has to be discarded after each purification. Regarding the sustainability, manual purifications require a greater use of materials such as plastics tips and TLC plates which contribute to the waste produced. Silica gel itself can cause harm to the environment and aquatic life and so must be disposed of safely. In addition, silica gel can cause respiratory issues and a dust mask must be worn at all times when using, packing, and discarding the silica during a manual column operation. However, this danger is eliminated for an automated purification as the RediSep pre-packed columns fully contain the silica such that the user is never exposed to it. The automated CombiFlash instrumentation air purges the solvent from the columns as waste at the end of a purification run, which allows for a significantly easier disposal of the column as compared to a manual column disposal. This decreases the risk involved and makes for a safer lab environment.

## Economic Analysis

On average each manual glass column performed consisted of the materials described in tables 1–3, and required on average the same quantities of materials. The automatic flash chromatography purification used solvents based on parameter settings and column size chosen for purification. In this case, 12 g and 4 g columns were used and materials and solvents utilised for one purification each are described below. Note, that the initial reusable equipment required, such as glassware for manual purification, and the Teledyne ISCO CombiFlash NextGen 300+ for the automatic purification, are not included in the price breakdown and comparisons.

The chemical product provider used in the following comparisons is Sigma Aldrich; therefore all prices listed are based on this supplier.

Materials Used	Price per quantity used (£)
70% hexane/30% EtOAc (600 mL)	49.59
230-400 mesh Silica Gel (100 g)	10.90
Dust mask	2.37
Sand (5 g)	0.39
TLC plates (7 total)	11.48
Pipette tips (26 total)	0.39
KMnO <sub>4</sub> (100 mL) (TLC plate detection)	4.39

**Total material cost for one purification: £79.51**

Table 1: Price breakdown of materials used for one manual glass-column purification

Materials Used	Price per quantity used (£)
Hexane (100 mL)	9.80
EtOAc (100 mL)	4.69
4 g RediSep Gold silica column	5.00
Hexane chaser (1 mL)	0.098
1 mL Syringe (2 total)	0.22

**Total material cost for one purification: £19.81**

Table 2: Price breakdown of materials used for one automatic flash purification using a 4 g column

Materials Used	Price per quantity used (£)
Hexane (300 mL)	29.40
EtOAc (200 mL)	9.38
12 g RediSep Gold silica column	5.00
Hexane chaser (3 mL)	0.29
1 mL Syringe (1 total)	0.11
10 mL Syringe (1 total)	0.52

**Total material cost for one purification: £44.70**

Table 3: Price breakdown of materials used for one automatic flash purification using a 12 g column

## Experimental

Powdered molecular sieves (0.28 g) and dry dichloromethane (15 mL) were added together and mixed whilst cooling to -10 °C. L-(+)-diethyl tartrate (0.13 mL) and titanium (IV) isopropoxide (0.15 mL) was added to the mixture, subsequently followed by an addition of t-butylhydroxide in decane solution (5.5 M, ca. 3 mL). The mixture was stirred at -10 °C for 10 min and then cooled to -20 °C. Geraniol (1.54 g) was dissolved in dry dichloromethane (1 mL) and added to the mixture ensuring temperature did not rise above -15 °C. After the addition, the mixture was stirred for 60 min at -15 to -20 °C. The mixture was warmed up to 0 °C and water (3 mL) was then added. When the solution warmed up to room temperature, a solution of sodium hydroxide (30 %, 0.7 mL) saturated with sodium chloride was added to the suspension. The mixture was stirred for 10 min. The aqueous layer was then extracted using dichloromethane (2 × 10 mL). The combined organic layers were dried over MgSO<sub>4</sub> and the residue was concentrated under reduced pressure to yield crude synthesised (2S,3S)-epoxygeraniol.

Instrumentation	Teledyne ISCO CombiFlash NextGen 300+	
Wavelengths	254 nm (red) 280 nm (purple)	
Mobile phases	Solvent A: Hexane Solvent B: Ethyl acetate	
Flow Rate	13 mL/min	
Equilibration Volume	7.0 CV	
Gradient	% Solvent B	Minute
	0.0	Initial
	0.0	0.5
	100.0	10.0
	100.0	3.5
	100.0	2.8
Run Length	11.4 min, not including equilibration time	
Notes	ELSD used	

Table 4: Experiment 4 (4 g column) fixed parameters used

Instrumentation	Teledyne ISCO CombiFlash NextGen 300+	
Wavelengths	254 nm (red) 280 nm (purple)	
Mobile phases	Solvent A: Hexane Solvent B: Ethyl acetate	
Flow Rate	30 mL/min	
Equilibration Volume	6.0 CV	
Gradient	% Solvent B	Minute
	0.0	Initial
	0.0	0.5
	100.0	10.0
	100.0	3.5
Run Length	8.3 min, not including equilibration time	
Notes	ELSD used	

Table 5: Experiment 5 (12 g column) fixed parameters used

## Conclusion

Synthesised (2S-3S)-epoxygeraniol was purified using automated and manual flash chromatography. It was determined that automated flash purification was more successful in purifying the crude product synthesised, than a manual purification, as a greater number of impurities and residual solvent peaks were removed from the product. This was established by analysing the NMR spectra acquired. Additionally, the efficiency of each purification technique was compared by analysing the yields obtained. It was found that a greater yield was achieved from the automated purification. Additionally, an automatic column purification was much less time consuming than a manual column, allowing for time maximisation in the laboratory. This removed the investment of labour required to complete a manual glass-column purification and removed the high potential of errors which could occur. The manual purification was found to be more expensive, less environmentally friendly, and posed more danger to the user than an automatic purification. Therefore, it can be concluded that an automatic purification instrumentation such as the Teledyne ISCO CombiFlash NextGen 300+ is a worthwhile investment, as it more efficient, purifies synthesised products with more success, and is a more economic and environmentally conscious investment. This conclusion can be applied to chemists both in professional settings, such as research or industry, and in undergraduate chemistry teaching facilities.

## Supplemental Information

### Experiment 4

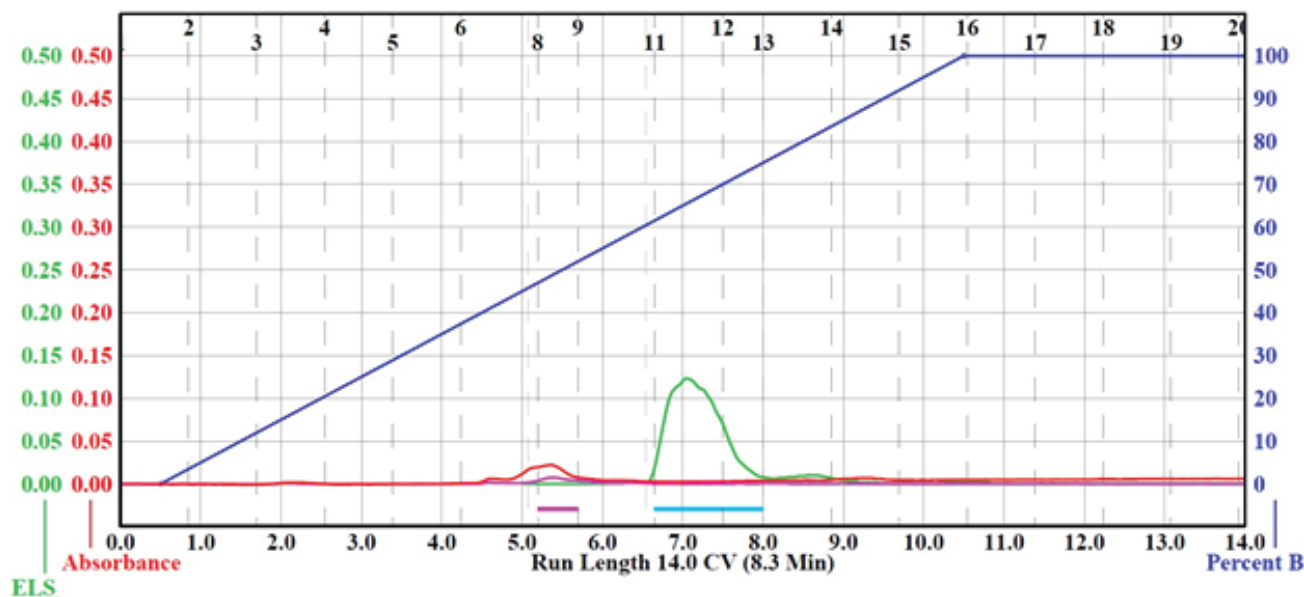
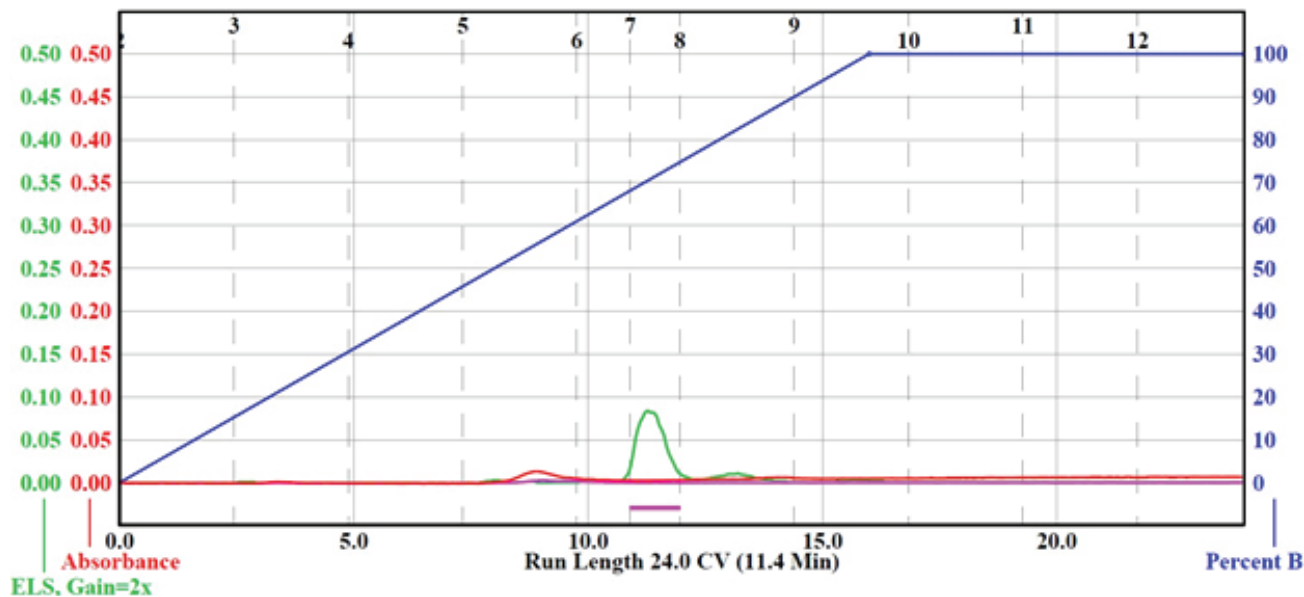
Crude product used for manual purification = 1.000 g  
 Pure manual purified product obtained = 0.2933 g  
 Yield =  $0.2933/1.000 \times 100 = 29.33\%$

Crude product used for automated purification = 0.4 g  
 Pure automated purified product obtained = 0.2114 g  
 Yield =  $0.2114/0.4 \times 100 = 52.85\%$

### Experiment 5

Crude product used for manual purification = 1.0441 g  
 Pure manual purified product obtained = 0.2855 g  
 Yield =  $0.2855/1.0441 \times 100 = 49.73\%$

Crude product used for automated purification = 1.0 g  
 Pure automated purified product obtained = 0.5614 g  
 Yield =  $0.5614/1.000 \times 100 = 56.14\%$



Automated flash runs from experiment 4( top, 4 g column) and experiment 5 (bottom, 12 g column)

1. Purification of Delicate Compounds with RediSep Gold® Diol and Cyano Columns <https://www.teledyneisco.com/en-us/liquidChromatography/Chromatography%20Documents/Application%20Notes/Purification%20of%20Delicate%20Compounds%20with%20RediSep%20Rf%20Gold%20Diol%20and%20Cyano%20Columns.pdf> Retrieved 19 Nov 2021

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