# Manual vs Automated Flash Chromatography

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**Chromatography Application Note AN122**

## Abstract

The use of automated flash chromatography was evaluated for use in undergraduate teaching laboratories and compared to manual glass columns. The automatic flash system was faster to use, safer, and generated less waste because fewer thin layer chromatography (TLC) plates were needed. The automated system allowed students to see how changing chromatographic parameters affected the resolution between peaks. Although the automation was evaluated for undergraduate teaching labs, the same advantages would apply to graduate work, as well.

## 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; in addition a large number of TLC plates are required 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

There is an initial investment cost to an Automated flash chromatography system which has to be considered. An ongoing investment in pre-packed columns is also required, alongside any maintenance costs associated with the equipment.

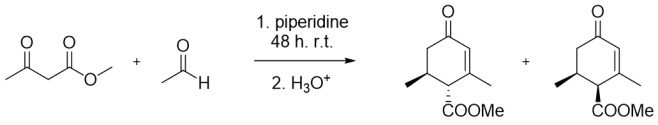


*Figure 1—The synthesis of the methyl 2,6-dimethyl-4-oxo-2-cyclohexene-1-carboxylate (2- trans and 3-cis ) through Knoevenagel Initiated Annelation Reaction.*

### Automated flash columns

The automated column provided insight into how different variables may affect the purification. During this project a total of four automated columns were conducted. The first column, Figure A, resulted in poor resolution. It was later realized that the column was conducted using too steep a gradient, and so another purification was performed using a shallow gradient followed by a steeper gradient to remove the final peaks. This resulted in great resolution, as reflected in the second run shown in Figure A.

All variables could be easily altered using the automated column. The impact of changing the method was made clear due to the UV detector graphing the progress of the column. If this was attempted using a manual column, TLCs would have to be conducted and it would not be so clear what impact the method had. Being able to easily change the variables of the automated column, and being able to immediately see the impact, makes the automated column a better education tool for seeing how changing specific variables may alter the flash column. (Nicholas)

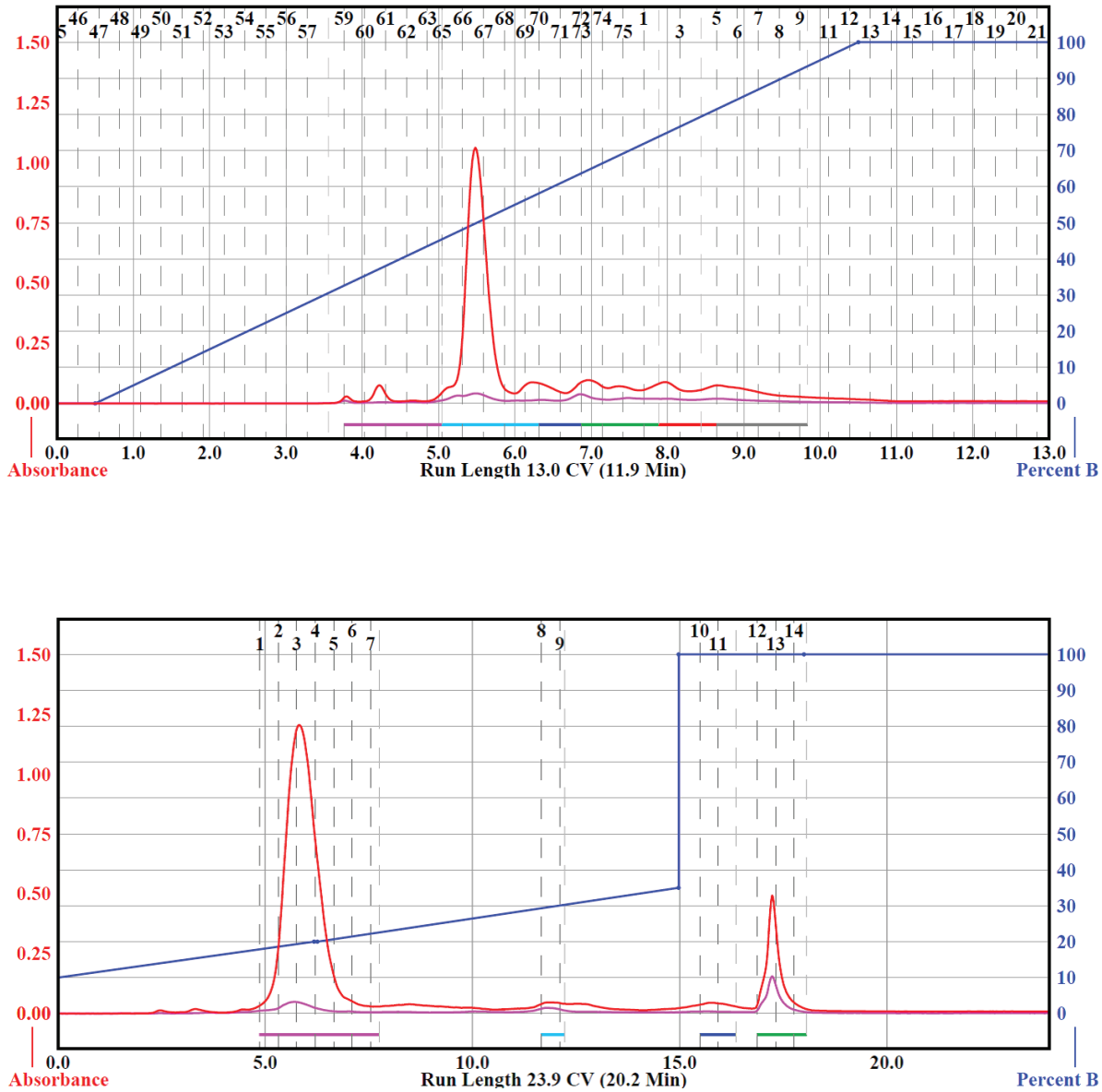


*Figure 2—Fast gradient on a 40 g RediSep Gold silica, and a flattened gradient run on a 24 g RediSep Gold silica.*

## Glass column

The students had concerns about safety when completing the manual column. Handling dry silica required an FFP2 mask causing the student's safety goggles to steam up, making it hard to see the apparatus. Furthermore, the hazards associated with the silica caused the student some anxiety. When cleaning the work area, it was found that a thin layer of silica had formed on the bottom of the fume hood. This was immediately wiped down and cleared by the student; however this shows that the fine silica powder is hard to contain and may pose a larger risk to less observant students.

| | Manual Column | Automated Flash System |
|---|---|---|
| Advantages | • Standard procedure—all undergraduate chemistry students will learn it during their degree<br>• Useful technique for many common experiments<br>• Cheaper than the automatic system | • Better purification<br>• Fast process<br>• Does not require supervision<br>• Minimal risks to the user<br>• Presence of product clearly indicated by a peak on the interface |
| Disadvantages | • Long process<br>• Requires constant supervision<br>• Consumes a lot of single-use materials (not eco-friendly)<br>• Poses health risks (respiratory system)<br>• The presence of the product must be decided using TLC which can sometimes be inconclusive or blurry<br>• Poorer quality purification than the automatic column<br>• Required TLC plate visualization, which generated more solid waste | • Not a standard procedure for undergraduate students—requires additional training. Automated flash is a standard procedure in industry.<br>• There is an initial investment cost for the instrument<br>• The solvent bottles are heavy |

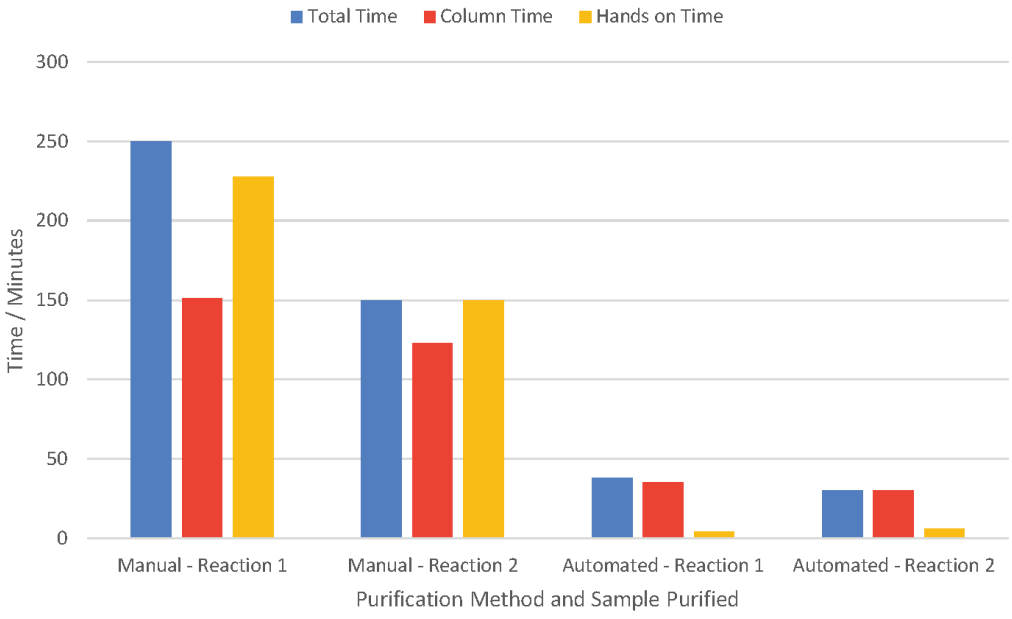


*Figure 3—Time required for manual column and automated flash column. Total Time (blue, left) corresponds to the total time spent from beginning of the purification through to end. Column Time (red, center) represents the time spent working on the column, including set up and running. Hands on Time (Yellow, right) represents the time that required constant presence from the student.*

In Figure B, the total time, in blue, corresponds to the total time needed for the purification. The manual column required constant attention from the student nearly the entire time (yellow bar). The second manual column required less time, suggesting a considerable learning curve is required to manage the column and fractions. The automated flash system required much less time to run and needed little attention by the student.

## Experimental

### Synthesis

4 samples containing 0.88 g (0.02 mol) of acetaldehyde, 4.65 g (0.04 mol) of methyl acetoacetate and 1.7 g (0.02 mol) of piperidine in 25 mL of 50% aqueous methanol each were left stirring at room temperature for 69 hours. Afterwards 10 mL of 6M hydrochloric acid was added and then extracted twice with 30 mL portions of ether. The extracts were dried over anhydrous magnesium sulfate, then gravity filtered, and the ether was removed using a rotary evaporator, leaving a yellow oil.

### Manual flash column

The solution mixture for the manual column was composed of 4 parts light petroleum 40-60 to 1-part ethyl acetate, while the automated column was conducted under a linear concentration gradient of ethyl acetate and hexane. The manual column used 90-100 mL silica. After the purification, the excess solvent from the extracts containing the product was evaporated using a rotary evaporator.

### Automated flash column

Two samples were run with the Combi*Flash*® NextGen 300+ (Teledyne ISCO, USA). One run used a gradient from 0 to 100% B over 13 column volumes as per the gradient in Figure A. The other run used a focused gradient based on the TLC plate data, from 10 to 35% B. Both runs used light petroleum 40-60 as the A solvent, and ethyl acetate as the B solvent.

## Conclusion

The automated column provided insight into how different variables may affect the purification. During this project a total of four automated columns were conducted. The first column (Figure A top) resulted in poor resolution. It was realized the column was conducted using too steep a gradient, and so another purification was conducted using a shallow gradient followed by a steeper gradient to remove the final peaks (Figure A, bottom). This resulted in great resolution, as reflected in appendix Figure 3. The final automated column was conducted by reusing this prepared method, and again resulted in great resolution as seen in appendix Figure 4.

All variables could be easily altered using the automated column. The impact of changing the method was made clear due to the UV detector graphing the progress of the column. If this were to be attempted using a manual column, TLCs would have to be conducted and it would not be so clear what impact the method had. Being able to easily change the variables of the automated column, and being able to immediately see the impact, allows the automated column to be an education tool to explain how changing specific variables may alter the flash column.

The flexibility of the Combi*Flash* system could be exploited for use in undergraduate labs. In order to help teach the principles of flash chromatography multiple columns could be conducted using a standard mixture and changing variables such as gradient, loading, column size, flow rate etc. The output chromatogram could then be analyzed by the student, teaching them the importance of each variable in chromatography.
An important final note is that automated flash chromatography is widely used in industry, especially within the pharmaceutical and biotech industries. Given that "nearly 40% of all chemistry graduates became science professionals or associate professionals and technicians with roles in research and development in agrochemicals, petrochemicals, pharmaceuticals, plastics and toiletries", it makes sense to prepare students with the skills of automated flash chromatography that they will likely meet during their career. Experience of automated flash would be beneficial for students applying for pharmaceutical / biotech internships. The AstraZeneca "Synthetic Organic Chemistry" summer internship program explicitly requests applicants have "some knowledge of organic chemistry and purification techniques". It stands to reason those students with a strong foundation in these techniques would be at an advantage when applying for these roles.

## Conflict of Interest Statement

Students were paid by Teledyne ISCO to complete this study. While they tried to remain totally impartial, it is possible some bias was involved in the study. The student is confident that the results of this study represent a true and fair comparison of both automated and manual columns. The student suggests that a following study be completed at a university where the Combi*Flash* system is bought for use in undergraduate teaching labs. Following completion of both a manual and automated column, students could be surveyed on their impressions of both systems and their overall preferences. This would result in an unbiased representation of both methods and may also provide more compelling evidence to future customers.

This study does not reflect the time commitments required to learn how to use the Combi*Flash* system. Due to the Covid-19 pandemic the students had not had access to chemistry labs for 18 months, which significantly impacted the students' lab skills and confidence. In order to ensure the students were ready for the project, Teledyne ISCO representatives offered a bonus two days of train-

ing covering the theory and application of chromatography systems. Included in this training was hands-on practice with the system; the time spent specifically learning how to use the automated column, however, was not recorded. In the opinion of the students, the system is so simple to use that the instructions could have been summarized in a short video of around 15 minutes. However, given the students would be the only people in the lab trained to use the apparatus, it was important they were thoroughly confident with all aspects of the system.

## Supplemental information

### NMR

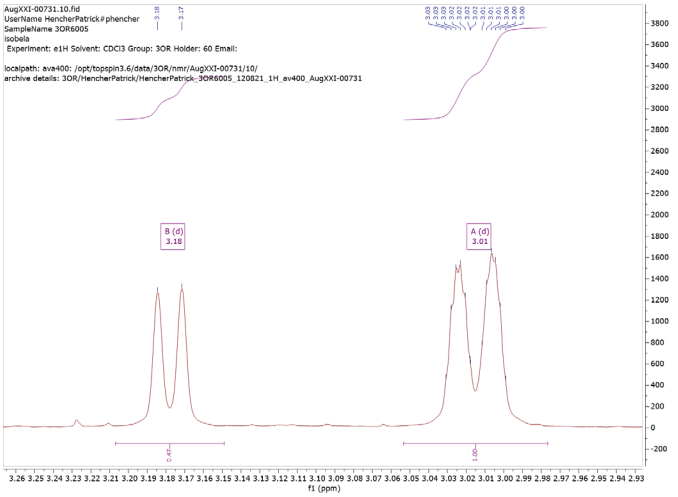


*Figure 4—The two doubles signalizing the presence of the methyl 2,6-dimethyl-4-oxo-2-cyclohexene-1-carboxylate (taken from the NMR spectrum of sample 3 A)*

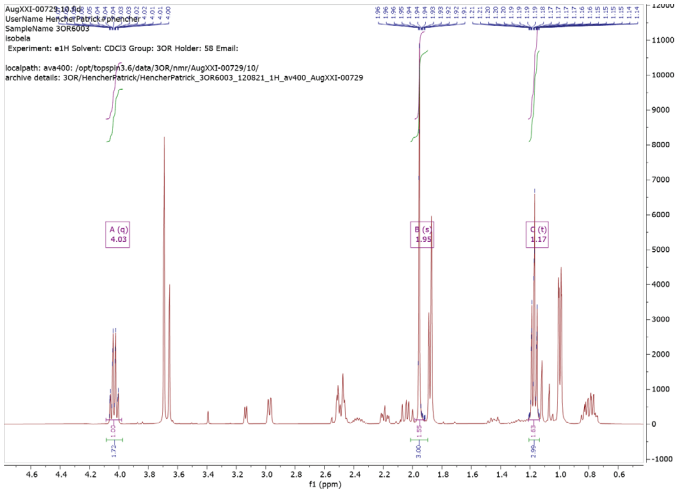


*Figure 5—The H1 NMR spectrum of the 2A sample, with the characteristic solvent peaks indicated—the most significant being the quartet between 4.0 and 4.2 ppm.*

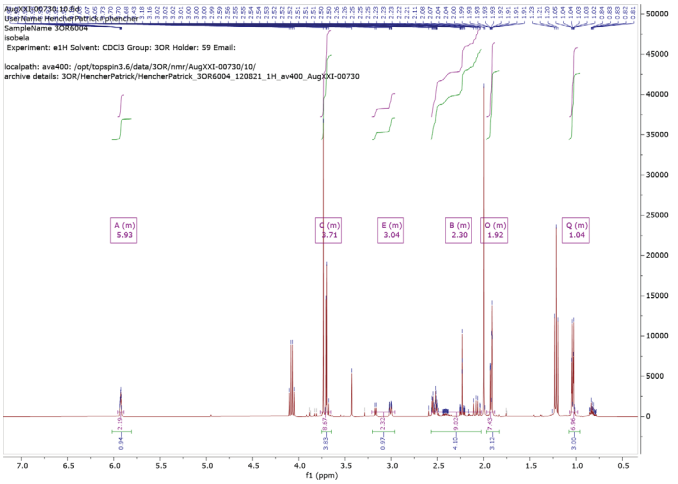


*Figure 6—The 2B spectrum with the product peaks integrated and their multiplets analyzed.*

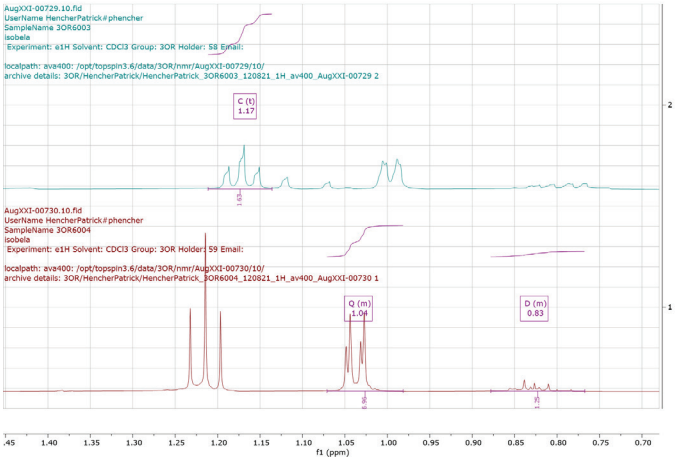


*Figure 7—The section of the 2A (blue) and 2B (red) spectra, showing the presence of additional, impurity peaks in the 2A sample and the sharpness of the peaks of the 2B sample.*

1. *LCGC North America-03-01-2020, Volume 38, Issue 3 https://luminate.prospects.ac.uk/what-do-science-graduates-do (accessed August 2021)*
2. *https://careers.astrazeneca.com/early-careers-internships (accessed August 2021)*

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