# Setting up flash chromatography in your lab – faster workflows for better purifications

## Introduction

**"Chemistry, unlike other sciences, sprang originally from delusions and superstitions, and was at its commencement exactly on a par with magic and astrology."** – *THOMAS THOMSON, 1773-1852, contributed to the early spread of Dalton's atomic theory*

In contrast with 18th century folklore, chemistry today is rapidly advancing with the growth of our digital world. No longer do we need to wait days, or even hours, for painstakingly long chromatography processes. Our 21st century chemistry methods were once the concept of space-age fantasy – now, with software technology guiding the way, producing simplified workflows and repeatable results have revolutionized the chemist's lab.

Among these complex processes, flash chromatography exists as the primary method for compound purification. A technique for reaction mixtures, synthetic products, medicinal compounds, natural products and more, flash is the standard for separating components of complex mixtures.

"Any synthetic organic or natural products lab today would not be complete without a flash purification system. The premise is simple – mixture in, compounds separated, fractions collected, purification complete. However more and more, labs are realizing the complexity behind a 'simple separation.' Identifying the compounds of interest in the reaction mixture, preparing your method, confirming your fractions – this all takes time and often goes unmentioned when speaking about the purification workflow," said Dr. Daniel Eikel, Director, Product Application Development & Customer Support, Advion Interchim Scientific.

Although reaction monitoring, compound identification, and purification are some of the cornerstones of a successful synthesis, they are also lengthy, labor-intensive processes that create workflow bottlenecks. Thanks to advanced software technology and fast, prep-free screening methods, several of these purification bottlenecks have been reduced or all-together eliminated from the workflow.

Using TLC, a smartphone application and a simple dipping probe for fraction ID, a novel, systematic approach to purification takes the standard operational timeline from hours to minutes. “That’s what we worked to create in our labs – one continuous process with the help of autonomous software fueled by technology that can help accelerate syntheses and purification.”

Here we showcase the steps to this novel workflow, highlighting the differences between new and traditional testing methods.

## Step 1: Reaction monitoring using TLC

Prior to purification, the compound of interest must be identified. One traditional way to do this is utilizing TLC/MS. Taking a developed TLC plate, you then scrape your spots of interest, extract the compound, purify it, concentrate it, and finally inject the compound into the mass spectrometer. This method is time consuming and difficult for the novice (and even expert) chromatographer.

A more modern method involves automated TLC analysis. New instruments allow for push-button analysis of TLC plates for results in <30 seconds. Here, using an Advion Interchim Scientific Plate Express® TLC plate reader, you simply align your spot of interest, and, at the push of a button, the head lowers and extracts the sample with a solvent to send the analyte directly to the mass spectrometer, thus showcasing the mass spectrum and identifying your compound of interest within seconds of pushing a button.

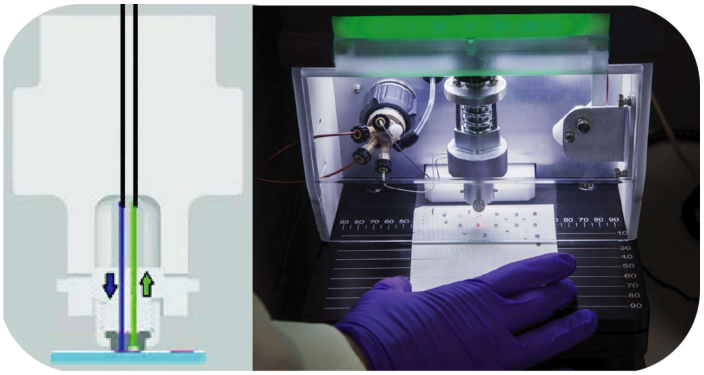


**Figure 1:** The TLC plate reader head lowers on to the spot of interest, and a flow of solvent extracts the silica and performs the perfect dilution for automatic transfer to the mass spectrometer.

## Step 2: Using smartphone technology to develop your flash method

No longer do you need to be an expert in flash to flawlessly carry out a successful purification. With a new software algorithm that enables your cell phone to create the method for you, it simply requires photographing your TLC plate and entering simple parameters. The full method development takes seconds, even suggesting the best column to optimize your run.

Here we review the Advion Interchim Scientific puriFlash® flash purification system, utilizing InterSoftX® "Genius" smartphone technology for method development.

**a.**

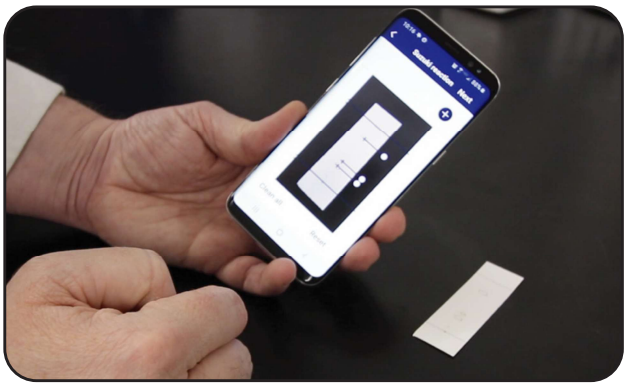
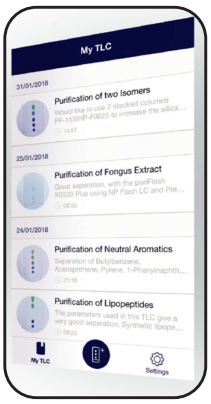


**Figure 2: a.** The method development process begins by naming your purification and photographing a developed TLC plate using a cell phone or tablet loaded with the "TLC to Flash" app, part of the Advion Interchim Scientific InterSoftX® "Genius" software suite.

**b.**

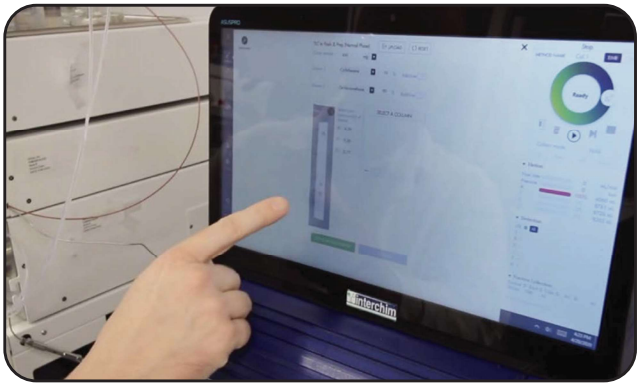



**c.**


**b.** TLC separations are captured automatically using the smartphone app, which calculates Rf and ΔCV (= ΔK).

**c.** Indicate the solvents, their proportions and your comments in the dedicated areas and send via Bluetooth to the puriFlash®.

**d.**




**d.** The TLC data appears on the InterSoftX® "Genius" software and the system suggests the best gradient conditions, injection type and ideal column to use

Essentially, all a researcher needs to do to purify a compound is to snap a photo of the TLC plate and send it to the flash purification system via a smartphone app built specifically for linking TLC data to a column separation method. The software installed in the flash system automatically generates a gradient method for purification on the column by analyzing the TLC data provided by the user.

"The software takes into account many things, like how much material you want to clean up, size of the column, and the type of material that the column is made of and it's particle size," Eikel says. "All those things are considered by the algorithm in suggesting a method that gives an optimal separation for your compound of interest."

### Step 3: Fraction ID in seconds

After the purification run is complete, collected fractions are located based on their UV profile. They need to be confirmed to ensure they eluted as expected. Traditionally, chemists perform fraction ID by direct injection into a mass spectrometer. This method is time consuming and the "dilute and shoot" method of sample introduction is messy, and for a novice, can cause trouble with high concentrations and lack of adequate sample preparation.

Using new sample introduction methods, a direct sampling probe for mass spectrometry is an ideal solution to optimize this workflow. The ASAP direct-probe sampling technique for the Advion Interchim Scientific expression® Compact Mass Spectrometer is the final step to confirm fractions in seconds by simply dipping the probe in the pre-identified tube, and inserting it directly into the mass spectrometer.

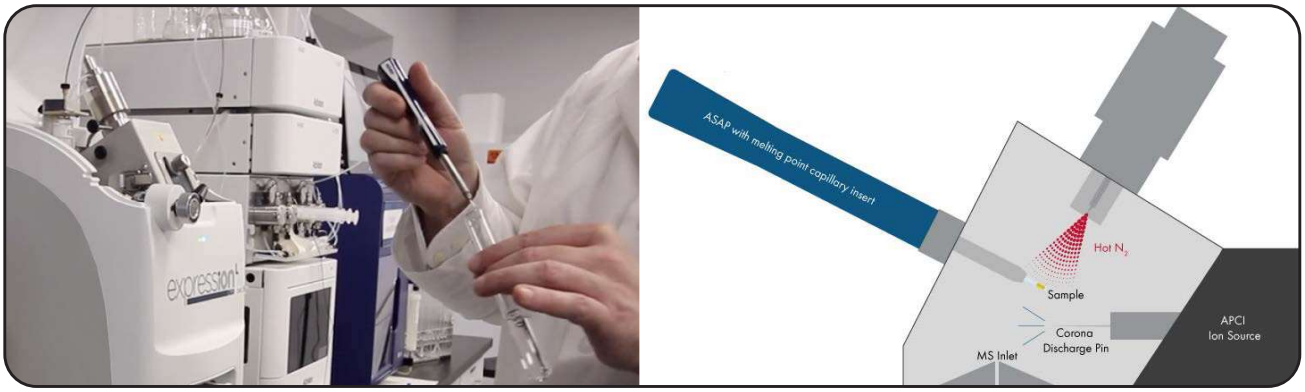



**Figure 3:** The Atmospheric Solids Analysis Probe (ASAP) is dipped into one of the identified fraction tubes and inserted into the mass spectrometer, where is it instantly analyzed by the APCI ion source. The APCI, or Atmospheric Pressure Chemical Ionization source, schematic is also shown, where the probe is quickly heated with hot nitrogen gas, instantly vaporizing the sample and ionizing it via corona discharge for preparation free sample introduction.

## A Streamlined, User-Friendly Purification Workflow

According to Eikel, the expectation a few decades ago was that an organic chemist could complete one synthesis step a day. This has now gone up to about two steps per day, but the purification has also become more rigorous. Most chemists clean up their solutions after every step to get a better yield. "I don't believe it's easy or even possible to clean up two steps in a day, actually, without a fast, streamlined workflow," Eikel says. "We've found that by using TLC/MS to monitor a reaction, you can save hours of reaction time. With an efficient flash purification system coupled to a mass probe, you can drive more synthesis steps per day."

When this type of streamlined workflow was developed, one of Advion Interchim Scientific's goals was to make it user friendly. For example, the instruments needed to be compact; the software features needed to be simple; and perhaps most importantly, must be built for the specific purpose of coupling and accelerating compound identification and purification. Eikel believes that the biggest advantage of this streamlined workflow and smartphone-guided flash system is how it combines multiple components that work well together to enhance the efficiency of organic synthesis workflows.

"Decades ago, it would not have been possible to achieve the speed and purity that is now made possible through integration and novel preparation free sampling techniques," said Eikel. "We see flash and preparative purification moving from the expert to the novice – no natural products, chemical synthesis or medicinal chemistry lab can exist without an automated separation system, and modern systems like the puriFlash® are well-equipped to support the described modern approach to separation science."

Today, if you have a cell phone if your pocket, you are well on your way to developing a purification method – no magic required.