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응용 자료

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Abstract

This application note describes techniques to simplify the choosing of an appropriate first-try purification method for a chemist's raw extract using an Open Access purification system.

Benefits

Simple method selection criteria (based on TLC analysis) allow separation methods to be tailored to an extract, as opposed to a single generic method for all extracts. This allows chemists to reduce the number of re-analysis and re-purification steps; ultimately, speeding up the entire purification process.

Introduction

Extraction of active medicinal compounds from plant materials is accomplished in many diverse ways. Ultimately, the chemist is left with a raw extract that contains potentially active compounds along with many other non-active constituents. Following extraction, the next logical step is purification or isolation of those compounds of interest. Preparatory liquid chromatography is often utilized to accomplish this task.

The diversity of compounds that may be extracted is large and an adequate purification requires access to different chromatographic methods for effective isolation. An Open Access liquid chromatography system that may have many methods available for use by walk-up chemists is, therefore, desirable. Methods can vary in column size, run time, gradient conditions, solvents, modifiers, and other variables. These options can make it

difficult for a chemist to choose the best method for their extract and delay the isolation and development of the compounds of interest.

To simplify the choosing of an appropriate first-try method for each chemist's extract, an Open Access system using simple method selection criteria (based on TLC analysis) is described.

By using separation methods tailored to their extracts, chemists are better able to obtain higher purity compounds. By selecting a suitable method, as opposed to a single generic method for all extracts, chemists essentially reduce the number of re-analysis and re-purification steps; ultimately, speeding up the entire process.

Experimental

Samples and Extraction

Three different plant materials were extracted for the trial; ground schisandra berries (*Schisandra Chinenis*), cinnamon pieces (*Cinnamomum Zeylanicum*), and kudzu root powder (*Pueraria lobata*). 10 grams of dry sample were shaken for one hour with a 50-mL mixture of 60:40 methanol/water. Extracts were centrifuged and the clear extract used without further manipulation. No attempt was made to optimize the extractions.

Thin Layer Chromatography

Extracts were separated on 5 x 20 cm silica gel TLC plates (Partisil LK6F Silica Gel 60A with fluorescence indicator, Whatman, Maidstone, England) using a mixture of hexane and ethyl acetate. Following development, samples were visualized by exposing the plate to UV light and observing the spots. Rf values were generated from these observations.

Preparative Liquid Chromatography

Preparatory HPLC analyses were performed using a system consisting of a Waters 2545 Quaternary Gradient Module, a 2489 UV/Visible Detector, a Preparative Chromatography Rack, and a Fraction Collector III using a SunFire Prep C_{18} OBD, 5 µm, 19 x 100 mm Column. The entire system was controlled using MassLynx Software with both FractionLynx and Open Access application managers. Method parameters, mobile phase components, and gradient conditions are outlined in Table 1, shown on page 3.

Gradient One			
Time	%A	%B	%C
0	85	10	5
2	85	10	5
10	45	50	5
12	0	95	5
14	0	95	5
16	85	10	5

Gradient Two			
Time	%A	%B	%C
0	85	10	5
2	65	30	5
10	25	70	5
12	0	95	5
14	0	95	5
16	85	10	5

Gradient Three			
Time	%A	%B	%C
0	85	10	5
2	35	60	5
10	0	95	5
12	0	95	5
14	0	95	5
16	85	10	5

Flow:	25 mL/min
Temp.:	Ambient
A:	Water
B:	Methanol
C:	2% Formic acid in water
Detection:	UV @ 254 nm
Injection vol.:	1 mL

Table 1. Preparative chromatography method parameters.

Results and Discussion

To simplify the process of deciding “Which separation method should I use?”, users must first gather some information about the sample. Ideally, a series of analytical scale HPLC separations would be run and a preparatory method derived, developed, and scaled up from these results. In many cases, this level of detail is too time-consuming and unnecessary. This is particularly true when dealing with one-time extracts in the earliest stages of investigation.

A simpler approach is to conduct an initial evaluation using thin layer chromatography, evaluate the results, and create an appropriate preparatory LC method based on the calculated Rf value of the compound of interest.

In this example, extracts were first analyzed by TLC as shown in Figure 1. With the TLC results, users can either make a method with specific separation characteristics or use a method already created on the HPLC system, as shown in Table 2.

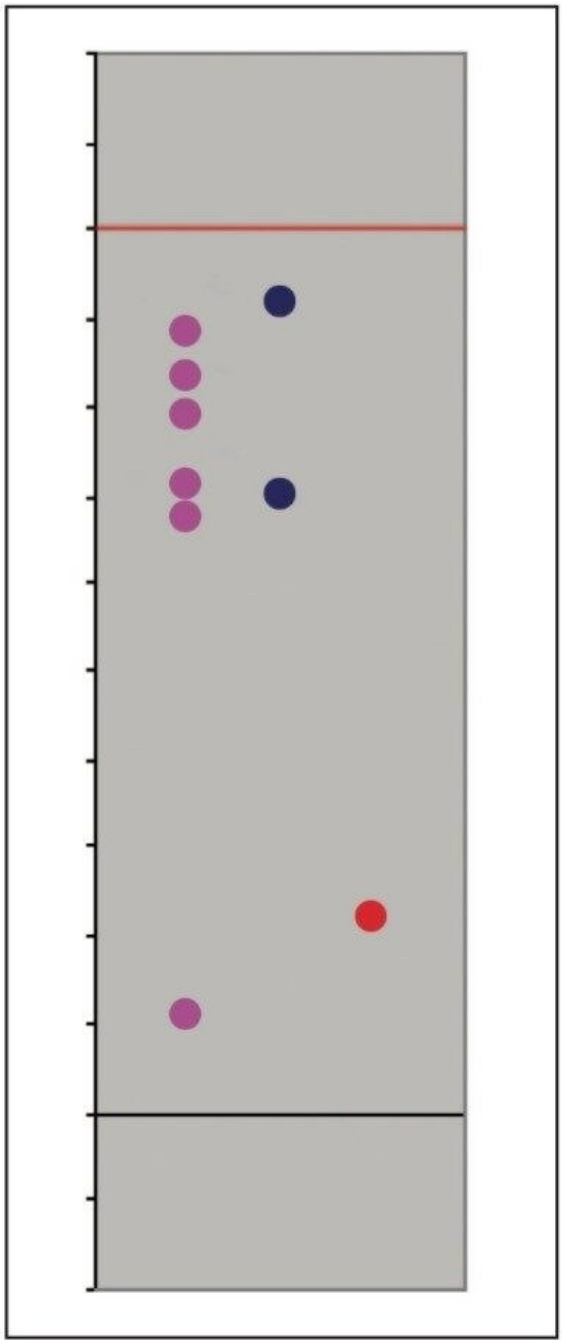

Figure 1. TLC results for (left to right), Schisandra berry, cinnamon, and kudzu extracts. Spots are indicative of UV active compounds, polar compounds toward the bottom, non-polar

compounds toward the top.

Gradient 1	Separation gradient 1 is for use when the Rf is less than 0.50
Gradient 2	Separation gradient 2 is for use when the Rf is greater than 0.50 and less than 0.75
Gradient 3	Separation gradient 3 is for use when the Rf is greater than 0.75 and less than 1.00

Table 2. Preparative gradients based upon Rf values.

To further simplify the HPLC purification process, method selection can be simplified using a single Open Access login page as displayed in Figure 2. After clicking the login button, users simply inject the sample via the manual injector. Results can be printed or emailed to users following completion of the run.

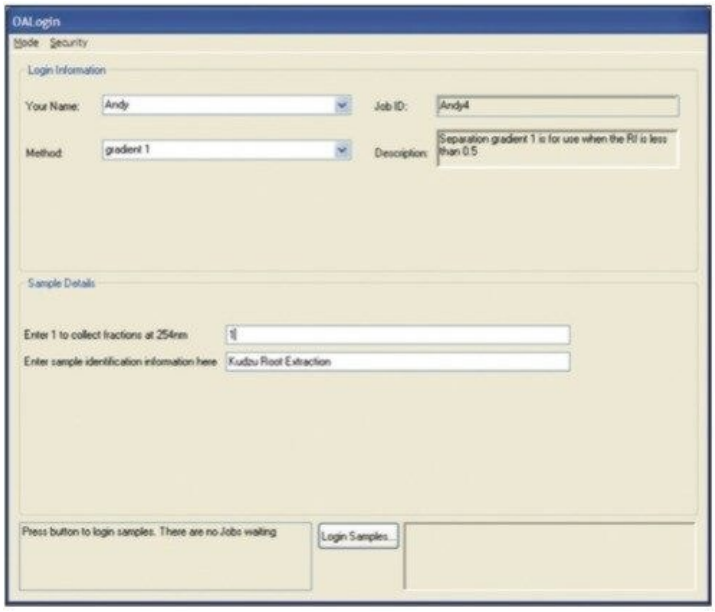

Figure 2. Open Access login page.

Gradients were designed to ensure that even if users choose a less-than-optimal method, all potential compounds of interest were eluted from the column and collected. An example of this can be seen in Figure 3 where a chromatogram shows the cinnamon extract separated using gradient 2. Two very late eluting compounds were collected long after the compound targeted by TLC.

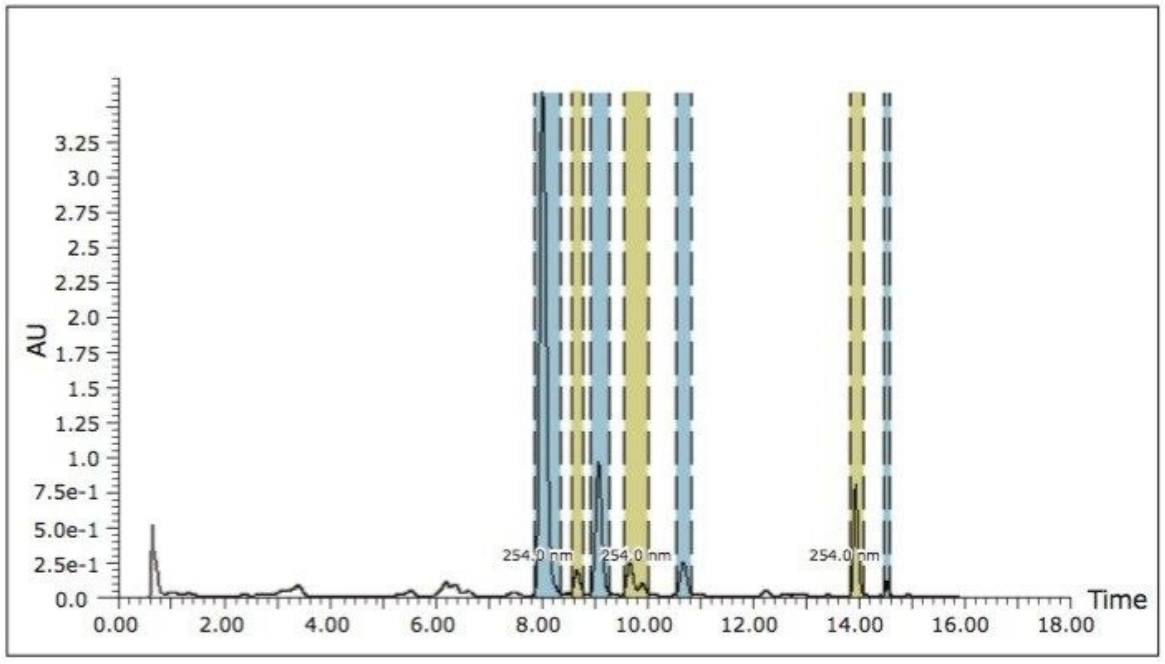

Figure 3. Cinnamon extract separated using gradient 2.

Figures 4 and 5 show both the kudzu root extract and schisandra berry extract separated using the described techniques. Chromatograms from the three natural product extracts are similar to TLC plate results, demonstrating that this progression of techniques provides a suitable workflow. As soon as the analysis is completed, sample results are emailed to users or printed as desired. System configuration and setup is enabled through a System Administrator who determines login access, method selection, and report generation. The System Administrator can also increase or decrease the number of methods available for analysis selection. The 2545 Quaternary Gradient Module allows four-solvent selection, providing the availability for selection of multiple buffers or alternative organic modifiers. The Preparative Chromatography Rack conveniently holds multiple columns and a pair of injectors to increase flexibility for the method selection choice.

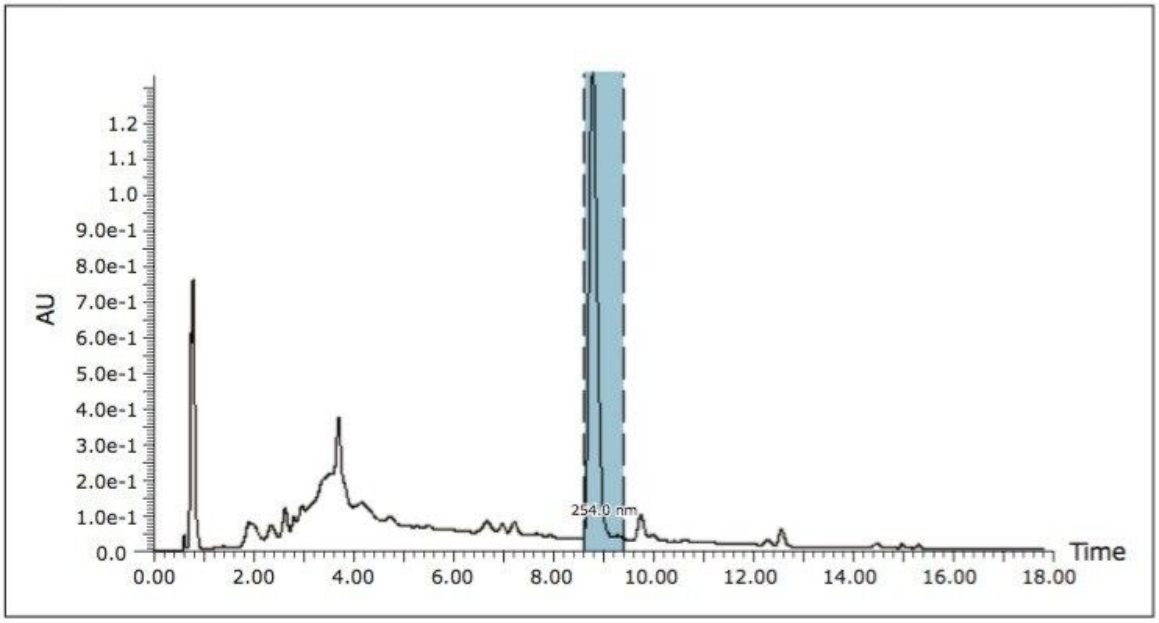

Figure 4. Kudzu root extract separated using gradient 1.

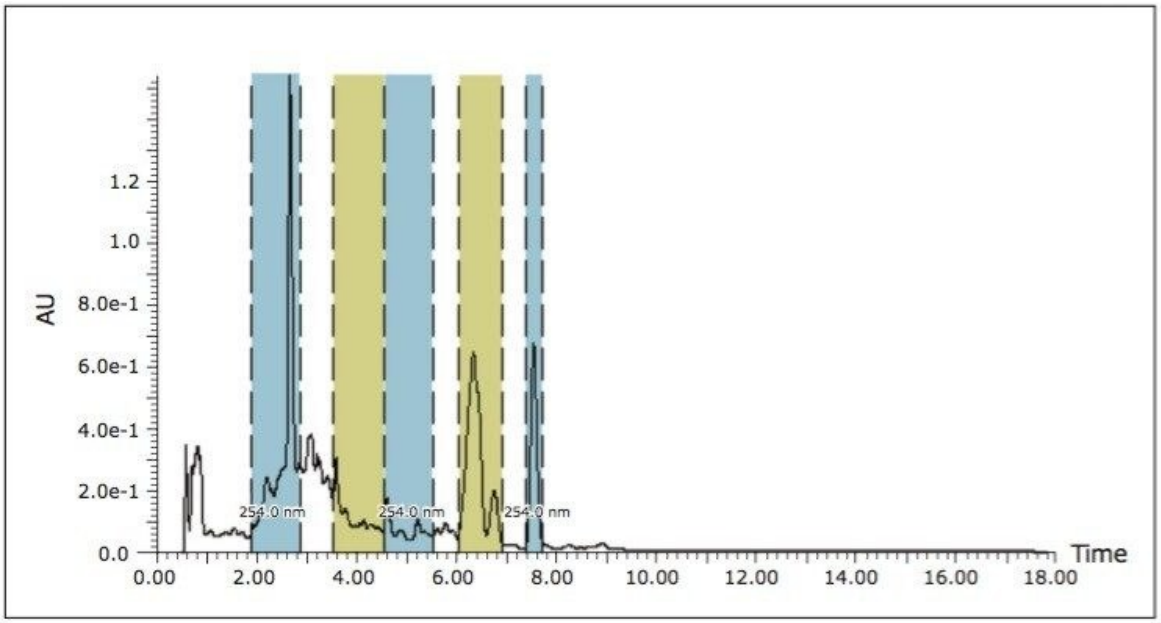

Figure 5. Schisandra berry extract separated using gradient 3.

As a final confirmation of the system functionality, the fraction collected from the kudzu root extract was re-analyzed using an analytical HPLC system, as shown in Figure 6. The results show a clean, single peak that was isolated and collected from the kudzu root, which can move to the next steps in the investigative process.

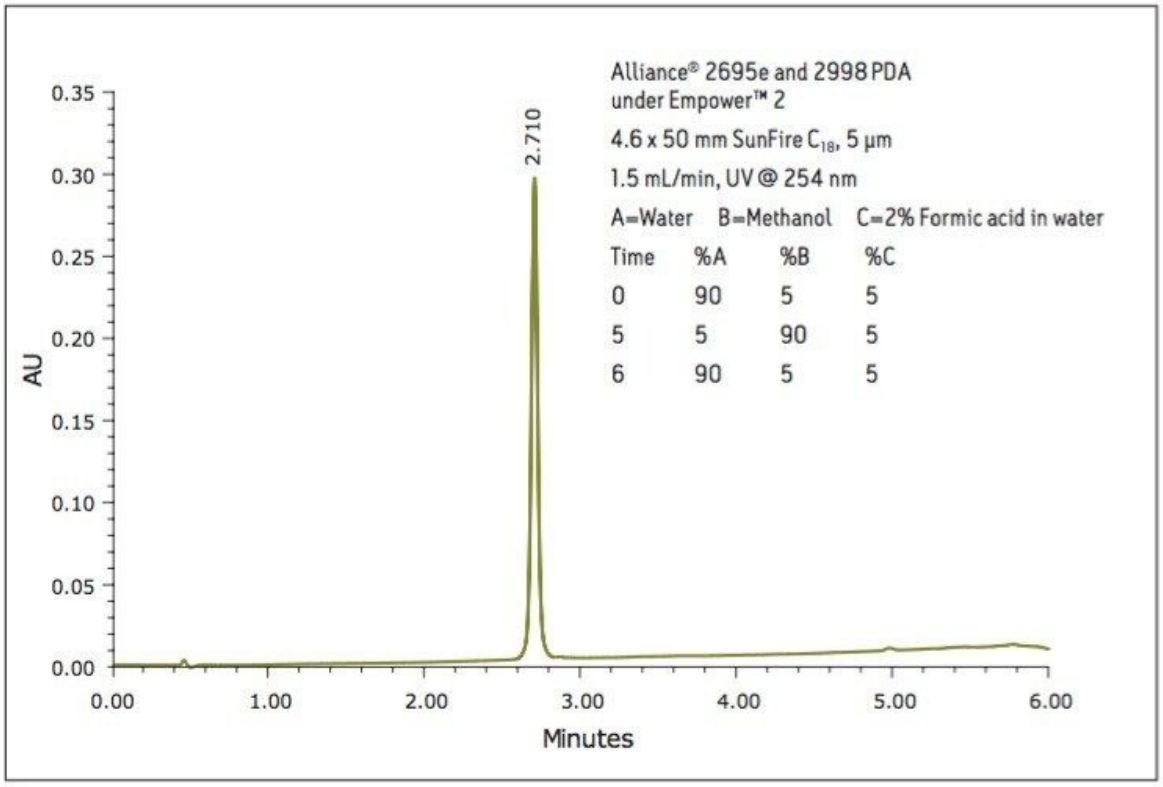

Figure 6. Single fraction collected from kudzu root extract separated using gradient 1.

Conclusion

- Waters Open Access systems give chemists the ability to analyze their own samples close to the point of production by simply walking up to the LC system, logging their sample information, injecting their samples, and walking away.
- Method parameters can be customized to suit the needs of the individual laboratory workflow.
- As many methods as required can be presented to an individual chemist, optimizing the collection of potential

active compounds.

- By using methods tailored to their compounds, chemists are able to obtain higher quality fractions from their mixtures in the shortest possible time; thereby, speeding up the investigation process.

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