Supercritical Fluid Chromatography (SFC) Replaces Chlorinated Solvents for Chinese Traditional Medicine Compound Purification!

Jack Silver • Teledyne LABS • 4700 Superior Street • Lincoln, NE 68504 • USA
Hui-hui Xiao • Department of Food Science and Nutrition • The Hong Kong Polytechnic University • Hong Kong • PR China

Abstract

Dichloromethane (DCM) and other chlorinated solvents are commonly used for chromatography because of their polarity, aprotic structure, and low boiling point. However, these solvents are considered hazardous, with their usage in the United States and elsewhere becoming restricted.[1] There has been much work to replace chlorinated solvents for chromatography, but because none are a drop-in replacement, several combinations of solvent need to be tested using thin layer chromatography plates to find the best combination of solvents.

Supercritical fluid chromatography (SFC) is a viable replacement for chromatography using chlorinated solvents. Although carbon dioxide is non-polar, it is polarizable and miscible with alcohols. Method development for SFC is very easy using scouting gradients. Most compounds only need methanol as a co-solvent, making method development easier.

Model compounds that were purified with liquid chromatography with dichloromethane and substituted mobile phases are run with SFC; examples of Chinese traditional Medicine compounds purified with chlorinated solvents are shown with SFC. In all cases, no chlorinated solvents were needed for SFC

Background

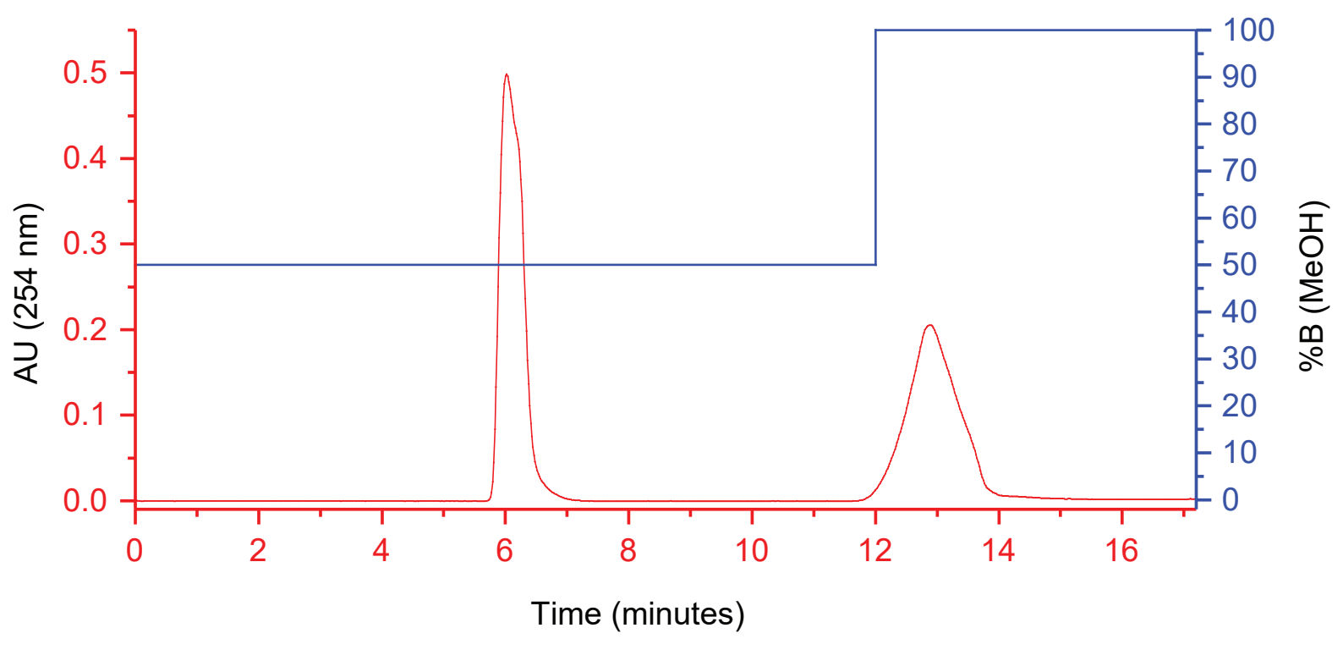
Purification of individual compounds in Chinese Traditional Medicines (CTM) is important for research and development, allowing the study of individual compounds leading to new therapeutic insights and applications. Purified compounds are also used as standard reference materials for markers to indicate that CTM raw materials contain the expected ingredients and the expected potency.

Many of these compounds have been purified using chlorinated solvents, which are toxic and their usage is increasingly restricted. Although various combinations of solvents are used as a replacement for chlorinated solvents for liquid chromatography, none of them represent a simple replacement for those solvents. These replacements need to be evaluated separately to determine if the compound(s) of interest are resolved from impurities, generally via thin layer chromatography (TLC).

TLC is limited to media readily available as TLC plates, while SFC can run various stationary phases that allow differing selectivity and the option to avoid solvent additives. SFC method development uses fast calibrated scouting gradients from which focused or isocratic gradient methods can be calculated.[2]

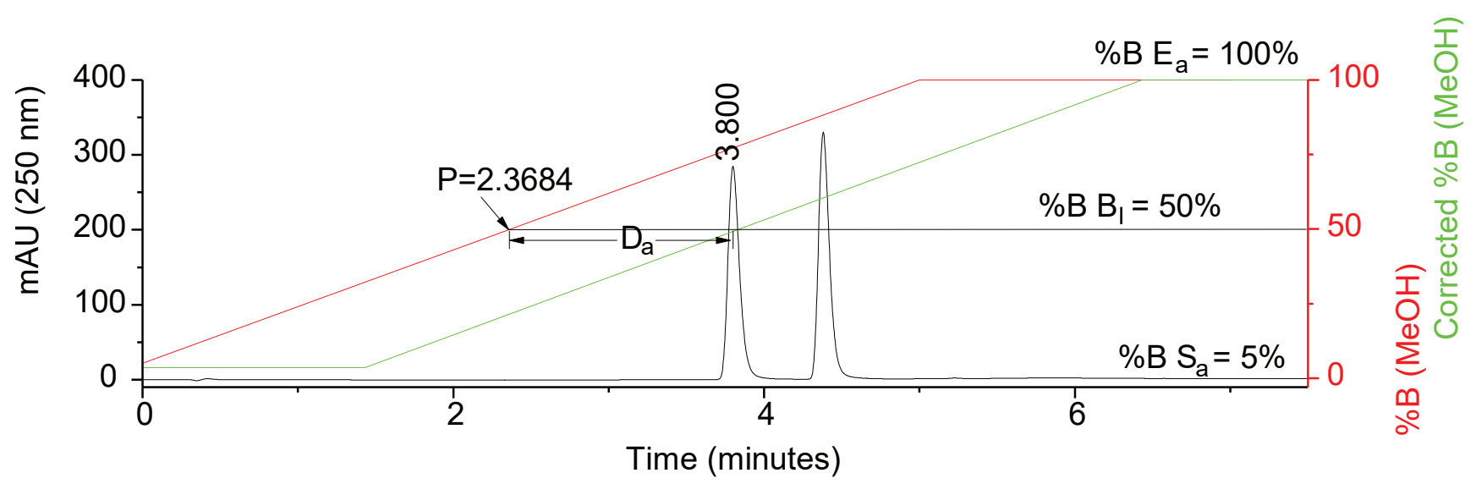
Experimental

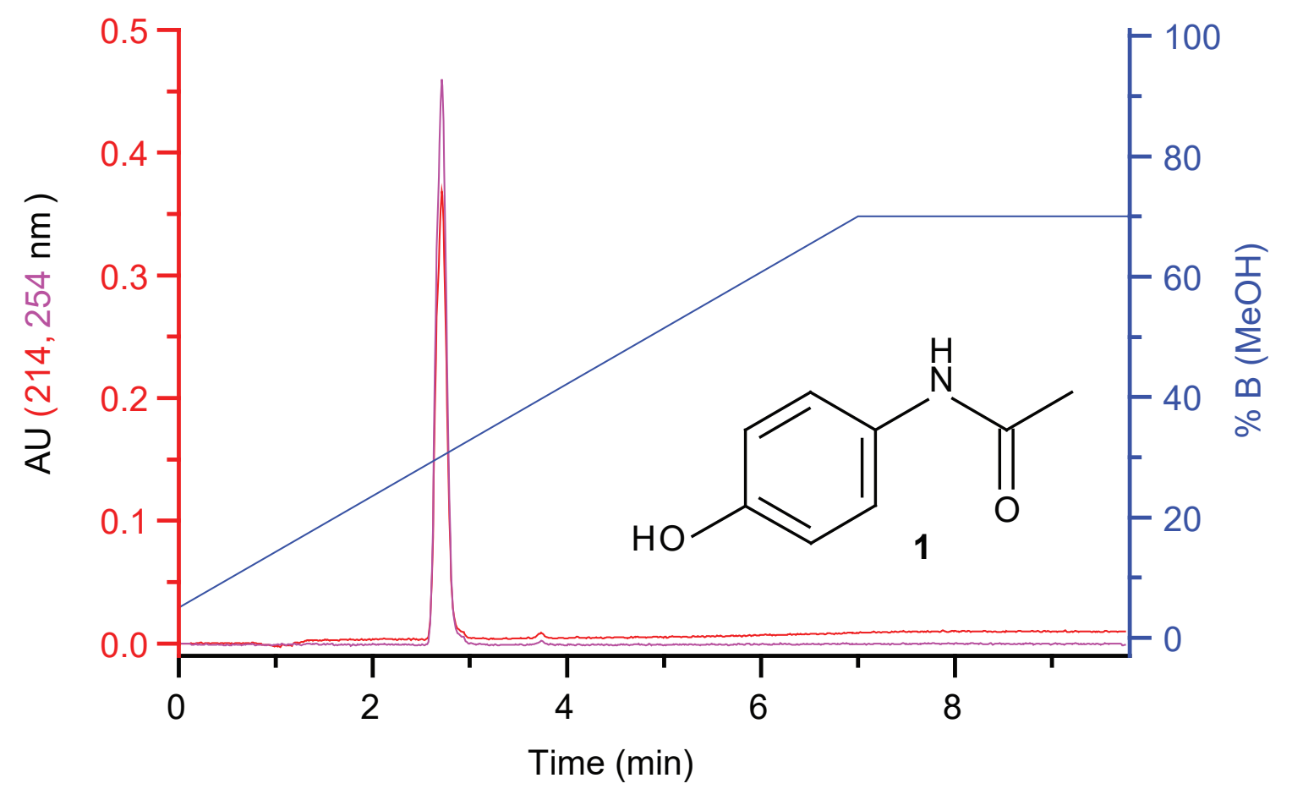
All preparative SFC and scouting runs used an ACCQ*Prep*® SFC and RediSep® Prep columns (20x150 mm) (both from Teledyne ISCO, Lincoln NE, USA). The column temperature was 40 °C, and the back pressure regulator (BPR) was set to 100 Bar. Scouting runs on the ACCQ*Prep* SFC employed the 20x150 mm columns that were then used for the preparative focused or isocratic gradient. Scouting gradients were run from 5 to 70% co-solvent. The flow rate was 60 mL/min, and the column oven temperature was set to 40 °C.

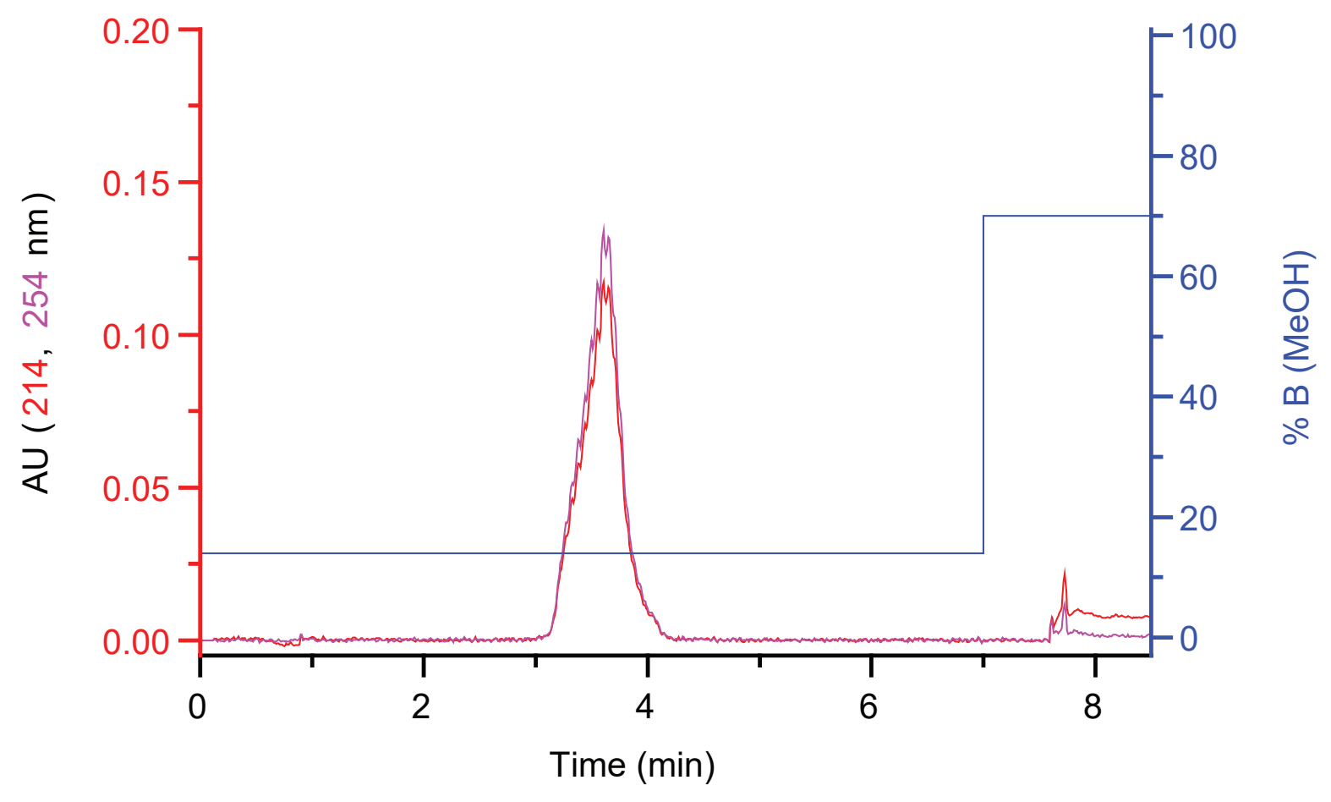


A model compound is run isocratically and the solvent composition is adjusted such that the compound elutes at the desired retention time. The solvent composition for this method is noted for the next step

The same model compound is run on a scouting gradient with a matching column in the same solvent system. The time required to elute the model is deemed to be the same solvent composition used for the isocratic elution. The Apparent Gradient Delay (D_a) is the time difference between the time the programmed gradient is at this solvent composition and the model elution time







The mixture to be purified is run using the same scouting gradient method and column size used for the calibration. Applying the D_a to the desired compound's elution time gives provides a solvent composition that can be used for an isocratic method or as the locus of a focused gradient. Acetaminophen is a compound commonly used to evaluate substitute solvents for dichloromethane. The scouting run allows calculation of a focused gradient.

- As long as the columns used for scouting are the same size, using the game gradient method, different types of columns can share the same calibration, allowing for column screening.
- Using the same gradient method for each solvent, different solvents and solvent additives can be tested.
- Table 1 contains the results of solvent and column screens and suggest the workflows below:

General workflow (all columns– solvent strength is a weaker solvent for each step):

1. Verify sample dissolves in co-solvent
2. Try methanol gradient (or weaker solvent if compound fails to dissolve in methanol)
3. If the compound elutes too early in #1, try 2-propanol
4. If the compound elutes too early in #2, try ethyl acetate

Silica column scouting workflow:

1. Basic compounds: Try isopropyl amine, triethyl amine, or another basic additive
2. Acidic compounds–add formic acid to the co-solvent
3. Neutral compounds: use co-solvent with no additives

Column scouting gradient screening workflow:

Column screening is useful for compounds that stick on silica and for avoiding additives.

1. Silica (may need additives)
2. Diol (may need additives)
3. PEI or diamine (no additive–basic column)
4. 4-ethyl pyridine

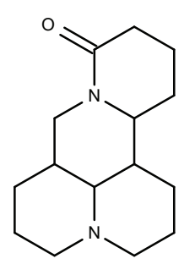
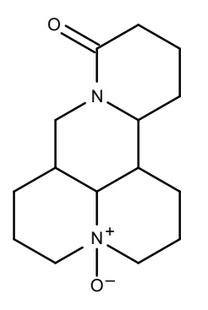
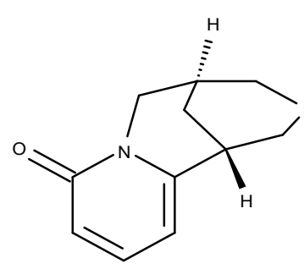
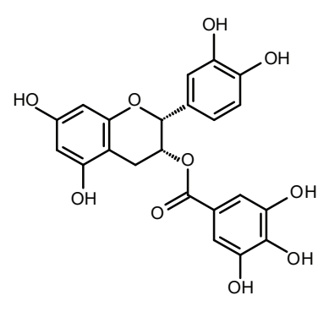
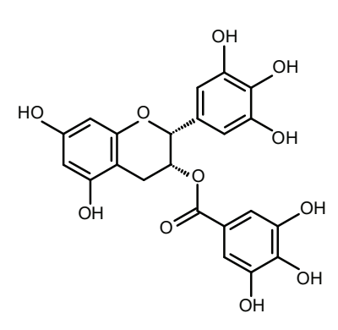
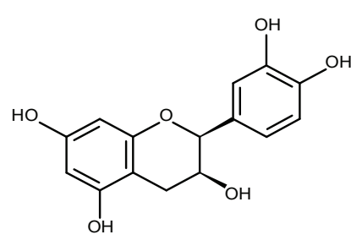
Compound	SFC Column Focus gradient range	Solvent	Chlorinated solvent used in original purification	Structure
Matrine	Silica Broad peak in scout run	Methanol, no additive	Dichloromethane with methanol	
	Diol Broad peak in scout run	Methanol, no additive		
	4-ethylpyridine 18.1–28.1	Methanol, no additive		
	PEI 26.6–36.6	Methanol, no additive		
	Silica 30.7–40.7	Methanol 0.5% isopropyl amine		
Oxymatrine	Silica No elution	Methanol	Dichloromethane with methanol	
	Diol Late elution, severe tailing	Methanol		
	4-ethylpyridine 34.3–44.3	Methanol		
	PEI Some tailing	Methanol		
	Silica 58.0–68.0	Methanol 0.5% isopropyl amine		
Cytisine	Silica–very broad peak	Methanol	dichloromethane with methanol	
	Diol Broad peak	Methanol		
	4-ethyl pyridine 17.5–27.5	Methanol		
	PEI 26.7–36.7	Methanol		
	Silica 28.4–38.4	Methanol 0.5% isopropyl amine		
(-)-Epicatechin 3-gallate	Silica 24.7–34.7	Methanol	Dichloromethane with methanol	
	Diol Peak tailed badly	Methanol		
(–)-Epigallocatechin 3-gallate	Silica Wide, tailing peak in scout	Methanol	Dichloromethane with methanol	
	Diol 38.4–48.4	Methanol		
	Silica 25.8–38.8	Methanol 0.5% formic acid		
Epicatechin	Silica 19.0–29.0	Methanol	This is found with other catechins and was run to demonstrate it could be resolved.	
	Silica 18.2–28.2	Methanol 0.5% formic acid		

Table 1— compounds purified via column chromatography using chlorinated solvents and run on SFC. Compounds showing acceptable peak shape for a column and co-solvent have a focused gradient range listed.

Conclusion

SFC is a useful way to purify compounds from Chinese Traditional Medicine and other natural products. As these are also small molecules, the methods described herein also apply to these compounds as well. All of the compounds were purified without chlorinated solvents, although the original liquid chromatography methods required these solvents. In addition to showing that chlorinated solvents aren't required to purify these compounds, the study also suggested two potential workflows; one uses only silica columns and the other makes use of a column screen. It is interesting that all compounds elute well from silica gel which suggests that silica can play the same role for SFC that C18 plays for reverse phase chromatography. Silica won't be able to purify all compounds within SFC but reverse phase chromatography also occasionally requires columns other than C18. Likewise, solvents such as toluene may be needed on occasion, particularly for aromatic compounds.

---

[1] Vasquez, K. C&EN, 2024; 102 (21); 22–27 July 15, 2024

[2] Silver, J. Overview of Analytical-to-Preparative Liquid Chromatography Method Development. *ACS Comb Sci* **2019**, *21* (9), 609-613. DOI: 10.1021/acscombsci.8b00187

Do you want a
copy of this poster
or additional
information?

SCAN ME

August 2025 GS Italy