

# Can you Purify Natural Products with SFC?

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## Abstract

Supercritical fluid chromatography (SFC) has long been associated with chiral purification. However, it is moving into mainstream purification for achiral purification as well. Modern preparative SFC systems allow this technique to be run as easily as HPLC. All major classes of natural products have been purified with SFC. Below, an example purification is shown from initial extraction to purified compound.

## Background

### Why SFC?

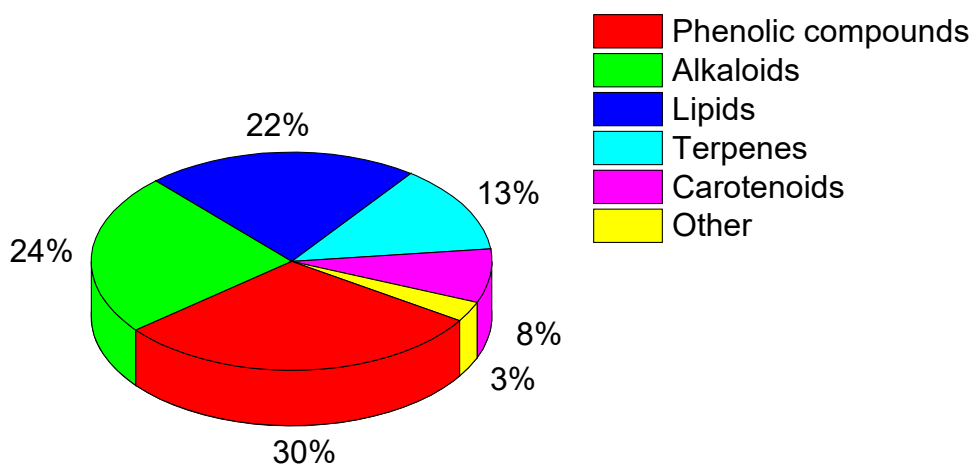
Supercritical fluid chromatography is a powerful tool for achiral purification in general, including natural products purification. SFC is a normal phase technique. Like hydrocarbons, carbon dioxide is non-polar and is the “weak” solvent. The “strong” solvent can be ethyl acetate, ethanol, or 2-propanol. Unlike hydrocarbons, solvents such as acetonitrile and methanol can be used as the cosolvent when using carbon dioxide, expanding the polarity range of potential eluting solvents. As of 2017, nearly all the major natural product classes were purified with SFC.<sup>1</sup>

Although now considered to be normal phase chromatography, SFC was originally considered an offshoot of gas chromatography, as “high pressure gas chromatography.” This different origin means that SFC terminology is slightly different from that used for regular chromatography. The “strong” or “B” solvent in SFC is termed “cosolvent,” while modifiers such as trifluoroacetic acid are called “additives.”

### Advantages

Some of the advantages of SFC over liquid chromatography:

- Less toxic—carbon dioxide is less toxic than typical organic solvents, while common cosolvents are low-toxicity alcohols.
- Less flammable—carbon dioxide is used to extinguish fires.
- Abundant—carbon dioxide is available as a byproduct of many industrial processes.

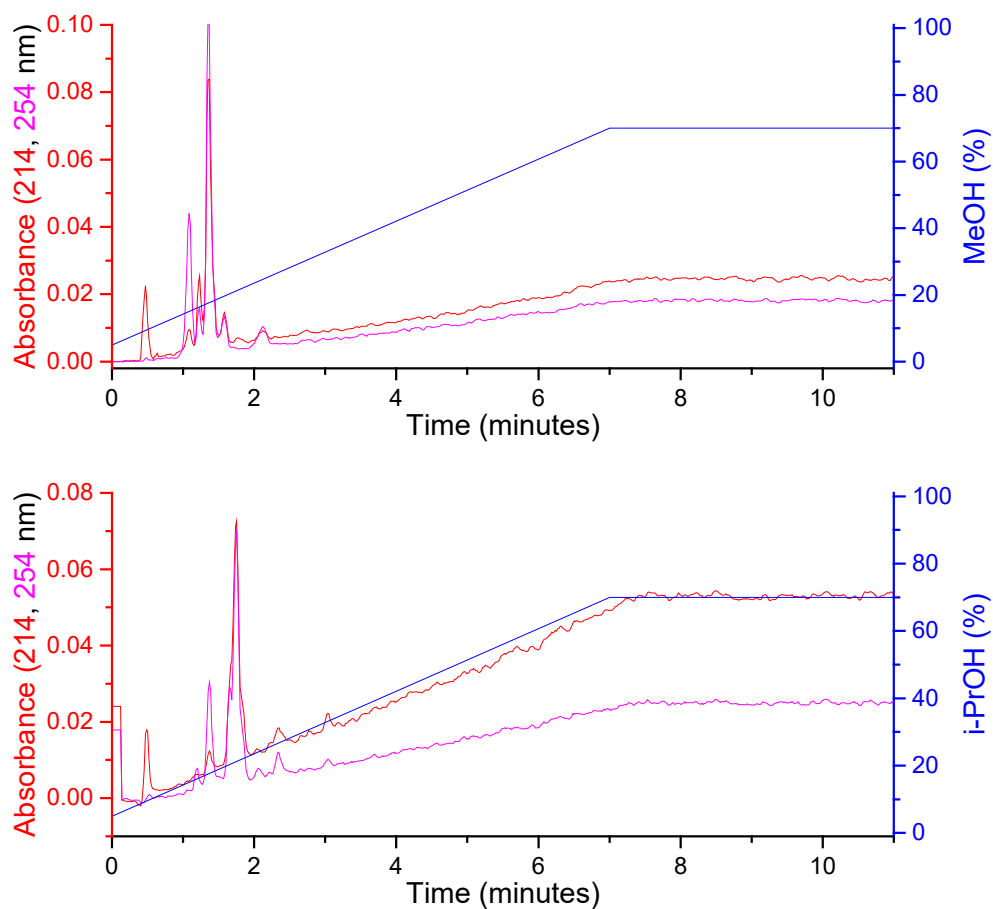


**Figure 1.** Classes of natural products purified by SFC.

## Purification of piperine

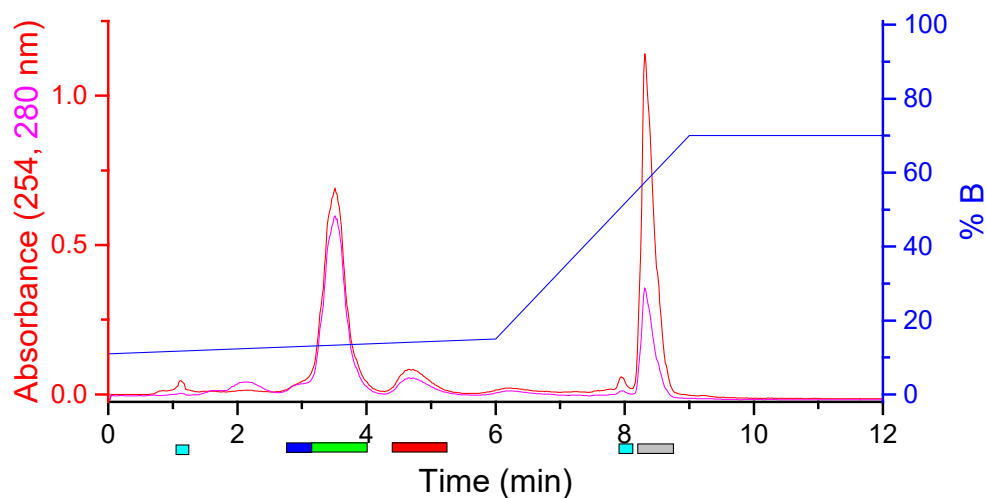
Piperine was used as an example from initial extraction from seeds through the final purification. Piperine was extracted from peppercorns, followed by extraction from dichloromethane into acidic water. This extract was adjusted to pH 10, and the alkaloids extracted into dichloromethane. The dichloromethane extract was purified with SFC.

A small amount of the extract was run with calibrated scouting runs for the Time-on-Target preparative gradient algorithm<sup>®</sup> with RediSep<sup>®</sup> silica columns (PN 692203826). The cosolvents tested were methanol and 2-propanol.



**Figure 2.** Scouting gradients for pure piperine in methanol (top) and 2-propanol (bottom).

The 2-propanol scouting run showed better resolution, so this solvent was used in the calculated gradient.



**Figure 3.** Piperine purified using a focused gradient calculated from the 2-propanol scouting run in Figure 2. Piperine eluted at ~3.5 minutes. Colored bars indicate collected fractions.

## Conclusion

Purification of natural products is easily done with SFC. There are a variety of normal phase columns and solvents allowing a variety of selectivities to better resolve compounds. In this case, sufficient resolution was achieved by changing from methanol to ethanol. The ACCQPrep® SFC PeakTrak™ software made purification as simple as it is for flash chromatography, while the fractions were contained in an inexpensive solvent that was easily evaporated compared to water in reverse phase chromatography.

## Notes

- Gibitz Eisath, N.; Sturm, S.; Stuppner, H. Supercritical Fluid Chromatography in Natural Product Analysis — an Update. *Planta Medica* **2017**, 84 (06/07), 361-371. <https://doi.org/10.1055/s-0037-1599461>.
- Silver, J. Overview of Analytical-to-Preparative Liquid Chromatography Method Development. *ACS Comb. Sci.* **2019**, 21 (9), 609-613. <https://doi.org/10.1021/acscombsci.8b00187>.