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Applikationsbericht

Extraction and Isolation of a Natural Product from Schisandra Berry Extract Using SFE and SFC

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Abstract

In this study, we focus on the feasibility of using the Prep 100q SFC System as a tool for isolating schisandrin A from dry schisandra berries extracted using supercritical fluid extraction (SFE).

Benefits

The Prep 100q SFC System is a highly reliable mass-directed preparative supercritical fluid chromatography instrument, suitable for compound isolation from natural product extracts.

Introduction

Berries of schisandra (*Schisandra chinensis*) have been widely used for medicinal purposes in traditional Chinese medicines for centuries. The berries contain a wide variety of organic compounds with dibenzo[a,c]cyclooctadiene lignans being of primary interest to many researchers. In this study, we focus on the feasibility of using the Prep 100q SFC System as a tool for isolating schisandrin A (CAS 61281-38-7, also known as deoxyschisandrin or wuweizisu A, Figure 1) from dry schisandra berries extracted using supercritical fluid extraction (SFE). Principles, techniques, and tools outlined here are applicable to the isolation of any compound from natural product samples.

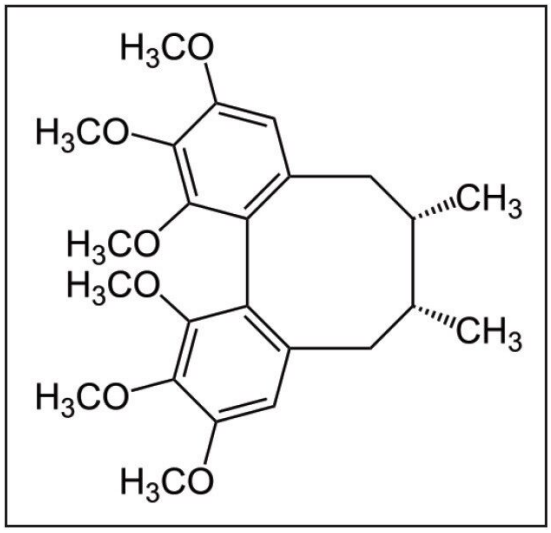

Figure 1. Chemical structure of Schisandrin A (Formula Wt. 416.5).

Experimental

Sample description and extraction

Fifty grams of dry, coarsely chopped Schisandra berries (StarWest Botanicals, Sacramento, CA, USA) were extracted for 60 minutes using an SFE 500 supercritical fluid extraction system fitted with a 100 mL extraction vessel. Extractions were performed dynamically at a total flow of 50 g/minute with an extraction pressure of 200 bar using a mixture of 99% CO_2 and 1% isopropyl alcohol. Extraction temperature was set to 40 °C. This extraction liberated approximately 30 mL of a dark yellow solution which was used in all subsequent experiments. For this kind of qualitative work, SFE provides a concentrated extract that contains no particulate matter. This removes the need for filtration, evaporation or other post extraction preparation which can greatly improve the overall workflow speed. No attempt was made to optimize the extraction conditions as it had been previously shown that with Schisandra, there appears to be little effect on extraction yield with changes of temperature or pressure.[1] The same authors also compared SFE of Schisandra to conventional solvent extractions (methanol, chloroform/methanol, hexane, and petroleum ether) and showed that there was little benefit to traditional solvent extractions compared to SFE.

SFC conditions

Preparative SFC system:	Prep 100q SFC System with an ACQUITY QDa Mass Detector
Analytical SFC system:	ACQUITY UPC^2 System with an ACQUITY QDa Mass Detector
Analytical column:	ACQUITY UPC^2 BEH Column, 130Å, 3.5 µm, 3 mm x 100 mm, Part Number 186006640
Preparative column:	Viridis BEH OBD Prep Column, 130Å, 5 µm, 19 mm x 150 mm, Part Number 186005733
Mobile phase A:	CO_2
Mobile phase B:	Acetonitrile/methanol 1
Gradient:	1 to 10% B over 5 min
Column temp.:	40 °C
Injection vol.:	2 µL analytical 120 µL prep
Flow rate:	2.5 mL/min analytical, 100 mL/min prep
ABPR:	1600 psi (110 bar) analytical, 1450 psi (100 bar)prep
Detection:	UV at 220 nm

ACQUITY QDa Detector conditions

Scan range:	150 – 600 *m/z*
Ionization:	ESi+

Data type:	Centroid
Cone voltage:	10 V
Sampling rate:	2 Hz
Probe temp.:	600 °C
ESI capillary voltage:	1.5 kV

Data management

Preparative SFC (Prep 100q SFC System) – MassLynx/FractionLynx

Analytical SFC (ACQUITY UPC^2 System) – Empower 3

SFE 500 – ChromScope v1.5

Results and Discussion

Although there are at least forty lignans of *Schisandra chinensis*,[2] Schisandrin A is abundant and its usefulness in medicinal extracts arises from its antiviral and anti-inflammatory effects.[3] Analytical chromatography was developed using the ACQUITY UPC^2 System on the crude Schisandra SFE extract (Figures 2 and 3) and showed a large peak at approximately 1.9 minutes. ACQUITY UPC^2-QDa data confirmed that this peak had an *m/z* of 417.3, consistent with that of Schisandrin A (M+H) and was the most abundant compound present. Schisandrin A is well resolved from neighboring peaks in the crude extract using a simple 1 to 10% gradient. The analytical separation was scaled to the Prep 100q SFC System using techniques previously described.[4]

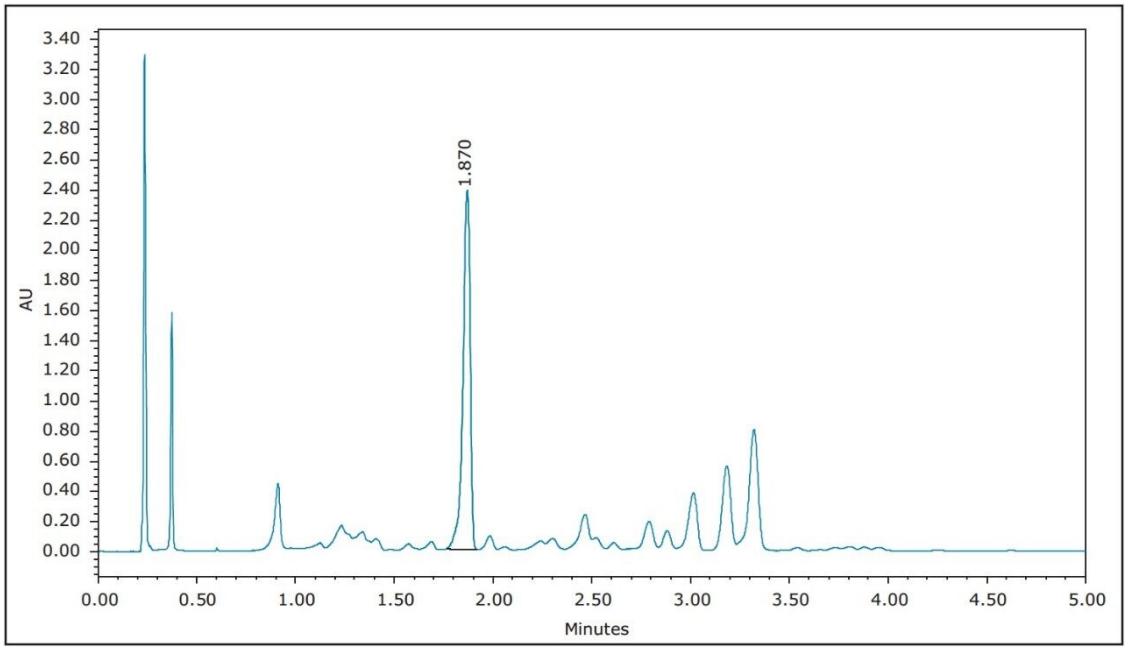

Figure 2. ACQUITY UPC^2 separation of Schisandra SFE extract. 2.5 mL/minute, 1%–10% gradient over 5 minutes, 1600 psi, 40 °C, 2 µL injection, UV at 220 nm.

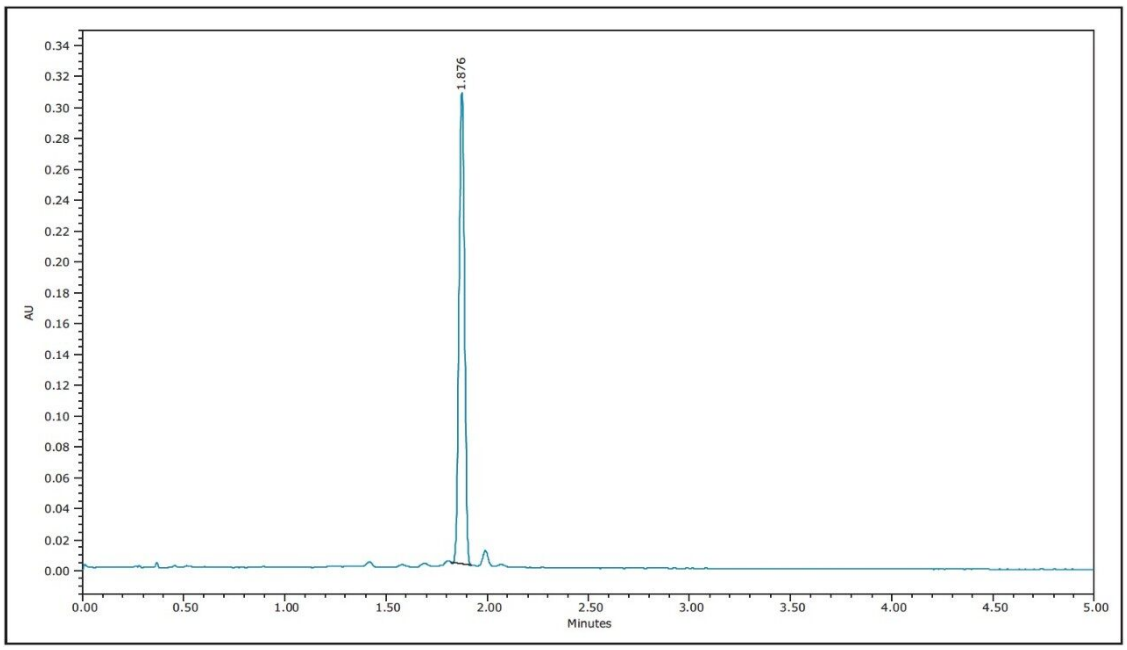

Figure 3. ACQUITY UPC^2 separation of Schisandra SFE extract. 2.5 mL/minute, 1%–10% gradient over 5 min, 1600 psi, 40 °C, 2 µL injection, total ion chromatogram 150–600 m/z. Inset, mass spectrum of peak at 1.9 min.

Schisandrin A was collected from the preparative runs by mass triggering, in this case *m/z* 417 (Figure 4). The collected fraction was removed, concentrated and analyzed using the previously described ACQUITY UPC^2 method. When using SFC as the purification tool, dry down or concentration of sample is faster than traditional HPLC as collections only contain organic solvent (residual co-solvent and make-up solvent, usually methanol). Analysis of the collected fraction showed significant improvement in purity moving from approximately 29% purity in the crude to just over 92% based on the UV 220 nm area counts (Figure 5).

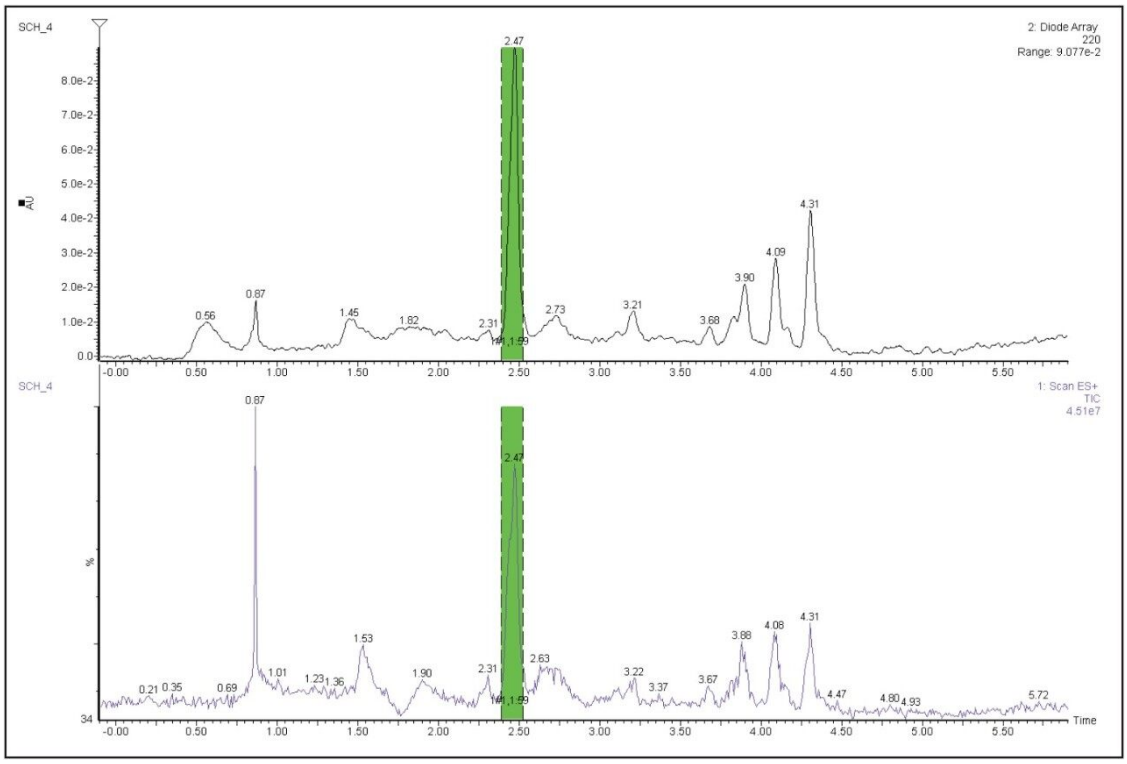

Figure 4. Prep 100q SFC System separation of Schisandra SFE extract. 100 mL/min, 1%–10% gradient over 5 min, 100 bar, 40 °C, 120 µL injection, Top – PDA at 220 nm, Bottom – ACQUITY QDa total ion chromatogram 150–600 m/z. Green bar indicates the area where collection occurred.

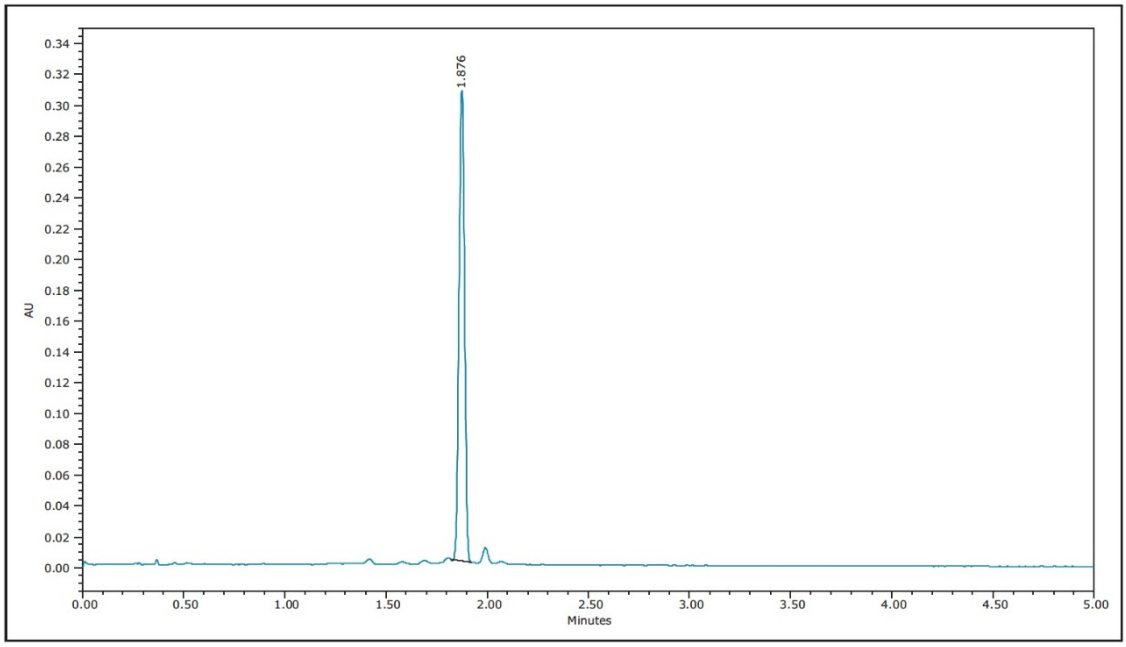

Figure 5. ACQUITY UPC^2 separation of collected fraction. 2.5 mL/min, 1%–10% gradient over 5 min, 1600 PSI, 40 °C, 0.5 µL injection, UV at 220 nm.

Conclusion

- Schisandra berries were successfully extracted using supercritical fluid extraction
- SFE extracts were analyzed using ACQUITY UPC^2 and a target compound (Schisandrin A) was selected for purification
- Purification of the SFE extract was achieved using a Prep 100q SFC System with collections triggered by mass
- A purified fraction was analyzed using ACQUITY UPC^2 and showed a significant increase in purity from 29% to 92%
- An extraction, analysis, purification, and reanalysis workflow using only supercritical fluid techniques was successfully demonstrated

References

1. Choi Y.H. et al. Optimum SFE Condition for Lignans of Schisandra chinensis Fruits. *Chromatographia* Vol. 48, No. 9/10, November 1998.
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4. Hudalla C.J. et al. UPC2 Strategy for Scaling SFC Methods: Applications for Preparative Chromatography. Waters Application Note, 2014, Part Number 720005064EN.

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