

Purification of Capsaicin from Habanero Peppers Using Centrifugal Partition Chromatography

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Capsaicin is the main component responsible for the spice, or “heat”, observed in several types of peppers. It is also found in many products such as pepper spray and in topical creams used to treat joint and muscle pain. Due to the many uses of capsaicin, it is advantageous to have methods for its purification on a preparatory scale. Presented here is a method that uses centrifugal partitioning chromatography to separate capsaicin from habanero pepper extract, and HPLC to analyze the purified fractions. Although this method focuses on the naturally occurring capsaicin in habanero peppers, it may also be used to purify the synthetically derived capsaicin found in commercial products.

Sample Preparation

Soak 1 habanero pepper inner membrane in 10 mL EtOH, 24 hrs.

Filter extract through 0.45 µm filter

Dry with N₂

Dissolve in 5 mL upper phase + 5 mL lower phase Arizona N

Prep: CPC Conditions

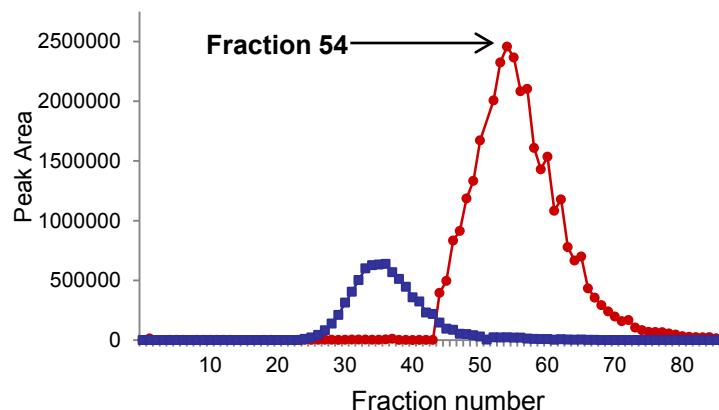
Instrument	Armen SpotPrep II/SCPC-250
Solvent System	Arizona N (1:1:1:1 Heptane/Ethyl Acetate/Methanol/Water)
Flow Rate	Elution: 8 mL/min. Extrusion: 30 mL/min.
Rotation Speed	1600 rpm
Mode	Ascending
Detection	280 nm
Fraction Volume	2 mL

Analytical: HPLC Chromaster Conditions

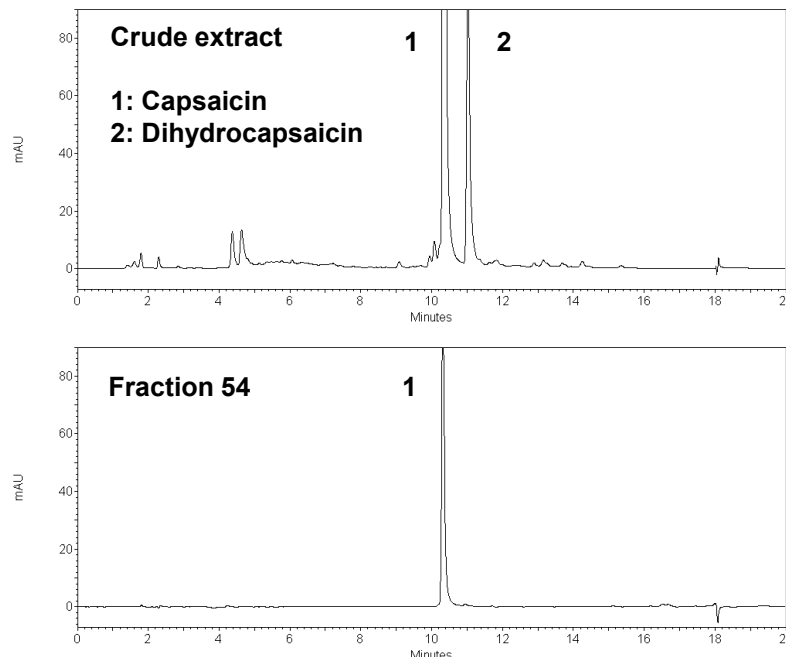
Pump (5110)	Mobile Phase A: (10/90) CH ₃ CN/H ₂ O Mobile Phase B: CH ₃ CN Flow Rate: 1 mL/min.
Autosampler (5210)	Injection Volume: 5 µL
Column Oven (5310)	30 °C
Detector (5430)	280 nm
Sample	Dry Fraction, redissolve in 85% CH ₃ OH

Results: Fraction Analysis

Blue = Dihydrocapsaicin, Red = Capsaicin



HPLC: Fraction Analysis



Discussion and Conclusion

The Armen Spot Prep II/CPC system is effective at separation of capsaicin from habanero pepper extract. Analysis of the fractions with the Hitachi Chromaster HPLC shows that high purity fractions can be attained. The fraction with the highest amount of capsaicin, Fraction 54, was 99% pure capsaicin (measured as % peak area at 280 nm).

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