SCPC-250: Purification of [6]-Gingerol



TECHNICAL NOTE TN207

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INTRODUCTION

Ginger, isolated from rhizome of *Zingiber officinale* Roscoe (Figure 1) is widely known for its use in flavoring foods and beverages, but it is also commonly used for medicinal, perfumery, and esthetic purposes as well. Natural products from ginger have been shown to exhibit several beneficial bioactivities, including chemopreventative, anti-inflammatory, antioxidant and anti-emetic.¹ The two main components contributing to the pungent fragrance and flavor exhibited by fresh ginger are gingerols and shogaols, with [6]-gingerol being the major phenol providing ginger's pungent characteristics (Figure 2). [6]-Gingerol is normally obtained as a pungent yellow oil and can even be found in a crystalline form under the right conditions.

This technical note highlights the ability of a centrifugal partition chromatography (CPC) system (Gilson SCPC-250) to obtain high purity fractions of an active compound from a small amount of crude extract using five times less solvent consumption than flash chromatography and prep HPLC. The Gilson SCPC-250 (Figure 3) was used to purify [6]-gingerol from a crude extract.

MATERIALS AND METHODS

Sample

Three separate analyses were performed using 0.5, 1, and 2 grams of crude extract of *Z. officinale* Roscoe rhizome.

Apparatus

A Gilson SCPC-250 was connected to a Gilson PLC 2050 Purification System equipped with a 50 mL/min quaternary gradient pump, UV/Vis detector, and a fraction collector. The purification analyses were performed using Gilson Glider CPC software to control the CPC with the PLC 2050 Purification System.

All HPLC work was performed using the LaChrom Elite HPLC system (VWR) equipped with a photodiode array detector (PDA) (200–800 nm).



Figure 1 Ginger (*Zingiber officinale Roscoe*) rhizome

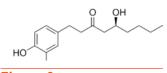


Figure 2 Structure of Gingerol



Figure 3 Gilson SCPC-250 Connected to a Gilson PLC 2050 Purification System



Analytical HPLC Method

The crude extract was first analyzed by HPLC under the conditions listed in Table 1. [6]-Gingerol is identified at RT=11.17 minutes with a peak area of 19.6% (Figure 3).

Table 1:

Analytical HPLC Method Conditions

HPLC Column	Purosphere RP18 (250*4.6 mm, 5 μm)			
Mobile Phase A	Water			
Mobile Phase B	Acetonitrile			
Gradient	0 min: 40% B 0-55 min: 40% B to 95% B 55-65 min: 95% B 65-70 min: 95% B to 40% B 70-80 min: 40% B			
Flow Rate	1 mL/min			
Injection Volume	2 μL			
Temperature	40°C			
Detection	210 nm			

CPC Method

The CPC solvent system used was determined by the shake flask method in order to obtain a K_a value near 1.

K_d = ~1 = <u>[HPLC peak area of gingerol]</u>_{stat} [HPLC peak area of gingerol]_{mobile}

Once the CPC solvent system was determined, three CPC analyses were performed on the Gilson SCPC-250 using 0.5, 1, and 2 g of crude extract. The crude extract sample was dissolved in 5 mL of upper phase and 5 mL of lower phase, filtered through a 0.45 μ L membrane filter, and then injected in the Gilson SCPC-250 according to the conditions described in Table 2. The fractions obtained as a result of the CPC analysis were analyzed by HPLC and grouped according to HPLC purity.

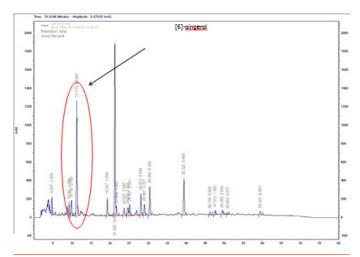


Figure 4

HPLC Analysis of Crude Extract

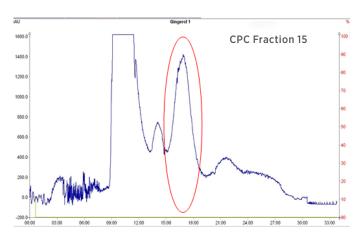


Figure 5

CPC chromatogram of 500 mg injection

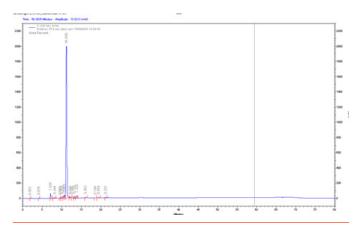


Figure 6 HPLC Analysis of CPC Fraction 15

Table 2

CPC Method Conditions

Column	Gilson SCPC-250
Column Volume	250 mL
Elution Flow Rate	10 mL/min
Extrusion Flow Rate	30 mL/min
Rotation Speed	2000 rpm
Solvent System	Hept/AcOEt/MeOH/W
Mode	Ascending
Samples	0.5 g crude extract in 5 mL lower + 5 mL upper 1 g crude extract in 5 mL lower + 5 mL upper 2 g crude extract in 5 mL lower + 5 mL upper
Detection	210 nm

RESULTS

ITERATION	EXTRACT INJECTED	RUN TIME	SOLVENT CONSUMPTION	[6]-GINGEROL RECOVERED	YIELD	HPLC PURITY
1	0.5 g	30 min	n 600 mL	31 mg	6.2%	90%
2	1 g			69 mg	6.8%	96%
3	2 g			87 mg	4.5%	92%

CONCLUSIONS

The combination of the Gilson SCPC-250 with the Gilson PLC 2050 Purification System allows for quick identification and separation of [6]-gingerol from a crude extract. Injections ranging from 0.5–2 g of crude extract were performed to evaluate the change in the amount of [6]-gingerol recovered. The injection of 2 g of crude extract did not significantly decrease the recovery of [6]-gingerol compared to the injection of 0.5 g. CPC provides a rapid one-step method for purification of natural products with minimal solvent consumption.

REFERENCES

1. Khodaie, L. and Sadeghpoor, O. (2015) Ginger From Ancient Times to the New Outlook. Jundishapur *Journal of Natural Pharmaceutical Products*, 10(1), e18402.

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