

# Purification of [6]-Gingerol from Ginger by Centrifugal Partition Chromatography (CPC) for Flavoring Industries



## APPLICATION NOTE AN1037

### APPLICATION BENEFITS

- Single step, quick production of highly pure [6]-gingerol standard
- Optimisation of the quantity injected for optimum recovery
- No loss or denaturation of the molecule
- Cost efficient method

### ADDRESSED ISSUES

- Natural product purification from complex extracts is always challenging
- Difficult to have a selective extraction process

G. AUDO AND C. LE QUEMENER | Gilson Purification Applications Laboratory  
S. HOLDERITH AND Y. MAESTRO | Chanel Parfums Beauté

### INTRODUCTION

Ginger, isolated from the 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. Natural products from ginger have been shown to exhibit several beneficial bioactivities, including chemo-preventative, anti-inflammatory, antioxidant, and anti-emetic.<sup>1</sup> 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, but can also be found in a crystalline form under the right conditions.

This study shows how centrifugal partition chromatography (CPC) can produce a high-quality standard of [6]-gingerol without the use of silica-based chromatography methods, such as FLASH and prep HPLC.

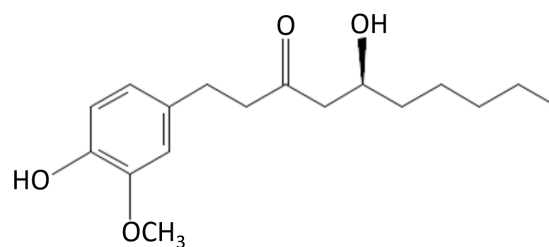
### MATERIALS AND METHODS

#### Sample

Three separate samples of 0.5, 1, and 2 g of crude extract of *Z. officinale roscoe* rhizome diluted in 5 mL of upper phase and 5 mL of lower phase of the CPC solvent system were prepared. Each sample was filtered through a 0.45  $\mu\text{m}$  membrane filter.



**Figure 1**  
Ginger (*Zingiber officinale roscoe*) rhizome



**Figure 2**  
Structure of [6]-Gingerol

## Apparatus

A Gilson CPC 250 connected to a PLC 2050 Purification System (Compact LC system) configured with a 50 mL/min quaternary gradient pump, UV/VIS detector, fraction collector, and Gilson Glider CPC control software was used for the purification step (Figure 3).

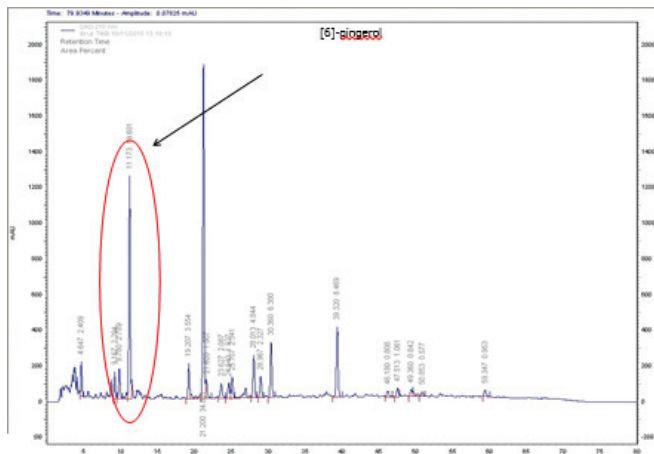


**Figure 3**

Gilson CPC 250 connected to a PLC 2050 Purification System (Compact LC 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% at 210 nm (Figure 4).



**Figure 4**

HPLC Analysis of Crude Extract

## CPC Method

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

$$K_d = -1 = \frac{[\text{HPLC peak area of gingerol}]_{\text{stat}}}{[\text{HPLC peak area of gingerol}]_{\text{mobile}}}$$

Once the CPC solvent system was determined, three CPC runs were performed on the CPC 250 using

0.5, 1, and 2 g of crude extract. Each sample as injected into the CPC 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.

**Table 1**

Analytical HPLC Method Conditions

HPLC Column	Purosphere RP18 (250*4.6 mm, 5 $\mu$ m)
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 $\mu$ L
Temperature	40°C
Detection	210 nm

**Table 2**

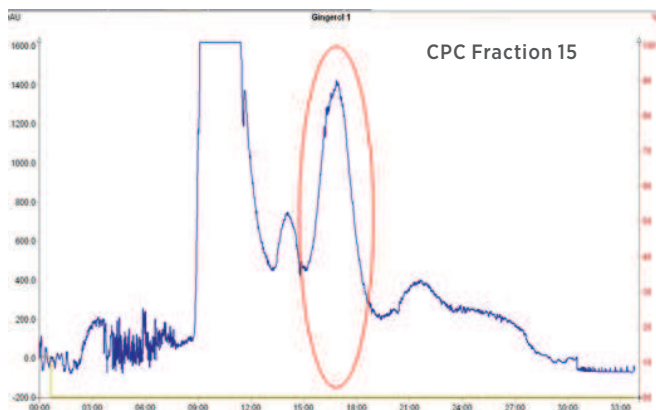
CPC Method Conditions

Column	VERITY CPC 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

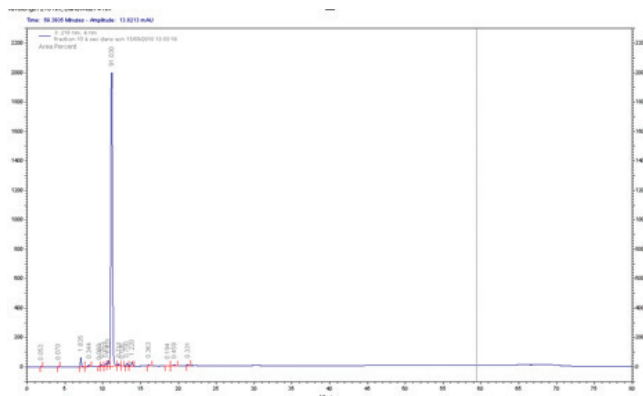
**Table 3**

CPC Purification Results

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

**Figure 5**

CPC chromatogram of 500 mg injection

**Figure 6**

HPLC Analysis of CPC Fraction 15

## RESULTS AND DISCUSSION

Figure 5 presents the CPC chromatogram of the 0.5 g injection. The peak between 15 to 18 min corresponding to fraction 15 was dried under vacuum and further analyzed by HPLC (Figure 6). This analysis shows a unique peak at Rt 11 min corresponding to [6]-Gingerol. 31 mg of [6]-Gingerol with an HPLC purity at 210 nm of 90% as obtained.

In Table 3, 1 g injection shows equivalent purity and recovery than the 0.5 g injection. 2 g injections shows a decrease of [6]-Gingerol recovery.

## CONCLUSIONS

The combination of the Gilson CPC 250 with the PLC 2050 purification system allows for quick production of highly pure [6]-Gingerol standard directly from a crude rhizome extract. Injections range from 0.5-2 g, with 0.5 and 1g showing similar results in terms of recovery and purity and 2 g injection showing a slight decrease of recovery.

Up to 87 mg of pure [6]-Gingerol were obtained with one step.

By using a larger scale CPC system such as the VERITY CPC Process, this feasibility study could be easily scaled up for multi kg to tons production per year.

## REFERENCES

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

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