# CPC 250:
# Purification of [6]-Gingerol from Ginger

## 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, but can also be found in a crystalline form under the right conditions.

This technical note highlights the ability of a centrifugal partition chromatography (CPC) system (Gilson CPC 250) to obtain high purity fractions of an active compound from a complex crude extract using five times less solvent than flash chromatography and prep HPLC.

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

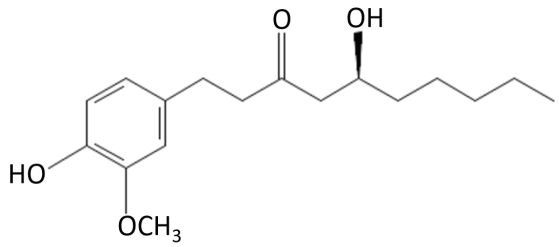


**Figure 2**
Structure of Gingerol

### MATERIALS AND METHODS

#### Sample

Three separate preparative runs were performed using 0.5, 1, and 2 grams of crude extract of *Z. officinale roscoe* rhizome.

#### Apparatus

A Gilson CPC 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 runs were performed using Gilson Glider CPC software to control the CPC with the PLC 2050 Purification System (Figure 3).

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

**Figure 3**
CPC 250 Connected to a
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% at 210 nm (Figure 4).

**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<br>0-55 min: 40% B to 95% B<br>55-65 min: 95% B<br>65-70 min: 95% B to 40% B<br>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 $K_d$ value near 1.

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

Once the CPC solvent system was determined, three CPC runs 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 µL membrane filter, and then injected in 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.

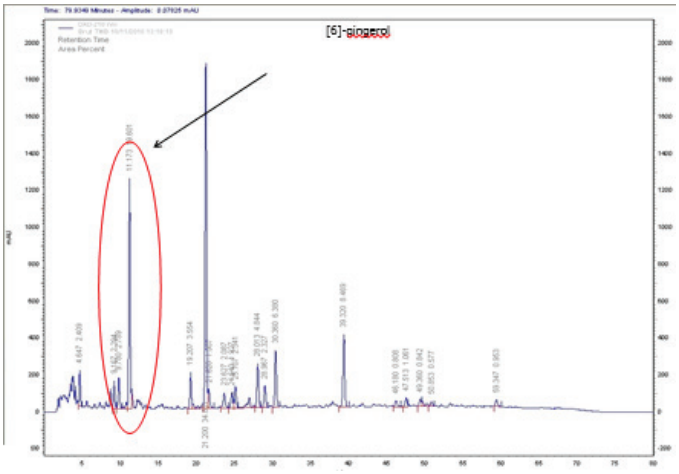


**Figure 4**
HPLC Analysis of Crude Extract

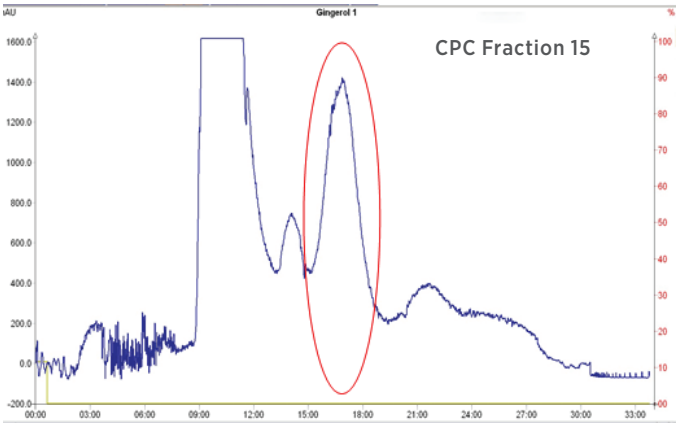


**Figure 5**
CPC chromatogram of 500 mg injection

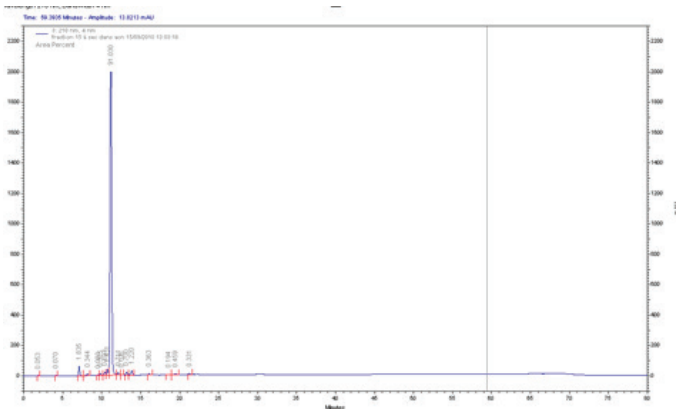

**Figure 6**
HPLC Analysis of CPC Fraction 15

**Table 2**
CPC Method Conditions

| | |
|---|---|
| Column | 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<br>1 g crude extract in 5 mL lower + 5 mL upper<br>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 | 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 CPC 250 with the 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.