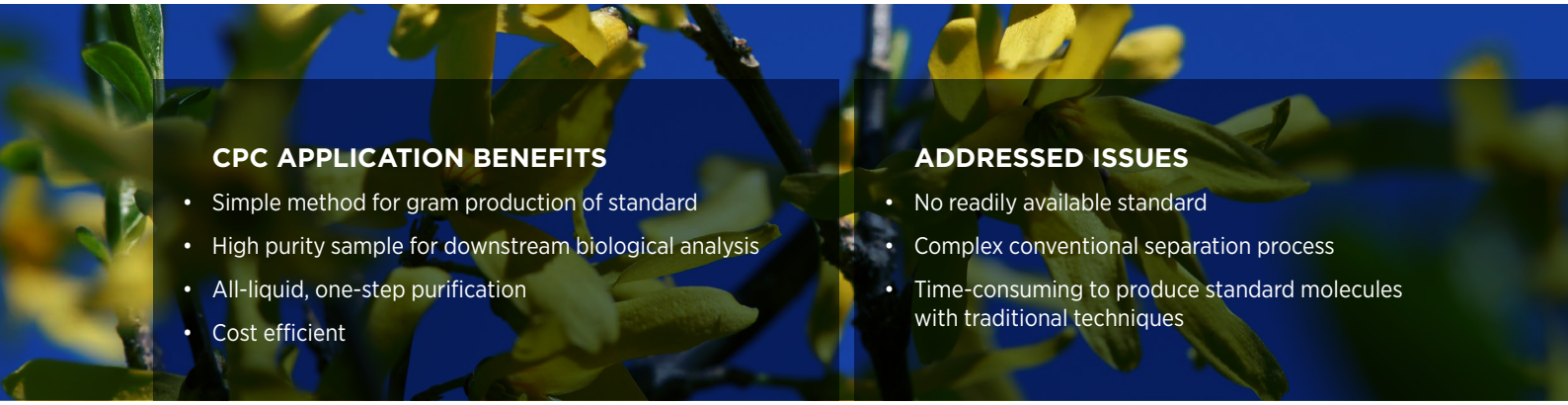
PURIFICATION OF FORSYTHIASIDE A FROM *FORSYTHIA SUSPENSA* BY CPC

APPLICATION NOTE AN1044



CPC APPLICATION BENEFITS

- Simple method for gram production of standard
- High purity sample for downstream biological analysis
- All-liquid, one-step purification
- Cost efficient

ADDRESSED ISSUES

- No readily available standard
- Complex conventional separation process
- Time-consuming to produce standard molecules with traditional techniques

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INTRODUCTION

Forsythia suspensa (Thunb.) Vahl. has been widely used in traditional medicines in China, Korea, and Japan to treat gonorrhea, erysipelas, inflammation, pyrexia, ulcer, and other diseases.[1] Forsythiaside A (Figure 1), a phenylethanoid glycoside, is one of the major active constituents extracted from the air-dried fruits of *Forsythia suspensa* (Figure 2) and has been reported to possess a wide range of pharmacological properties, including anti-inflammatory and antioxidant activities.[2]

Large quantities are needed as reference substances and in related research based on Forsythiaside A. Scientists have searched for a simple, yet effective method of isolation for this potent compound. In the past, open column chromatography was used but this incurred high time and solvent costs. Now however, the use of centrifugal partition chromatography (CPC) can minimize these costs.

CPC is a support-free, all liquid partition chromatography with no irreversible adsorption, high sample recovery, high sample loading capacity, and predictive scale up from lab to industrial scale.[3] This paper presents the utilization of CPC for the isolation of Forsythiaside A.

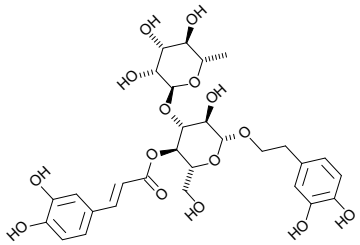

Figure 1
Structure of Forsythiaside A

Figure 2
Fruit of *Forsythia suspensa*

MATERIALS AND METHODS

Instrumentation

A Gilson VERITY® CPC Lab System consists of two components, a CPC 250 and a PLC 2050 Purification System including a 50 mL/min quaternary gradient pump, UV/VIS detector, fraction collector and Gilson Glider control software (Figure 3).

Preparation of crude extract

100% Methanol extract of the fruits of *Forsythia suspensa* was suspended in water. The aqueous solution was partitioned between methylene chloride, ethyl acetate (EtOAc), and water saturated *n*-butanol (*n*-BuOH), respectively. The *n*-BuOH fraction was freeze-dried and used for CPC purification.

CPC separation procedure

A CPC solvent system was chosen and tested on a laboratory scale according to the solvent system described by Yang M. *et. al*.[1] A mixture of EtOAc-*n*-BuOH-methanol-water (4:0.5:0.5:5, v/v) was shaken in a separating funnel and separated at room temperature. The upper phase was used as the stationary phase and the lower phase was used as the mobile phase. The butanol fraction of the fruits of *F. suspensa* (1st run 1 g; 2nd run 3 g; 3rd run 2.85 g) was dissolved in 4 mL of solvent mixture (2 mL upper phase and 2 mL lower phase of the solvent system) and was filtered with PTFE filter (hydrophobic; diameter: 13 mm, pore size: 0.45 µm). The 250 mL CPC column was filled with stationary phase at a flow rate of 30 mL/min and the rotation speed was maintained at 500 rpm. The lower phase was then pumped into the column at a flow rate of 8 mL/min and the rotation speed was set to 1800 rpm. After the hydrodynamic equilibrium was achieved, the sample (4 mL) was loaded into the injection valve. The effluent from the column was monitored at the wavelength of 254 nm, 280 nm and 330 nm and the fractions were collected according to the 330 nm wavelength absorption (Table 1).

Analysis of Fractions from CPC

Fractions collected from CPC were subjected to HPLC analysis with the HPLC conditions tabulated in Table 2.

Figure 3
CPC 250 connected to a PLC 2050 Purification System

Table 1
CPC Method Conditions

PARAMETERS	SETTINGS
Column	CPC 250
Column volume	250 mL
Elution flow rate	8 mL/min
Extrusion flow rate	30 mL/min
Rotation speed	1800 rpm
Solvent system	Ethyl acetate-*n*-butanol-methanol-water (4:0.5:0.5:5, v/v)
Mode	Descending
Detection	254, 280 and 330 nm

Table 2
Analytical HPLC Method Conditions

PARAMETERS	SETTINGS
HPLC column	YMC-Pack ODS-AQ (250 x 4.6 mm, 5µm)
Mobile phase A	Water with 0.1% formic acid
Mobile phase B	Methanol
Gradient	0 min: 25% B 0-10 min: 25% B to 35% B 10-35 min: 35% B 35-55 min: 35% B to 70% B 55-60 min: 70% B to 25% B 60-70 min: 25% B
Flow rate	2 mL/min
Injection volume	20 µL
Temperature	rt
Detection	254, 280 and 330 nm

RESULTS AND DISCUSSION

CPC injections

Once the CPC solvent system was determined, three CPC injections were performed on CPC 250 and PLC 2050 using 1 g, 3 g, and 2.85 g of samples. Figure 4 shows the chromatogram of the first run (1 g). Tabulated in Table 3, three combined fractions' yield, in which fractions were combined based on the Forsythiaside A peaks of the respective chromatograms. A total of 1.96 g of Forsythiaside A was obtained from the combined 6.85 g of crude *n*-butanol fraction, which corresponds to 28.61% of the total injection.

HPLC analysis of fractions from CPC

Figure 5 shows the HPLC analysis of fractions 11-16 obtained from batch 1, using the method described in Table 2. The figure shows fractions 11-16 were obtained with a purity of 94.56%.

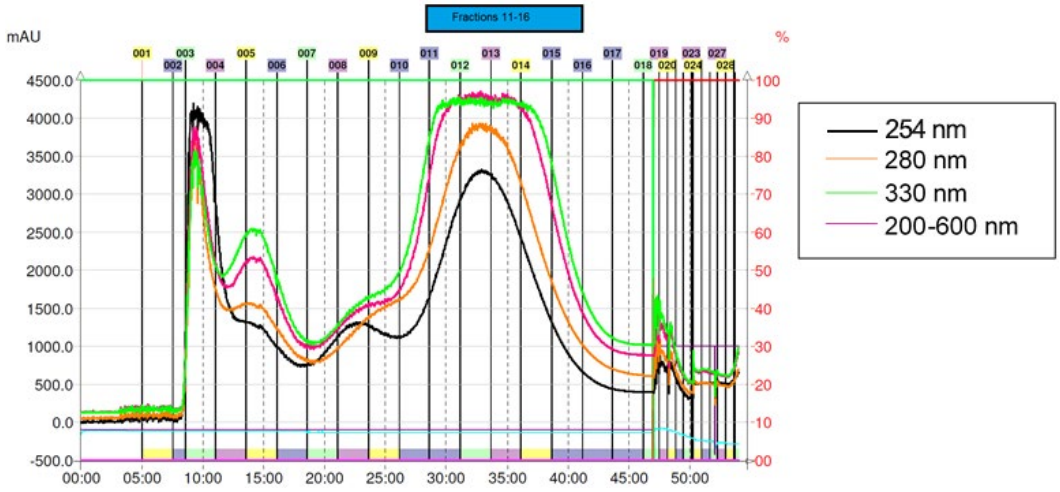

Figure 4

CPC chromatogram of the *n*-butanol fraction (1 g) of the fruits of *F. suspensa*. Fractions 11-16 were combined, dried and weighed.

Table 3

Yield of Forsythiaside A.

BATCH NO.	LOADING AMOUNT	COMBINED FRACTIONS	YIELD (G)	YIELD (%)
1	1.00	11-16	-	-
2	3.00	12-18	-	-
3	2.85	13-21	-	-
Total	6.85	-	1.96	28.61

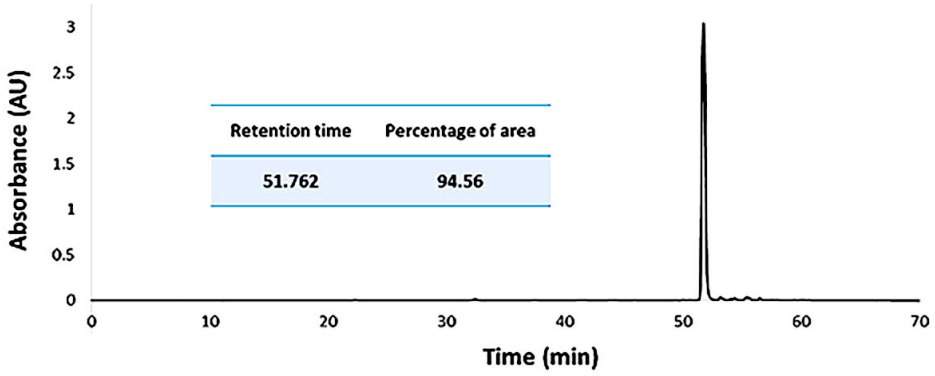

Figure 5

HPLC chromatogram of fractions 11-16 from CPC run batch 1.

CONCLUSIONS AND BENEFITS

A CPC method was successfully used for the isolation of Forsythiaside A from *Forsythia suspensa* extract through a one-step purification solution. With a total of 6.85 g of crude injected into CPC, 1.96 g of Forsythiaside A were obtained, with a yield of 28.61%. Compared to previous separation techniques, such as open column chromatography, CPC had demonstrated a large quantity of sample being injected into CPC 250, to be purified in a short period of time, while still retaining the desired purity, and by using less solvent (380 mL of lower mobile phase in elution for 1g of injection). The established method was simple, fast, and efficient to prepare pure compounds for related research such as bioassay-guided study, bioactivity, quality control, and pharmacology.

REFERENCES

1. Yang M., Xu X., Xie C., Huang J., Xie Z. and Yang D. Isolation and purification of forsythoside A and suspensaside A from *Forsythia suspensa* by high-speed counter-current chromatography. J. Liq. Chrom. Relat. Tech., 36, 2895-2904 (2013).
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3. Hostettman K., Marston A., Hostettman M. Preparative Chromatography Techniques: Applications in Natural Product Isolation, Springer, Berlin, 1998.