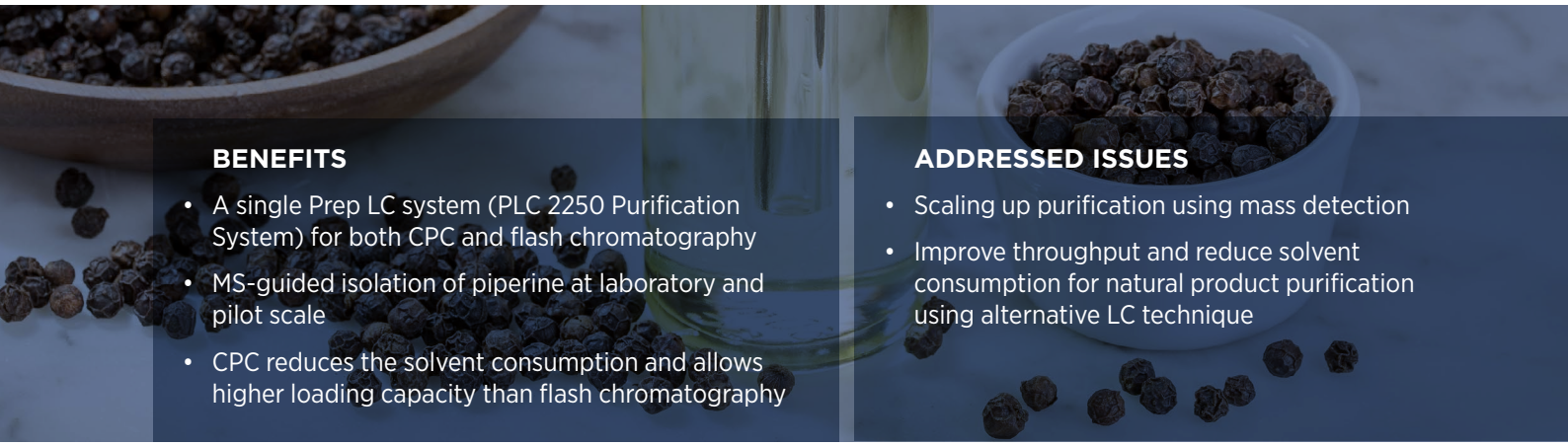
A COMPARATIVE STUDY BETWEEN CPC AND FLASH CHROMATOGRAPHY FOR NATURAL PRODUCT PURIFICATION

APPLICATION NOTE AN1048



BENEFITS

- A single Prep LC system (PLC 2250 Purification System) for both CPC and flash chromatography
- MS-guided isolation of piperine at laboratory and pilot scale
- CPC reduces the solvent consumption and allows higher loading capacity than flash chromatography

ADDRESSED ISSUES

- Scaling up purification using mass detection
- Improve throughput and reduce solvent consumption for natural product purification using alternative LC technique

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INTRODUCTION

Piperine, an alkaloid that gives black pepper its pungent taste (Piper nigrum), is also associated with several bioactivities and can be used as a spray in personal defense devices (Figure 1).[1] The preparative separation and purification of piperine is usually performed by conventional methods. Among them is centrifugal partition chromatography (CPC), which is a type of liquid-liquid chromatography using two immiscible liquid phases. The compounds undergoing separation are subjected to a continuous partition process between these two phases in a column space free of solid support.[2] In contrast, flash chromatography is a solid-liquid chromatography using relatively high flow rates with low pressure either by normal or reverse phase separation.[3] Flash chromatography is also considered a comparatively effective technique to achieve high yields using low solvent and raw material input.[4]

In this study, a crude extract of black pepper is used to purify piperine and to compare the performance of CPC versus flash chromatography. Piperine purity, separation yield, solvent consumption, and quantity injected are compared between both techniques. This study also underlines the capacity of the PLC 2250 to work with different types of chromatographic columns and detection systems (i.e., UV and MS detectors).

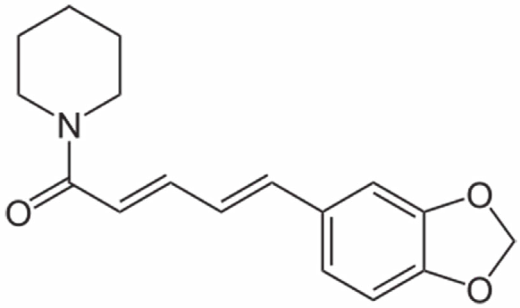

Figure 1

Structure of piperine

MATERIALS AND METHODS

Sample

Solid-liquid extraction of ground black pepper was carried out in dichloromethane (DCM) with a dry mass/solvent ratio of 1:5. Two extraction cycles of 3h each were necessary to recover as much piperine as possible. Both extracts were combined and concentrated to dryness.

Systems

CPC and flash chromatography columns were both connected to a single PLC 2250 (Figure 2). This compact system is equipped with both injection and back flush valves, a quaternary pump, a UV/Vis detector scanning wavelengths from 200 to 600 nm, and a fraction collector. An additional Gilson VERITY® 1920 Mass Spectrometer was connected to follow the piperine separation. The whole system was controlled by the Gilson GLIDER Software.

CPC Method

CPC separations were performed on a CPC 250 (column capacity 250 mL) and a CPC 1000 PRO (column capacity of 1000 mL). Piperine was purified in ascending mode using the elution-extrusion technique. Separation was realized using the Arizona P solvent system, which consists of heptane-ethyl acetate-methanol-water (6:5:6:5, v/v/v/v). Optimal elution parameters are described in Table 1 for both columns. Samples were first diluted in a mixture of upper and lower phases (50/50, v/v) and then warmed at 40°C for 20 min. The solutions were then ultrasonicated for 5 min and filtered (0.45 µm) before injection into the CPC system.

Figure 2

CPC 250, flash cartridge, and VERITY® 1920 Mass Spectrometer connected to a PLC 2250 Purification System

Table 1

Description of optimal CPC parameters used for piperine purification on the two CPC systems

	Loading flow rate (mL/min)	Loading rotation speed (rpm)	Elution flow rate (mL/min)	Extrusion flow rate (mL/min)	Elution and extrusion rotation speed (rpm)	Centrifugal acceleration (g force)
CPC 250	30	500	8	30	2000	304
CPC 1000 PRO	100	500	100	150	2000	452

Systems

Flash chromatography was performed on Scorpius silica gel 25 g and Scorpius silica gel 330 g cartridges (30 µm, 60 Å, BGB® Analytik) at 30 mL/min and 160 mL/min, respectively. Heptane and ethyl acetate were selected as elution solvents. Optimal separation was obtained with the isocratic proportion of heptane and ethyl acetate (80/20, v/v).

Injection Strategy

The amount of pepper extract injected at laboratory scale ranged from 100 mg to 500 mg, and from 5 g to 20 g at pilot scale. In CPC, samples were first diluted in a mixture of upper and lower phases (50/50, v/v) and then warmed at 40°C for 20 min (Table 2). The solutions were then ultrasonicated for 5 min and filtered (0.45 µm) before injection into the CPC system. With flash chromatography, the sample was first adsorbed to silica gel Si60, due to the low solubility of the pepper extract in the mobile phase (heptane/ethyl acetate), and then introduced into a precolumn connected to the top of the cartridge (Table 2).

Mass-directed Fraction Collection

To follow piperine purification, mass-directed fraction collection was performed using a single quadrupole VERITY 1920 MS. Ionization was performed by electrospray (ESI) in positive mode. The use of an active split enabled sampling a constant volume of effluent at very short time intervals, transferred via an auxiliary pump to the MS detection system. The sample was introduced to the MS using a mixture of water/MeOH (50:50, v/v) with 0.1% formic acid at a flow rate of 0.2 mL/min (split ratio 1:33 and 1:5 at laboratory and pilot scales, respectively). To detect the presence of piperine, an extract ion chromatogram from m/z 285 to m/z 287 was recorded.

HPLC Analysis

Purity and yields were determined by HPLC-DAD. Sample analyses were performed on a Chromaster HPLC system (VWR) equipped with a diode array detector (DAD) and piloted by EZChrome Elite software. Separations were achieved on a Lichrospher® 100 RP18 Endcapped column (250x4 mm, 5 µm, Merck) at 40°C. The injection volume was set at 5 µL and the flow rate at 1 mL/min. The mobile phase consisted of isocratic water-methanol both acidified with 1% acetic acid (35:65, v/v). Wavelength was selected at 280 nm for piperine samples diluted in methanol (0.5 mg/mL).

Table 2

Description of the different injection strategies used at laboratory and pilot scales for both CPC and flash chromatography

	Mass injected	Injection strategy	Injection volume	Adsorption on silica gel Si60
CPC 250	From 100 mg to 500 mg	Liquid	5 mL	-
CPC 1000 PRO	From 1 g to 20 g		50 mL	
Flash 25 g	From 100 mg to 500 mg	Solid	-	Ratio 1/3 (extract/Si60)
Flash 330g	From 1 g to 20 g			

RESULTS AND DISCUSSION

Typical chromatograms obtained using the CPC 250 or flash cartridge (25 g) at laboratory scale are depicted in Figure 3. Dual detection (UV and MS) enables us to demonstrate the difference in selectivity between CPC and flash chromatography. In the same way, chromatograms obtained at pilot scale (Figure 4) highlight the benefits of MS monitoring (green line), since the UV signals (blue and orange lines) saturate when gram quantities are injected into the CPC.

Both techniques were further compared in terms of solvent consumption and the amount injected in one day. Regardless of scale, flash chromatography consumes more solvent than CPC. Solvent consumption (including the conditioning step) is double with flash chromatography at laboratory scale and is multiplied by three at the pilot scale (Table 3). Regarding the quantity of extract that can be injected in one day, CPC can treat more material, especially at pilot scale. Only 15 g can be injected per day using flash, while 200 g can be treated by CPC. The large quantity of solvent used in flash chromatography has a major impact on the cost of separation, which is multiplied by ten. As a result, CPC at pilot scale is the best choice to purify large amounts of piperine with a reduced cost and consumption of solvent.

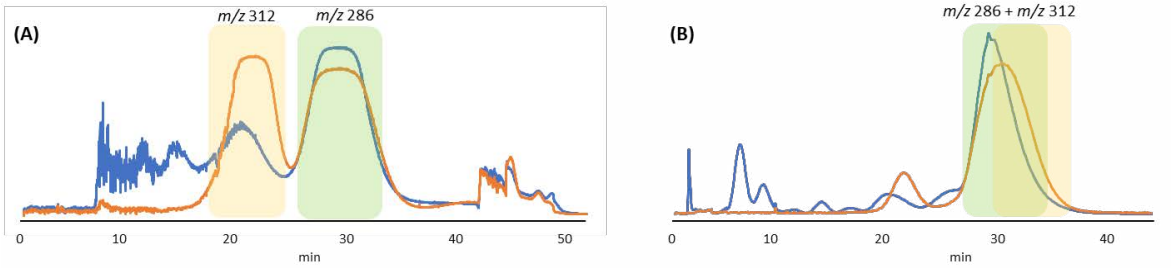

Figure 3

Illustration of the difference of selectivity between (A) CPC and (B) flash chromatography. Blue line: chromatogram at 254 nm. Orange line: chromatogram at 366 nm. m/z 286 corresponds to piperine; m/z 312 corresponds to impurity.

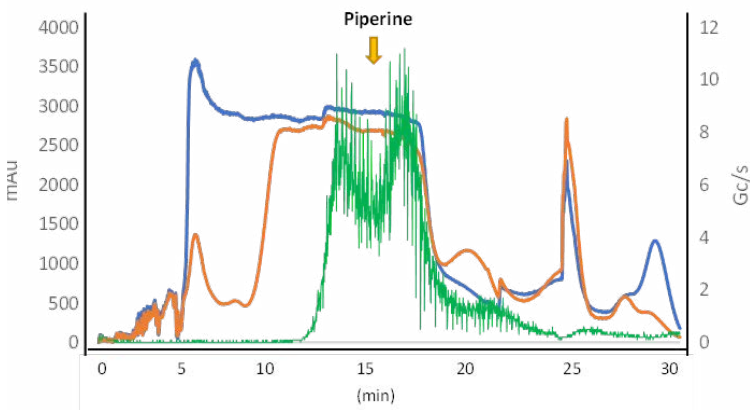

Figure 4

Illustration of piperine purification from extract of P. nigrum (10 g) using CPC-UV-MS (CPC 1000 PRO) and heptane-ethyl acetate-methanol-water (6:5:6:5, v/v/v/v) as solvent system. Separation was performed in ascending mode at 100 mL/min and 2000 rpm. Extrusion starts at 25 min. Blue line: CPC-UV at 254 nm. Orange line: CPC-UV at 366 nm. Green line: CPC-MS (EIC, m/z 285-287).

Table 3

Solvent consumption, productivity, and cost of separation at pilot scale. Data was calculated based on the value obtained at pilot scale (10 g and 20 g injection of piper extract with both techniques).

	Solvent volume (L)/g injected	Productivity: injected extract (g)/day	Cost for 100g of extract injected (10 injections)
CPC 1000 PRO	0.5	200	430 € (only solvents)
Flash (330 g)	1.55	15	4460€ (solvents + 10 cartridges)

CONCLUSIONS

CPC is the best option at both laboratory and pilot scale for piperine purification in comparison to flash chromatography. CPC allows higher purity than flash chromatography. A larger quantity of sample can be injected in CPC, with lower solvent consumption and no solid waste to treat (i.e., silica cartridge). Therefore, this work recommends CPC for purification of natural products compared to flash chromatography. The results also highlight the efficient and easy use of the PLC system in combination with CPC, flash chromatography, and MS detection.

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