CPC 250: Mass-Directed Purification of Piperine from *Piper nigrum*

TECHNICAL NOTE TN208

GILSON APPLICATIONS LABORATORY

INTRODUCTION

Piperine (Figure 1) is a pungent alkaloid that confers the biting taste to black pepper. Black pepper has long been used in traditional medicine, and recent studies have shown that piperine is associated with a number of bioactivities including chemopreventive, antioxidant, anti-inflammatory, and immunomodulatory activities.[1] This natural product is also reported to increase pancreatic activity, contribute to the reduction of blood cholesterol, triglycerides, and glucose, enhance the bioavailability of other therapeutics, and provide protection against radiation for chemotherapy patients.[2]

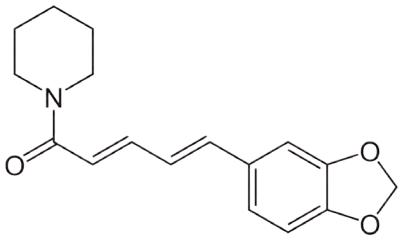

Figure 1
Structure of Piperine

Peppercorns, the dried fruits from *Piper nigrum*, contain approximately 2%–9% piperine by mass. Gilson has developed a purification method for piperine using centrifugal partition chromatography (CPC) with mass-directed fraction collection (Figure 2). The method is rapid and reproducible, recovers close to 100% of the piperine from a complex crude extract, requires less solvent than other LC methods, and can be easily scaled up from milligram to multi-kilogram scale.

* Please note the Verity 1900 MS Has been updated with the Verity 1920 MS

Figure 2
Instrumentation used for purification of piperine includes Gilson CPC, PLC, and VERITY® 1900 MS

Centrifugal partition chromatography (CPC), also known as counter current chromatography (CCC), is a silica-free, liquid-liquid chromatographic technique for preparative- and industrial-scale purifications. Two non-miscible liquid phases are used: one as the mobile phase or the eluent and the other as the stationary phase maintained by a centrifugal field. The affinity of the solute for each phase, measured by their partition coefficient, gives their order of elution.

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MATERIALS AND METHODS

A Gilson CPC 250 column was run with an elution rate of 10 mL/min, an extrusion flow rate of 20 mL/min, and a rotation speed of 2000 rpm. The CPC column and VERITY® 1900 MS Detector were controlled by a PLC 2250 Purification System equipped with a 250 mL/min quaternary gradient pump, UV/Vis detector, and fraction collector. Gilson Glider CPC software was used to control the instrumentation. Fraction collection was automatically triggered based on MS XIC channel when a molecular peak of 286 m/z was detected. For each 1 g sample of crude black pepper extract, 0.5 L of solvent was consumed during a separation time of 50 min. All organic solvents were analytical or HPLC reagent grade.

RESULTS AND DISCUSSION

In this study, 1 g of crude extract of black pepper was subjected to centrifugal partition centrifugation and fractions were collected based on mass spectra. CPC relies on a rotating chromatography column (rotor) with a stack of discs composed of twin cells (Figure 3). This design permits better retention of the stationary phase, allowing for a higher flow rate and faster separations. Since no solid support (silica or resin) is required for separation of compounds, CPC can be performed without concern for loss of material due to adsorption to the matrix. The method requires only ~20% the volume of solvent required for more traditional preparative chromatography, and the metal CPC column can be cleaned and reused.

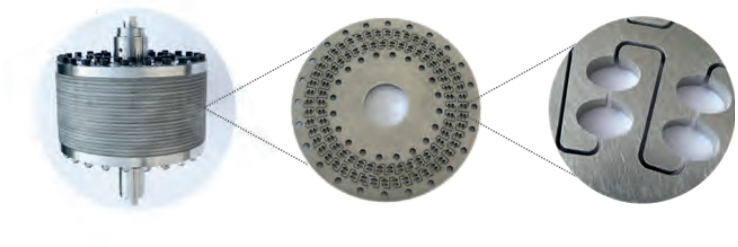

Figure 3
A typical CPC rotor (left) contains a stack of discs (center) composed of twin cells (right).

Gilson Glider CPC software controlled the fraction collection based on data from the VERITY® 1900 MS Detector and the UV detector integrated into the PLC 2250 Purification System. PLC purification systems from Gilson include fully integrated pumping systems (binary or quaternary) and a fraction collector with space for up to three racks. These customizable instruments can be used with HPLC and flash chromatography columns, as well as CPC. Detector options include UV, DAD, ELSD, and MS. Gilson Glider Prep software is integrated into the touchscreen control of PLC Purification Systems, and can also be installed on a PC to facilitate method development. Users can easily edit methods and specify solvents, columns, gradient conditions, detector settings, and more. Parameters can be easily modified during a run.

When a peak of 286 m/z was detected above a certain threshold, fractions were collected into tubes on the deck of the PLC 2250 Purification System (Figure 3). This one-step purification method resulted in clean separation of piperine from other compounds. The final product had a purity of over 95% as shown by HPLC analysis (254 nm). Purity was also assessed using thin layer chromatography (TLC) (data not shown). The VERITY 1900 MS Detector employs miniaturized chip-based mass spectrometry, which consumes less power, conserves bench space, does not require a nitrogen generator, and produces less noise than a traditional MS. Whether coupled with a PLC purification system or a GX-series liquid handler, this versatile detector allows researchers to collect fractions based on target mass.

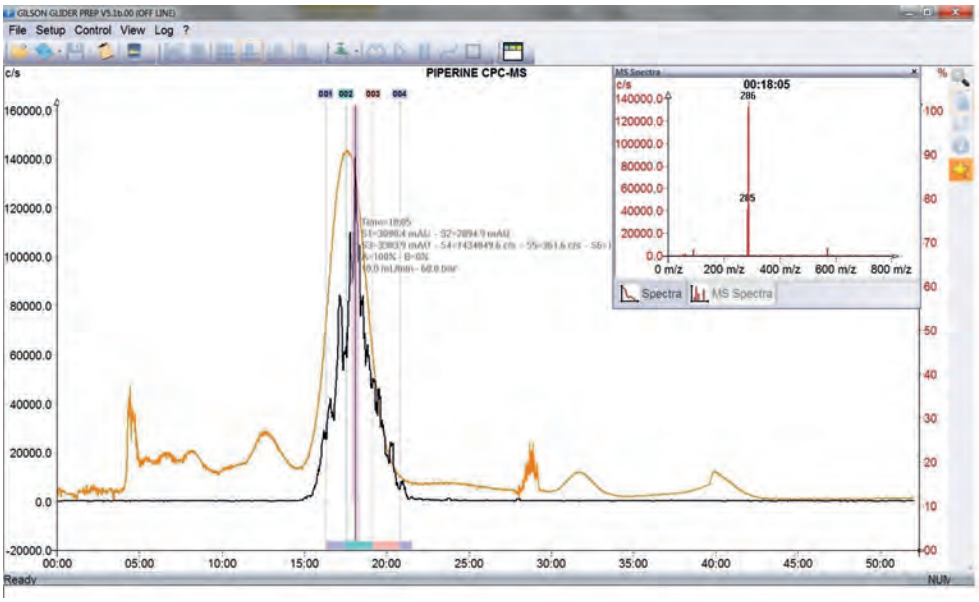

Figure 4
Screenshot from Gilson Glider Prep software showing HPLC chromatogram (280 nm), MS spectra, and peak tracking (fraction collection). Fractions were automatically collected when criteria for UV absorption and mass spectra were met. The four fractions collected are designated 001–004.

CONCLUSIONS

Mass-directed fraction collection offers huge time savings by minimizing the number of fractions collected, thereby reducing the time required for sample analysis. This approach has the added advantage of coupling high recovery with direct identification of the target compounds. Co-purification of unwanted compounds is reduced and the resulting product is cleaner. Since CPC uses a silica-free liquid-liquid chromatographic technique, there is no irreversible adsorption of the sample to the matrix and therefore no sample loss, resulting in higher yields. The method described in this work is adaptable for purification of a wide range of natural compounds from complex extracts.

REFERENCES

1. Srinivasan (2007) Black pepper and its pungent principle – piperine: a review of diverse physiological effects. Critical Reviews in Food Science and Nutrition 47(8): 725–748. http://dx.doi.org/10.1080/10408390601062054
2. Gorgani, L., Mohammadi, M., Najafpour, G. D. and Nikzad, M. (2017), Piperine – the bioactive compound of black pepper: from isolation to medicinal formulations. Comprehensive Reviews in Food Science and Food Safety 16: 124–140. doi:10.1111/1541-4337.12246