VERITY® CPC-250: TWO STEP PURIFICATION OF ALKALOID WITH ANTIMALARIAL ACTIVITY

APPLICATION NOTE AN1034

APPLICATION BENEFITS

- Bioassay-guided isolation
- New lead structure for antimalarial activity
- Alkaloid as challenging compound
- Common plant material, wide use

ADDRESSED ISSUES

- All-liquid separation scheme
- Only three steps from extract to pure compound
- Cost-effective and modular setup
- ARIZONA solvent systems for common use

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INTRODUCTION

O-tigloylcyclovirobuxeine-B (Figure 1), a *nor*-cycloartane alkaloid from *Buxus sempervirens* L. (European Box; Buxaceae), has shown promising and selective *in vitro* activity against *Plasmodium falciparum*, a causative agent of Malaria tropica. The target compound occurs in a complex matrix consisting of numerous congeneric alkaloids. Hence, an efficient separation scheme is required. As the drug resistance of malaria parasites increases, the development of innovative and effective antimalaria drugs is of high importance. Natural products provide a vast field of new lead structures that are effective in fighting the disease.

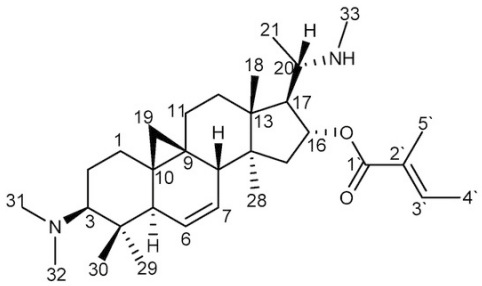

Figure 1
Chemical structure formula of *O*-tigloylcyclovirobuxeine-B

METHODOLOGY

This technical application note emphasizes the use of centrifugal partition chromatography (CPC, Figure 2) to purify a natural compound from a complex starting extract, replacing the traditional column. CPC does not require a solid support like silica. Instead two immiscible liquid phases are used. One serves as the mobile phase or the eluent, and the other as the stationary phase. The stationary phase is retained in the column by a centrifugal field. The affinity of the solute for each phase can be measured by their partition coefficient, (K_c), that in turn dictates the order of elution for each compound. There are various advantages in excluding solid stationary phases during isolation, such as achieving a high recovery of injected sample, low solvent consumption (less waste and expense), minimized tailing of eluted peaks, no irreversible loss of sample due to chemisorption, high reproducibility, and high purification levels.

Figure 2
VERITY® CPC-250

GILSON®

MATERIALS AND METHODS

The CPC run was performed on a Gilson VERITY CPC-250 instrument in a modular setup with an elution flow rate of 2.5 – 3 mL/min, extrusion flow rate of 5 mL/min, and a rotation speed of 1200 – 1300 rpm. The solvent system was Hexane/EtOAc/MeOH/Water in ascending mode for crude purification and Hexane/Acetonitrile/Dichloromethane in ascending mode for final purification. Further, isocratic pump and fraction collector were used. Fractions were collected by time and analysed by LC-MS.

The analytical HPLC was performed on an Ultimate 3000 RS Liquid Chromatography System (Dionex, Sunnyvale, USA) with a Bruker Daltonics microOTOF-QII time-of-flight mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). Detailed methods are described in Table 1 (HPLC) and Table 2 (CPC), and results are summarized in Table 3.

The alkaloid fraction was obtained by acid/base extraction of the crude dichloromethane (DCM) extract of *B. sempervirens* L. leaves. The alkaloid fraction was separated into 20 fractions by CPC. Fraction 2 contained *O*-tigloylcyclovirobuxeine-B as a main constituent, further purified in a successive CPC run.

Table 1

Analytical LC/MS Method Conditions

PARAMETER	DESCRIPTION
HPLC Column	Acclaim RSLC 120 RP18 (2.1 × 100 mm, 2.2 µm)
Mobile Phase A	Water (with 0.1 % formic acid)
Mobile Phase B	Acetonitrile (with 0.1 % formic acid)
Gradient	0 - 1.88 min: 15 % B to 30 % B 1.88 - 7.88 min: 30 % B to 33 % B 7.88 - 9.9 min: 33 % B to 50 % B 9.9 - 9.93 min: 50 % B to 100 % B 9.93 - 15.88: isocratic 100 % B 15.88 - 15.98 min: 100 % B to 15 % B 15.98 - 20.0 min: isocratic 15 % B
Flow Rate	0.4 mL/min
Injection Volume	2 µL
Temperature	40°C
Mass range *m/z*	50-1500

Table 2

CPC Method Conditions

PARAMETER	ALKALOID FRACTION	FRACTION 2
Column	VERITY CPC-250	VERITY CPC-250
Column Volume	250 mL	250 mL
Elution Flow Rate	3 mL/min	2.5 mL/min
Extrusion Flow Rate	5 mL/min	5 mL/min
Rotation Speed	1200 rpm	1300 rpm
Solvent System	Hexane/Ethyl Acetate/MeOH/Water	Hexane/Acetonitrile/Dichloromethane
Mode	Ascending	Ascending
Samples	4 g (sample concentration: 250 mg in 6 mL upper + 3 mL lower phase)	310 mg (in 6 mL upper + 1.5 mL lower phase)

Table 3

Results from CPC runs

	ELUTION VOLUME RANGE OF *O*-TIGLOYLCYCLOVIROBUXEINE-B	*O*-TIGLOYLCYCLOVIROBUXEINE-B RECOVERED	RECOVERY RATE
Alkaloid fraction	54 to 96 mL	39.6 mg	86.8%
Fraction 2	93.75 to 106.25 mL	35.0 mg	96.2%

RESULTS

Preliminary small-scale tests were carried out by the shake flask method to determine a suitable phase system with a K_c value near 1 for the fractionation.

$$K_c = \frac{[O-\text{tigloylcyclovirobuxeine}-B]_{stat}}{[O-\text{tigloylcyclovirobuxeine}-B]_{mob}}$$

The first CPC run was performed using the alkaloid fraction (LC/MS profile, Figure 3). The sample was dissolved in 6 mL of upper phase, 3 mL of lower phase and filtered through a 0.45 µm syringe filter. *O*-tigloylcyclovirobuxeine-B was detected in fraction 2 (337.7 mg). For final purification, 310 mg of fraction 2 were again subjected to the CPC and yielded 35 mg of *O*-tigloylcyclovirobuxeine-B after crystallization from acetonitrile. The purity of *O*-tigloylcyclovirobuxeine-B was 91.1% as estimated from the LC/MS analysis.

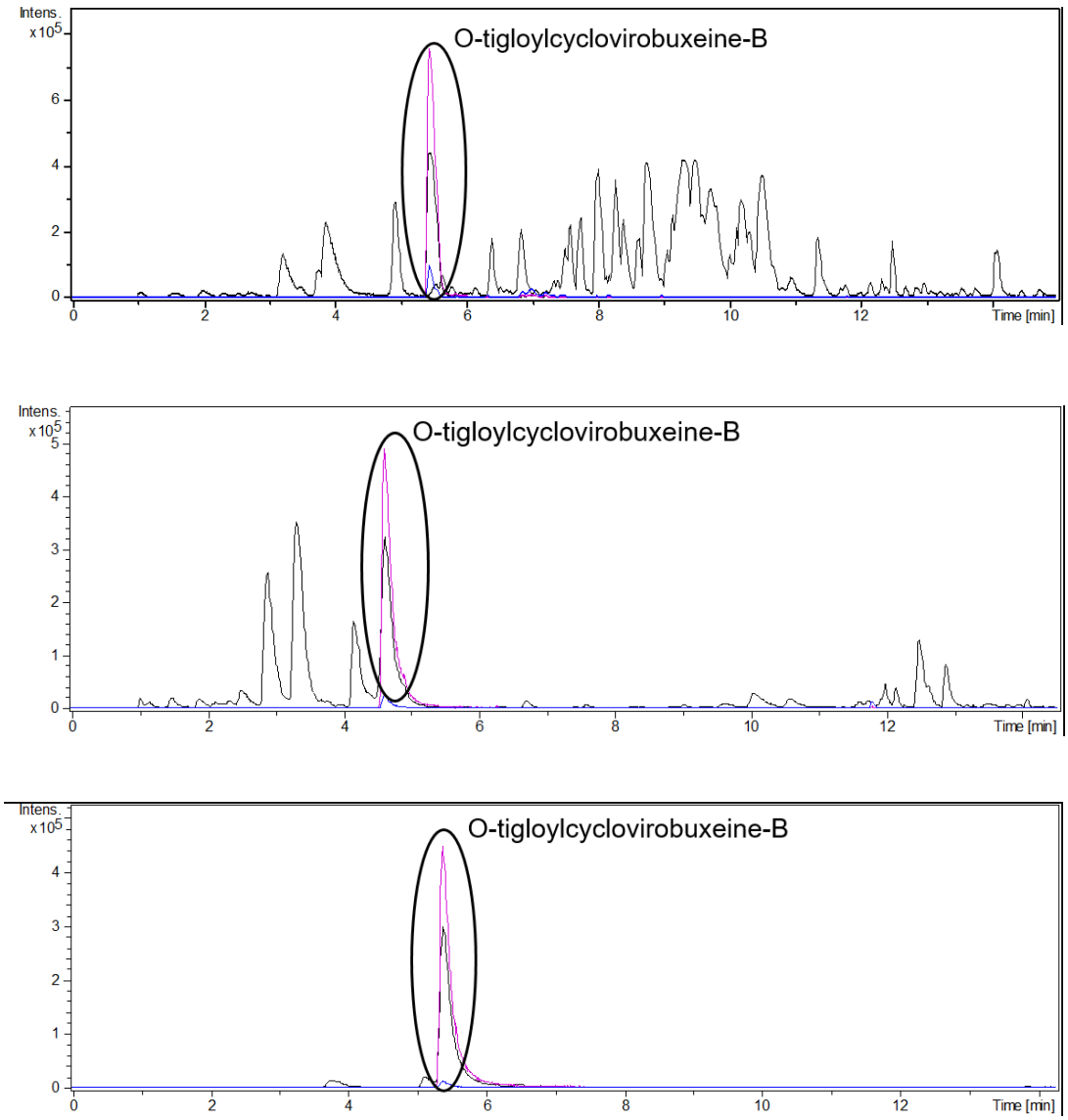

Figure 3

UHPLC/+ESI-QqTOF-MS chromatograms. Top: alkaloid fraction from extract. Middle: Fraction 2 containing target compound *O*-tigloylcyclovirobuxeine B. Bottom: Pure *O*-tigloylcyclovirobuxeine B.

CONCLUSIONS

CPC is an efficient isolation method for *O*-tigloylcyclovirobuxeine-B from a complex extract from leaves of *B. sempervirens* L. In contrast to other isolation techniques with a solid stationary phase, this all-liquid separation scheme uses minimal solvent consumption and affords a high purity and recovery of the target compound. The purification workflow from the crude extract involves only three steps. Taking into account that the purification of alkaloids is challenging due to their chemical properties, the results achieved in this work are remarkable and remove the need of solid stationary phases. For isolation of natural products, CPC is an advantageous chromatographic technique as demonstrated in the case of amino *nor*-cycloartane-type alkaloids from *Buxus*. The lead structure of *O*-tigloylcyclovirobuxeine-B will serve as a starting point for derivatives with improved bioactivity and toxicity profiles.

REFERENCES

Szabó, L.U.; Schmidt, T.J. Target-Guided Isolation of *O*-tigloylcyclovirobuxeine-B from *Buxus sempervirens* L. by Centrifugal Partition Chromatography. Molecules 2020, 25, 4804, DOI: 10.3390/molecules25204804.