TARGETED PURIFICATION OF AN ANTIMALARIAL COMPOUND FROM *ARTEMISIA ANNUA L.* EXTRACT USING THE VERITY® CPC LAB MS SYSTEM

APPLICATION NOTE AN1042

CPC APPLICATION BENEFITS

- Fast and cost-effective isolation of pure molecule from complex natural extract
- High recovery level based on both centrifugal partition chromatography (CPC) technology and mass spectrometer (MS) direct detection
- One software to control all modules including CPC column, Prep LC system, and MS detector

ADDRESSED ISSUES

- Targeted MS collection of artemisinin, a molecule that does not provide a UV signal
- One step purification of artemisinin from a highly complex natural extract with the VERITY® CPC 250
- Reduced fraction numbers, treatments and post analysis with online VERITY® 1920 MS Detector

C. LE QUEMENER, A. BICHON, G. AUDO AND R. HERRY | GILSON PURIFICATION

INTRODUCTION

Artemisinin (Figure 1) is a sesquiterpene lactone obtained from sweet wormwood, *Artemisia Annua L.* (Figure 2). The extract of the leaves of *Artemisia Annua L.* is used in ancient Chinese herbal therapy for malarial fevers. Artemisinin has been identified as an active antimalarial compound. It has been recently found to have potent activity against many forms of malarial organisms, including chloroquine-resistant *Plasmodium falciparum*. It is used as an antimalarial for the treatment of multi-drug resistant strains of falciparum malaria.

In this application note, the use of CPC coupled with a preparative liquid chromatography system equipped with UV-DAD and mass spectrometry (MS) detectors, illustrates the success of such technologies to isolate active compounds from complex mixtures. Mass directed CPC purification allows individual targeting and selective collection of molecules based on their mass to charge ratio, even for those that do not provide a UV signal. Because the VERITY CPC Lab MS System does not denature fragile molecules, it is an ideal choice for natural extract fractionation and natural compound purification[1].

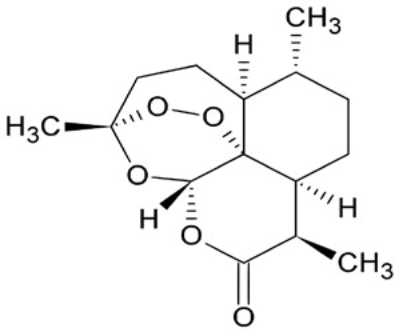

Figure 1
Structure of artemisinin

Figure 2
Artemisia Annua L. leaf

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MATERIALS AND METHODS

Artemisinin purification was performed on a VERITY CPC LAB MS (Figure 3). It was composed of a Gilson VERITY CPC 250 column connected to a PLC 2250 Purification System (Compact LC system) which was configured with a 250 mL/min quaternary gradient pump, automatic injection valve, backflush valve, UV/VIS detector, fraction collector, and GLIDER control software.

The VERITY 1920 MS Detector was set with a splitting kit, composed of a VERITY® 3011 Isocratic Pump with 5 mL head and an MRA valve, to divert a small amount of the flow for mass detection.

CPC solvent system:

The Arizona R solvent system composed of n-heptane, ethyl acetate, methanol and water was used to perform the separation by CPC. The lower phase or the upper phase were automatically generated online using GLIDER software and the PLC 2250 low pressure gradient valves.

Sample preparation:

250 mg of crude extract was prepared from dried *Artemisia Annua L.* Plant extract was dissolved in 10 mL of upper phase of Arizona R and filtered on a 10 µm syringe filter.

The sample solution was loaded into a 10 mL loop on the PLC 2250 and automatically injected onto the VERITY CPC 250 using GLIDER software.

CPC separation method:

All steps of the separation method were programed into GLIDER software:

The column was first filled in ascending mode with the aqueous mobile phase of the solvent system at 50 mL/min and 500 rpm. The organic mobile phase of the solvent system was pumped at 12 mL/min and 2000 rpm.

After injection, elution with mobile phase was performed for the duration of 35 min, then an extrusion step was applied to recover any remaining compounds from the sample in the stationary phase. During this last step, the fresh aqueous phase was pumped at 50 mL/min and 2000 rpm.

CPC effluent was monitored by UV detection at 280 nm and 335 nm, and in scan mode from 200 to 600 nm. In addition, MS detection was performed according to the total ion chromatogram (TIC) and to the extracted ion chromatogram of the artemisinin (XIC) (see Table 1). 12mL fraction volumes were collected according to the XIC signal threshold.

The purity of the fractions was controlled by thin layer chromatography (TLC). After solvent evaporation, the mass of purified artemisinin was determined.

Figure 3
VERITY CPC LAB MS

Mass Spectrometry settings:

The VERITY 1920 MS was equipped with the Electrospray ionization (ESI) probe. The ionization parameters are described in the table below. During the CPC separation, an aliquot of the CPC effluent was transferred to the VERITY 1920 MS using the VERITY MRA valve with a split factor set into GLIDER software. The make-up VERITY 1910 pump was set at 0.2 mL/min to pump the aliquot into the ESI source. In order to get UV and MS signal synchronisation, a tubing kit was installed between the MRA and the PLC.

Table 1
MS parameters set during the CPC separation

PARAMETERS	SETTINGS
Make-up solution	methanol / water (50/50) 0.1% formic acid (v/v)
Make-up flowrate	0.2 mL/min
Tubing Kit	1
Split factor	15
Ionisation source	ESI
Ionisation mode	POSITIVE
Ionisation parameters	Fragmentation: typical Temperature: medium
MS detection	TIC: 10-2000 m/z XIC of artemisinin: 282-284 m/z ([M+H+] = 283 m/z)

TLC Analytical method:

TLC analysis of the fractions were performed on silica gel 60 F254 using a heptane/ethyl acetate (60/40 v:v) eluent mixture. The artemisinin detection was observed after derivatization with anisaldehyde-sulfuric acid reagent at 366 nm with an Rf of 0.52 (Figure 4).

RESULTS AND DISCUSSION

CPC separation of the *Artemisia Annua L.* crude extract resulted in a single-step purification of artemisinin and of global fractionation of the sample. As shown in the CPC chromatogram in Figure 5, UV signal could not be used to follow the elution of artemisinin, whereas based on its molecular ion, the MS detection allowed a direct detection of the targeted molecule from 25 min to 36 min with the most intense signal between 30 to 36 min.

The threshold level on the XIC signal from the MS was fixed at 200 Mcts/s to collect only the artemisinin fraction. Triggering fraction collection drastically limits the number of fractions and so post-separation fraction treatment. TLC analysis confirmed that artemisinin was recovered in fractions 6 to 11 (Figure 6).

Artemisinin fractions were then pooled and the solvent remove by evaporation to obtain 20 mg of pure molecule.

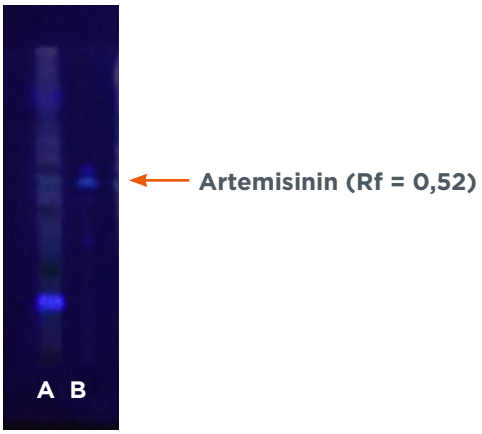

Figure 4

Crude extract (A) and artemisinin standard (B) TLC analysis at 366 nm

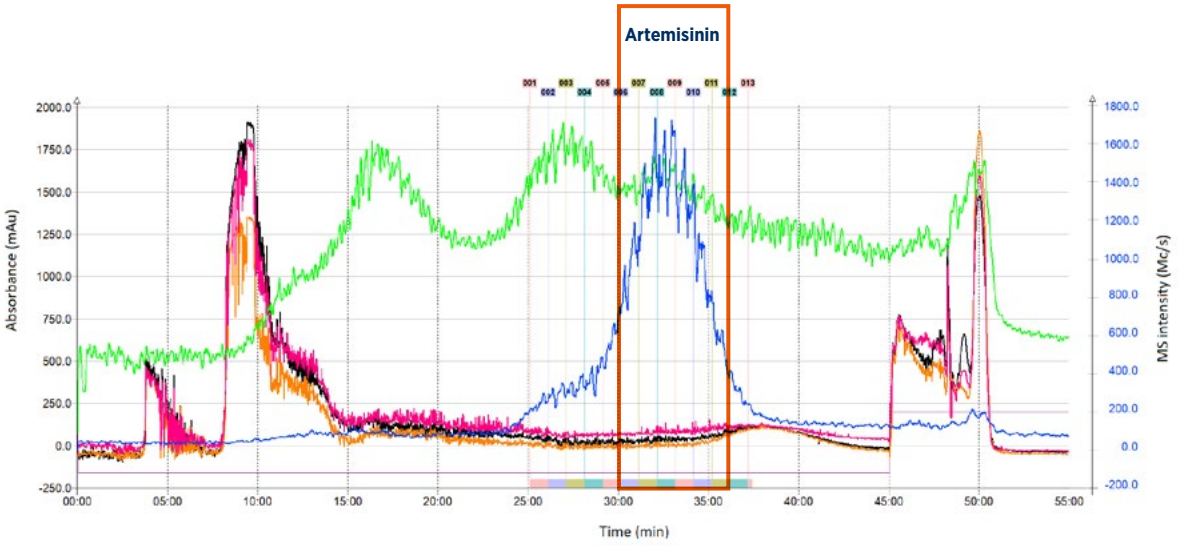

Figure 5

CPC chromatogram based on UV and MS detection with MS target collection (280 nm in black, 335 nm in orange, scan 200-600 nm in pink, TIC in green, XIC 282-284 m/z in blue)

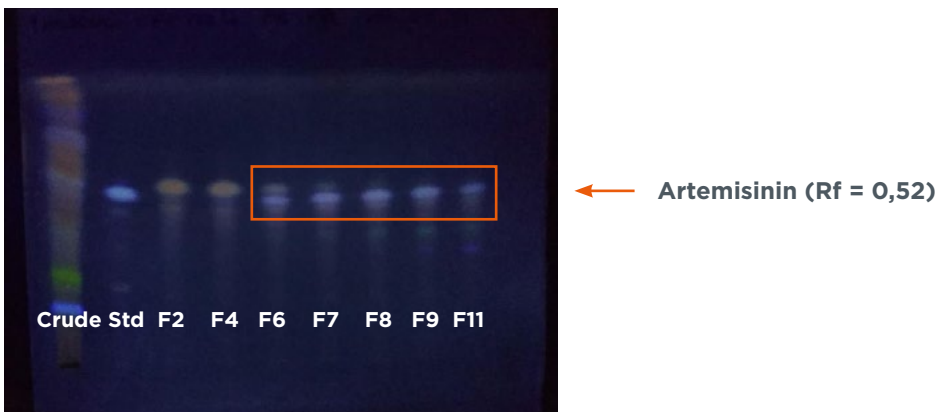

Figure 6

TLC analysis of fractions obtained after CPC separation based on MS signal target collection

CONCLUSIONS AND BENEFITS

In this study, the use of the combination of CPC technology and online MS detection with the Gilson VERITY CPC Lab MS System achieved two goals in a single step:

- The global fractionation of a complex plant extract and the purification of a target compound in one step. By using CPC, a silica-free liquid-liquid chromatography technology, the sample preparation is softer than Flash or Preparative HPLC, reducing the risk of loosing compounds. Another benefit of CPC is its capability to easily scale-up to pilot and production range.
- The triggering of fraction collection using mass-directed purification to reduce fraction number and treatments such as dry down, reconstitution, and analysis.

Isolation of biologically active compounds such as artemisinin from natural products is very interesting for multiple research industries looking for purification of polyphenols, lipids, vitamins, terpenes, phytocannabinoids, etc.

Phytochemistry provides a huge resource of natural active compounds but natural product extracts are usually complex and the combination of mass detection with CPC allows quick and direct purification of mg to g of pure molecule for further structural analysis, biological testing or used as an analytical standard.

REFERENCES

1. https://fr.gilson.com/FRFR/system-verity-compact-cpc-ms-system.html