APPLICATION DATA SHEET

CPC application note N°3

Artemisinin purification from the leaves of *Artemisia Annua L.* with SCPC-250

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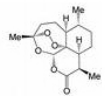
Introduction

The extract of the leaves of *Artemisia Anua L.* are used in Chinese medicinal for treat attacks of malaria, since many years. Artemisinin is identified as the active antimalarial compound.
Two trials were done on CPC equipped with 250ml column to follow resolution of the separation between 0.5 and 5g injections of crude extract to purify artemisinin. Purity is controlled by TLC [1]



Apparatus

An **Armen SCPC-250** connect to an **Armen Spot prep II** system equipped with 50 ml/mn quaternary gradient pump, UV/Vis detector, fraction collector and **AGCPC software** was used.

Sample

Figure 1 Crude extract of *Artemisia annua* L. And TLC of crude mixture and standard of artemisinin

Table 1 TLC conditions

TLC Plate	:Alugram Silica gel 60 F_{254}
Sample	:0.4 µL in toluene
Developing solvent	:Cyclohexane, ethyl acetate, acetic acid (20:20:1)
Derivatization reagent	:Anysaldehyde reagent. 20 mL acetic acid, 4 mL sulfuric acid, 2 mL Anisaldehyde are add to a mixture of 100 mL of ethanol with 80 mL of water
Derivatization	:Plate immersed in the reagent for 1s. After 1 mn the plate is heated at 100 °C for 12 min
Evaluation	:UV 366 nm

Crude extract is first analyzed by TLC [**Fig. 1**]. Artemisinin is identify at Rf=0.4 as a yellow brown spot after derivatization with anisaldehyde reagent.

Results

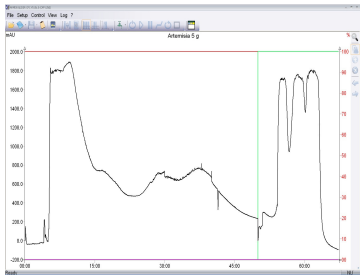


Figure 2. CPC chromatogram of 5 g injection of crude mixture

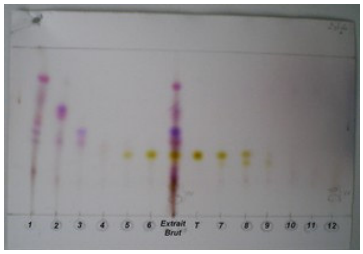


Figure 3. TLC plate of CPC fraction with crude mixture and standard artemisinin deposit

Table 2 CPC conditions

CPC column volume	:250 mL
Elution flow rate	:12 mL/mn
Extrusion flow rate	:20 mL/mn
Rotation speed	:2000 rpm
Solvent system	:Hept/AcOEt/MeOH/W 2/1/2/1
Mode	:Ascending, upper phase as mobile phase
Sample	:0.5 g in 10 mL upper phase for run N°1and 5 g in 30 mL upper phase for run N°2
Detection	:280 nm

CPC solvent system is determined with shake flask method with TLC to get a Kd=(Spot intensity of Artemisinin)$_{stat}$/(Spot intensity of Artemisinin)$_{mobile}$ close to one. The sample is dissolved in upper phase, filtered through a 0.45 µm membrane filter and injected in CPC according to conditions describe in table 2. Two CPC runs are done with 0.5 and 5 g [**Fig 2&3**] of crude extract. Extrusion with stationary phase is done at 50 mn. CPC Fractions obtained are analyzed by TLC and grouped according to TLC purities. Results are resumed in table 3

Table 3 results

CPC run N°	Mass injected	Run time	Solvent consumption	Artemisinin recovered	Yield (w/w)
1	500 mg	45 mn	1L	37 mg	7.4%
2	5 g			268 mg	5.3%

Conclusion

Artemisinin was purified from a very crude extract of *Artemisia Annua L.* Injection up to 5 gr was carry out to evaluate the capacity of a 250 ml column on this application. 5 g injection do not decrease significantly recovery of artemisinin compared to 0.5 g injection. .

1] Camag application notes A-86.1

Notes : This application note has been produced and edited using information that was available when the data was acquired for each article. This application note is subject to revision without prior notice

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