



Anthocyanins purification with Centrifugal Partition Chromatography SCPC-100

Application Note 03

Introduction

Anthocyanins, which are found in high concentration in the black currant, give to the edible berry its characteristic dark purple color.

Centrifugal Partition Chromatography (CPC) also known as **Counter Current Chromatography (CCC)** is a preparative, pilot and industrial liquid purification technique that does not require traditional solid supports.

CPC was used to purify few mg of anthocyanins from a crude powder extract.



Materials and Methods

A Gilson [SCPC-100](#) connects to a Gilson [PLC2250](#) system equipped with 50 mL/min quaternary gradient pump, UV/Vis detector, fraction collector and AGCPC software was used.

CPC solvent system is determined with shake flask method to get a $K_d = \frac{[\text{HPLC peak area of anthocyanin}]_{\text{stat}}}{[\text{HPLC peak area of anthocyanin}]_{\text{mobile}}}$ closed to one.





Table 1: CPC conditions

CPC column volume	: 100 mL
Elution flow rate	: 8 mL/min
Extrusion flow rate	: 30 mL/min
Rotation speed	: 2000 rpm
Solvent system	: BuOH/AcOEt/TFA 0.1%
Mode	: Ascending
Mass injected	: 0.5 g
Sample	: in 2 mL lower + 2 mL upper
Detection	: 520 nm

Results and Discussion

Crude extract was first analyzed by HPLC to identify target anthocyanin to be purified. 4 major peaks (A, B, C, and D) are detected at 520 nm:

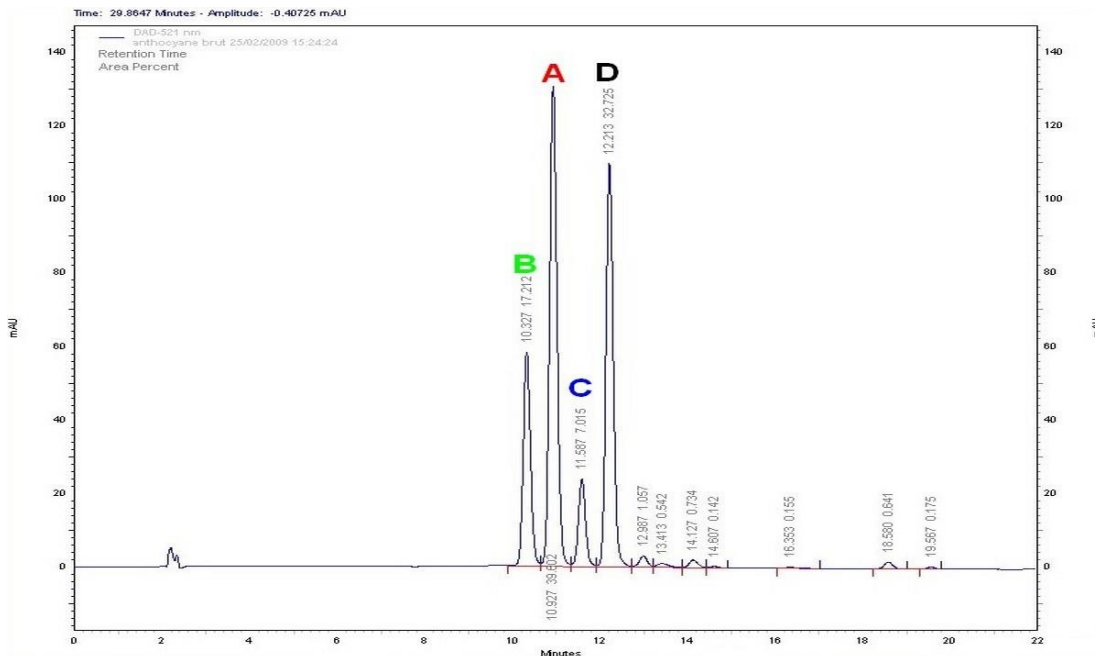
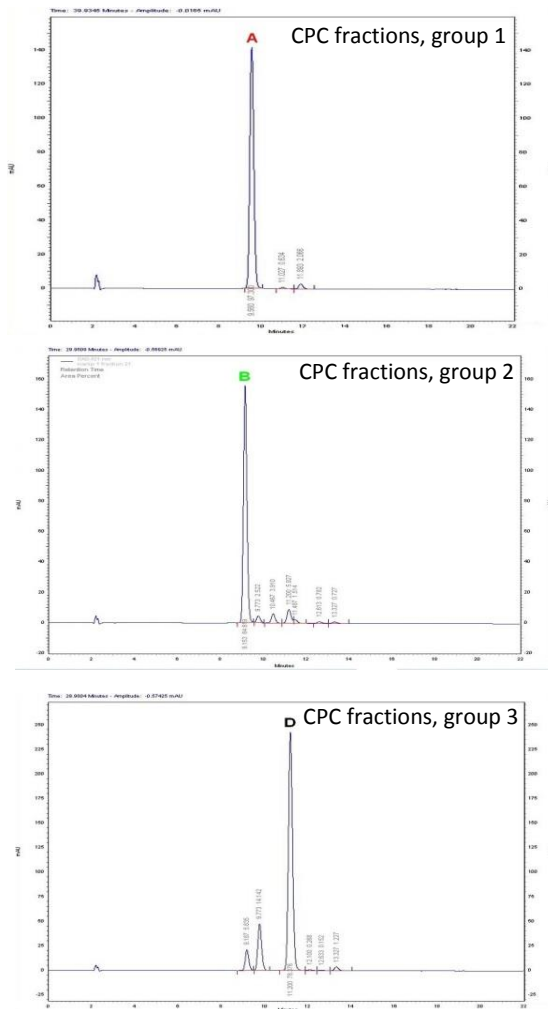


Figure 1: HPLC analysis 520 nm of crude black currant extract



0.5 g of sample is injected in CPC according to the table 1 conditions. CPC fractions obtained are analyzed by HPLC and grouped according to anthocyanin purity.

Results are resumed in table 2.

Table 2: Results

Duration	: 30 min
Solvent consumption	: 500 mL
Purity HPLC 520 nm	: Group 2, 97% Anthocyanin A Group 1, 84% Anthocyanin B Group 3, 78% Anthocyanin D

Conclusions

100 mL CPC column allows injection of 0.5g crude black currant mixture to get few mg of pure anthocyanin A. In addition the same run also permit to **purify 2 others anthocyanins**. Therefore, multi gram injections could be perform on 250 mL or 1L CPC column for **scale up and small production** of pure anthocyanins.