



# Anthocyanins purification from Blackcurrant with SCPC-100

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## Introduction

The black currant is the edible berry of a shrub, which usually grows to 1-2 meters and can be found in Northern/Central Europe and Asia. Anthocyanins are found in high concentrations in the fruit and give its characteristic dark purple color. Delphinidin 3-O-glucoside, delphinidin 3-O-rutside, cyanidin 3-O-glucoside and cyanidin 3-O-rutside, made up more than 97% of anthocyanins in the plant [1].

500 mg of powder extract of blackcurrant is injected by CPC (Centrifugal Partition Chromatography) to purify anthocyanins.



## Apparatus



An **Armen SCPC-100** 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.

HPLC was performed on **LaChrom Elite HPLC system** (VWR) equipped with Photodiode Array Detector (PDA) (200-800 nm).

## Sample

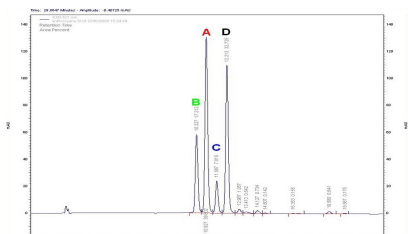


Figure 1. HPLC analysis 520 nm of crude blackcurrant extract.

Table 1 Analytical HPLC conditions

HPLC column	:Purosphere RP18, 250X4.6mm, 5µm
Mobile phase A	:10% formic acid
Mobile phase B	:MeOH
Time program	:5%B (0.00 min)-60%B(20 min)- 100%B(25 min)-100%B(30 min)-5%B(35 min)-5%B(40 min)
Flow rate	:1 mL/mn
Injection volume	:10 µL
Temperature	:30°C

Crude extract was first analyzed by HPLC [Fig.1] to identify target anthocyanin to be purified. 4 major peaks A, B, C and D are detected at 520 m with the following peak area: 39% A, 17% B, 7% C and 32 % D. A is the target compound to purify by CPC.

## Results

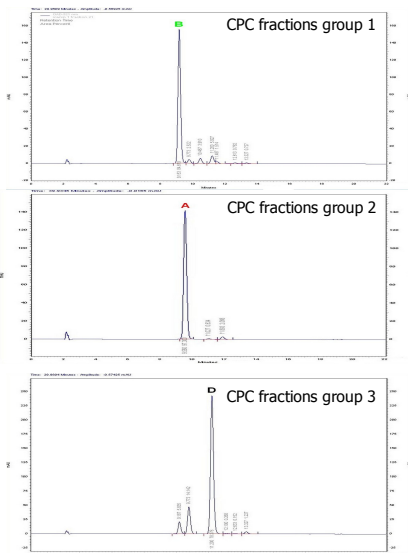


Figure 2. HPLC analysis 520 nm of CPC fraction groups 1, 2 and 3

Table 2 CPC conditions

CPC column volume	:100 mL
Elution flow rate	:8 mL/mn
Extrusion flow rate	:30 mL/mn
Rotation speed	:2000 rpm
Solvent system	:BuOH/AcOEt/TFA 0.1%
Mode	:Ascending
Injection volume	:4 mL
Sample	:0.5 g in 2 mL upper + 2 mL lower
Detection	:520 nm

CPC solvent system is determined with shake flask method to get a  $K_d = \frac{[\text{HPLC peak area of A}]_{\text{stat}}}{[\text{HPLC peak area of A}]_{\text{mobile}}}$  closed to one. 0.5 g of sample are dissolved in 2 ml of upper phase and 2 ml of lower phase, filtered through a 0.45 µm membrane filter and injected in CPC according to conditions describe in table 2. CPC Fractions obtained are analyzed by HPLC and grouped according to HPLC purity of A, B, C and D. The grouped fractions are analyzed by HPLC [Fig.2]. Results are resumed in table 3

Table 3 results

<b>Duration</b>	<b>:30 mn</b>
<b>Solvent consumption</b>	<b>:500 mL</b>
<b>Purity HPLC 520 nm</b>	<b>:Group 2, 97% anthocyanin A</b>
	<b>:Group 1, 84% anthocyanin B</b>
	<b>:Group 3, 78% anthocyanin D</b>

## Conclusion

100 mL CPC column allows injection of 0.5 g of crude blackcurrant 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 250ml or 1L CPC column for scale up and small production of pure anthocyanin.

[1] Slimestad, R.; Solheim, H. J. Agric. Food Chem. 2002, 50(11), 3228-3231. "Anthocyanins from Black Currants (Ribes nigrum L.)"

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