Qualitative and quantitative determination of anthocyanins and anthocyanidins in bilberry preparations

Instrument parameters

HPLC Shimadzu LC10A system, controller SCL10A, column oven CTO10A, autosampler SIL10A, UV/VIS detector SPD10A, 2 pumps LC10AT and Waters-Inline-Degasser AF

Stationary phase: Discovery C18, 180 Å, 5 µm, 4.6 × 250 mm (Supelco-Merck).

Mobile phase: A. water and formic acid (91.5:8.5, v/v)

B. formic acid, acetonitrile, methanol and water (8.5:22.5:22.5:41.5, v/v/v/v).

Injection volume: 10 µL, temperature: 30 °C, detection wavelength: λ = 535 nm.

Gradient elution

[min]	Flow [mL/min]	% A	%B
0	1.0	87.5	
35	1.0	75	
45	1.0	35	
46	1.0	0	100
56	1.0	0	100
56.1	1.0	87.5	
60	1.0	87.5	

Sample preparation

The contents of 10 capsules (selected at random) are mixed. The average filling mass of one capsule is accurately weighed and dissolved in 25.0 mL of a mixture of methanol/hydrochloric acid = 98:2 (v/v). A total of 5.0 mL of this solution is diluted to 20.0 mL with phosphoric acid in water (10 %, m/m).

Quantification

The quantification was done according to the European Pharmacopoeia[1]. The total anthocyanin content is calculated and expressed as cyanidin-3-O-glucoside chloride. All content data refer to the quantification from three independent analyses.

[1] Myrtilli fructus recentis extractum siccum raffinatum et normatum. European Pharmacopoeia, 9th ed.

Structural formulae and sample chromatograms

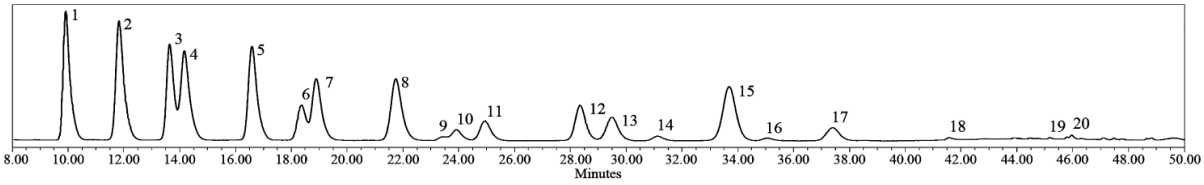
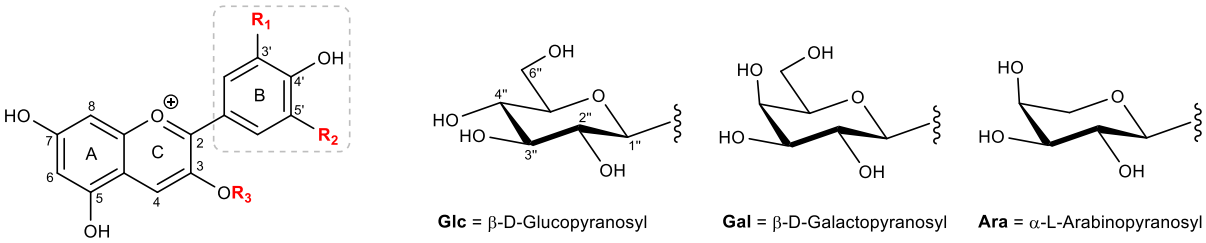


Fig. 1 Representative HPLC chromatogram (fingerprint). Authentic bilberry extract (V. myrtillus, "HRS extract").



Anthocyanidins (= aglyka of anthocyanins)

R_1	R_2	R_3	Nr.	Name
OH	H	H	**16**	Cyanidin (Cy)
OH	OH	H	**9**	Delphinidin (Dp)
OCH_3	H	H	**19**	Peonidin (Pn)
OCH_3	OH	H	**18**	Petunidin (Pt)
OCH_3	OCH_3	H	**20**	Malvidin (Mv)

Anthocyanins (= glycosides of anthocyanidins)

R_1/R_2	R_3	No.	Name	No.	Name	No.	Name
see left	Glc/Gal/Ara	**5**	Cy-3-*O*-glucoside	**3**	Cy-3-*O*-galactoside	**7**	Cy-3-*O*-arabinoside
	Glc/Gal/Ara	**2**	Dp-3-*O*-glucoside	**1**	Dp-3-*O*-galactoside	**4**	Dp-3-*O*-arabinoside
	Glc/Gal/Ara	**12**	Pn-3-*O*-glucoside	**10**	Pn-3-*O*-galactoside	**14**	Pn-3-*O*-arabinoside
	Glc/Gal/Ara	**8**	Pt-3-*O*-glucoside	**6**	Pt-3-*O*-galactoside	**11**	Pt-3-*O*-arabinoside
	Glc/Gal/Ara	**15**	Mv-3-*O*-glucoside	**13**	Mv-3-*O*-galactoside	**17**	Mv-3-*O*-arabinoside

Fig. 2 Structural formulae of anthocyanidins and anthocyanins in bilberry extract. Numbers correspond to the elution order.

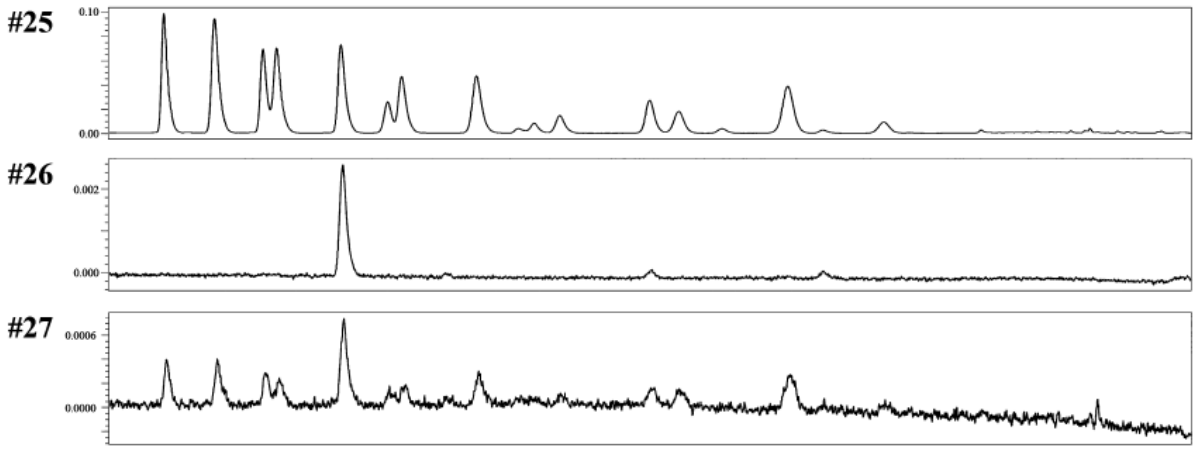


Fig. 3 Three chromatograms of food supplements of different quality.

#25 "Blueantox® Nature"	**category 1**
#26 "Myrtillus plus capsules"	**category *3/4***
#27 "Bilberries Myrtillus capsules"	**category 3**