HPLC-DAD/ESI-MS identification of the compounds from the fruits of *R. idaeus* and *R. occidentalis*

In the optimized HPLC conditions, 12 anthocyanin standard compounds and sanguiin H-6 were resolved. However, delphinidin 3-O-glucoside and pelargonidin 3,5-di-O-glucoside could not be separated and the resolution of cyanidin 3-O-sambubioside and cyanidin 3-O-glucoside was not satisfactory (Tab. 1, Fig. 1).
In *R. occidentalis* 'Litacz' an unknown compound was found eluting at t_R 18,1 min (C_1), which was identified basing on UV and MS spectra, as cyanidin 3-O-(2^G-xylosyrutinoside) (C_1) (Tab. 1).
In cultivars 'Ljulin' and 'Veten' an unknown compound (C_2) was found eluting at t_R 17,7 min, and was identified, on the basis of UV and MS spectra, as pelargonidin 3-O-sophoroside (C_2) (Tab. 1).

Validation of HPLC methods used for quantitative analysis of the compounds in the fruit extracts of *R. idaeus* and *R. occidentalis*

The developed HPLC method for purposes of quantitative analysis was validated by determining the calibration curves, linear regression, PLOQ and recovery of biologically active compounds (Tab. 2), except pelargonidin 3-O-sophoroside and cyanidin 3-O-(2^G-xylosyrutinoside), which amount was calculated corresponding to cyanidin 3-O-glucoside. In *R. occidentalis* 'Litacz' cyanidin 3-O-sambubioside and cyanidin 3-O-glucoside could not be separated (Figure 1), therefore their content was determined using HPLC-ESI-MS (Tab. 3).

Figure 1. HPLC chromatogram of the mixture of standard compounds: UV detection, A: λ = 280 nm, B: λ = 520 nm

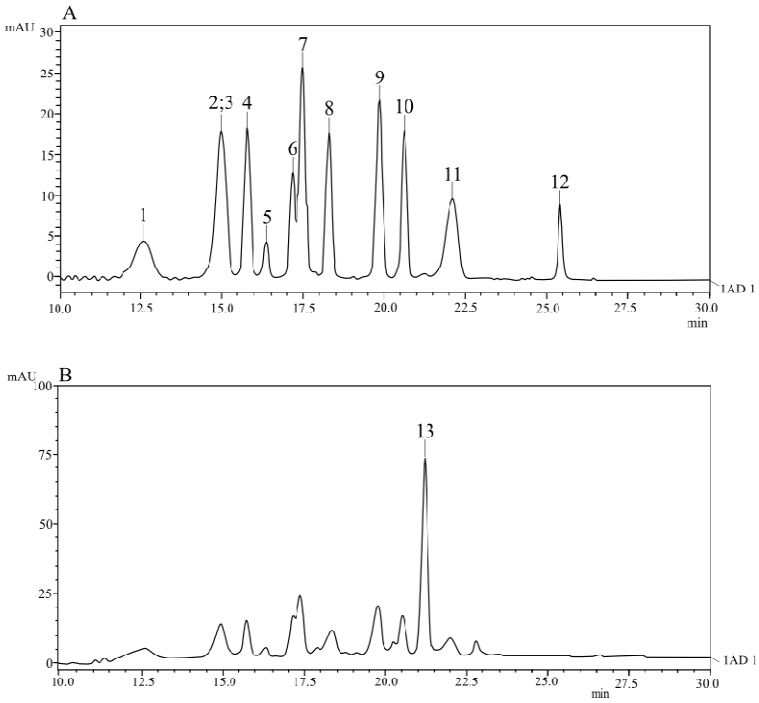

Table 1. HPLC-DAD/ESI-MS data of the compounds identified in the fruits of *R. idaeus* and *R. occidentalis*

HPLC peak	Compounds	t_R (min)	λ_{max} (nm)	$M^{+/-}$ (m/z)	Fragment ions (m/z)
1	cyanidin 3,5-di-O-glucoside	12.6	276, 513	611^+	287^+
2	delphinidin 3-O-glucoside	14.9	274, 523	465^+	303^+
3	pelargonidin 3,5-di-O-glucoside	14.9	269, 496	596^+	271^+
4	cyanidin 3-O-sophoroside	15.7	279, 514	611^+	287^+
5	cyanidin 3-O(2^G-glucosylrutinoside)	16.3	279, 518	757^+	287^+
6	cyanidin 3-O-sambubioside	17.1	280, 517	581^+	287^+
7	cyanidin 3-O-glucoside	17.4	279, 517	449^+	287^+
8	cyanidin 3-O-rutinoside	18,3	279, 517	595^+	287^+
9	pelargonidin 3-O-glucoside	19.8	269, 504	433^+	271^+
10	pelargonidin 3-O-rutinoside	20.6	275, 502	579^+	271^+
11	malvinidin 3-O-glucoside	22.1	274, 527	493^+	331^+
12	cyanidin	25.4	273, 523	287^+	
13	sanguiin H6	21.3	206, 251	1870^-	934^-, 935^-
C_1	cyanidin 3-O-(2^G-xylosyrutinoside)	18.1	279, 517	727^+	287^+
C_2	pelargonidin 3-O-sophoroside	17.7	279, 504	595^+	271^+

Table 2. Validation parameters of the developed HPLC method for quantitative analysis of anthocyanins in the fruits

Compounds	Calibration curve	R^2	PLOQ (μg/1ml)	Recovery
cyanidin 3,5-diglucoside	y=0.000221148*x+0.760168	0.999	1.0	104.6±5.2
cyanidin 3-O-sophoroside	y=0.000166372*x+0.157799 y=0.000150383*x+1.45536	0.999 0.999	0.75	108.1±3.9
cyanidin 3-O(2^G-glucosylrutinoside)	y=0.00011316*x+0.01275 y=0.000112415*x+0.11805	0.999 0.999	0.5	99.6±8.2
cyanidin 3-O-sambubioside	y=0.000267489* x-0.00044298	0.999	1.0	102.8±2.7
cyanidin 3-O-glucoside	y=0.00011316*x+0.01275 y=0.000112415*x+0.11805	0.999 0.999	0.5	99.6±8.2
cyanidin 3-O-rutinoside	y=0.000381367*x+0.189157 y=0.000330891*x+3.96089	0.999 0.999	1.5	98.6-±5.2
pelargonidin 3-O-glucoside	y=0.000140779*x+0.646178	0.999	0.75	103.8±4.5
pelargonidin 3-O-rutinoside	y=0.000177942*x+0.287662	0.999	0.75	104.9±6.9
cyanidin	y=0.0000563588*x+ 0.00621106	0.996	0.2	113.4±16.8
sanguiin H6	y=0.000575157*x+5.52713	0.999	1.5	94.3±4.9

*PLOQ – pooled limit of quantification

Table 3. Validation parameters of HPLC-ESI-MS method for quantitative analysis of cyanidin 3-O-sambubioside and cyanidin 3-O-glucoside in the fruits of *R. occidentalis*

Compounds	Calibration curve	r^2	PLOQ (μg/1ml)	Recovery
cyanidin 3-O-sambubioside	y=0.000026945*x+0.978	0.999	1.83	99.3±7.4
cyanidin 3-O-glucoside	y=0.00001275*x-0.0759	0.999	3.71	128.5±10.4