Supplementary data

Pharmacokinetic study of *Aronia melanocarpa* anthocyanins bound to amylopectin nanoparticles using high-performing liquid chromatography-tandem mass spectrometry

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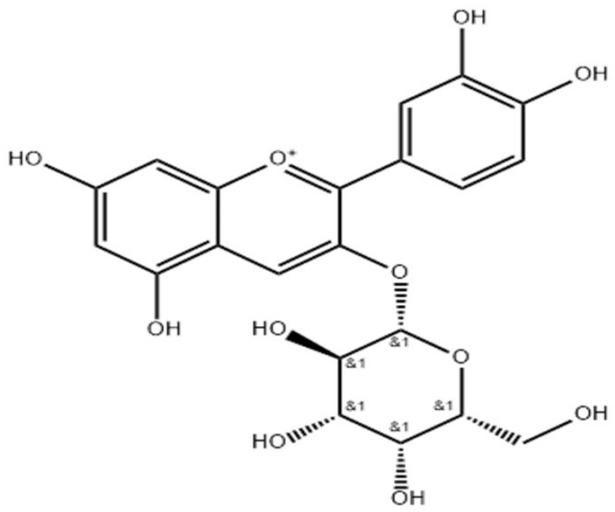
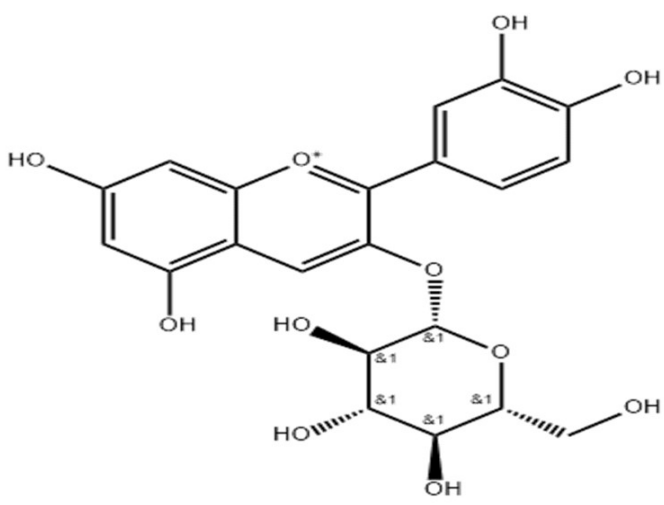


Cyanidin-3-O-galactoside



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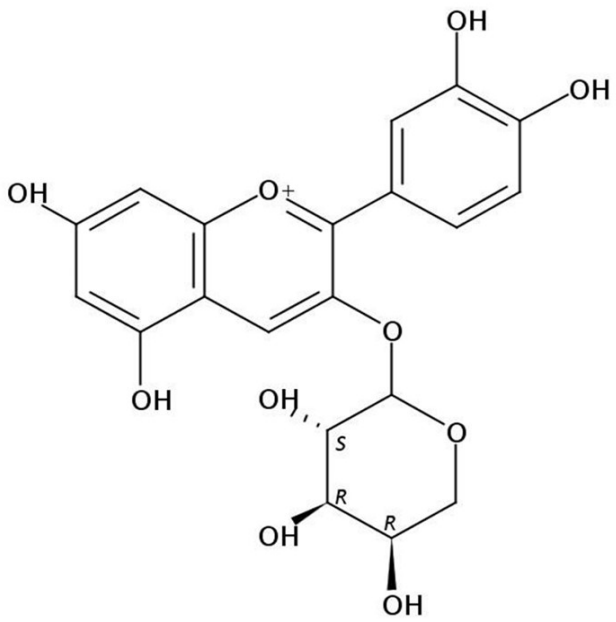
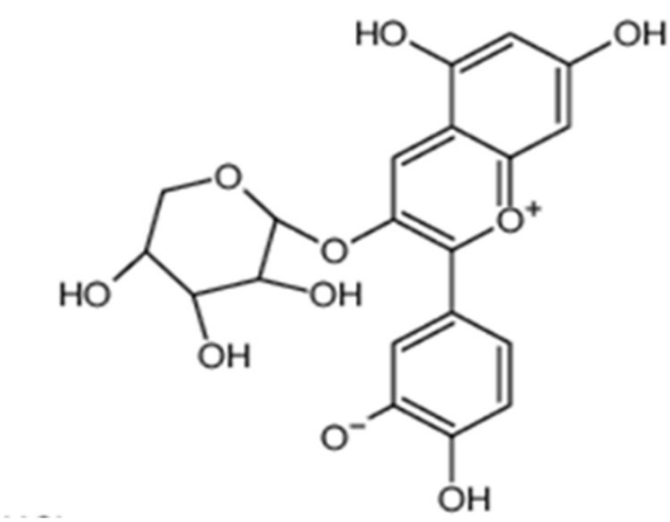


Cyanidin-3-O-arabinoside

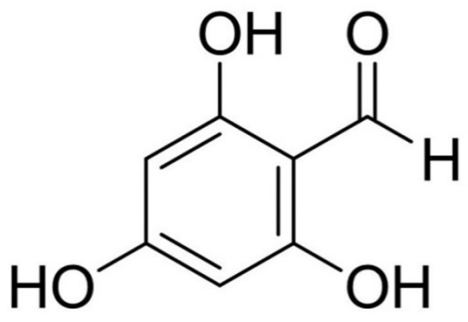


Cyanidin-3-O-xyloside

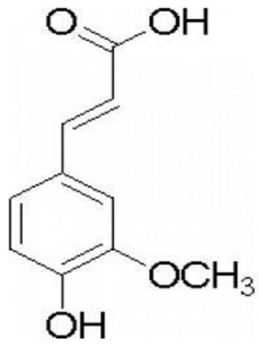
34

35 Fig. S1. Chemical structure of four main anthocyanins in Aronia melanocarpa.

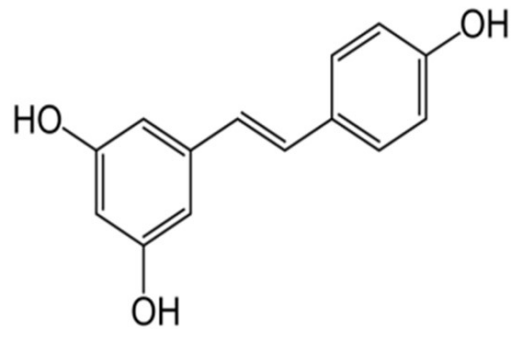
36



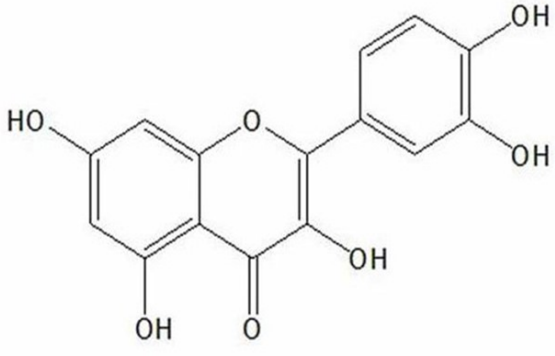
2,4,6-trihydroxybenzaldehyde



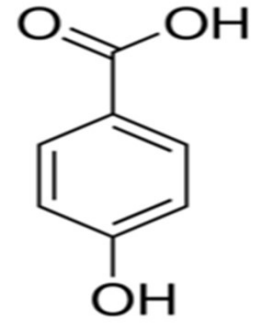
4’-Hydroxy-3’-methoxycinnamic acid



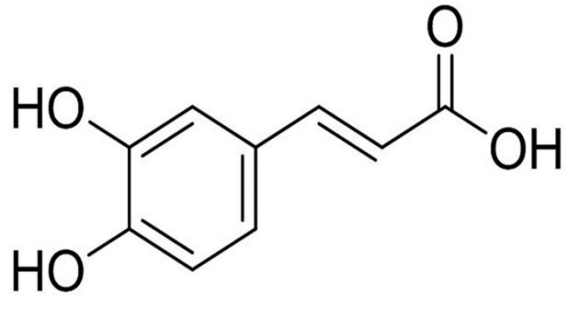
Resveratrol



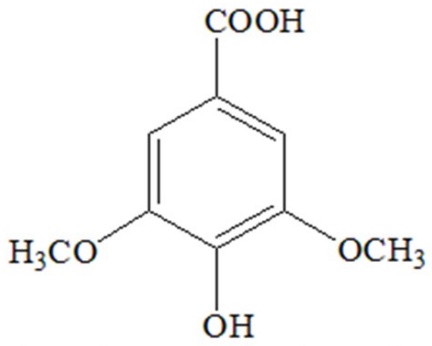
Quercetin



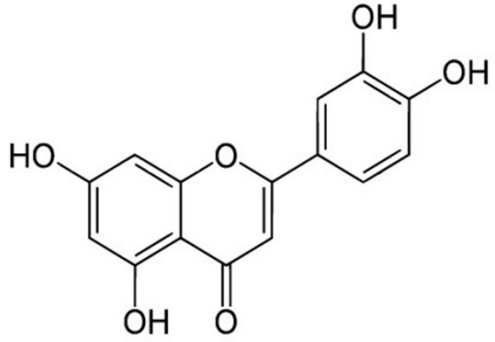
4-Hydroxybenzoic acid



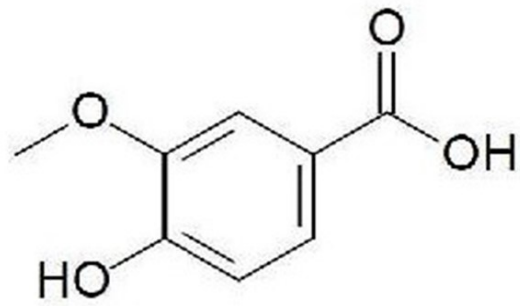
3’,4’-Dihydroxycinnamic acid



3,5-Dimethoxy-4-hydroxybenzoic acid



Luteolin



4-Hydroxy-3-methoxybenzoic acid

37

38 Fig. S2. Chemical structure of twelve main metabolites in in the rat plasma after oral administration.

39

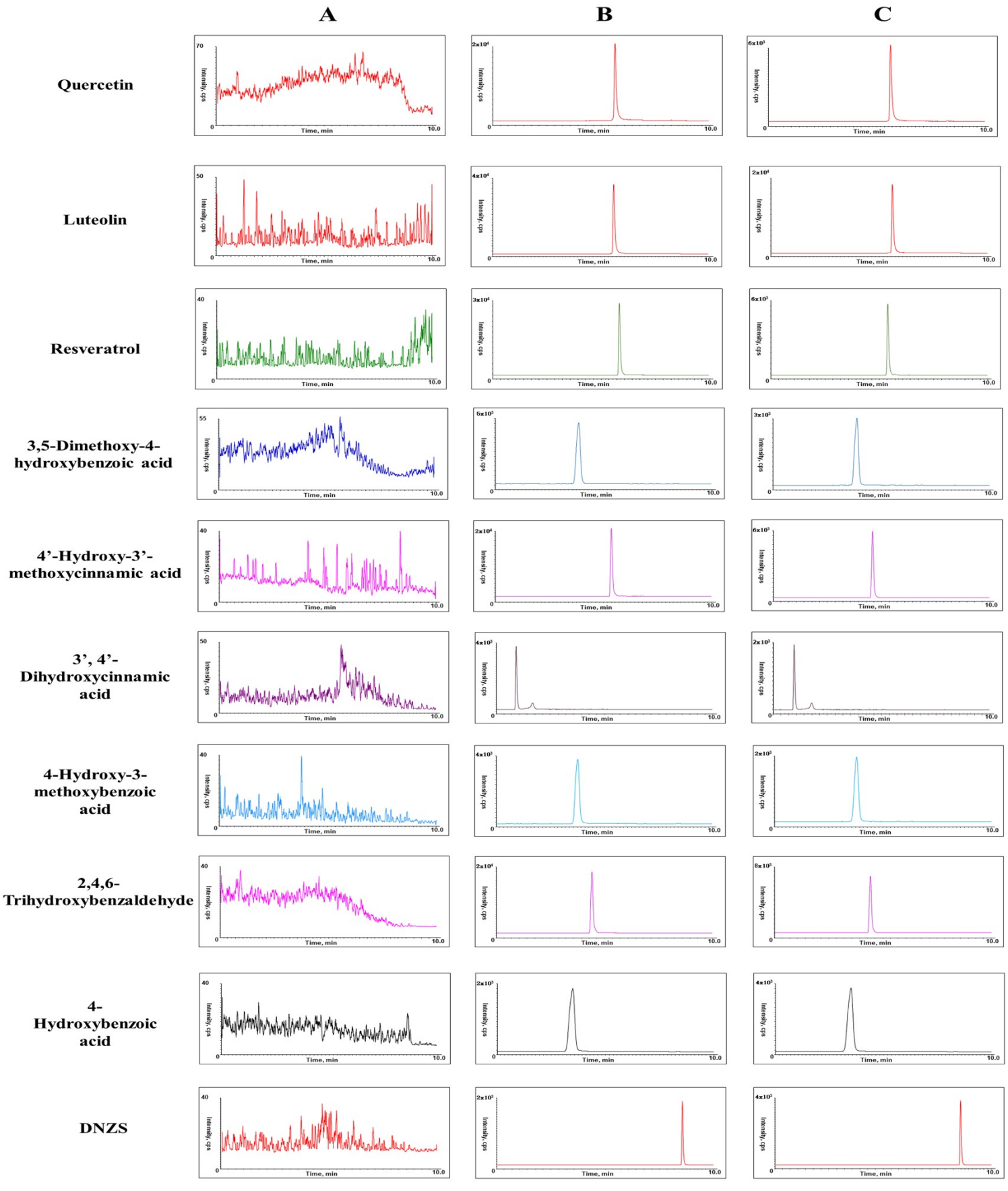

A
B
C
Quercetin
Luteolin
Resveratrol
3,5-Dimethoxy-4-hydroxybenzoic acid
4'-Hydroxy-3'-methoxycinnamic acid
3', 4'-Dihydroxycinnamic acid
4-Hydroxy-3-methoxybenzoic acid
2,4,6-Trihydroxybenzaldehyde
4-Hydroxybenzoic acid
DNZS
Intensity, cps
Time, min
10.0

Fig. S3. Representative chromatograms of (a) blank plasma, (b) blank plasma spiked with analytes and internal solution (IS), and (c) plasma samples obtained after oral administration.

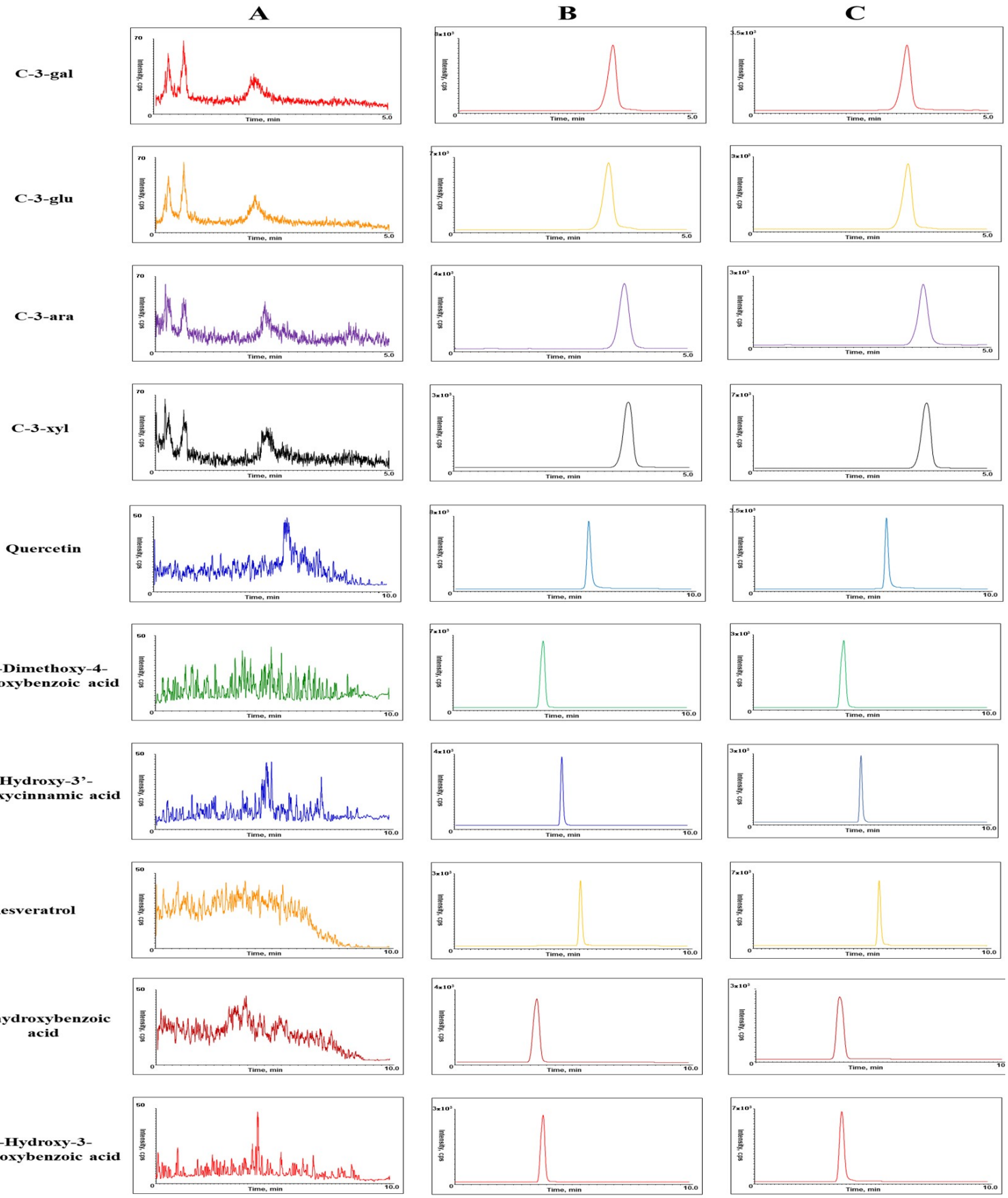
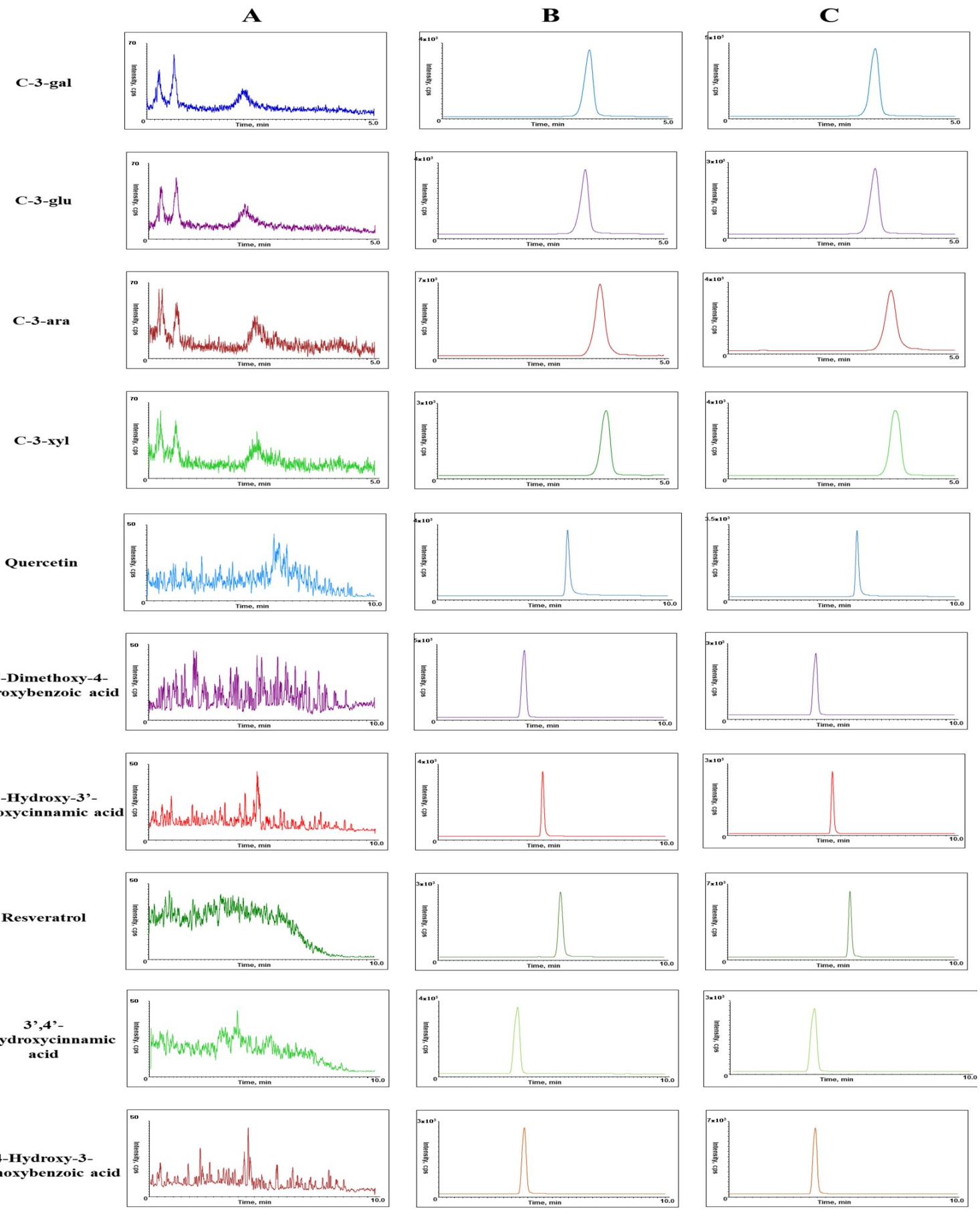
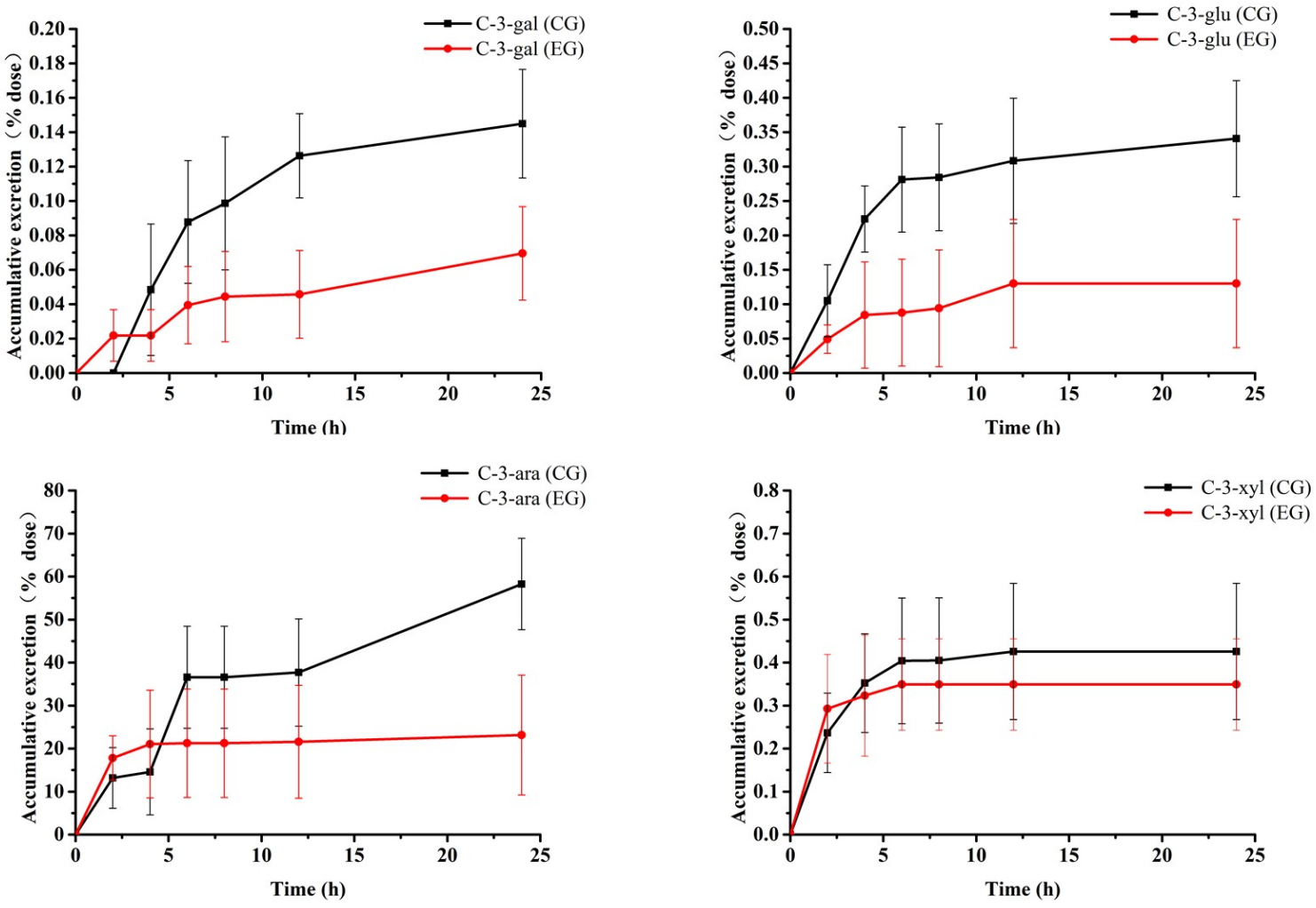

A
B
C
C-3-gal
C-3-glu
C-3-ara
C-3-xyl
Quercetin
3,5-Dimethoxy-4-hydroxybenzoic acid
4'-Hydroxy-3'-methoxycinnamic acid
Resveratrol
4-hydroxybenzoic acid
4-Hydroxy-3-methoxybenzoic acid
Intensity, cps
Time, min

Fig. S4. Representative chromatograms of (a) blank urine, (b) blank urine spiked with analytes, and (c) urine samples obtained after oral administration of anthocyanins.


A
B
C
C-3-gal
C-3-glu
C-3-ara
C-3-xyl
Quercetin
3,5-Dimethoxy-4-hydroxybenzoic acid
4'-Hydroxy-3'-methoxycinnamic acid
Resveratrol
3',4'-Dihydroxycinnamic acid
4-Hydroxy-3-methoxybenzoic acid
Intensity, cps
Time, min

47 Fig. S5. Representative chromatograms of (a) blank feces, (b) blank feces spiked with analytes, and (c) feces samples obtained

48 after oral administration of anthocyanins.



49

50 Fig. S6. Cumulative excretion of four anthocyanins in rat urine after oral administration of anthocyanins (CG) and

51 anthocyanins bound to amylopectin nanoparticles (EG).

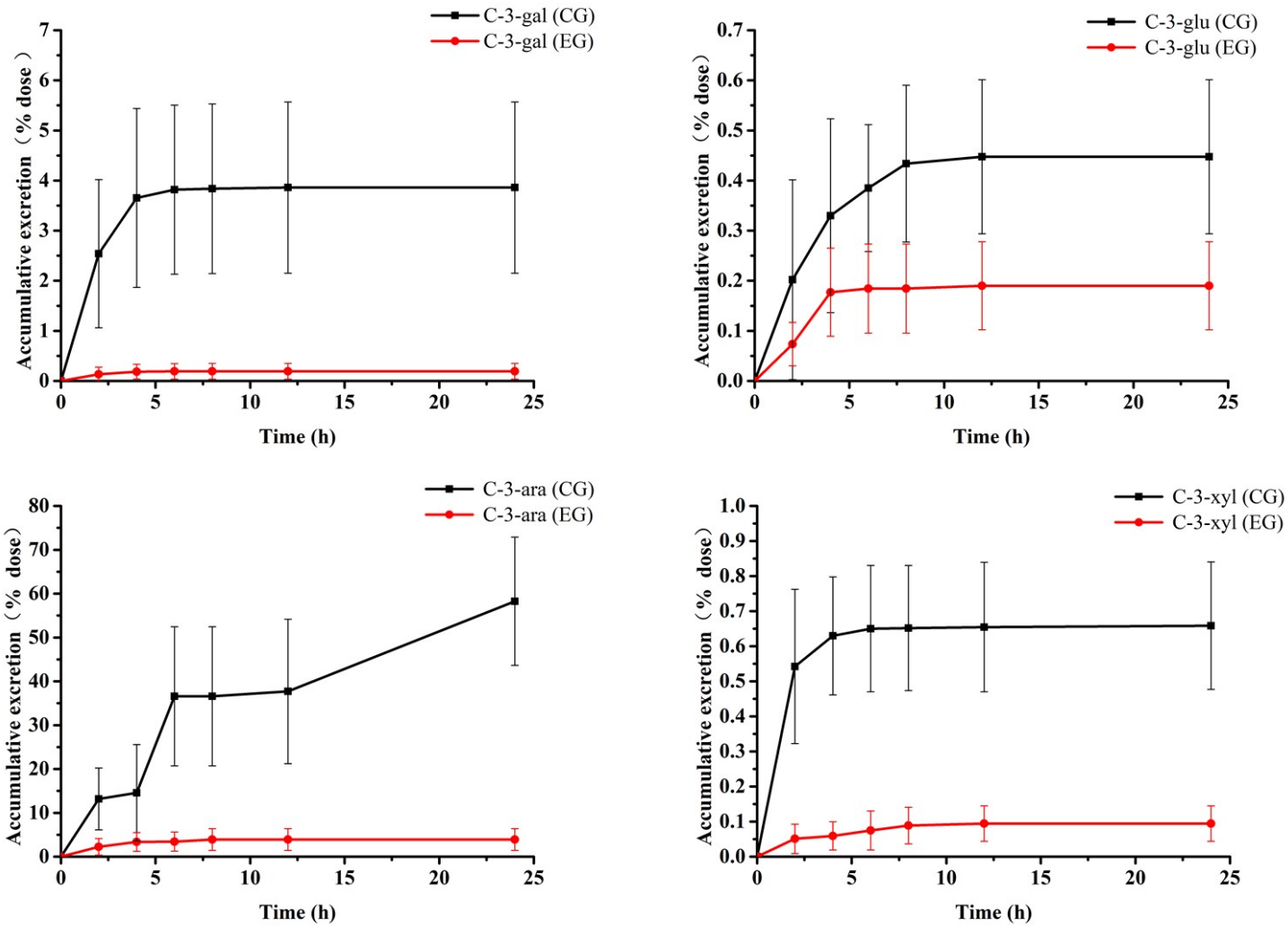
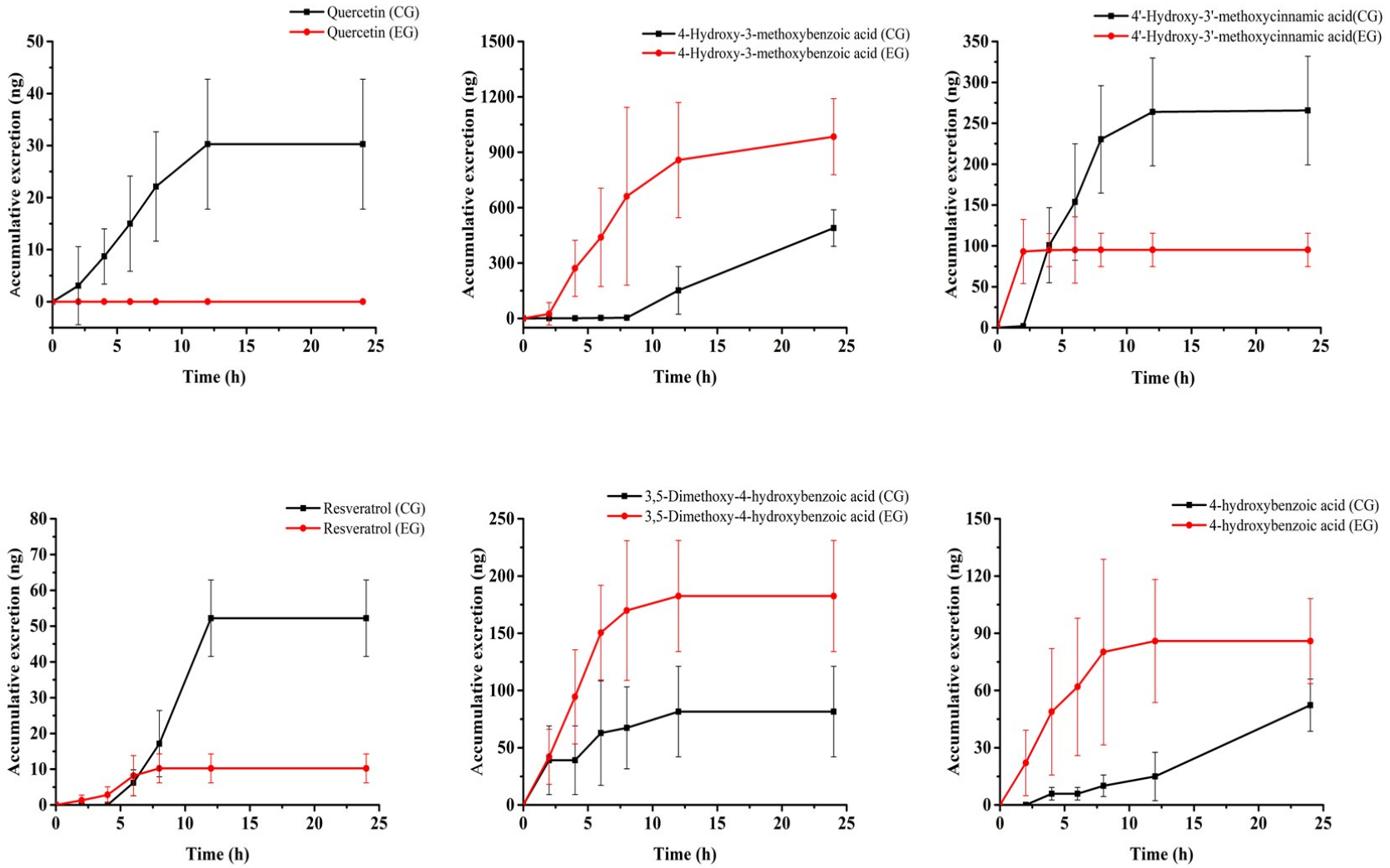


Fig. S7. Cumulative excretion of four anthocyanins in rat feces after oral administration of anthocyanins (CG) and anthocyanins bound to amylopectin nanoparticles (EG).

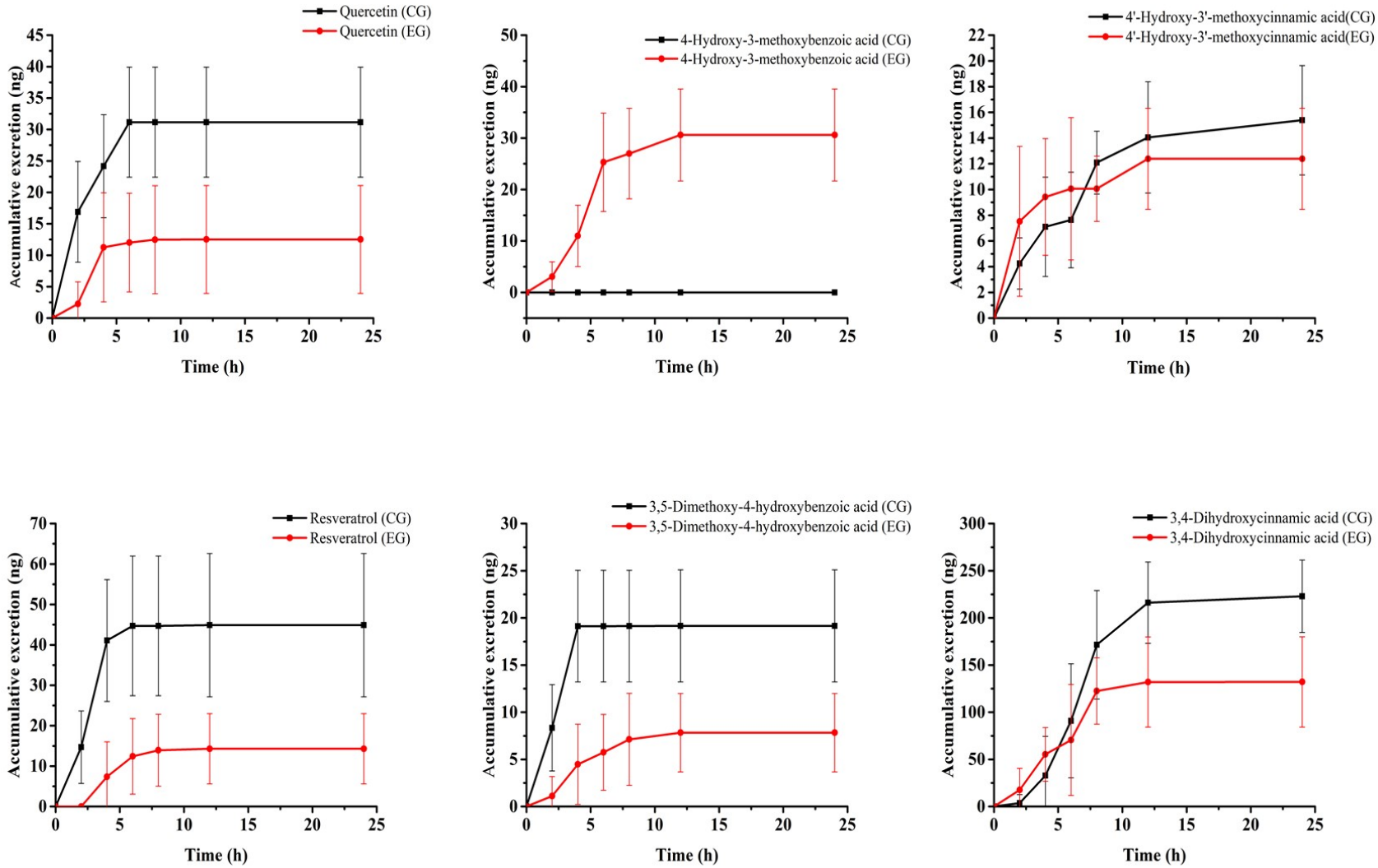


55

56 Fig. S8. Cumulative excretion of six metabolites in rat urine after oral administration of anthocyanins (CG) and anthocyanins

57 bound to amylopectin nanoparticles (EG).

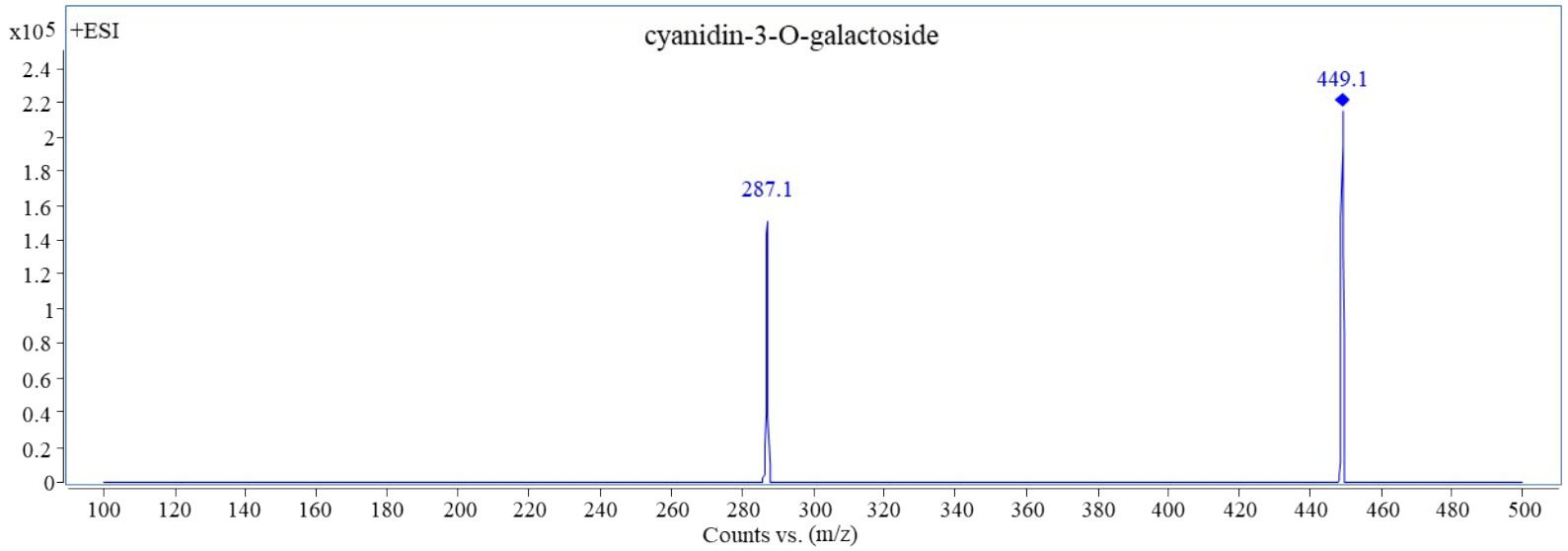
58



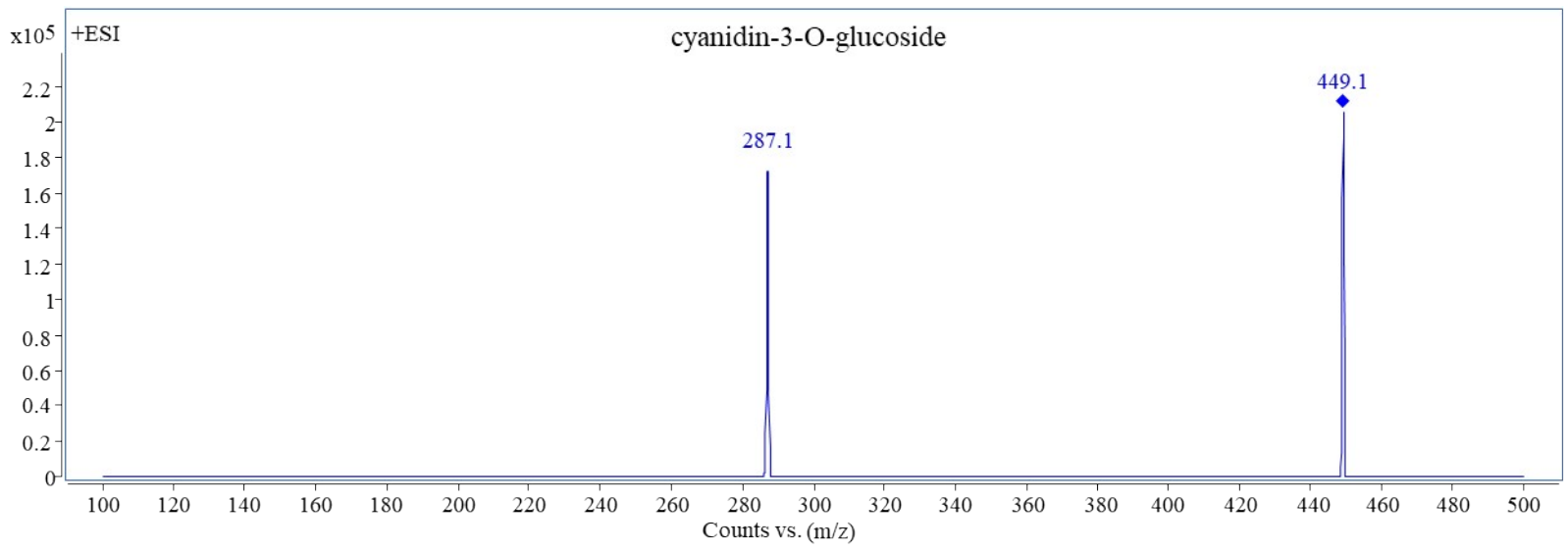
59

60 Fig. S9. Cumulative excretion of six metabolites in rat feces after oral administration of anthocyanins (CG) and anthocyanins

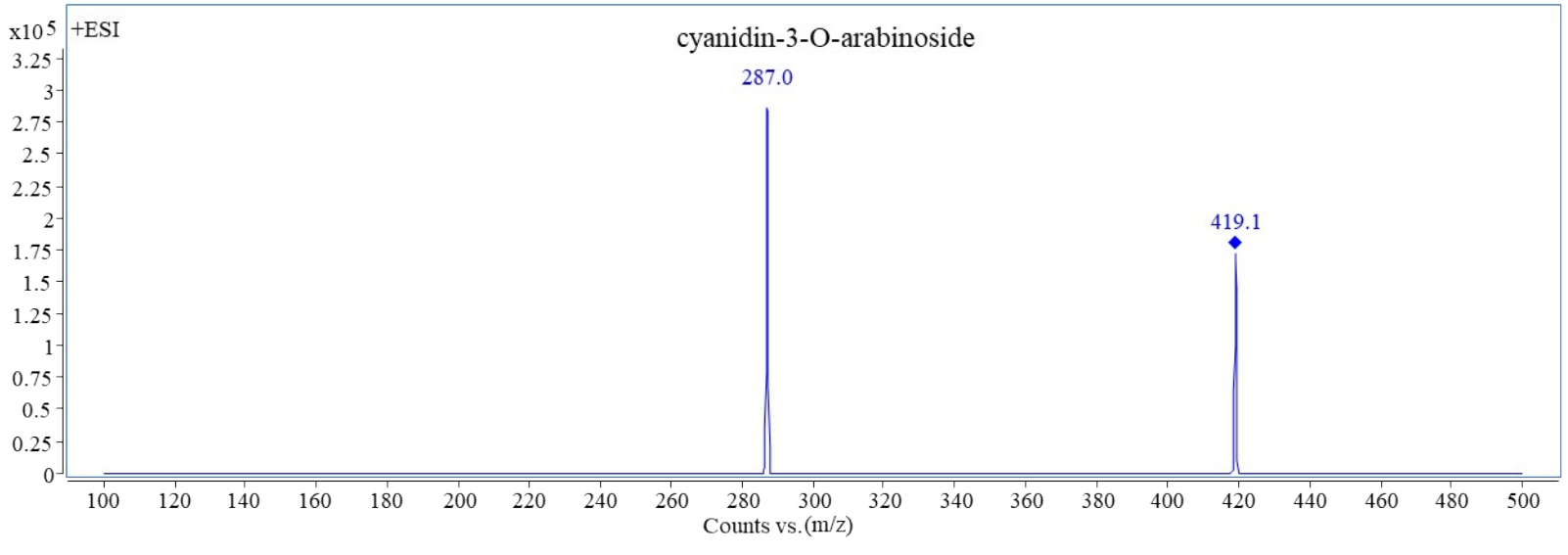
61 bound to amylopectin nanoparticles (EG).


x10^5
+ESI
cyanidin-3-O-galactoside
449.1
287.1
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
Counts vs. (m/z)

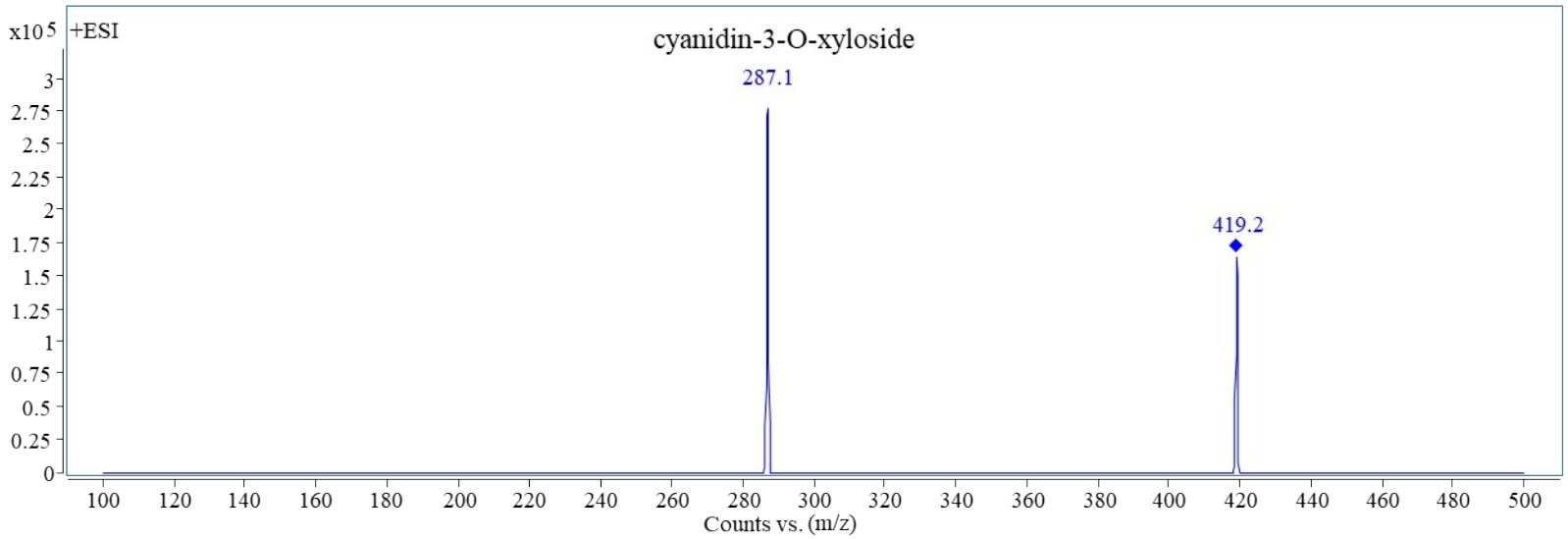
62


x10^5
+ESI
cyanidin-3-O-glucoside
449.1
287.1
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
Counts vs. (m/z)

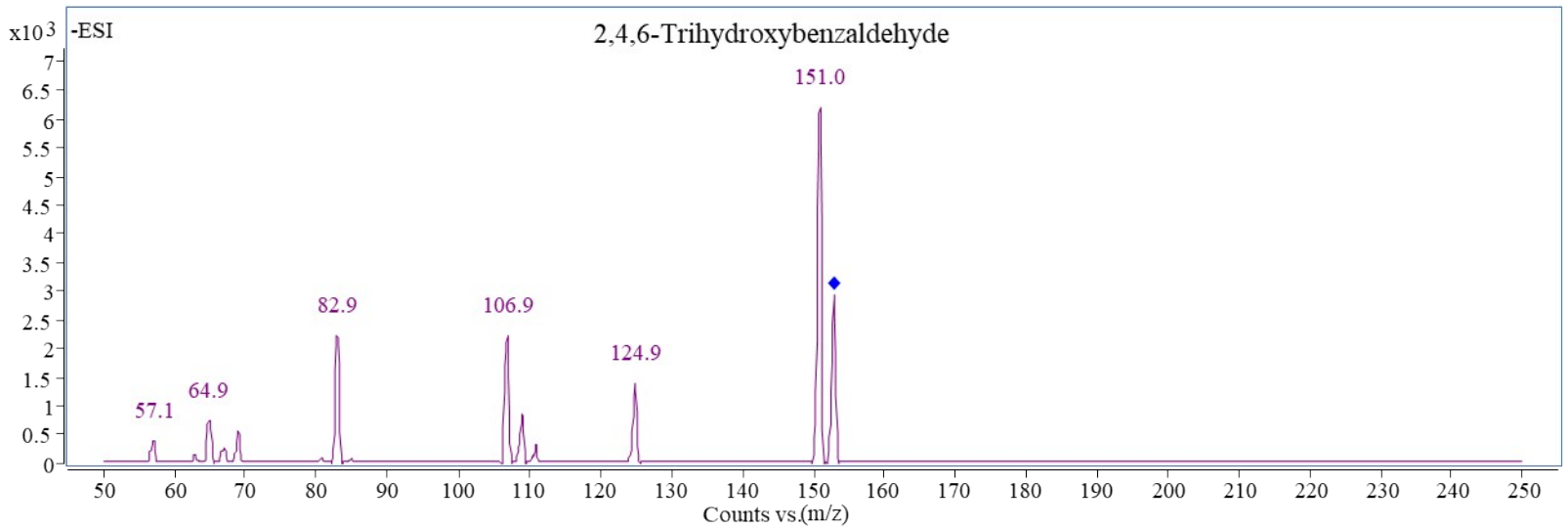
63


x10^5
+ESI
cyanidin-3-O-arabinoside
287.0
419.1
3.25
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500
Counts vs. (m/z)

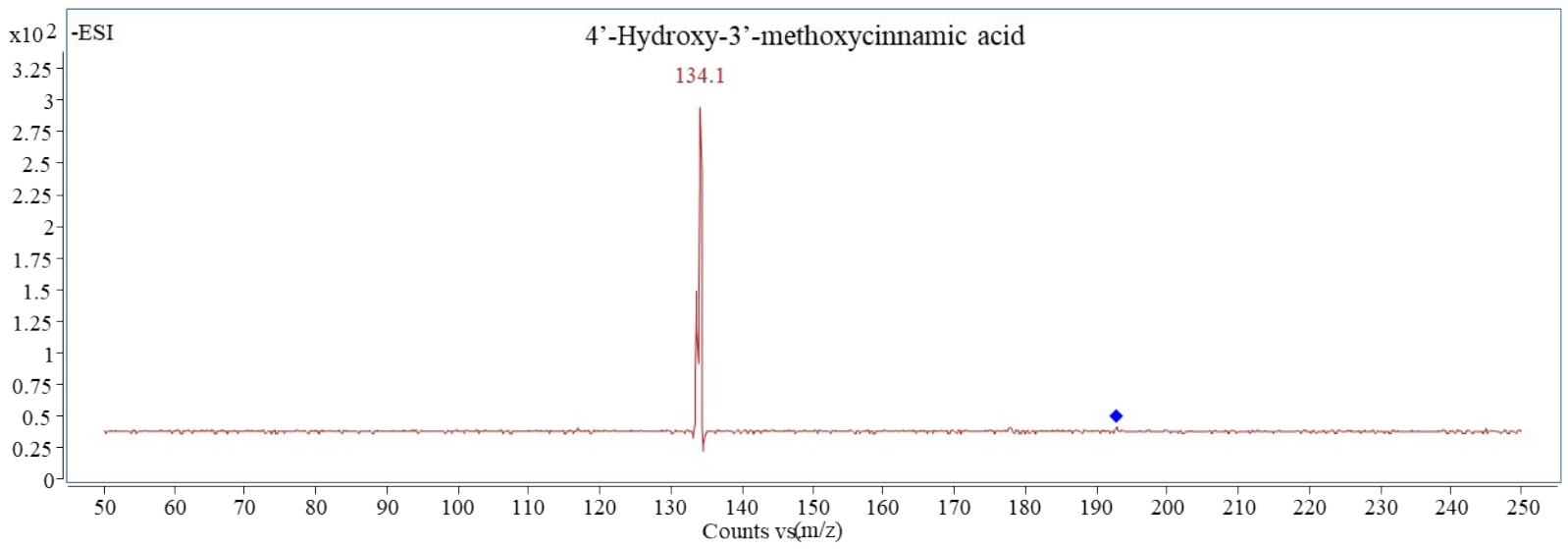
64


x10 5
+ESI
cyanidin-3-O-xyloside
287.1
419.2
Counts vs. (m/z)

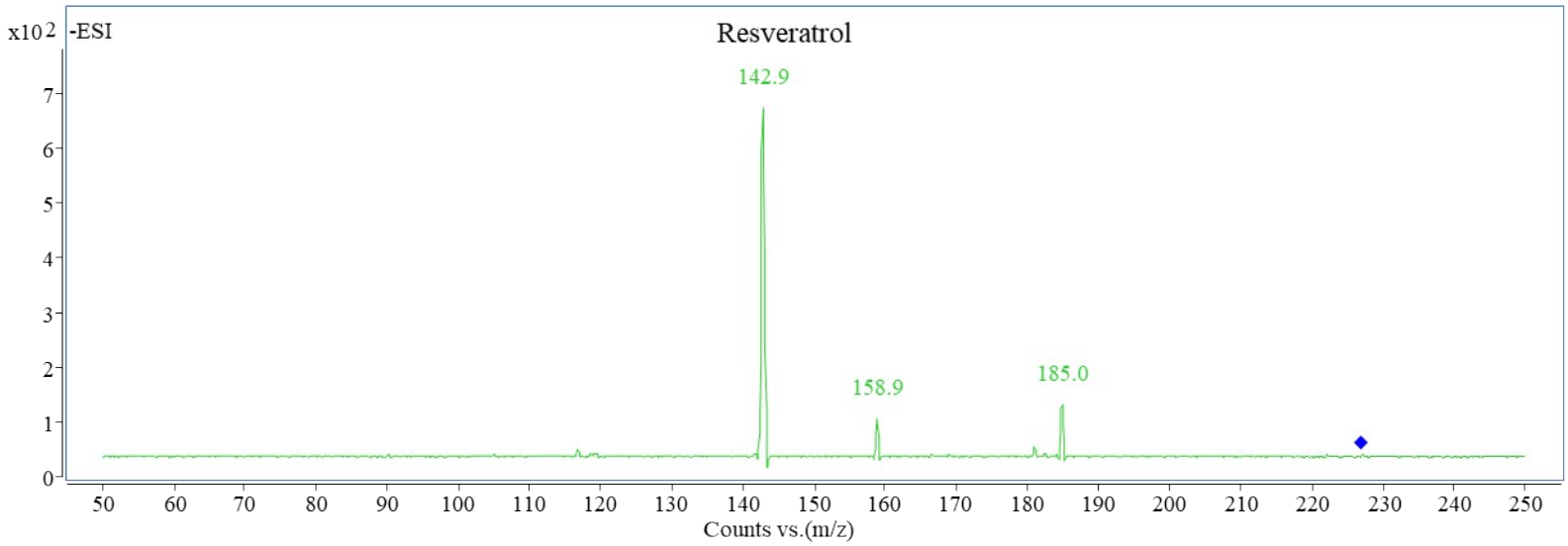
65


x10 3
-ESI
2,4,6-Trihydroxybenzaldehyde
151.0
82.9
106.9
124.9
64.9
57.1
Counts vs.(m/z)

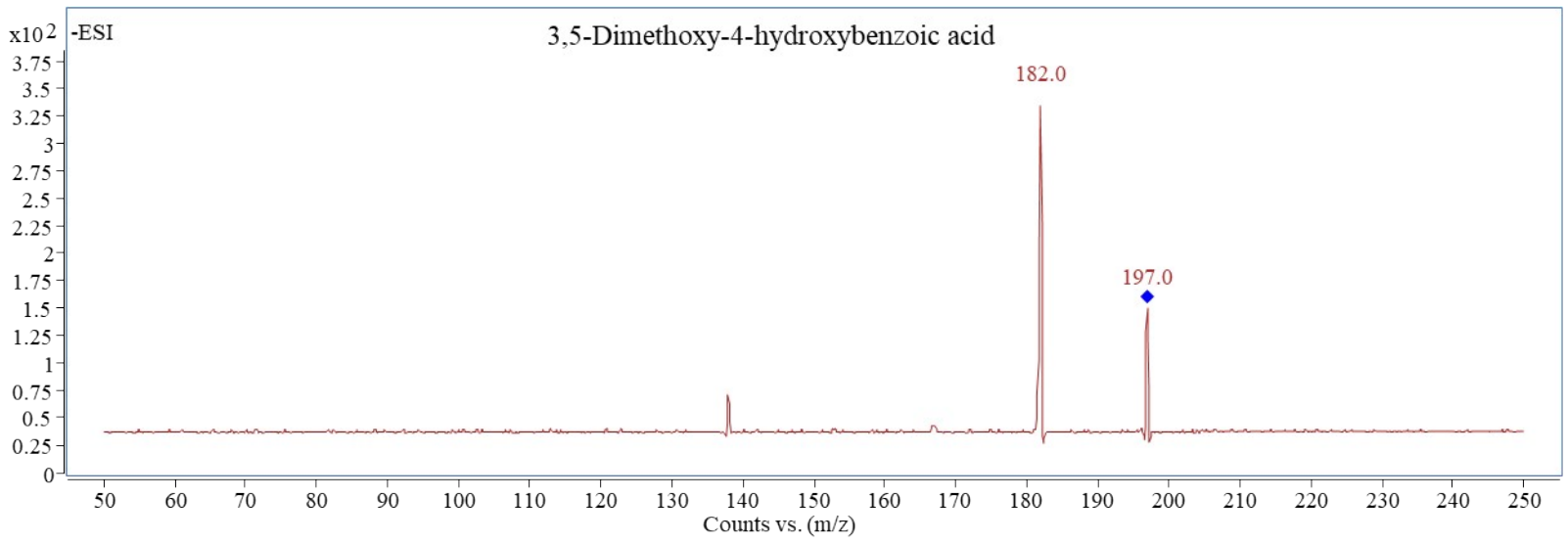
66


x10 2
-ESI
4'-Hydroxy-3'-methoxycinnamic acid
134.1
Counts vs(m/z)

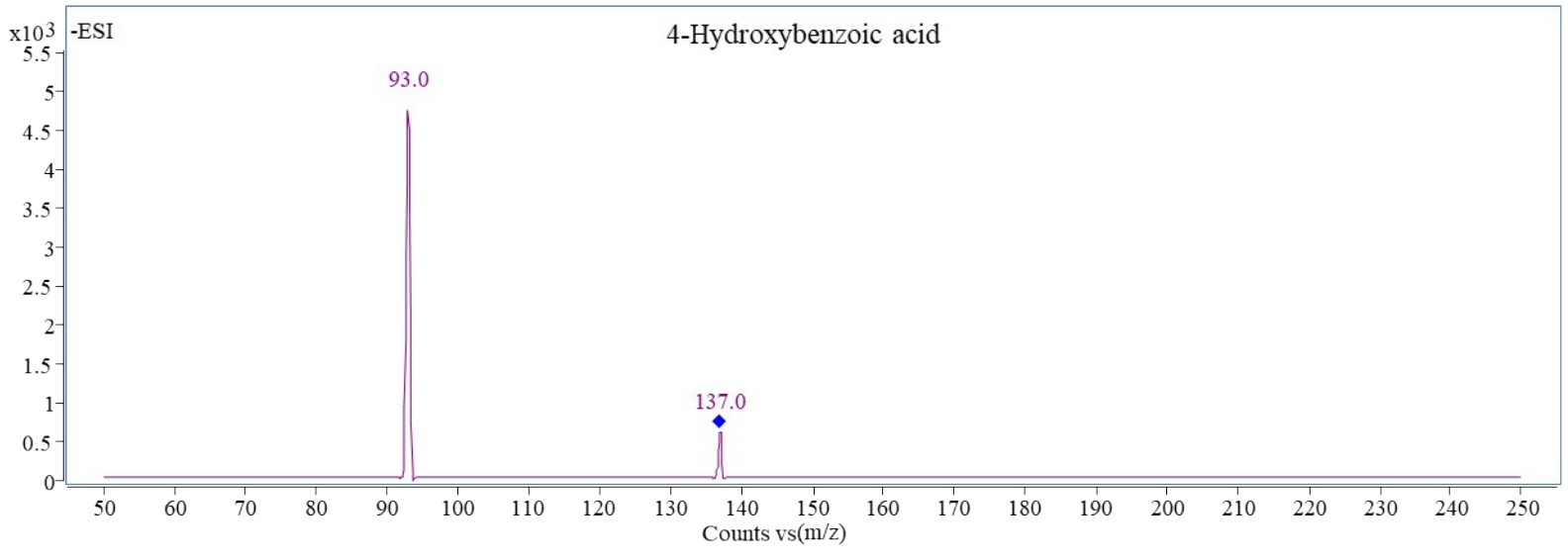
67


x10 2
-ESI
Resveratrol
142.9
158.9
185.0
Counts vs.(m/z)

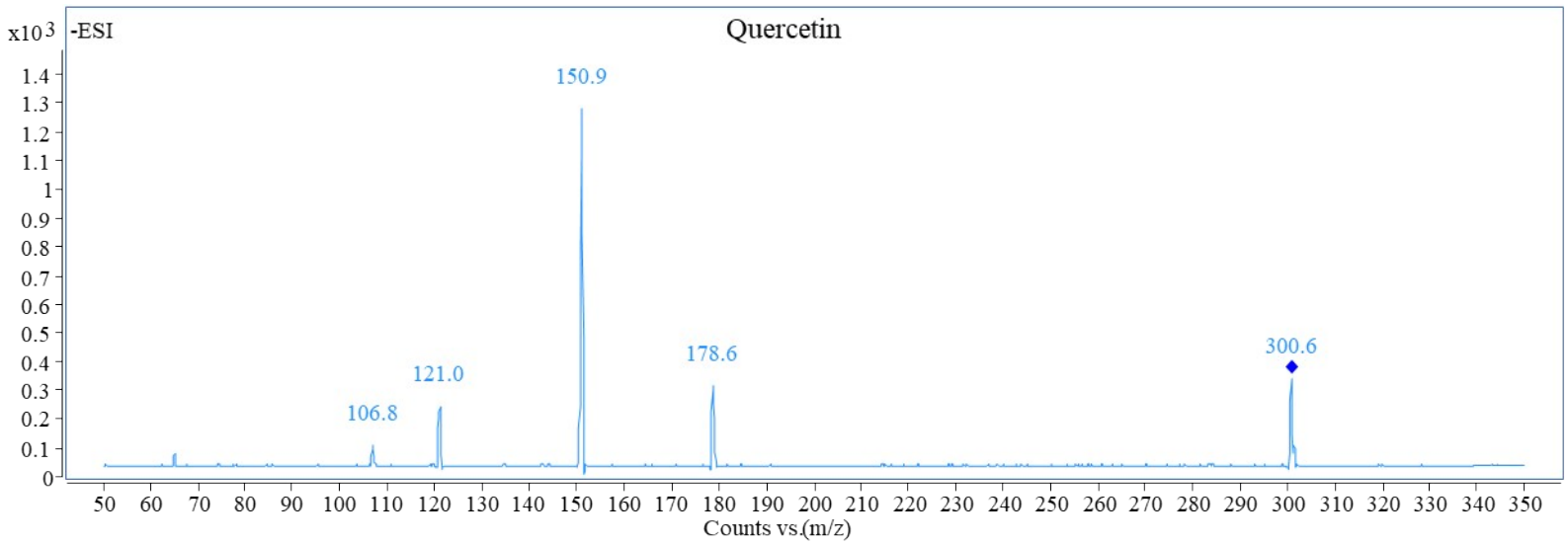
68


x10 2
-ESI
3,5-Dimethoxy-4-hydroxybenzoic acid
182.0
197.0
Counts vs. (m/z)

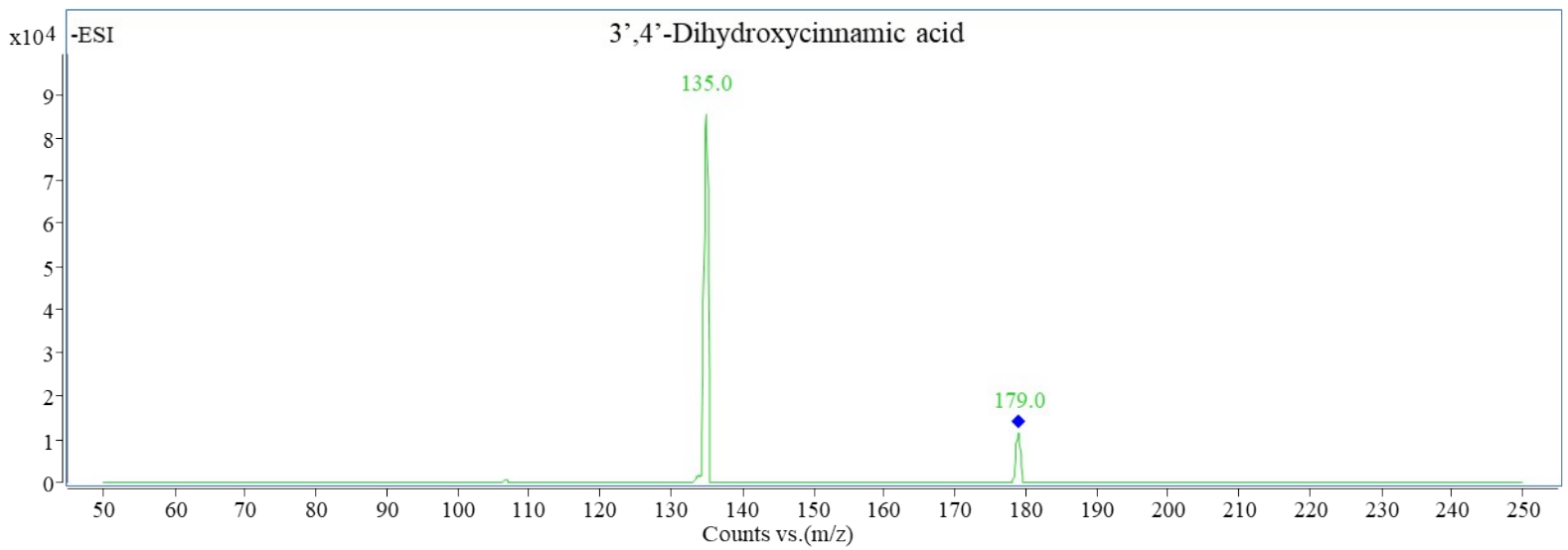
69


x10 3
-ESI
4-Hydroxybenzoic acid
93.0
137.0
Counts vs(m/z)

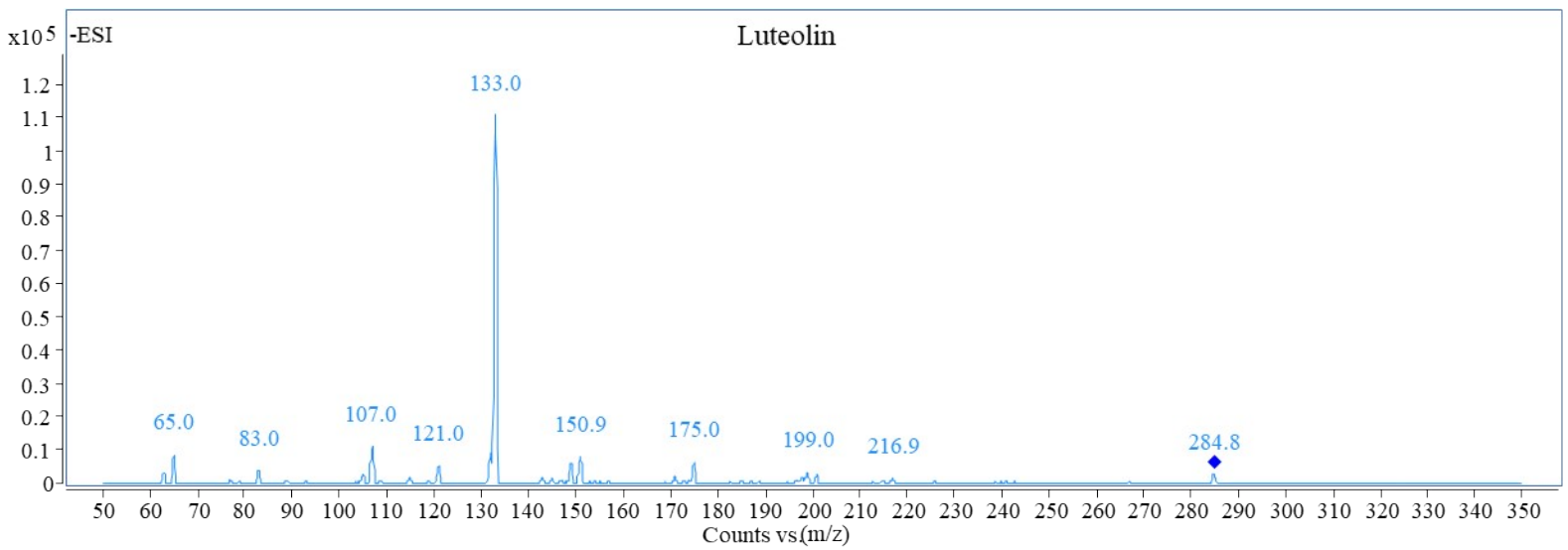
70


x10 3
-ESI
Quercetin
150.9
178.6
121.0
300.6
106.8
Counts vs.(m/z)

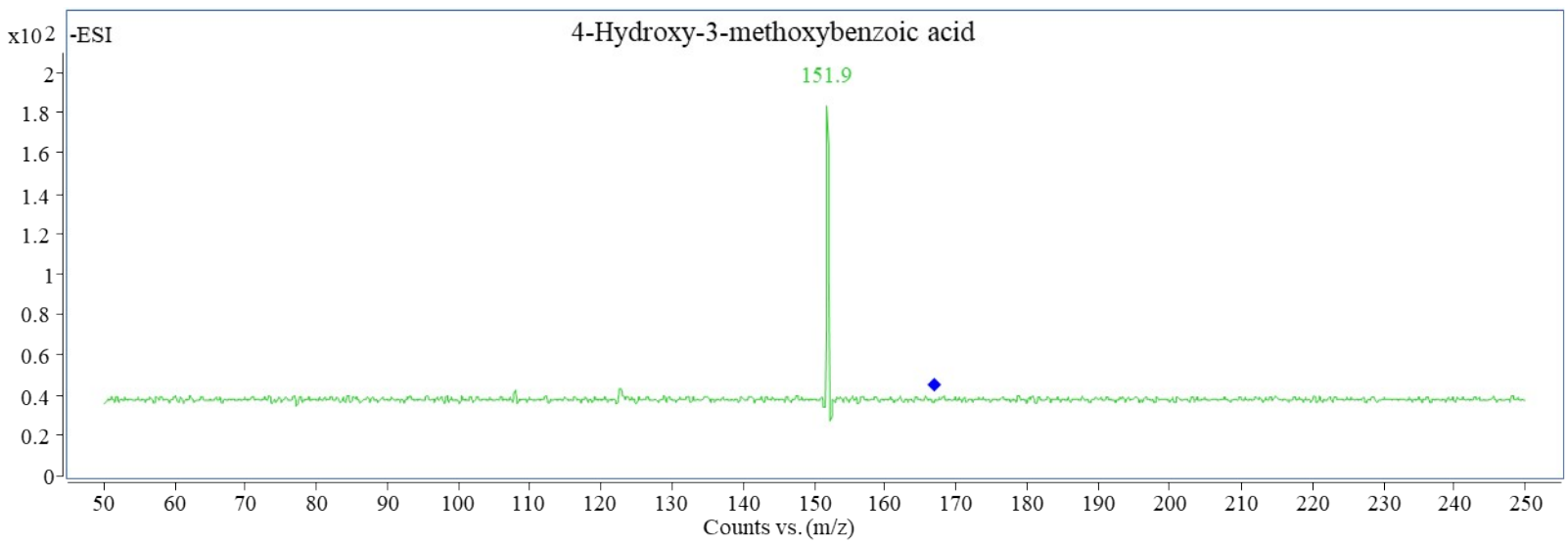
71


x10 4
-ESI
3',4'-Dihydroxycinnamic acid
135.0
179.0
Counts vs.(m/z)

72


x10 5
-ESI
Luteolin
133.0
65.0
83.0
107.0
121.0
150.9
175.0
199.0
216.9
284.8
Counts vs.(m/z)

73



74

75 Fig. S10. The MRM transitions and MS2 spectrum of four Aronia melanocarpa anthocyanins and nine metabolites.

76 Table S1. Dosage of four main anthocyanins in Aronia melanocarpa.

77

Dosage (μg kg-1)	C-3-gal	C-3-glu	C-3-ara	C-3-xyl
Anthocyanins-APNPs	237.5	197.9	88.3	96.5
Anthocyanins	251.6	189.2	101.4	111.2

78 APNPs, amylopectin nanoparticles; C-3-gal, cyanidin-3-O-galactoside; C-3-glu, cyanidin-3-O-glucoside; C-3-ara, cyanidin-3-

79 O-arabinoside; C-3-xyl, cyanidin-3-O-xyloside.

80

81 Table S2. Parameters for the detection of four *Aronia melanocarpa* anthocyanins and nine metabolites.

82

Compounds	MW	MRM (m/z)	tR (min)	Fragmentor (V)	Collision energy (eV)
C-3-gal	449.4	449.1/287	3.302	105	20
C-3-glu	484.8	449/287	3.297	100	20
C-3-ara	454.8	419.3/287	3.714	95	16
C-3-xyl	454.8	419.3/287	3.695	90	16
2,4,6-Trihydroxybenzaldehyde	154	153/151.1	5.113	80	12

4’-Hydroxy-3’-methoxycinnamic acid	194	193/134.1	5.374	80	16
Resveratrol	228	227/143.1	5.850	100	24
3,5-Dimethoxy-4-hydroxybenzoic acid	198	197/182.1	4.685	80	12
4-Hydroxybenzoic acid	138	137/93.1	4.171	70	12
Quercetin	302	301/151.1	6.163	135	16
3’,4’-Dihydroxycinnamic acid	180	179/135.1	4.590	110	12
Luteolin	286	285/133.1	6.135	150	32
4-Hydroxy-3-methoxybenzoic acid	168	167/152.1	4.544	70	12
DNZS (IS)	410	409/351.1	8.701	80	16

MW, molecular weight; MRM, multiple reaction monitoring; tR, retention time; C-3-gal, cyanidin-3-O-galactoside; C-3-glu, cyanidin-3-O-glucoside; C-3-ara, cyanidin-3-O-arabinoside; C-3-xyl, cyanidin-3-O-xyloside; DNZS, a-mangostin (IS, internal solution).

Table S3. Intra- and inter-day accuracy and precision of four *Aronia melanocarpa* anthocyanins in the rat plasma.

Compounds	Concentration (ng mL-1)	Intra-day			Inter-day		
		mean ± SD	RSD (%)	RE (%)	mean ± SD	RSD (%)	RE (%)
C-3-gal	5	4.86±0.34	7.07	-2.71	4.88±0.30	6.14	-2.32
	200	198.07±7.40	3.74	-0.96	189.91±5.52	2.91	-5.04
	20000	19748.58±1475.73	7.47	-1.26	19583.42±1320.13	6.74	-2.08
C-3-glu	5	5.06±0.34	6.65	1.12	5.08±0.18	3.52	1.53
	200	201.13±8.07	4.01	0.56	196.27±8.82	4.49	-1.86
	20000	20514.35±1051.64	5.13	2.57	19927.36±1116.06	5.60	-0.36
C-3-ara	5	4.78±0.27	5.61	-4.35	4.85±0.32	6.49	-2.91

	200	193.32±8.14	4.21	-3.34	191.53±7.99	4.17	-4.24
	20000	20086.42±1189.71	5.92	0.43	19044.05±1051.51	5.52	-4.78
C-3-xyl	5	4.87±0.23	4.76	-2.62	4.89±0.13	2.71	-2.12
	200	190.54±8.30	4.36	-4.73	194.35±8.65	4.45	-2.82
	20000	19200.10±831.49	4.33	-3.99	19407.88±1092.70	5.63	-2.96

89 SD, standard deviation; RSD, relative standard deviation; RE, relative error; C-3-gal, cyanidin-3-O-galactoside; C-3-glu,
90 cyanidin-3-O-glucoside; C-3-ara, cyanidin-3-O-arabinoside; C-3-xyl, cyanidin-3-O-xyloside.

91

92 Table S4. Mean recovery and matrix effects of four *Aronia melanocarpa* anthocyanins in the rat plasma.

93

Compounds	Concentration (ng mL-1)	Recovery (%)	Matrix effect (%)
C-3-gal	5	92.85±1.08	95.93±6.82
	200	88.09±6.04	101.97±9.04
	20000	87.41±8.70	98.88±5.25
C-3-glu	5	94.94±6.71	92.34±6.36
	200	93.26±3.73	100.61±6.07
	20000	87.10±4.45	96.57±3.29
C-3-ara	5	98.75±4.96	92.93±6.13
	200	92.12±9.80	103.70±5.28
	20000	94.71±5.72	93.55±2.35
C-3-xyl	5	94.95±3.69	100.49±9.74
	200	95.09±8.85	96.19±1.94

	20000	88.05±1.97	102.55±9.56

94 C-3-gal, cyanidin-3-O-galactoside; C-3-glu, cyanidin-3-O-glucoside; C-3-ara, cyanidin-3-O-arabinoside; C-3-xyl, cyanidin-3-

95 O-xyloside.

96 Table S5. Stability of four *Aronia melanocarpa* anthocyanins in the rat plasma.

Compounds		C-3-gal			C-3-glu			C-3-ara			C-3-xyl		
Concentration (ng mL-1)		5	200	20000	5	200	20000	5	200	20000	5	200	20000
2 h at 20°C	mean ± SD	5.11±0.26	201.74±9.51	20226.68±1096.21	4.95±0.33	189.02±10.05	19921.42±1128.43	5.09±0.25	194.77±11.21	20339.53±1427.32	4.93±0.34	200.62±14.65	20018.13±1365.91
	RSD (%)	5.13	4.71	5.42	6.74	5.32	5.66	4.97	5.75	7.02	6.94	7.30	6.82
	RE (%)	2.17	0.87	1.13	-0.93	-5.49	-0.39	1.78	-2.62	1.70	-1.41	0.31	0.09
Three freeze (-20°C)-thaw (20°C) cycles	mean ± SD	5.09±0.29	196.58±13.72	18998.81±1332.54	5.05±0.27	197.66±4.93	20481.05±911.94	4.77±0.32	206.73±10.12	19754.13±1398.20	4.92±0.29	195.08±9.24	19764.05±1515.37
	RSD (%)	5.74	6.98	7.01	5.31	2.49	4.45	6.81	4.89	7.08	5.85	4.73	7.67
	RE (%)	1.85	-1.71	-5.01	1.09	-1.17	2.41	-4.57	3.63	-1.23	-1.62	-2.46	-1.18
1 month at -80°C	mean ± SD	4.93±0.25	204.21±9.73	19867.66±1087.01	4.79±0.37	203.29±12.95	20007.82±1178.09	5.10±0.26	195.62±10.33	20503.28±952.59	5.07±0.22	186.41±4.84	20760.02±1347.26
	RSD (%)	5.03	4.76	5.47	7.66	6.37	5.89	5.02	5.28	4.65	4.42	2.60	6.49
	RE (%)	-1.46	2.11	-0.66	-4.29	1.65	0.04	2.07	-2.19	2.52	1.44	-6.80	3.80
12 h in autosampler vials	mean ± SD	4.84±0.45	195.00±7.47	19334.82±841.66	5.13±0.26	199.79±12.25	19449.58±1156.36	4.89±0.33	192.61±9.94	20881.51±1046.80	4.95±0.16	191.96±6.38	19801.21±1123.48
	RSD (%)	9.21	3.83	4.35	5.11	6.13	5.95	6.79	5.16	5.01	3.25	3.33	5.67
	RE (%)	-3.15	-2.50	-3.33	2.52	-0.11	-2.75	-2.11	-3.70	4.41	-1.06	-4.02	-0.99

97 SD, standard deviation; RSD, relative standard deviation; RE, relative error; C-3-gal, cyanidin-3-O-galactoside; C-3-glu,
98 cyanidin-3-O-glucoside; C-3-ara, cyanidin-3-O-arabinoside; C-3-xyl, cyanidin-3-O-xyloside.

99

100 Table S6 Lower limits of quantification (LLOQs), linear ranges, calibration curves, and correlation coefficients (*r*) of four
101 *Aronia melanocarpa* anthocyanins and 6 metabolites in the rat urine.

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Compounds	LLOQ (μg·mL-1)	linear ranger (μg·mL-1)	Linear regression equation	Correlation coefficient(r)
C-3-gal	0.0024	0.0024-24.000	y=2.5312x+0.0292	0.9971
C-3-glu	0.0024	0.0024-24.000	y=5.7492x-0.0029	0.9980
C-3-ara	0.0024	0.0024-24.000	y=1.3617x+0.0103	0.9952
C-3-xyl	0.0024	0.0024-24.000	y=6.7870x+0.0009	0.9939
Quercetin	0.025	0.025-13.750	y=0.0854x-0.0107	0.9966

3,5-Dimethoxy-4-hydroxybenzoic acid	0.064	0.064-4.060	y=0.0149x+0.0228	0.9971
4’-Hydroxy-3’-methoxycinnamic acid	0.064	0.064-4.060	y=0.0771x+0.0060	0.9929
Resveratrol	0.098	0.098-6.250	y=0.0535x-0.0027	0.9960
4-Hydroxybenzoic acid	0.029	0.029-3.750	y=0.1687x-0.0081	0.9952
4-Hydroxy-3-methoxybenzoic acid	0.066	0.0659-8.438	y=0.0257x+0.0119	0.9987

Table S7 Lower limits of quantification (LLOQs), linear ranges, calibration curves, and correlation coefficients (*r*) of four *Aronia melanocarpa* anthocyanins and 6 metabolites in the rat feces.

Compounds	LLOQ (ng·mL-1)	linear ranger (μg·mL -1)	Linear regression equation	Correlation coefficient(r)
C-3-gal	2	0.0024-24.000	y=1.9478x-0.0013	0.9967
C-3-glu	2	0.0024-24.000	y=3.6455x-0.0057	0.9991
C-3-ara	2	0.0024-24.000	y=1.2431x+0.0229	0.9933
C-3-xyl	2	0.0024-24.000	y=5.7538x+0.0116	0.9982
Quercetin	10	0.025-13.750	y=0.1043x-0.0365	0.9990
3,5-Dimethoxy-4-hydroxybenzoic acid	50	0.064-4.060	y=0.0378x+0.0222	0.9962
4’-Hydroxy-3’-methoxycinnamic acid	50	0.064-4.060	y=0.0235x+0.0341	0.9981
Resveratrol	50	0.098-6.250	y=0.0671x+0.0078	0.9939
3’,4’-Dihydroxycinnamic acid	50	0.053-6.875	y=0.1936x-0.0023	0.9973
4-Hydroxy-3-methoxybenzoic acid	50	0.0659-8.438	y=1.0678x-0.0117	0.9988

Table S8. Intra- and inter-day accuracy and precision of four *Aronia melanocarpa* anthocyanins in the urine.

Compounds	Concentration (ng mL-1)	Intra-day			Inter-day		
		mean ± SD	RSD (%)	RE (%)	mean ± SD	RSD (%)	RE (%)
C-3-gal	5	4.95±0.27	5.47	-0.97	4.92±0.30	6.15	-1.58

	200	194.91±12.54	6.43	-2.54	202.98±11.20	5.55	1.49
	20000	19796.36±773.39	3.91	-1.02	20190.55±811.37	4.02	0.95
C-3-glu	5	5.11±0.22	4.21	2.24	4.80±0.23	4.80	-3.99
	200	193.96±10.86	5.60	-3.02	194.89±13.27	6.81	-2.57
	20000	19699.81±931.72	4.73	-1.50	20760.00±1219.01	5.87	3.80
C-3-ara	5	4.88±0.20	4.12	-2.38	5.07±0.27	5.39	1.48
	200	205.52±8.72	4.24	2.76	196.72±11.40	5.79	-1.64
	20000	19977.66±1341.14	6.71	-0.11	19877.35±1027.39	5.17	-0.61
C-3-xyl	5	4.94±0.30	6.16	-1.29	4.88±0.34	7.03	-2.35
	200	203.45±10.70	5.26	1.73	192.59±8.78	4.56	-3.71
	20000	20992.13±1049.99	5.00	4.96	19858.61±1446.53	7.28	-0.71

Table S9. Mean recovery and matrix effects of four *Aronia melanocarpa* anthocyanins in the urine.

Compounds	Concentration (ng mL-1)	Recovery (%)	Matrix effect (%)
C-3-gal	5	92.10±3.19	95.93±5.55
	200	100.22±2.45	97.12±8.03
	20000	89.73±4.16	93.03±6.35
C-3-glu	5	95.02±5.33	93.66±1.94
	200	102.70±2.34	91.17±7.57
	20000	100.13±5.48	104.55±1.77
C-3-ara	5	92.20±8.88	99.30±9.11
	200	86.22±4.68	103.81±9.81
	20000	99.67±5.05	98.42±3.77
C-3-xyl	5	101.97±2.83	101.35±9.73
	200	94.78±8.43	97.94±6.86
	20000	101.99±8.95	85.14±2.75

115 Table S10. Stability of four *Aronia melanocarpa* anthocyanins in the urine.

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Compounds		C-3-gal			C-3-glu			C-3-ara			C-3-xyl		
Concentration (ng mL-1)		5	200	20000	5	200	20000	5	200	20000	5	200	20000
2 h at 20°C	mean ± SD	4.85±0.21	201.68±13.18	20081.45±1027.69	4.88±0.28	194.95±5.36	20046.70±1465.76	5.27±0.15	201.23±15.37	20206.13±1176.88	5.14±0.19	208.86±10.65	20393.58±1059.64
	RSD (%)	4.33	6.54	5.12	5.75	2.75	7.31	2.85	7.64	5.82	3.69	5.10	5.20
	RE (%)	-3.04	0.84	0.41	-2.40	-2.53	0.23	5.49	0.62	1.03	2.78	4.43	1.97
Three freeze (-20°C)-thaw (20°C) cycles	mean ± SD	4.73±0.23	199.45±15.02	20248.30±1240.64	4.99±0.32	195.00±14.90	20437.98±1325.19	5.11±0.35	205.73±11.98	21044.93±981.32	5.00±0.39	202.21±7.96	20286.68±916.31
	RSD (%)	4.77	7.53	6.13	6.38	7.64	6.48	6.80	5.82	4.66	7.88	3.94	4.52
	RE (%)	-5.38	-0.27	1.24	-0.14	-2.50	2.19	2.11	2.87	5.22	0.05	1.11	1.43
1 month at -80°C	mean ± SD	5.09±0.23	199.02±7.23	19663.92±1411.43	4.92±0.34	197.99±12.20	19922.51±935.68	4.88±0.37	202.03±9.85	19918.28±1054.10	5.01±0.24	201.33±13.52	19178.38±720.04
	RSD (%)	4.57	3.63	7.18	6.82	6.16	4.70	7.61	4.87	5.29	4.82	6.71	3.75
	RE (%)	1.71	-0.49	-1.68	-1.61	-1.00	-0.39	-2.50	1.01	-0.41	0.29	0.67	-4.11
12 h in autosampler vials	mean ± SD	4.84±0.25	197.83±8.49	21114.39±179.77	4.84±0.28	200.69±12.95	20512.35±1244.53	5.23±0.23	196.10±6.96	19688.10±1468.37	4.99±0.28	193.33±8.02	19728.02±1080.71
	RSD (%)	5.07	4.29	8.51	5.82	6.45	6.07	4.31	3.55	7.46	5.63	4.15	5.48
	RE (%)	-3.20	-1.08	5.57	-3.28	0.35	2.56	4.62	-1.95	-1.56	-0.14	-3.34	-1.36

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118 Table S11. Intra- and inter-day accuracy and precision of four *Aronia melanocarpa* anthocyanins in the feces.

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Compounds	Concentration (ng mL-1)	Intra-day			Inter-day		
		mean ± SD	RSD (%)	RE (%)	mean ± SD	RSD (%)	RE (%)
C-3-gal	5	5.16±0.22	4.32	3.27	4.88±0.12	2.47	-2.43
	200	195.42±13.08	6.69	-2.29	204.20±14.84	7.27	2.10
	20000	21047.29±1437.12	6.83	5.24	19502.93±1085.39	5.57	-2.49
C-3-glu	5	4.92±0.30	0.06	-1.60	5.22±0.20	3.74	4.34
	200	198.23±13.52	6.82	-0.89	201.25±15.45	7.68	0.63

	20000	19661.03±731.50	3.72	-1.69	20620.12±617.12	2.99	3.10
C-3-ara	5	5.03±0.32	6.89	0.69	4.94±0.37	7.54	-1.16
	200	206.46±10.96	5.31	3.23	195.33±13.46	6.89	-2.33
	20000	20716.64±1255.06	6.06	3.58	21063.28±987.40	4.69	5.32
C-3-xyl	5	4.81±0.19	3.90	-3.8	5.04±0.31	6.18	0.71
	200	206.88±12.12	5.86	3.44	200.15±13.18	6.59	0.08
	20000	20104.61±1028.67	5.12	0.52	20354.42±1027.65	5.05	1.77

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121 Table S12. Mean recovery and matrix effects of four *Aronia melanocarpa* anthocyanins in the feces.

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Compounds	Concentration (ng mL-1)	Recovery (%)	Matrix effect (%)
C-3-gal	5	89.92±9.14	100.90±9.73
	200	93.49±4.76	99.58±2.48
	20000	103.65±9.55	96.80±1.50
C-3-glu	5	97.85±6.16	93.41±9.18
	200	92.74±8.82	92.57±3.92
	20000	86.78±3.80	96.52±4.87
C-3-ara	5	101.57±2.68	101.65±9.30
	200	99.79±8.03	95.77±8.72
	20000	87.48±6.05	86.43±9.19
C-3-xyl	5	89.29±9.69	90.19±1.21
	200	88.02±6.99	94.81±8.63
	20000	102.08±3.42	94.21±4.63

123

124 Table S13. Stability of four *Aronia melanocarpa* anthocyanins in the feces.

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Compounds	C-3-gal	C-3-glu	C-3-ara	C-3-xyl

Concentration (ng mL-1)		5	200	20000	5	200	20000	5	200	20000	5	200	20000
2 h at 20°C	mean ± SD	5.19±0.32	196.96±12.30	20242.23±1312.36	5.14±0.28	202.97±5.26	20171.39±1224.34	5.16±0.19	202.42±12.14	20811.02±878.58	4.83±0.25	195.90±13.03	20456.65±1273.25
	RSD (%)	6.16	6.24	6.48	5.47	2.59	6.01	3.64	6.00	4.22	5.26	6.65	6.22
	RE (%)	3.74	-1.52	1.21	2.86	1.49	0.86	3.20	1.21	4.06	-3.47	-2.05	2.28
Three freeze (-20°C)-thaw (20°C) cycles	mean ± SD	4.83±0.30	190.73±10.62	20728.07±1408.78	5.04±0.34	193.61±11.35	20735.41±1028.50	5.08±0.36	202.26±10.56	19616.05±900.37	4.97±0.40	203.69±11.30	19004.11±567.71
	RSD (%)	6.14	5.57	6.80	6.72	5.86	4.96	7.06	5.22	4.59	8.02	5.55	2.99
	RE (%)	-3.40	-4.64	3.64	0.78	-3.19	3.68	1.53	1.13	-1.92	-0.50	1.84	-4.97
1 month at -80°C	mean ± SD	4.82±0.27	196.08±11.46	20037.00±1635.22	4.88±0.35	190.48±8.03	19796.55±990.81	4.97±0.36	197.10±10.46	19471.05±1321.33	4.94±0.20	207.54±9.83	19784.40±849.11
	RSD (%)	5.53	5.84	8.16	7.15	4.21	5.00	7.30	5.31	6.79	4.02	4.74	4.29
	RE (%)	-3.59	-1.96	0.19	-2.31	-4.76	-1.02	-0.65	-1.45	-2.64	-1.21	3.77	-1.08
12 h in autosampler vials	mean ± SD	4.82±0.34	191.08±10.71	19532.43±669.95	4.94±0.35	204.65±13.36	20401.66±1755.95	4.96±0.24	194.56±7.71	19993.79±1286.07	5.06±0.30	193.27±7.22	19601.33±1396.01
	RSD (%)	7.01	5.60	3.43	7.18	6.53	8.61	4.80	3.96	6.43	5.93	3.74	7.12
	RE (%)	-3.55	-4.46	-2.34	-1.24	3.23	2.01	-0.71	-2.72	-0.03	1.23	-3.37	-1.99

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