Recovering Phytochemicals from a Brewery By-product: A Sustainable Reuse Proposal Using a Lactic Acid-Based Deep Eutectic Solvent

Klycia Fidélis Cerqueira e Silva[1*], Paula Virginia de Almeida Pontes[2], Patrícia Tonon de Souza[2], Monique Martins Strieder[3], Eduardo Augusto Caldas Batista[2], Miriam Dupas Hubinger[1*]

[1]Laboratory of Process Engineering (LEP), Department of Food Engineering and Technology, School of Food Engineering (DETA), University of Campinas (UNICAMP), 13083-862, Campinas, São Paulo, Brazil.

[2]Laboratory of Extraction, Applied Thermodynamics and Equilibrium (EXTRAE), Department of Food Engineering and Technology (DETA), School of Food Engineering, University of Campinas (UNICAMP), 13083-862, Campinas, São Paulo, Brazil.

[3]Multidisciplinary Laboratory of Food and Health (LabMAS), School of Applied Sciences (FCA), University of Campinas (UNICAMP), Rua Pedro Zaccaria, 1300, 13484-350, Limeira, São Paulo, Brazil.

Author Note
[*]Corresponding author.
Telephone number: +55(19)3521-4036
E-mail address: klycia.fidelis@gmail.com; mhub@unicamp.br

Table S1. Polyphenol references for mass spectrometer scanning via UHPLC-PAD/MS-ESI.

Compound (Molecular mass)	Molecular formula	$[M^{+}H]^{+}$	Compound (Molecular mass)	Molecular formula	$[M^{+}H]^{+}$
Tyrosol (138.16 g/mol)	$C_8H_{10}O_2$	139.1717	(-)-Catechin (290.27 g/mol)	$C_{15}H_{14}O_6$	291.2760
Protocatechuic acid (154.12 g/mol)	$C_7H_6O_4$	155.1281	Hesperetin (302.28 g/mol)	$C_{16}H_{14}O_6$	303.2867
Gentisic acid (154.12 g/mol)	$C_7H_6O_4$	155.1281	Quercetin (302.23 g/mol)	$C_{15}H_{10}O_7$	303.2436
2,6-Dihydroxybenzoic acid (154.12 g/mol)	$C_7H_6O_4$	155.1281	Morin (202.23 g/mol)	$C_{15}H_{10}O_7$	303.2436
Gallic acid (170.12 g/mol)	$C_7H_6O_5$	171.1275	(+)-Gallocatechin (306.27 g/mol)	$C_{15}H_{14}O_7$	307.2754
p-coumaric acid (164.16 g/mol)	$C_9H_8O_3$	165.1660	Myricetin (318.23 g/mol)	$C_{15}H_{10}O_8$	319.2430
3-Coumaric acid (164.16 g/mol)	$C_9H_8O_3$	165.1660	Isocohumulone (332.4 g/mol)	$C_{20}H_{28}O_4$	333.4419
o-Coumaric acid (164.16 g/mol)	$C_9H_8O_3$	165.1660	Desmethylxanthohumol (340.4 g/mol)	$C_{20}H_{20}O_5$	341.3777
Caffeic acid (180.16 g/mol)	$C_9H_8O_4$	181.1654	Cohumulone (348.4 g/mol)	$C_{20}H_{28}O_5$	349.4413
Ferulic acid (194.18 g/mol)	$C_{10}H_{10}O_4$	195.1919	Chlorogenic acid (354.31 g/mol)	$C_{16}H_{18}O_9$	355.3167
Sinapic acid (224.21 g/mol)	$C_{11}H_{12}O_5$	225.2179	Xanthohumol (354.4 g/mol)	$C_{21}H_{22}O_5$	355.4043
Daidzein (254.24 g/mol)	$C_{15}H_{10}O_4$	255.2454	Isoxanthohumol (354.4 g/mol)	$C_{21}H_{22}O_5$	355.4043
Formononetin (268.26 g/mol)	$C_{16}H_{12}O_4$	269.2720	Isohumulone (362.5 g/mol)	$C_{21}H_{30}O_5$	363.4678
Apigenin (270.24 g/mol)	$C_{15}H_{10}O_5$	271.2448	Humulone (362.5 g/mol)	$C_{21}H_{30}O_5$	363.4678
Genistein (270.24 g/mol)	$C_{15}H_{10}O_5$	271.2448	Adhumulone (376.5 g/mol)	$C_{22}H_{32}O_5$	377.4944
Naringenin (272.25 g/mol)	$C_{15}H_{12}O_5$	273.2607	Prehumulone (376.5 g/mol)	$C_{22}H_{32}O_5$	377.4944
Biochanin A (284.26 g/mol)	$C_{16}H_{12}O_5$	285.2714	Postlupulone (386.5 g/mol)	$C_{24}H_{34}O_4$	387.5323
Glycitein (284.26 g/mol)	$C_{16}H_{12}O_5$	285.2714	Colupulone (400.5 g/mol)	$C_{25}H_{36}O_4$	401.5589
Kaempferol (286.24 g/mol)	$C_{15}H_{10}O_6$	287.2442	Lupulone (414.6 g/mol)	$C_{26}H_{38}O_4$	415.5855
Luteolin (286.24 g/mol)	$C_{15}H_{10}O_6$	287.2442	Adlupulone (414.6 g/mol)	$C_{26}H_{38}O_4$	415.5855
(-)-Epicatechin (290.27 g/mol)	$C_{15}H_{14}O_6$	291.2760	Naringin (580.5 g/mol)	$C_{27}H_{32}O_{14}$	581.5425

$[M^{+}H]^{+}$ - relative molecular mass

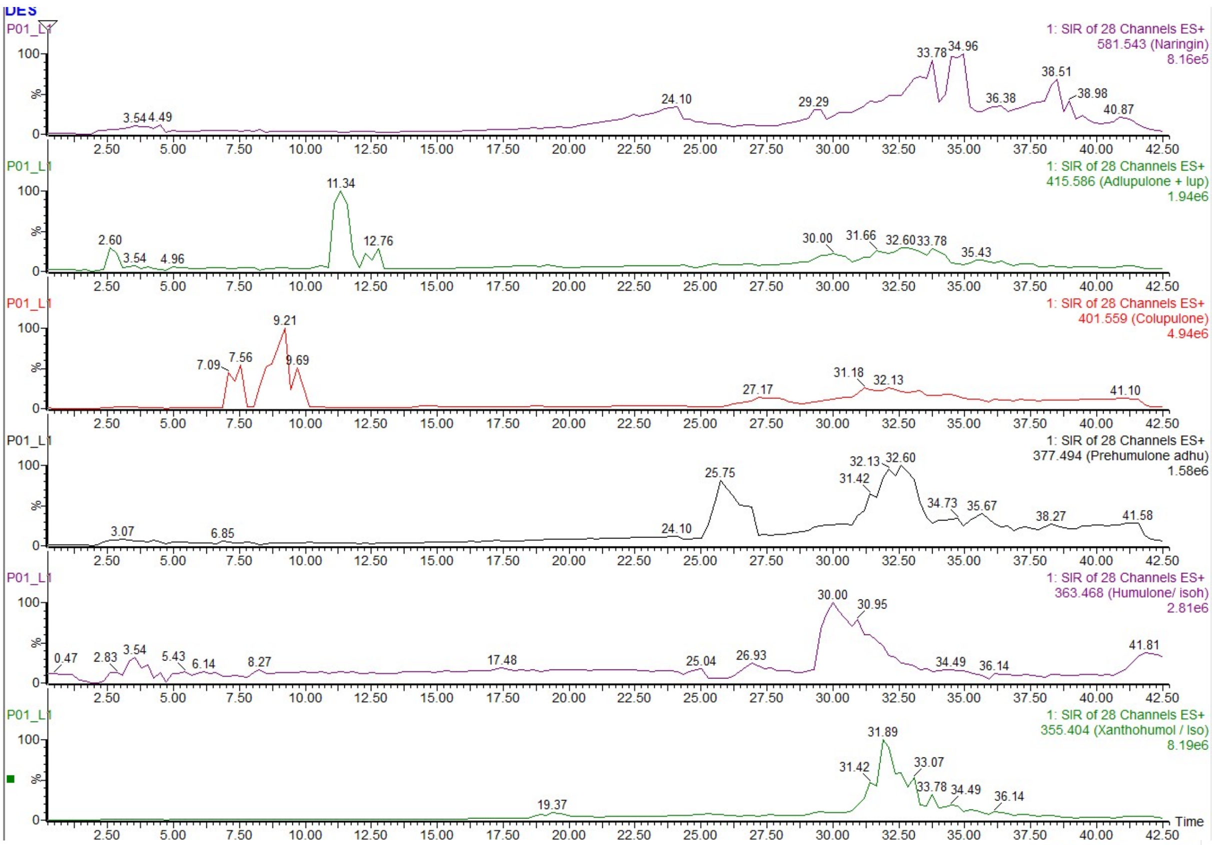
Table S2. ANOVA of the models for XN and antioxidant activity (FRAP) using DES extraction data at p-value < 0.05, 7 essays (n = 7), and 5 levels (m = 5).

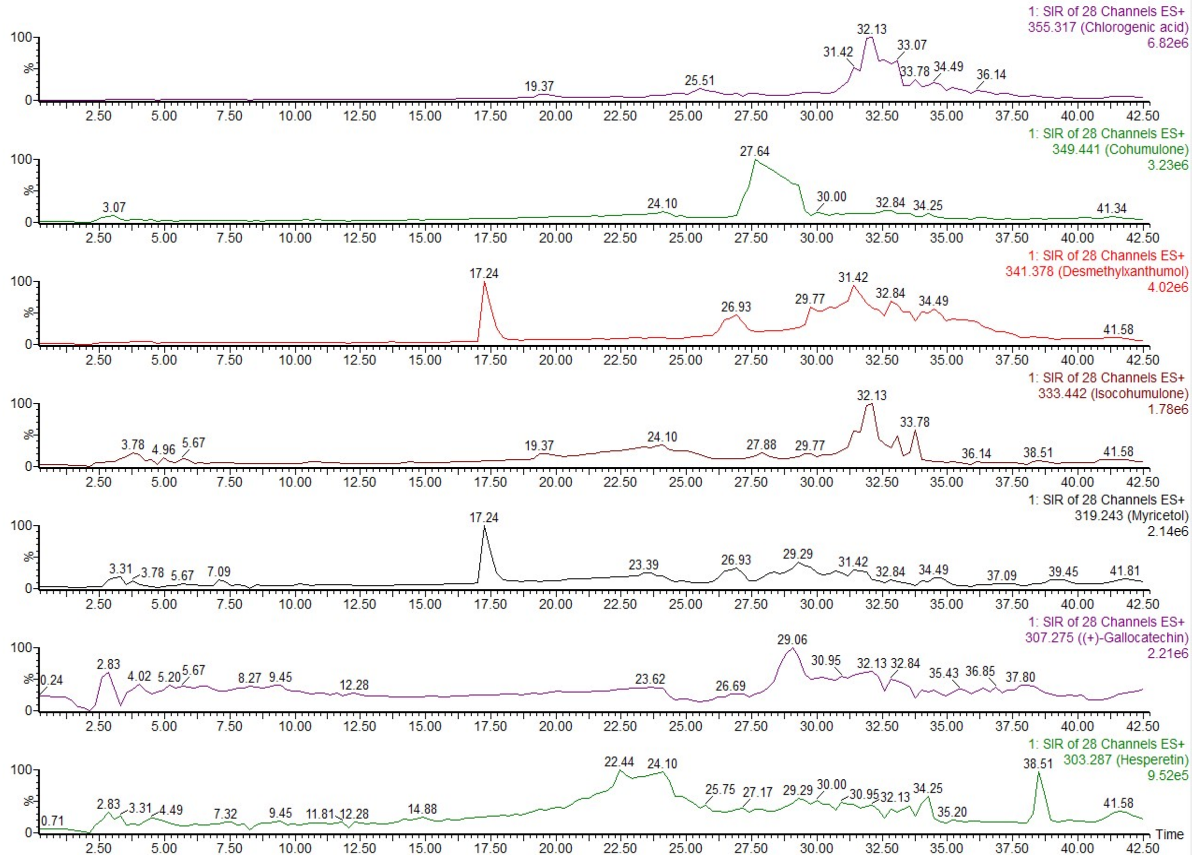
Source of variation	**SQ***	**DF***	**MS***	F_{calc}	F_{table}
XN (Y_1) − 03 parameters (p = 3)					
Regression (Reg)	536.60	2	268.30	MQ_{Reg}/MQ_{Res}= 64.42	$F_{2,4}$= 6.94
Residuals (Res)	16.66	4	4.16		
Lack of fit (LF)	4.62	2	2.31	MQ_{LF}/MQ_{PE} = 0.38	$F_{2,2}$= 19.00
Pure error (PE)	12.04	2	6.02		
Total	553.26	6			
Explained variance (R^2)		0.97			
Maximum of explained variance		0.98			
p-value (lack of fit)		0.72			
FRAP (Y_2) − 03 parameters (p = 3)					
Regression (Reg)	10.40	2	5.20	MQ_{Reg}/MQ_{Res}= 10.18	$F_{2,4}$ = 6.94
Residuals (Res)	2.04	4	0.51		
Lack of fit (LF)	1.78	2	0.89	MQ_{LF}/MQ_{PE} = 6.68	$F_{2,2}$ = 19.00
Pure error (PE)	0.27	2	0.13		
Total	12.44	6			
Explained variance (R^2)		0.84			
Maximum of explained variance		0.98			
p-value (lack of fit)		0.13			

*SQ= Sum of the square; DF= Degrees of freedom; MS= Mean square

Table S3. Path2Green metric scores of each principle.

Principles	Attributed Weight[a]	Score	Reason
Principle 1: Biomass	6.0	+1.0	Hot trub is a low-cost agro-industrial by-product obtained during beer processing.
Principle 2: Transport	5.0	0.37	Based on a 70 km transport distance (from Piracicaba to Campinas), the score corresponds to 0.37.
Principle 3: Pre-treatment	2.50	+1.0	Non-pretreatment was employed to prepare the hot trub for the extraction.
Principle 4: Solvent	6.00	+1.0	Solvents (DES and ethanol) are recommended, aligning with green chemistry principles.
Principle 5: Scaling	5.00	-1.0	The process was carried out in batch mode.
Principle 6: Purification	2.50	+0.5	Purification is required to eliminate ethanol before the application of the extract.
Principle 7: Yield	4.00	+1.0	An exhaustive extraction was performed.
Principle 8: Post-treatment	2.50	+1.0	No additional post-treatment is required for the application of extracts.
Principle 9: Energy	5.00	-0.50	Low-energy extraction techniques use renewable energy.
Principle 10: Application	4.5	+1.0	The hot trub extracts are suitable for applications in food, nutraceuticals, cosmetics, and pharmaceuticals.
Principle 11: Repurposing	6.00	+1.0	Non-virgin material (hot trub) was employed as raw material.
Principle 12: Waste management	6.00	-0.13	The residual biomass, with potential for further valorization after extraction, was approximately 13%.


DES
1: SIR of 28 Channels ES+
581.543 (Naringin)
8.16e5
1: SIR of 28 Channels ES+
415.586 (Adlupulone + lup)
1.94e6
1: SIR of 28 Channels ES+
401.559 (Colupulone)
4.94e6
1: SIR of 28 Channels ES+
377.494 (Prehumulone adhu)
1.58e6
1: SIR of 28 Channels ES+
363.468 (Humulone/ isoh)
2.81e6
1: SIR of 28 Channels ES+
355.404 (Xanthohumol / Iso)
8.19e6
Time


1: SIR of 28 Channels ES+
355.317 (Chlorogenic acid)
6.82e6
1: SIR of 28 Channels ES+
349.441 (Cohumulone)
3.23e6
1: SIR of 28 Channels ES+
341.378 (Desmethylxanthumol)
4.02e6
1: SIR of 28 Channels ES+
333.442 (Isocohumulone)
1.78e6
1: SIR of 28 Channels ES+
319.243 (Myricetol)
2.14e6
1: SIR of 28 Channels ES+
307.275 ((+)-Gallocatechin)
2.21e6
1: SIR of 28 Channels ES+
303.287 (Hesperetin)
9.52e5
Time

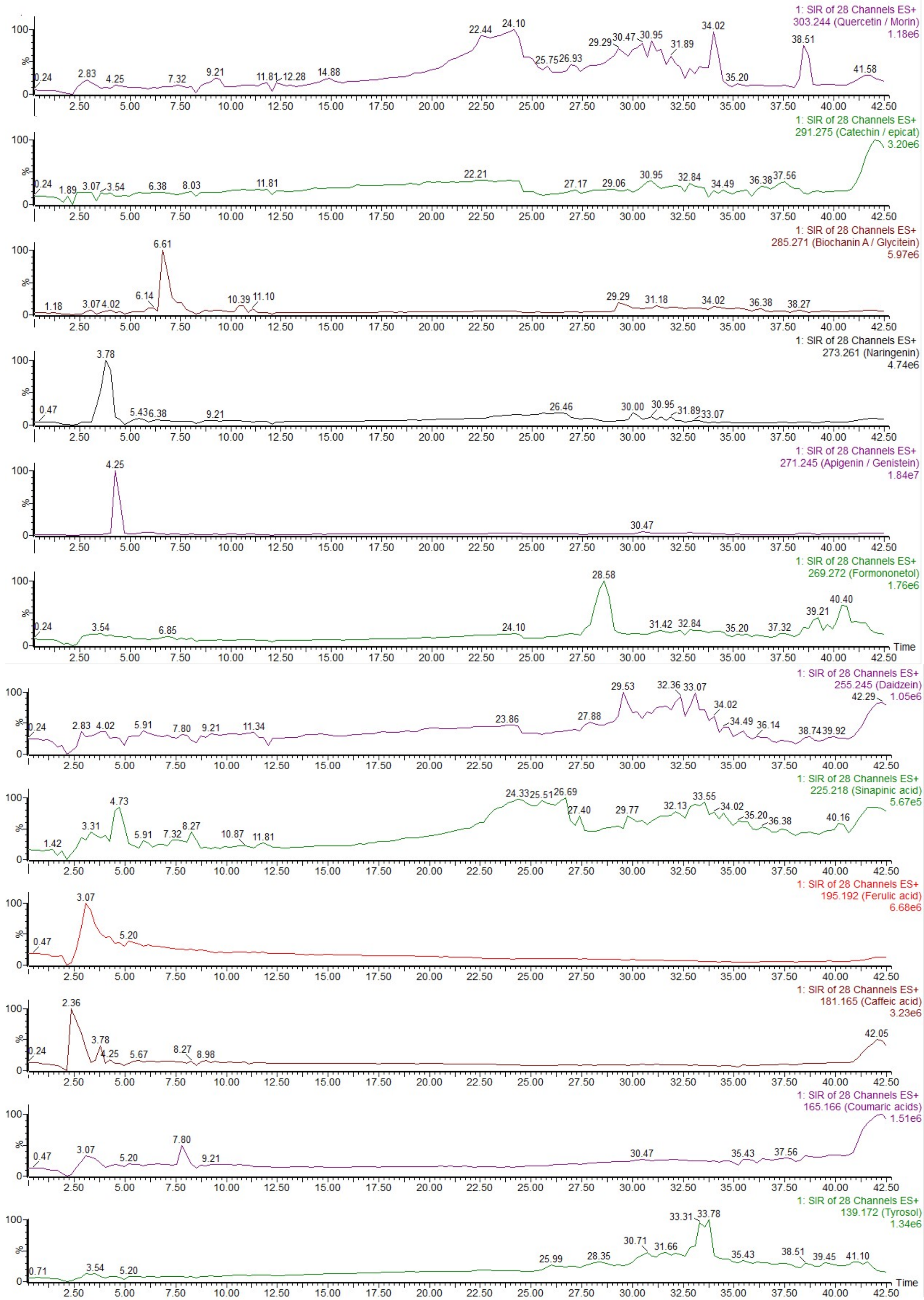
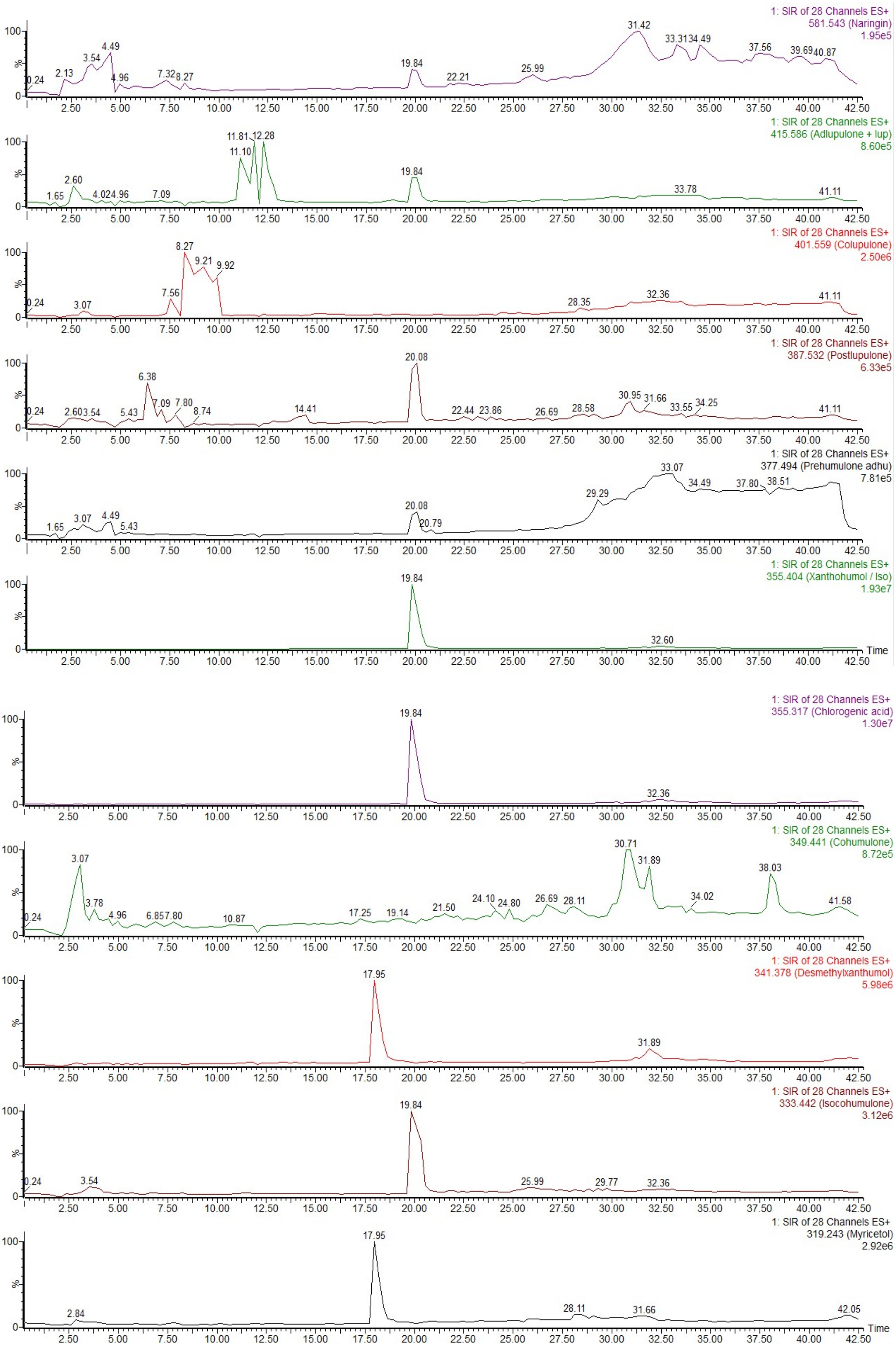


Figure S1. The total ion current chromatograms of polyphenolic scanning via a mass sensor on the DES extraction regarding CCRD during run 01


1: SIR of 28 Channels ES+
581.543 (Naringin)
1.95e5
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415.586 (Adlupulone + lup)
8.60e5
1: SIR of 28 Channels ES+
401.559 (Colupulone)
2.50e6
1: SIR of 28 Channels ES+
387.532 (Postlupulone)
6.33e5
1: SIR of 28 Channels ES+
377.494 (Prehumulone adhu)
7.81e5
1: SIR of 28 Channels ES+
355.404 (Xanthohumol / Iso)
1.93e7
Time
1: SIR of 28 Channels ES+
355.317 (Chlorogenic acid)
1.30e7
1: SIR of 28 Channels ES+
349.441 (Cohumulone)
8.72e5
1: SIR of 28 Channels ES+
341.378 (Desmethylxanthumol)
5.98e6
1: SIR of 28 Channels ES+
333.442 (Isocohumulone)
3.12e6
1: SIR of 28 Channels ES+
319.243 (Myricetol)
2.92e6
Time

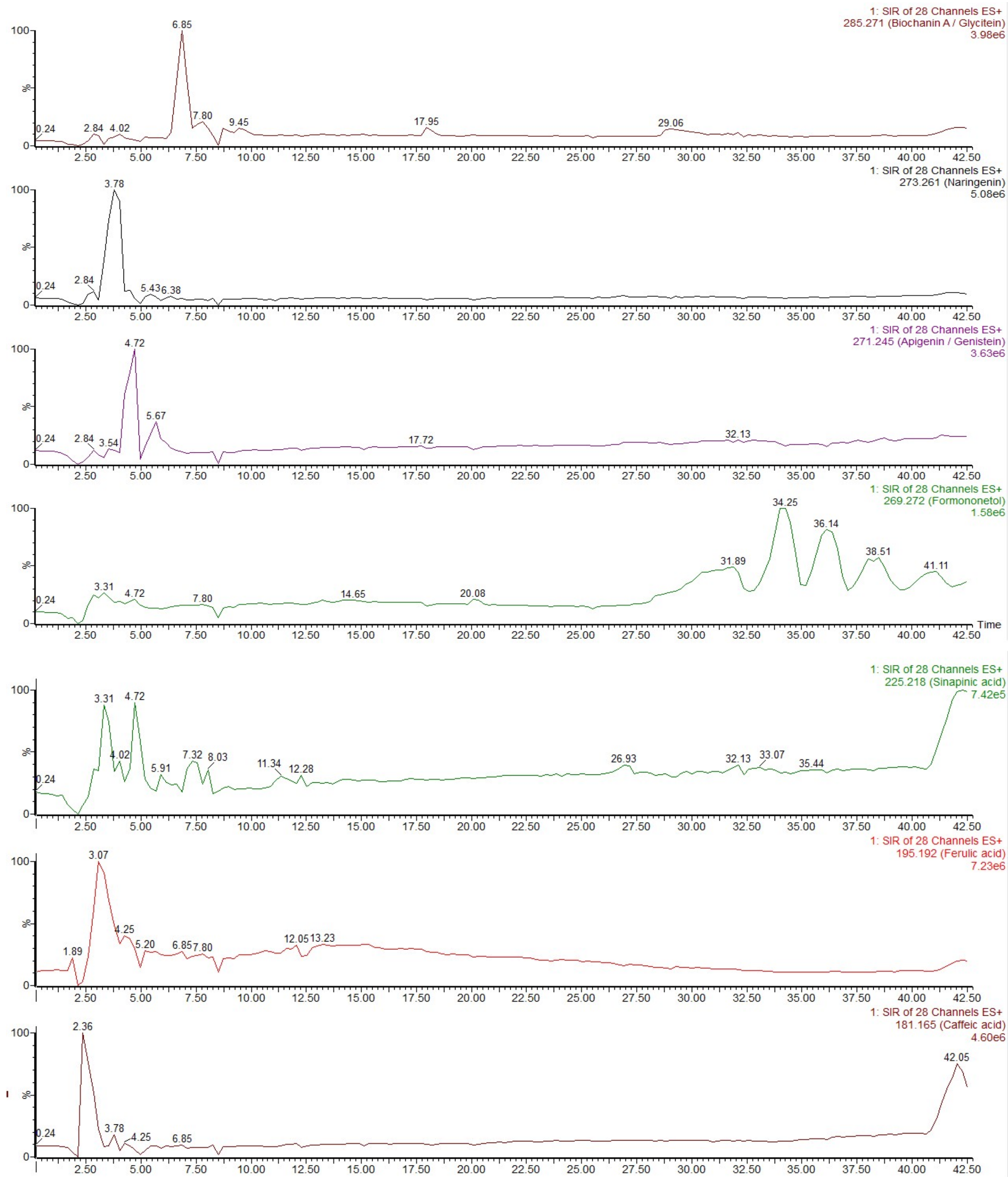
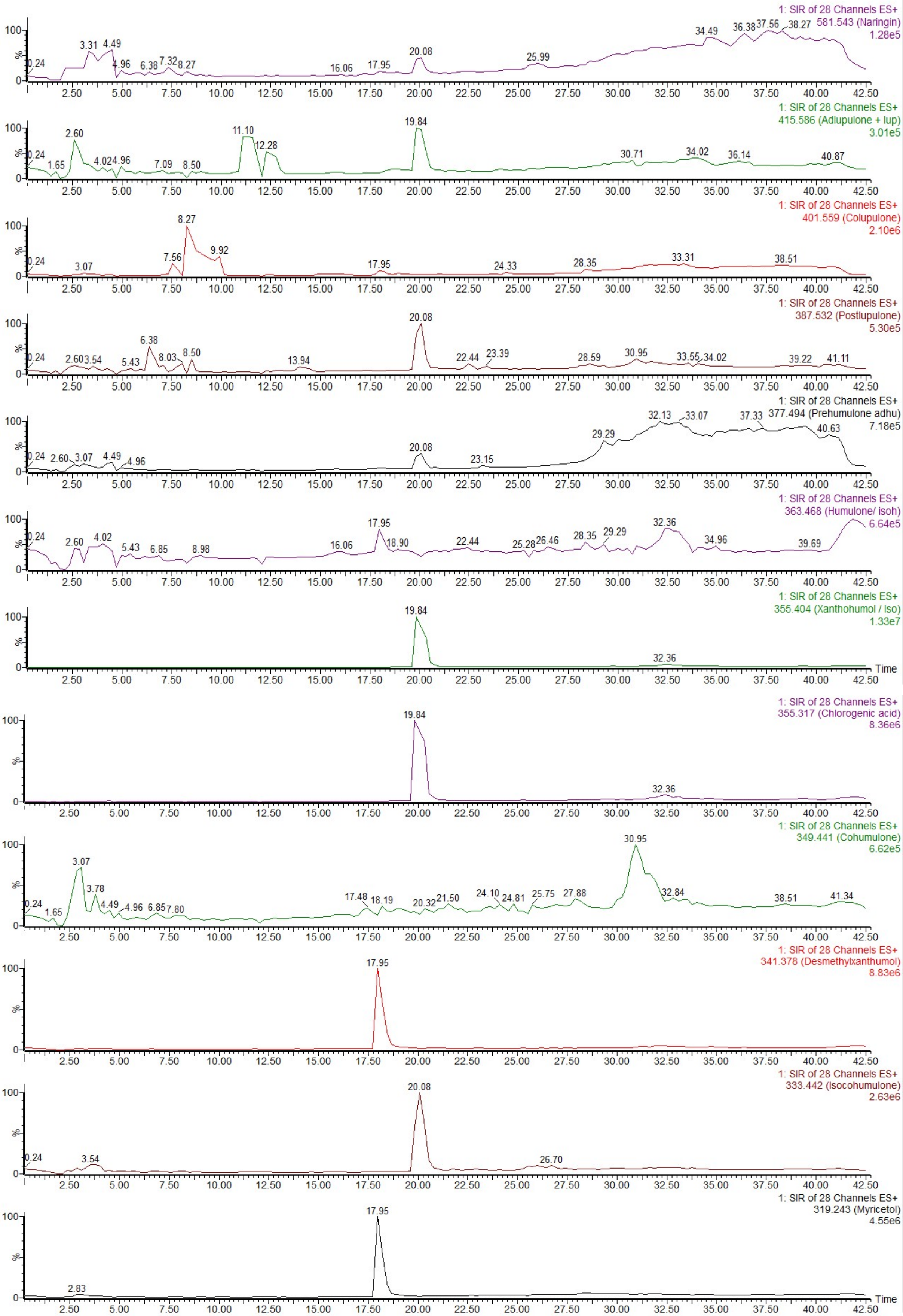


Figure S2. The total ion current chromatograms of polyphenolic scanning via a mass sensor on the DES extraction regarding CCRD during run 04



1: SIR of 28 Channels ES+
581.543 (Naringin)
1.28e5
1: SIR of 28 Channels ES+
415.586 (Adlupulone + lup)
3.01e5
1: SIR of 28 Channels ES+
401.559 (Colupulone)
2.10e6
1: SIR of 28 Channels ES+
387.532 (Postlupulone)
5.30e5
1: SIR of 28 Channels ES+
377.494 (Prehumulone adhu)
7.18e5
1: SIR of 28 Channels ES+
363.468 (Humulone/ isoh)
6.64e5
1: SIR of 28 Channels ES+
355.404 (Xanthohumol / Iso)
1.33e7
Time
1: SIR of 28 Channels ES+
355.317 (Chlorogenic acid)
8.36e6
1: SIR of 28 Channels ES+
349.441 (Cohumulone)
6.62e5
1: SIR of 28 Channels ES+
341.378 (Desmethylxanthumol)
8.83e6
1: SIR of 28 Channels ES+
333.442 (Isocohumulone)
2.63e6
1: SIR of 28 Channels ES+
319.243 (Myricetol)
4.55e6
Time

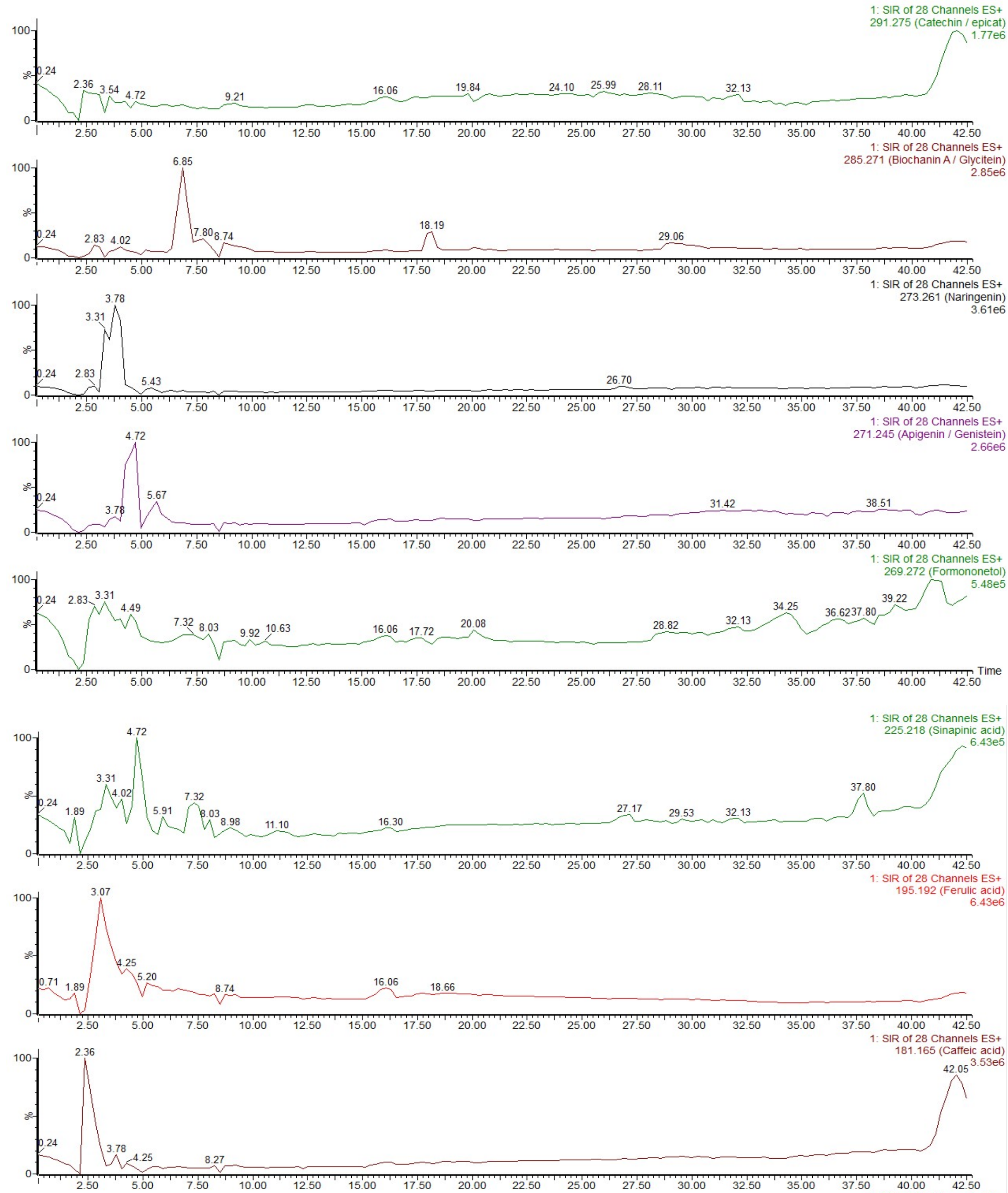
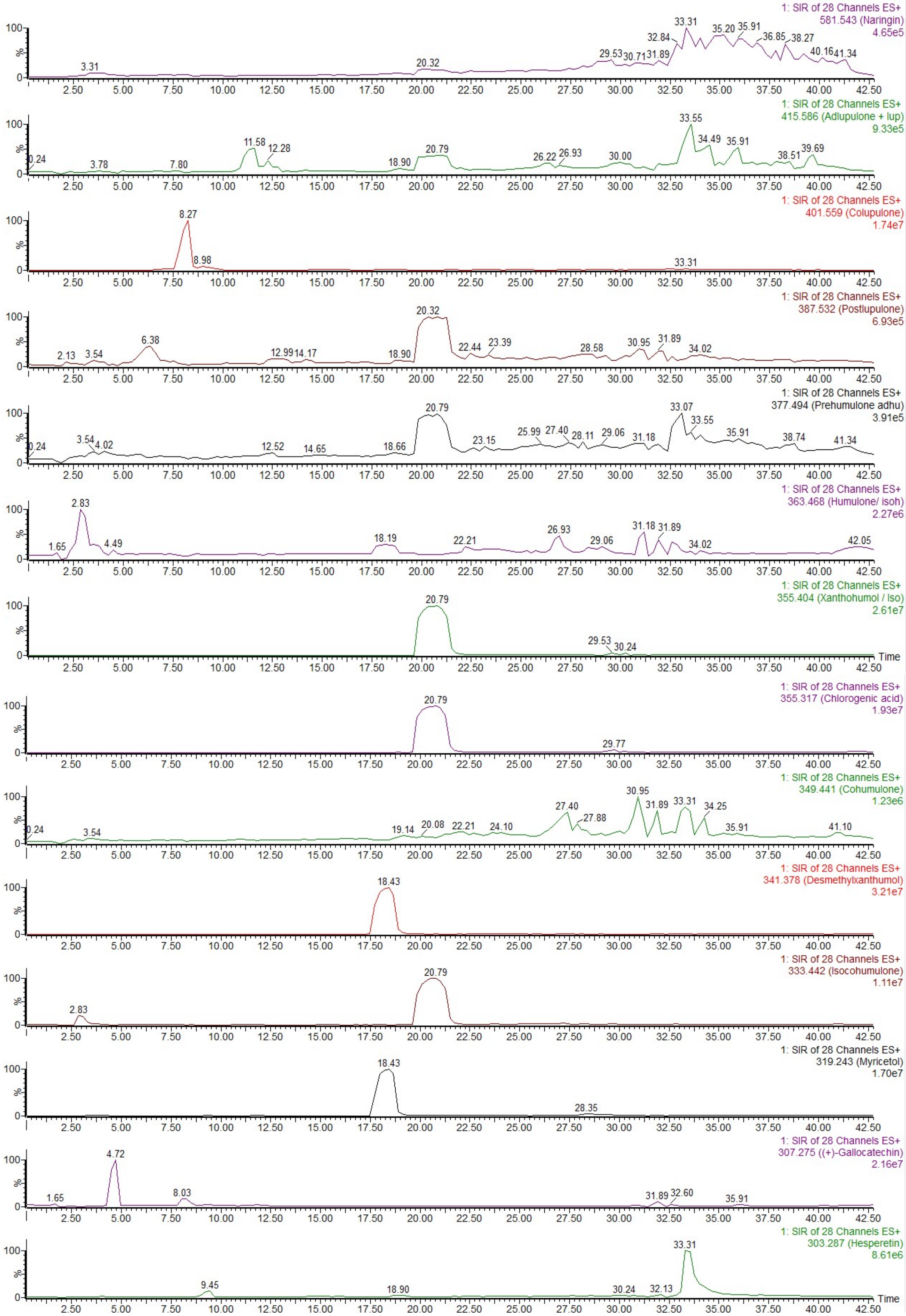


Figure S3. The total ion current chromatograms of polyphenolic scanning via a mass sensor on the DES extraction regarding CCRD during run 10 (Central point)



1: SIR of 28 Channels ES+
581.543 (Naringin)
4.65e5
3.31
20.32
29.53
30.71
31.89
32.84
33.31
35.20
35.91
36.85
38.27
40.16
41.34
1: SIR of 28 Channels ES+
415.586 (Adlupulone + lup)
9.33e5
0.24
3.78
7.80
11.58
12.28
18.90
20.79
26.22
26.93
30.00
33.55
34.49
35.91
38.51
39.69
1: SIR of 28 Channels ES+
401.559 (Colupulone)
1.74e7
8.27
8.98
33.31
1: SIR of 28 Channels ES+
387.532 (Postlupulone)
6.93e5
2.13
3.54
6.38
12.99
14.17
18.90
20.32
22.44
23.39
28.58
30.95
31.89
34.02
1: SIR of 28 Channels ES+
377.494 (Prehumulone adhu)
3.91e5
0.24
3.54
4.02
12.52
14.65
18.66
20.79
23.15
25.99
27.40
28.11
29.06
31.18
33.07
33.55
35.91
38.74
41.34
1: SIR of 28 Channels ES+
363.468 (Humulone/ isoh)
2.27e6
1.65
2.83
4.49
18.19
22.21
26.93
29.06
31.18
31.89
34.02
42.05
1: SIR of 28 Channels ES+
355.404 (Xanthohumol / Iso)
2.61e7
20.79
29.53
30.24
Time
1: SIR of 28 Channels ES+
355.317 (Chlorogenic acid)
1.93e7
20.79
29.77
1: SIR of 28 Channels ES+
349.441 (Cohumulone)
1.23e6
0.24
3.54
19.14
20.08
22.21
24.10
27.40
27.88
30.95
31.89
33.31
34.25
35.91
41.10
1: SIR of 28 Channels ES+
341.378 (Desmethylxanthumol)
3.21e7
18.43
1: SIR of 28 Channels ES+
333.442 (Isocohumulone)
1.11e7
2.83
20.79
1: SIR of 28 Channels ES+
319.243 (Myricetol)
1.70e7
18.43
28.35
1: SIR of 28 Channels ES+
307.275 ((+)-Gallocatechin)
2.16e7
1.65
4.72
8.03
31.89
32.60
35.91
1: SIR of 28 Channels ES+
303.287 (Hesperetin)
8.61e6
9.45
18.90
30.24
32.13
33.31
Time
100
%
0
2.50
5.00
7.50
10.00
12.50
15.00
17.50
20.00
22.50
25.00
27.50
30.00
32.50
35.00
37.50
40.00
42.50

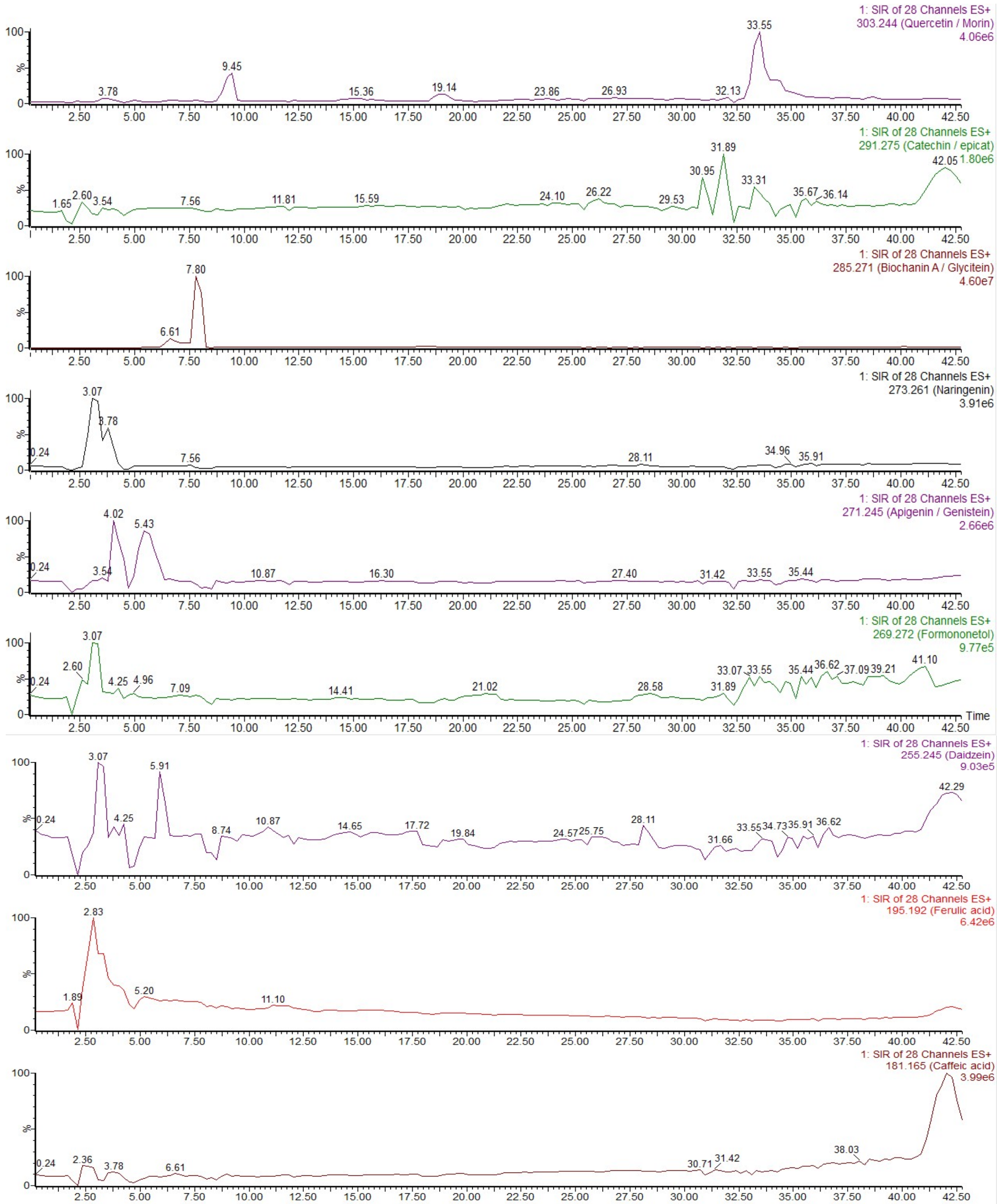
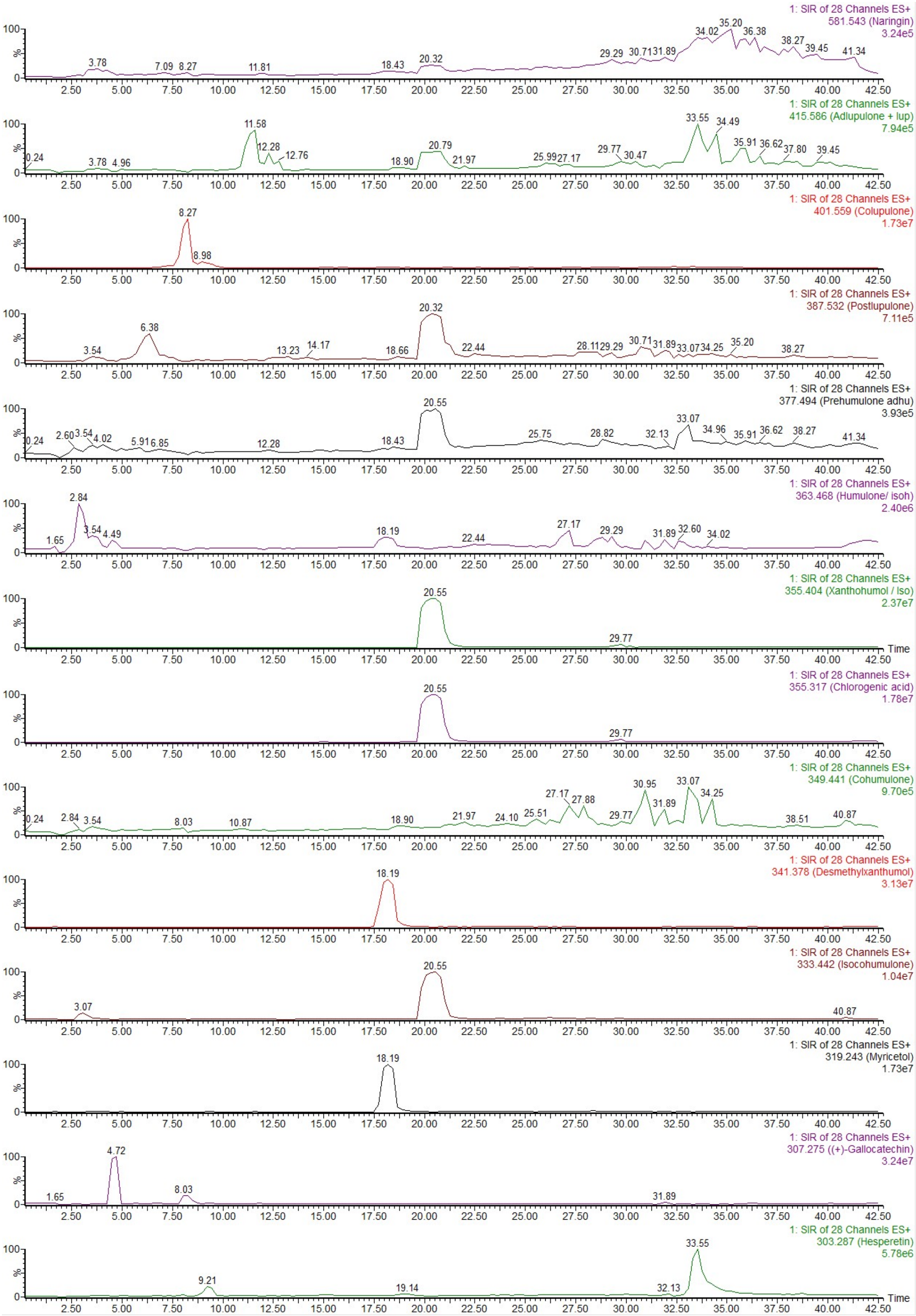


Figure S4. The total ion current chromatograms of polyphenolic scanning via a mass sensor on the ethanolic extraction regarding CCRD during run 01



1: SIR of 28 Channels ES+
581.543 (Naringin)
3.24e5
3.78 7.09 8.27 11.81 18.43 20.32 29.29 30.71 31.89 34.02 35.20 36.38 38.27 39.45 41.34
1: SIR of 28 Channels ES+
415.586 (Adlupulone + lup)
7.94e5
0.24 3.78 4.96 11.58 12.28 12.76 18.90 20.79 21.97 25.99 27.17 29.77 30.47 33.55 34.49 35.91 36.62 37.80 39.45
1: SIR of 28 Channels ES+
401.559 (Colupulone)
1.73e7
8.27 8.98
1: SIR of 28 Channels ES+
387.532 (Postlupulone)
7.11e5
3.54 6.38 13.23 14.17 18.66 20.32 22.44 28.11 29.29 30.71 31.89 33.07 34.25 35.20 38.27
1: SIR of 28 Channels ES+
377.494 (Prehumulone adhu)
3.93e5
0.24 2.60 3.54 4.02 5.91 6.85 12.28 18.43 20.55 25.75 28.82 32.13 33.07 34.96 35.91 36.62 38.27 41.34
1: SIR of 28 Channels ES+
363.468 (Humulone/ isoh)
2.40e6
1.65 2.84 3.54 4.49 18.19 22.44 27.17 29.29 31.89 32.60 34.02
1: SIR of 28 Channels ES+
355.404 (Xanthohumol / Iso)
2.37e7
20.55 29.77
1: SIR of 28 Channels ES+
355.317 (Chlorogenic acid)
1.78e7
20.55 29.77
1: SIR of 28 Channels ES+
349.441 (Cohumulone)
9.70e5
0.24 2.84 3.54 8.03 10.87 18.90 21.97 24.10 25.51 27.17 27.88 29.77 30.95 31.89 33.07 34.25 38.51 40.87
1: SIR of 28 Channels ES+
341.378 (Desmethylxanthumol)
3.13e7
18.19
1: SIR of 28 Channels ES+
333.442 (Isocohumulone)
1.04e7
3.07 20.55 40.87
1: SIR of 28 Channels ES+
319.243 (Myricetol)
1.73e7
18.19
1: SIR of 28 Channels ES+
307.275 ((+)-Gallocatechin)
3.24e7
1.65 4.72 8.03 31.89
1: SIR of 28 Channels ES+
303.287 (Hesperetin)
5.78e6
9.21 19.14 32.13 33.55
100
%
0
2.50 5.00 7.50 10.00 12.50 15.00 17.50 20.00 22.50 25.00 27.50 30.00 32.50 35.00 37.50 40.00 42.50
Time

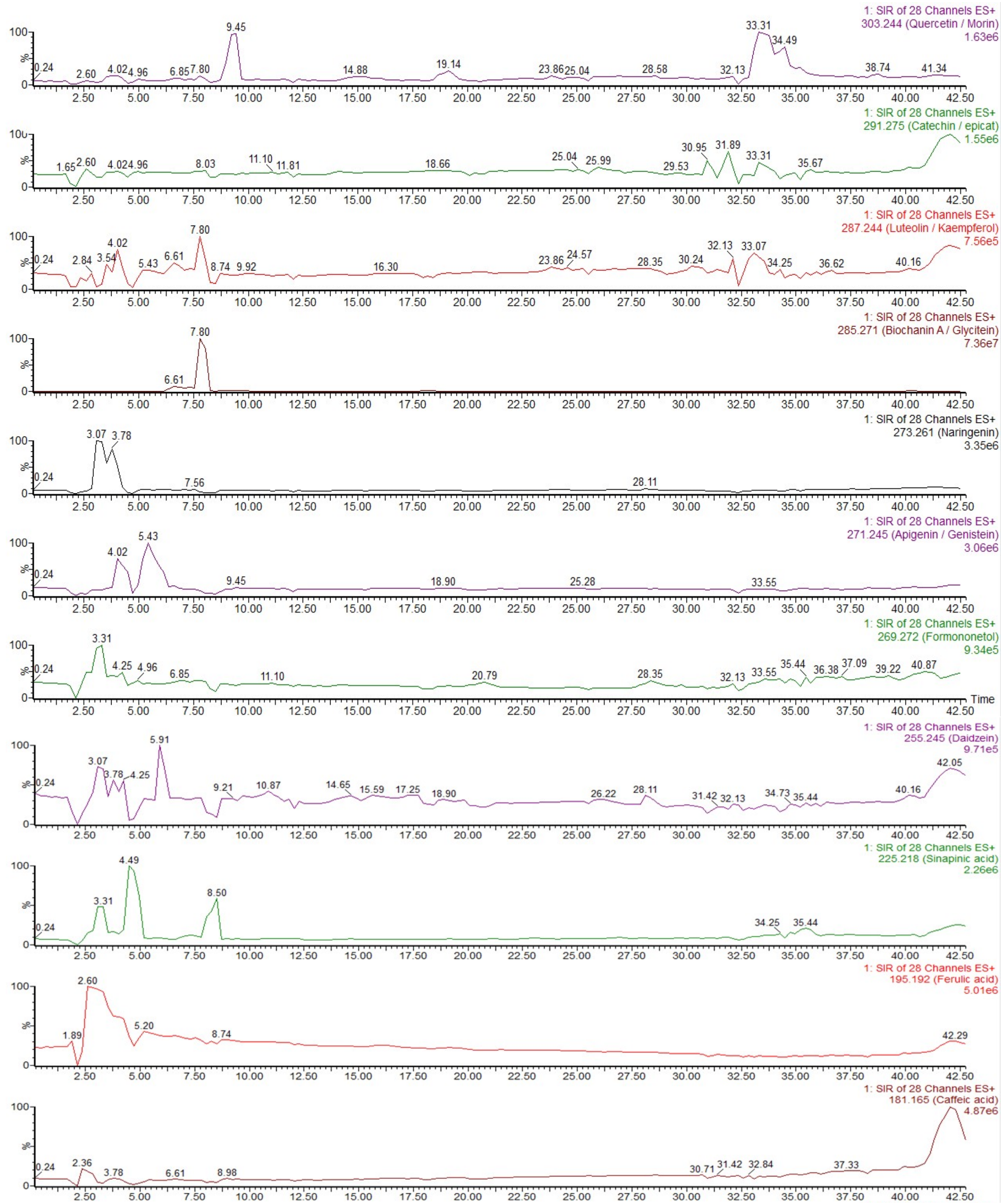
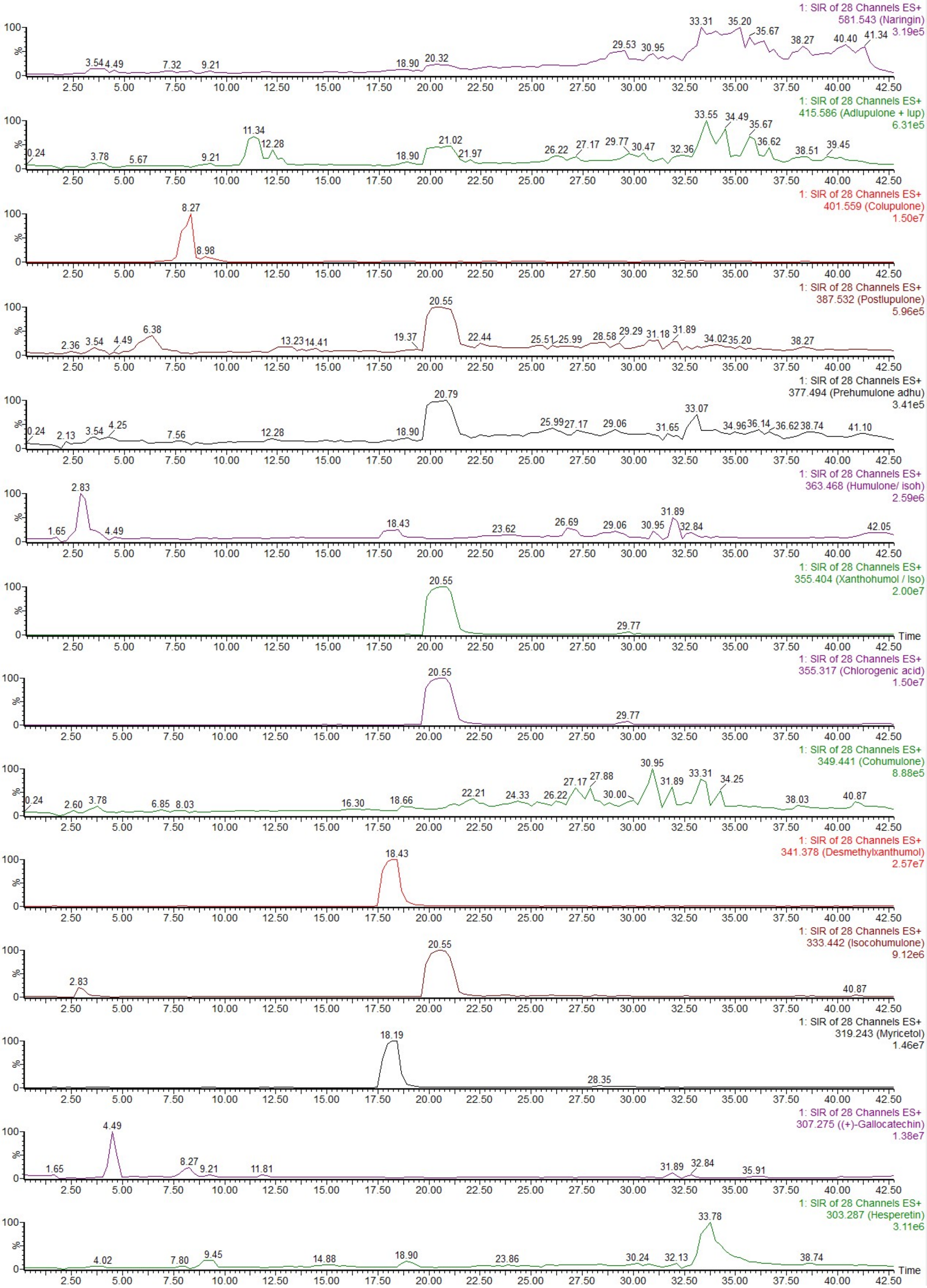


Figure S5. The total ion current chromatograms of polyphenolic scanning via a mass sensor on the ethanolic extraction regarding CCRD during run 04


1: SIR of 28 Channels ES+
581.543 (Naringin)
3.19e5
1: SIR of 28 Channels ES+
415.586 (Adlupulone + lup)
6.31e5
1: SIR of 28 Channels ES+
401.559 (Colupulone)
1.50e7
1: SIR of 28 Channels ES+
387.532 (Postlupulone)
5.96e5
1: SIR of 28 Channels ES+
377.494 (Prehumulone adhu)
3.41e5
1: SIR of 28 Channels ES+
363.468 (Humulone/ isoh)
2.59e6
1: SIR of 28 Channels ES+
355.404 (Xanthohumol / Iso)
2.00e7
1: SIR of 28 Channels ES+
355.317 (Chlorogenic acid)
1.50e7
1: SIR of 28 Channels ES+
349.441 (Cohumulone)
8.88e5
1: SIR of 28 Channels ES+
341.378 (Desmethylxanthumol)
2.57e7
1: SIR of 28 Channels ES+
333.442 (Isocohumulone)
9.12e6
1: SIR of 28 Channels ES+
319.243 (Myricetol)
1.46e7
1: SIR of 28 Channels ES+
307.275 ((+)-Gallocatechin)
1.38e7
1: SIR of 28 Channels ES+
303.287 (Hesperetin)
3.11e6
Time

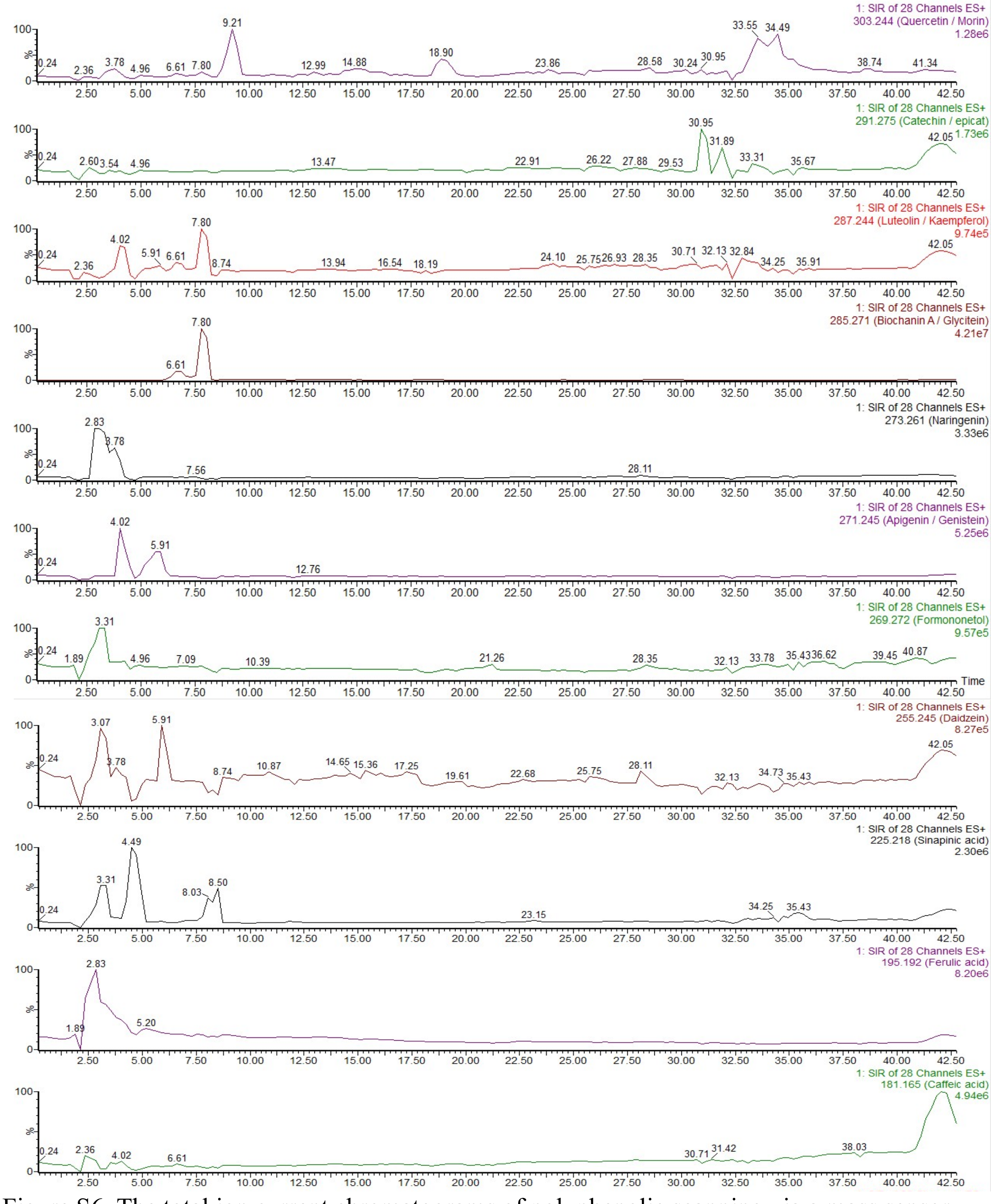


Figure S6. The total ion current chromatograms of polyphenolic scanning via a mass sensor on the ethanolic extraction regarding CCRD during run 10