

Table S1: Additional optimized transitions and parameters in dynamic multiple reaction monitoring (DMRM) mode for identification of compounds in human blood cells. Electron multiplier voltage (EMV) was set to +500 V in ESI negative and +1000 V in ESI positive mode. Following intracellular metabolites of M1 were monitored: Conjugation products of M1 and glutathione (M1-GSH), cysteine (M1-CYS) and oxidized glutathione (M1-GSSG); an acetylated form of M1 (M1-acetylated), the open-chained ester form of M1 (M1-COOH) and another metabolite, respectively hydroxybenzoic acid.

Compound	Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	FV ^a [V]	CE ^b [V]	CAV ^c [V]	MS 1 Resolution	MS 2 Resolution	R _T ^d [min]	R _T Window (DMRM)	ESI mode
M1-GSH	514.0	514.0	95	0	1	Widest	Widest	3.50	0.50	positive
	514.0	385.0	95	13	1	Widest	Widest	3.50	0.50	positive
	514.0	130.0	95	23	1	Widest	Widest	3.50	0.50	positive
M1-CYS	328.0	328.0	100	0	1	Widest	Widest	3.60	0.50	positive
	328.0	155.0	70	15	7	Widest	Widest	3.60	0.50	positive
	328.0	85.0	100	25	7	Widest	Widest	3.60	0.50	positive
M1-GSSG	410.0	410.0	150	0	3	Widest	Widest	3.10	0.40	positive
	410.0	232.0	135	15	7	Widest	Widest	3.10	0.40	positive
M1-acetylated	251.0	251.0	100	0	7	Widest	Widest	5.40	0.50	positive
M1-COOH	225.0	123.0	100	10	3	Widest	Widest	3.60	0.40	negative
	225.0	101.0	100	5	3	Widest	Widest	3.60	0.40	negative

^aFV. Fragmentor voltage. ^bCE. Collision energy. ^cCAV. Cell accelerator voltage. ^dR_T. Retention time.

Table S2: Optimized transitions and parameters in dynamic multiple reaction monitoring (DMRM) employing negative ESI ionization mode for LC-MS/MS analysis of prepared synovial fluid samples. Electron multiplier voltage (EMV) was set to +750 V. Cycle time was 1000 ms.

Compound	Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	FV ^a [V]	CE ^b [V]	CAV ^c [V]	MS 1 Resolution	MS 2 Resolution	R _T ^d [min]	R _T Window (DMRM)
(+) -Catechin	289.1	245.0 ^e	76	9	7	Widest	Widest	3.60	0.45
	289.1	203.0	76	17	7	Widest	Widest	3.60	0.45
M1	207.0	163.1 ^e	115	13	5	Widest	Widest	4.00	0.45
	207.0	122.0	115	17	5	Widest	Widest	4.00	0.45
Caffeic acid	179.0	135.1 ^e	90	13	5	Widest	Widest	4.00	0.50
	179.0	134.0	90	25	5	Widest	Widest	4.00	0.50
Taxifolin	303.1	285.0 ^e	95	9	4	Widest	Widest	4.55	0.45
	303.1	125.0	95	21	4	Widest	Widest	4.55	0.45
Ferulic acid	193.1	134.1 ^e	80	13	1	Widest	Widest	4.65	0.45
	193.1	178.0	80	9	1	Widest	Widest	4.65	0.45
Hydrocaffeic acid (IS)	181.2	137.0 ^e	85	9	4	Wide	Widest	3.85	0.50
	181.2	109.0	85	9	4	Wide	Widest	3.85	0.50

^aFV. Fragmentor voltage. ^bCE. Collision energy. ^cCAV. Cell accelerator voltage. ^dR_T. Retention time. ^eQuantifier. transition for quantification

Table S3: Calibration range, calibration function and correlation coefficients of the five analytes extracted from human pooled synovial fluid (n= 3).

Analytes	Range [ng/mL]	Slope $\pm$ SD	y-intercept	Correlation coefficient R
(+)-Catechin	2.14 - 34.93	0.0195 ± 0.006	-0.0175	0.9977
Ferulic acid	1.53 - 24.93	0.1184 ± 0.024	0.0693	0.9979
M1	0.117 - 1.900	1.6016 ± 0.243	0.0330	0.9976
Taxifolin	0.080 - 1.307	0.7518 ± 0.010	-0.0388	0.9992
Caffeic acid	3.07 - 49.95	0.6752 ± 0.113	-0.8800	0.9988

Table S4: Intraday accuracy and precision of the analytes in human pooled synovial fluid (n= 3).

Analytes and spiked concentration [ng/mL]	Calculated concentration Mean $\pm$ SD [ng/mL]	Accuracy Mean $\pm$ SD [%]	Precision [%]
<i>Catechin</i>			
2.14	2.24 $\pm$ 0.15	104.55 $\pm$ 6.78	6.48
2.68	2.63 $\pm$ 0.43	98.24 $\pm$ 16.24	16.50
8.18	7.47 $\pm$ 0.79	91.40 $\pm$ 9.67	10.58
12.77	10.86 $\pm$ 0.41	85.04 $\pm$ 3.21	3.77
19.96	17.62 $\pm$ 0.71	88.25 $\pm$ 3.54	4.01
<i>Ferulic acid</i>			
1.53	1.70 $\pm$ 0.05	111.13 $\pm$ 3.45	3.10
1.91	1.98 $\pm$ 0.14	103.36 $\pm$ 7.18	6.95
5.83	5.13 $\pm$ 0.19	87.85 $\pm$ 3.28	3.73
9.12	8.60 $\pm$ 0.73	94.33 $\pm$ 7.97	8.45
14.24	13.27 $\pm$ 0.95	93.14 $\pm$ 6.68	7.17
<i>M1</i>			
0.117	0.129 $\pm$ 0.016	110.51 $\pm$ 13.43	12.16
0.146	0.152 $\pm$ 0.014	103.84 $\pm$ 9.37	9.02
0.445	0.457 $\pm$ 0.049	102.64 $\pm$ 10.97	10.69
0.695	0.770 $\pm$ 0.030	110.82 $\pm$ 4.25	3.84
1.086	1.014 $\pm$ 0.080	93.39 $\pm$ 7.37	7.89
<i>Taxifolin</i>			
0.080	0.084 $\pm$ 0.016	105.70 $\pm$ 19.48	18.43
0.100	0.096 $\pm$ 0.015	95.91 $\pm$ 15.48	16.14
0.306	0.270 $\pm$ 0.016	88.35 $\pm$ 5.11	5.78
0.478	0.427 $\pm$ 0.020	89.36 $\pm$ 4.27	4.77
0.747	0.653 $\pm$ 0.020	87.40 $\pm$ 2.72	3.12
<i>Caffeic acid</i>			
3.07	3.55 $\pm$ 0.18	115.73 $\pm$ 5.74	4.96
3.83	3.64 $\pm$ 0.13	95.11 $\pm$ 3.47	3.65
11.69	10.42 $\pm$ 0.70	89.15 $\pm$ 5.97	6.70
18.27	18.65 $\pm$ 1.99	102.10 $\pm$ 10.91	10.69
28.54	27.85 $\pm$ 2.17	97.57 $\pm$ 7.59	7.78

Table S5: Interday accuracy and precision of the analytes in human pooled synovial fluid (n= 3).

Analytes and spiked concentration [ng/mL]	Calculated concentration Mean $\pm$ SD [ng/mL]	Accuracy Mean $\pm$ SD [%]	Precision [%]
<i>Catechin</i>			
2.14	2.16 $\pm$ 0.33	100.72 $\pm$ 15.43	15.32
2.68	2.77 $\pm$ 0.16	103.53 $\pm$ 5.85	5.65
8.18	7.97 $\pm$ 0.53	97.51 $\pm$ 6.51	6.68
12.77	11.28 $\pm$ 0.40	88.34 $\pm$ 3.15	3.56
19.96	17.96 $\pm$ 0.39	89.98 $\pm$ 1.94	2.16
<i>Ferulic acid</i>			
1.53	1.51 $\pm$ 0.16	98.91 $\pm$ 10.71	10.82
1.91	1.88 $\pm$ 0.09	98.43 $\pm$ 4.63	4.71
5.83	5.60 $\pm$ 0.43	95.97 $\pm$ 7.31	7.62
9.12	8.76 $\pm$ 0.18	96.08 $\pm$ 2.02	2.11
14.24	13.04 $\pm$ 0.39	91.53 $\pm$ 2.71	2.97
<i>M1</i>			
0.117	0.119 $\pm$ 0.009	101.47 $\pm$ 7.86	7.75
0.146	0.143 $\pm$ 0.008	97.62 $\pm$ 5.59	5.73
0.445	0.446 $\pm$ 0.010	100.11 $\pm$ 2.32	2.32
0.695	0.682 $\pm$ 0.077	98.17 $\pm$ 11.03	11.23
1.086	0.975 $\pm$ 0.035	89.80 $\pm$ 3.21	3.58
<i>Taxifolin</i>			
0.080	0.080 $\pm$ 0.004	100.56 $\pm$ 4.92	4.89
0.100	0.096 $\pm$ 0.006	96.23 $\pm$ 6.29	6.53
0.306	0.286 $\pm$ 0.015	93.50 $\pm$ 4.93	5.28
0.478	0.441 $\pm$ 0.013	92.29 $\pm$ 2.64	2.86
0.747	0.675 $\pm$ 0.020	90.31 $\pm$ 2.66	2.94
<i>Caffeic acid</i>			
3.07	3.06 $\pm$ 0.42	99.90 $\pm$ 13.81	13.82
3.83	3.67 $\pm$ 0.16	95.89 $\pm$ 4.06	4.23
11.69	11.23 $\pm$ 0.86	96.08 $\pm$ 7.36	7.66
18.27	17.39 $\pm$ 1.30	95.19 $\pm$ 7.11	7.46
28.54	24.66 $\pm$ 4.35	93.19 $\pm$ 4.11	4.41

Table S6: Robustness of the developed method at two concentrations (n= 3) with human pooled synovial fluid which was intentionally contaminated with 1% human whole blood.

Analytes and spiked concentration [ng/mL]	Calculated concentration Mean $\pm$ SD [ng/mL]	Accuracy Mean [%]	Precision [%]
<i>Catechin</i>			
8.18	8.68 $\pm$ 0.55	106.18	6.34
19.96	20.99 $\pm$ 1.98	105.16	9.42
<i>M1</i>			
0.445	0.478 $\pm$ 0.02	107.37	4.55
1.086	1.033 $\pm$ 0.08	95.12	8.17
<i>Caffeic acid</i>			
11.69	11.30 $\pm$ 1.40	96.65	12.37
28.54	24.69 $\pm$ 0.82	86.50	3.33
<i>Taxifolin</i>			
0.306	0.314 $\pm$ 0.04	102.77	13.50
0.747	0.776 $\pm$ 0.11	103.88	14.06
<i>Ferulic acid</i>			
5.83	6.50 $\pm$ 0.12	111.40	1.91
12.24	15.70 $\pm$ 0.49	110.21	3.11

Table S7: Post-preparative stability: autosampler stability of the analytes after 6 h and 12 h at room temperature (RT) after previous LC/MS/MS analysis (n= 3).

Analytes and spiked concentration [ng/mL]	Autosampler stability: 6 h - RT - in darkness			Autosampler stability: 12 h - RT - in darkness		
	Calculated concentration Mean $\pm$ SD [ng/mL]	RSD [%]	Δ [%] ¹ Mean $\pm$ SD	Calculated concentration Mean $\pm$ SD [ng/mL]	RSD [%]	Δ [%] ¹ Mean $\pm$ SD
<i>(+)-Catechin</i>						
2.68	2.72 $\pm$ 0.34	12.54	1.35	2.78 $\pm$ 0.34	12.32	3.71
8.18	7.84 $\pm$ 1.20	15.28	-4.16	7.21 $\pm$ 0.72	9.96	-11.87
12.77	10.72 $\pm$ 1.51	14.04	-16.04	10.92 $\pm$ 1.50	13.72	-14.50
19.96	17.71 $\pm$ 1.11	6.27	-11.25	16.10 $\pm$ 0.83	5.17	-19.36
<i>M1</i>						
0.146	0.151 $\pm$ 0.02	14.28	3.09	0.157 $\pm$ 0.01	7.21	7.24
0.445	0.457 $\pm$ 0.04	9.07	2.66	0.437 $\pm$ 0.01	3.06	-1.90
0.695	0.700 $\pm$ 0.08	11.06	0.73	0.749 $\pm$ 0.07	9.70	7.72
1.086	1.172 $\pm$ 0.06	4.70	7.96	1.097 $\pm$ 0.10	8.71	1.04
<i>Caffeic acid</i>						
3.83	3.73 $\pm$ 0.02	0.47	-2.76	3.71 $\pm$ 0.25	6.77	-3.14
11.69	11.26 $\pm$ 1.71	15.18	-3.66	10.15 $\pm$ 0.84	8.25	-13.17
18.27	15.86 $\pm$ 0.08	5.07	-13.16	15.71 $\pm$ 0.59	3.74	-13.99
28.54	26.80 $\pm$ 2.60	9.69	-6.10	25.24 $\pm$ 2.30	9.12	-11.58
<i>Taxifolin</i>						
0.100	0.107 $\pm$ 0.01	13.99	6.75	0.104 $\pm$ 0.01	12.53	4.06
0.306	0.268 $\pm$ 0.03	11.48	-12.57	0.277 $\pm$ 0.03	9.41	-9.46
0.478	0.398 $\pm$ 0.08	2.10	-16.70	0.430 $\pm$ 0.06	15.09	-10.06
0.747	0.744 $\pm$ 0.04	5.01	-0.46	0.666 $\pm$ 0.04	6.59	-10.80
<i>Ferulic acid</i>						
1.91	1.87 $\pm$ 0.29	15.63	-2.19	1.71 $\pm$ 0.19	10.86	-10.61
5.83	5.57 $\pm$ 0.79	14.20	-4.49	4.95 $\pm$ 0.25	5.02	-15.15
9.11	9.01 $\pm$ 0.51	5.68	-1.15	8.49 $\pm$ 0.83	9.80	-6.82
14.24	13.50 $\pm$ 0.83	6.12	-5.18	12.91 $\pm$ 0.26	2.00	-9.37

¹: (calculated concentration mean $\pm$ SD [ng/mL] / (spiked concentration [ng/mL]) - 1) * 100

Table S8: Post-preparative stability: stability of the analytes after one freeze-thaw cycle (n= 3).

Analytes and spiked concentration [ng/mL]	Freeze-thaw stability: 1 cycle -20 °C h/at least 12 h - RT/1h		
	Calculated concentration Mean ± SD [ng/mL]	RSD [%]	Δ [%] ¹ Mean ± SD
<i>(+)-Catechin</i>			
2.68	2.09 ± 0.16	7.81	-21.82
8.18	7.42 ± 0.59	7.52	-9.29
12.77	10.63 ± 0.34	3.21	-16.76
19.96	17.78 ± 2.66	14.96	-10.93
<i>M1</i>			
0.146	0.209 ± 0.00	0.99	42.87
0.445	0.564 ± 0.05	8.22	26.63
0.695	0.737 ± 0.06	8.64	6.02
1.086	1.180 ± 0.176	14.95	8.66
<i>Caffeic acid</i>			
3.83	4.04 ± 0.37	9.18	5.58
11.69	9.92 ± 0.43	4.38	-15.13
18.27	15.50 ± 0.39	2.51	-15.14
28.54	23.55 ± 1.85	7.87	-17.50
<i>Taxifolin</i>			
0.100	0.107 ± 0.01	5.56	7.45
0.306	0.352 ± 0.02	6.12	14.98
0.478	0.466 ± 0.07	14.48	-2.48
0.747	0.742 ± 0.12	15.95	-0.70
<i>Ferulic acid</i>			
1.91	2.24 ± 0.12	5.51	16.91
5.83	6.11 ± 0.59	9.64	4.80
9.11	9.94 ± 0.95	9.55	9.04
14.24	13.29 ± 1.89	14.20	-6.72

¹: (calculated concentration mean ± SD [ng/mL] / (spiked concentration [ng/mL])-1)*100

Table S9: Lower limit of quantification (LLOQ) and related accuracy of the five analytes extracted from human pooled synovial fluid (n= 3).

Analytes	LLOQ [ng/mL]	Accuracy _{LLOQ} [%] Mean $\pm$ SD
(+)-Catechin	2.14	101.41 $\pm$ 17.00
Ferulic acid	1.53	93.39 $\pm$ 14.38
M1	0.117	97.24 $\pm$ 17.38
Taxifolin	0.080	103.15 $\pm$ 12.49
Caffeic acid	3.07	106.85 $\pm$ 8.31

Table S10: Recovery, matrix effects and process efficiency of the five analytes extracted from human pooled synovial fluid at three concentrations (n= 3).

Analytes and spiked concentration [ng/mL]	Recovery [%]			Process efficiency [%]			Matrix effects [%]		
	Mean	± SD	RSD [%]	Mean	± SD	RSD [%]	Mean	± SD	RSD [%]
<i>Catechin</i>									
8.18	39.24	3.04	7.75	29.03	2.09	7.21	-26.00	0.71	2.72
12.77	45.16	8.01	17.74	33.91	4.75	14.02	-24.91	2.91	11.70
19.96	40.81	2.41	5.90	32.37	2.62	8.10	-20.68	3.11	15.05
<i>Taxifolin</i>									
0.306	63.24	6.12	9.67	95.25	7.56	7.94	50.62	1.40	2.76
0.478	87.63	7.10	8.12	122.00	11.85	9.72	39.23	1.78	4.53
0.747	71.76	7.24	10.08	109.86	10.86	9.88	53.10	3.21	6.05
<i>M1</i>									
0.445	45.76	4.16	9.10	165.99	11.92	7.18	262.77	15.11	5.75
0.695	49.17	9.72	19.78	172.14	14.08	8.18	250.13	27.75	11.09
1.086	55.43	3.23	5.82	192.04	11.09	5.78	246.45	5.67	2.30
<i>Ferulic acid</i>									
5.83	52.47	7.32	13.94	149.25	19.75	13.23	184.42	2.45	1.33
9.11	58.14	8.00	13.75	155.37	17.05	10.97	167.22	5.40	3.23
14.24	57.54	1.35	2.34	148.15	3.44	2.32	157.48	0.42	0.27
<i>Caffeic acid</i>									
11.69	59.92	1.59	2.66	76.51	4.20	5.50	27.69	3.90	14.07
18.27	68.30	11.71	17.15	88.63	13.96	15.75	29.77	1.94	6.50
28.54	67.48	7.65	11.33	84.63	9.67	11.43	25.41	2.84	11.16

Table S11: Recovery, matrix effects and process efficiency of the five analytes extracted from three lots of synovial fluid at two concentrations (n= 3).

Analytes and spiked concentration [ng/mL]	Recovery [%]			Process efficiency [%]			Matrix effect [%]		
	Mean	± SD	RSD [%]	Mean	± SD	RSD [%]	Mean	± SD	RSD [%]
<i>Catechin</i>									
8.18	43.46	6.41	14.74	31.23	3.49	11.19	-27.79	4.97	17.87
19.96	48.17	3.24	6.72	36.92	1.97	5.34	-23.30	2.21	9.47
<i>Taxifolin</i>									
0.306	58.16	13.15	22.61	87.37	17.02	19.48	51.13	8.12	15.87
0.747	69.25	2.62	3.78	110.24	7.98	7.24	59.05	5.97	10.11
<i>M1</i>									
0.445	47.45	11.15	23.51	164.55	47.69	28.98	243.83	21.48	8.81
1.086	59.38	0.68	1.14	200.23	17.52	8.75	237.04	26.50	11.18
<i>Ferulic acid</i>									
5.83	46.82	7.58	16.19	130.57	17.40	13.33	180.61	27.99	15.49
14.24	48.47	3.45	7.13	128.48	9.16	7.13	165.07	3.01	1.82
<i>Caffeic acid</i>									
11.69	63.83	8.23	12.89	80.69	11.38	14.10	26.30	1.49	5.65
28.54	62.98	6.85	10.88	81.68	11.75	14.38	29.36	5.28	18.00

Table S12: Internal standard (IS; hydrocaffeic acid) normalised matrix factor (MF) at human pooled synovial fluid in three concentrations (n= 3) and in three lots of synovial fluid at two concentrations (n= 3).

Analytes and spiked concentration [ng/mL]	IS-normalised MF in human pooled synovial fluid			IS-normalised MF in three lots of human synovial fluid		
	Mean	± SD	RSD [%]	Mean	± SD	RSD [%]
<i>Catechin</i>						
8.18	-0.236	0.01	3.08	-0.252	0.05	21.05
12.77	-0.229	0.04	18.11			
19.96	-0.195	0.03	16.68	-0.215	0.03	14.46
<i>Taxifolin</i>						
0.306	0.459	0.02	3.48	0.459	0.05	10.92
0.478	0.360	0.04	11.09			
0.747	0.501	0.05	9.66	0.540	0.03	6.18
<i>M1</i>						
0.45	2.380	0.21	8.61	2.207	0.28	12.74
0.70	2.297	0.28	12.29			
1.09	2.326	0.14	6.17	2.174	0.26	11.84
<i>Ferulic acid</i>						
5.830	1.671	0.08	4.97	1.638	0.32	19.45
9.110	1.536	0.09	5.82			
14.240	1.486	0.06	3.95	1.514	0.05	3.56
<i>Caffeic acid</i>						
11.69	0.251	0.03	11.11	0.237	0.00	1.41
18.27	0.273	0.01	2.60			
28.54	0.240	0.03	13.93	0.269	0.05	17.20

Figure S1: Example chromatograms for identification of intracellular metabolites of M1 in blood cells of study participants after multiple dosing of 200 mg/day Pycnogenol® over the course of three weeks (P+, V3). After initially smoothing (Gaussian, 5 points) the signal-to-noise ratio (SNR; peak-to-peak height) was calculated.

- A. M1-COOH. The SNR was 47 based on calculation of the transition 223 > 123 (ESI negative).
- B. M1-GSH. The SNR was 5 based on calculation of the transition 514 > 385 (ESI positive).

