

Analysis of Green Tea Extract Using UPLC-MS/MS and LC/UV

Tim Cristy

Battelle

505 King Ave
Columbus, OH 43201

May 2023

Common Botanical Name	CAS No.	Lot No.	Container ID	Net Weight
Green Tea	NA	20S0293200	NA	5 kg

Sample storage condition until analysis
-20 °C

Quantitative Method:

LC/UV Method

LC Conditions	UV Conditions														
System: Agilent 1260	System: Agilent 1260														
Column: Thermo Scientific Accucore aQ (4.6 x 150 mm, 2.6 μm)	Wavelength: 274 nm														
Mobile phase A: 97:3 H₂O:CH₃CN + 0.1% FA															
Mobile phase B: CH₃CN + 0.1% FA															
Flow rate: 1 mL/min															
Column temperature: 40 °C															
Gradient															
<table><tr><th>Time (min)</th><th>Mobile phase B (%)</th></tr><tr><td>0</td><td>0</td></tr><tr><td>40</td><td>10</td></tr><tr><td>50</td><td>30</td></tr><tr><td>60</td><td>30</td></tr><tr><td>61</td><td>0</td></tr><tr><td>70</td><td>0</td></tr></table>	Time (min)	Mobile phase B (%)	0	0	40	10	50	30	60	30	61	0	70	0	
Time (min)	Mobile phase B (%)														
0	0														
40	10														
50	30														
60	30														
61	0														
70	0														

Quantitative Results

Compound	Concentration in extract (mg/g) $\pm$ s
Caffeine	1.12 ± 0.02
Catechin [C]	6.72 ± 0.68
Epicatechin [EC]	85.1 ± 0.7
Epigallocatechin [EGC]	147 ± 2
Epigallocatechin gallate [EGCG]	440 ± 4
Epicatechin gallate [ECG]	84.5 ± 0.7
Galocatechin gallate [GCG]	23.8 ± 0.4
Galocatechin [GC]	14.8 ± 0.2
Gallic acid [GA]	6.21 ± 0.11
Theobromine	1.43 ± 0.05
Theophylline	0.148 ± 0.043

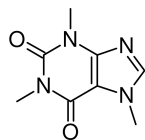
Standards

Name	Supplier	CAS Number	Catalog Number	Purity %	Batch/Lot number
Caffeine	Sigma-Aldrich	58-08-2	C1778	99.6%	SLCJ5558
(-)-Catechin [C]	Sigma-Aldrich	18829-70-4	PHL80992	100.00%	112218378
(-)-Epicatechin [EC]	Sigma-Aldrich	490-46-0	PHL89192	99.73%	99627492
(-)-Epigallocatechin [EGC]	Sigma-Aldrich	970-74-1	PHL89655	98.47%	87929981

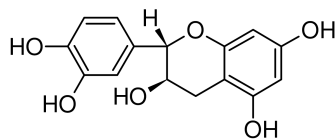
(-)-Epigallocatechin-3-O-gallate [EGCG]	Sigma-Aldrich	989-51-5	1236700	92%	R088T0
(-)-Epicatechin gallate [ECG]	Sigma-Aldrich	1257-08-5	E3893	99%	WXBD0103V
(-)-Gallocatechin gallate [GCG]	Sigma-Aldrich	4233-96-9	G6782	99%	SLCH7064
(-)-Gallocatechin [GC]	Sigma-Aldrich	3371-27-5	01388	99.6%	WXBD3189V
Gallic acid [GA]	Sigma-Aldrich	149-91-7	PHL89198	100.00%	111238103
Theobromine	Sigma-Aldrich	83-67-0	T4500	99.4%	BCCF0961
Theophylline	Sigma-Aldrich	58-55-9	1653004	99.9%	R084X0

Chemical structures of standards used for quantitative analysis

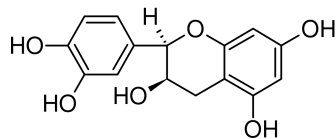
Caffeine



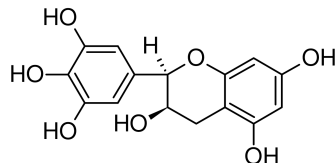
Catechin



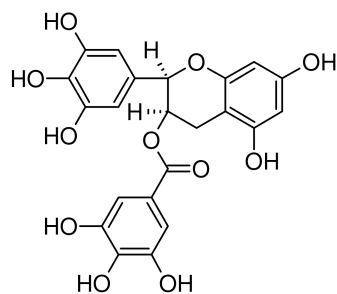
Epicatechin



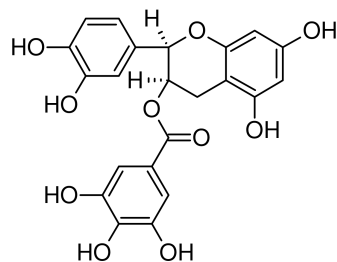
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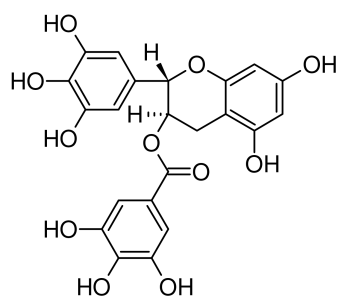
Epigallocatechin Gallate



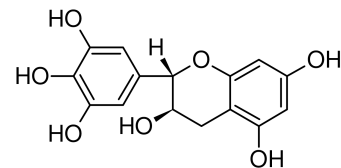
Epicatechin Gallate



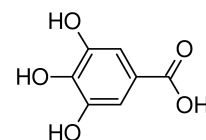
Gallocatechin gallate



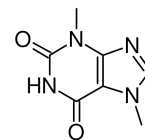
Gallocatechin



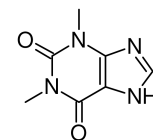
Gallic Acid



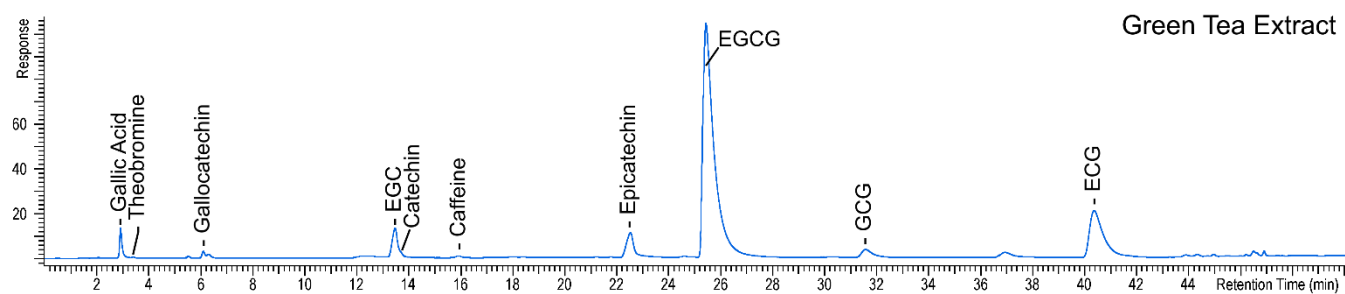
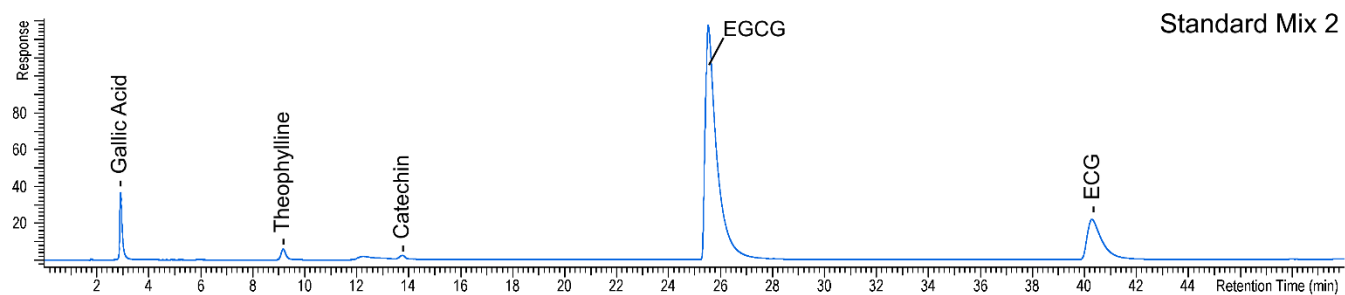
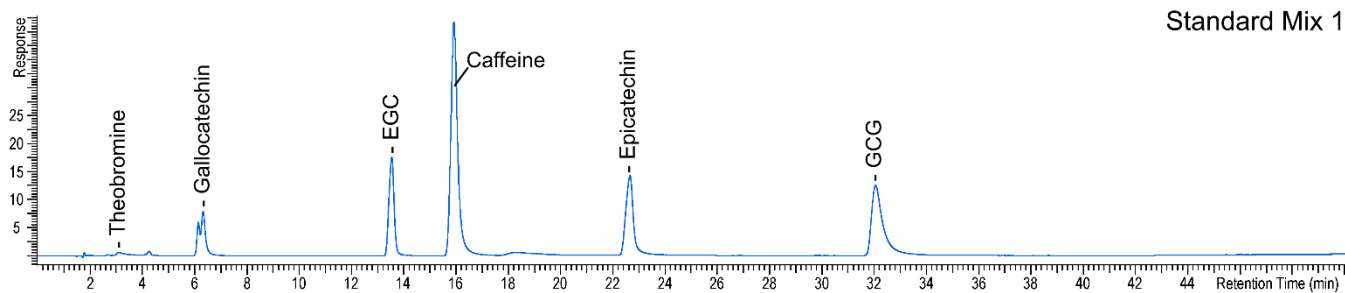
Theobromine



Theophylline



Chromatograms



Calibration

Compound	Lower limit of quantitation (LLOQ) ug/mL	Calibration range (ug/mL)	Number of Calibration Points	R ²	Concentration mg/g in extract (n = 3)
Caffeine	0.20	0.20 – 10	3	1.0000	1.12 ± 0.02
Catechin [C]	0.20	0.20 – 2.0	3	0.9992	6.72 ± 0.68
Epicatechin [EC]	1.4	1.4 – 20	3	0.9998	85.1 ± 0.7
Epigallocatechin [EGC]	2.0	2.0 – 40	3	0.9999	147 ± 2
Epigallocatechin gallate [EGCG]	8.0	8.0 – 120	3	0.9993	440 ± 4
Epicatechin gallate [ECG]	1.0	1.0 – 20	3	0.9999	84.5 ± 0.7
Gallocatechin gallate [GCG]	2.0	2.0 – 16	3	0.9928	23.8 ± 0.4
Gallocatechin [GC]	1.0	1.0 – 10	3	0.9997	14.8 ± 0.2
Gallic acid [GA]	0.80	0.80 – 3.0	3	0.9999	6.21 ± 0.11
Theobromine	0.10	0.10 – 1.0	3	0.9709	1.43 ± 0.05
Theophylline	0.10	0.10 – 1.0	3	1.0000	0.148 ± 0.043

Targeted and Untargeted Identification Method:

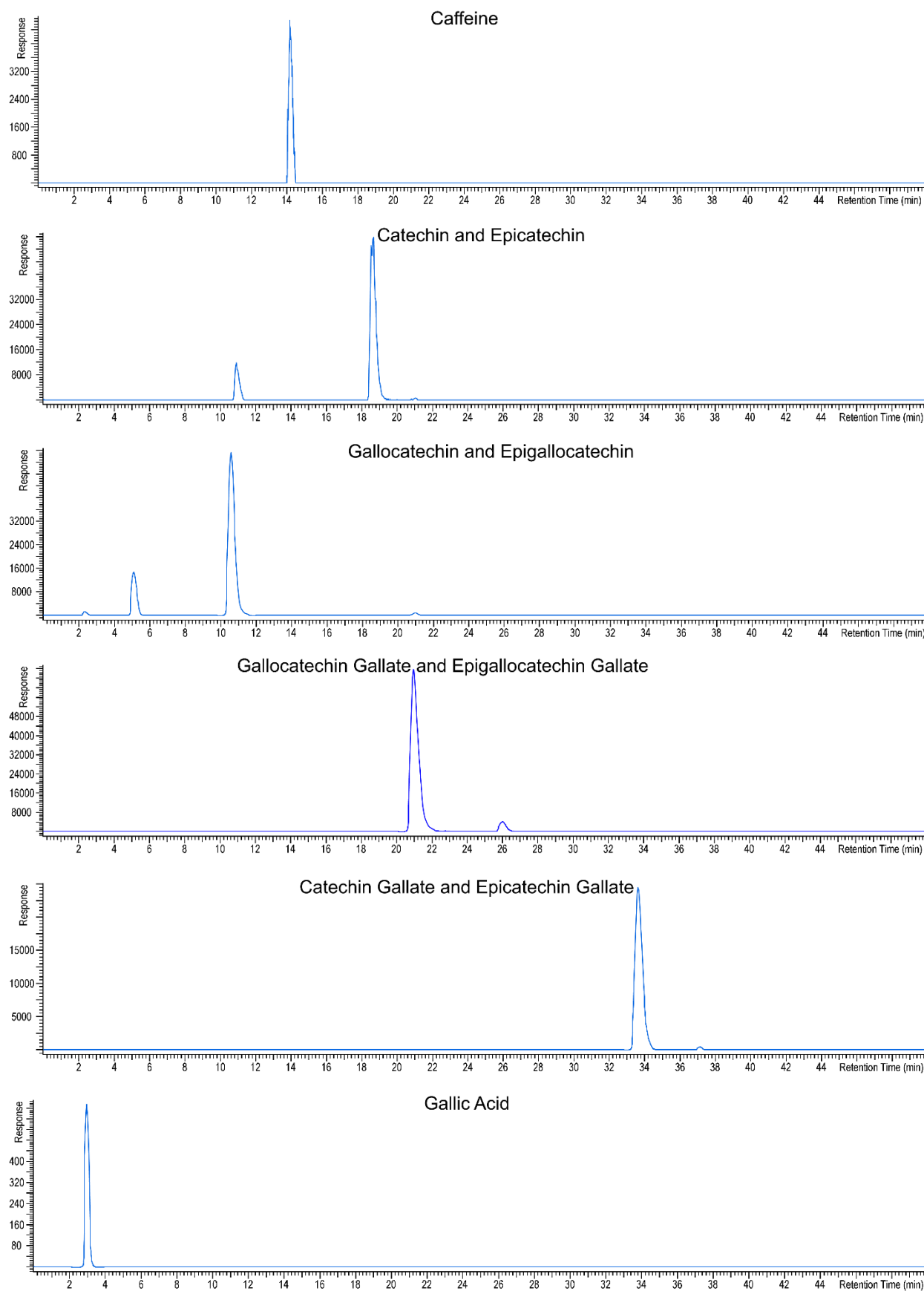
UPLC/MS/MS Method

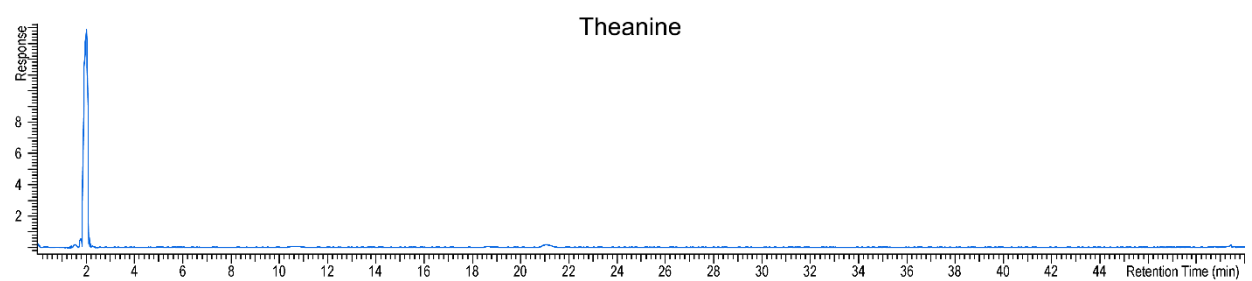
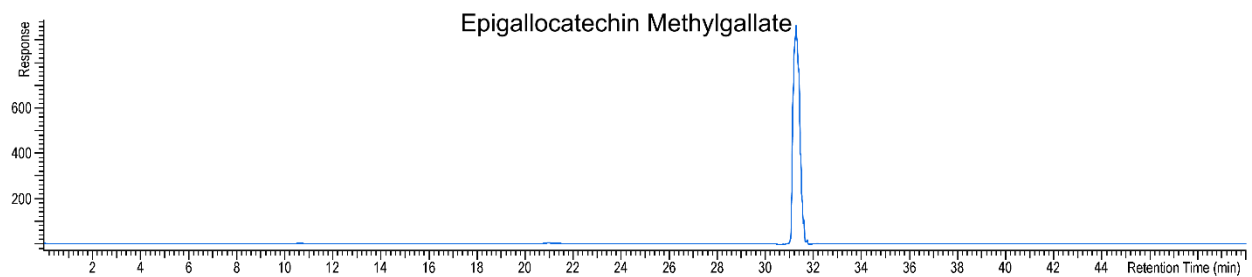
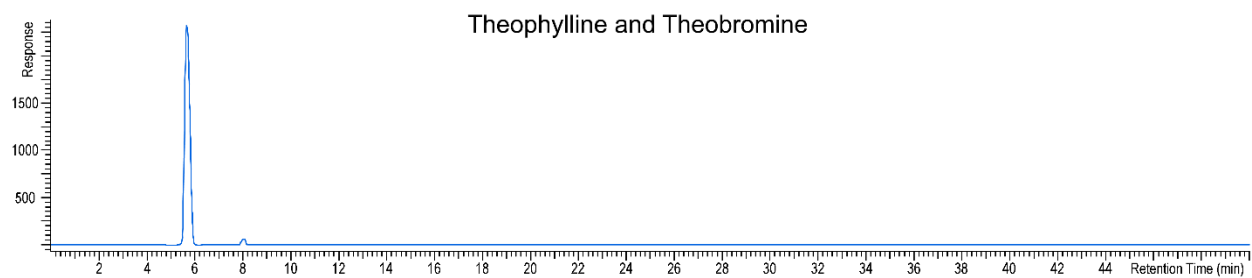
UPLC Conditions	MS Conditions														
System: Waters Acquity I-Class	System: Sciex Triple TOF 5600														
Column: Thermo Scientific Accucore aQ (4.6 x 150 mm, 2.6 um)	Ionization: Electrospray (ESI)														
Mobile phase A: 97:3 H₂O:CH₃CN + 0.1% FA	Polarity: Positive														
Mobile phase B: CH₃CN + 0.1% FA	Source Settings: · Nebulizer Gas: 30 psi · Heater Gas: 50 psi · Curtain Gas: 35 psi · Ion Source Voltage: 5500 V · Source Temperature: 450 °C														
Flow rate: 1 mL/min	MS Settings: · TOF-MS Scan Range: m/z 50 – 1000 · TOF-MS Collision Energy: 10 V · IDA MS/MS Scan Range: m/z 20 – 1000 · IDA MS/MS Collision Energy: 25 V														
Column temperature: 40 °C															
Gradient															
<table><tr><th>Time (min)</th><th>Mobile phase B (%)</th></tr><tr><td>0</td><td>0</td></tr><tr><td>40</td><td>10</td></tr><tr><td>50</td><td>30</td></tr><tr><td>60</td><td>30</td></tr><tr><td>61</td><td>0</td></tr><tr><td>70</td><td>0</td></tr></table>	Time (min)	Mobile phase B (%)	0	0	40	10	50	30	60	30	61	0	70	0	
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0	0														
40	10														
50	30														
60	30														
61	0														
70	0														

Table 1: Proposed identification of constituents of Green Tea

Peak No.	RT (min)	<i>m/z</i>	Mass accuracy (ppm)	Proposed ID's (Confirmed with Std in green) (Structure with correlated MS ² spectra in orange) (Most probable ID is yellow)	Molecular Formula
1	14.18	195.087	-5	Caffeine	C ₈ H ₁₀ N ₄ O ₂
2	10.95	291.087	0	Catechin [C]	C ₁₅ H ₁₄ O ₆
3	18.62	291.087	0	Epicatechin [EC]	C ₁₅ H ₁₄ O ₆
4	10.60	307.083	3	Epigallocatechin [EGC]	C ₁₅ H ₁₄ O ₇
5	20.94	459.092	-2	Epigallocatechin gallate [EGCG]	C ₂₂ H ₁₈ O ₁₁
6	33.62	443.094	-9	Epicatechin gallate [ECG]	C ₂₂ H ₁₈ O ₁₀
7	25.99	459.089	-9	Galocatechin gallate [GCG]	C ₂₂ H ₁₈ O ₁₁
8	5.09	307.083	3	Galocatechin [GC]	C ₁₅ H ₁₄ O ₇
9	2.97	171.030	6	Gallic acid [GA]	C ₇ H ₆ O ₅
10	5.65	181.071	-1	Theobromine	C ₇ H ₈ N ₄ O ₂
11	8.09	181.073	0	Theophylline	C ₇ H ₈ N ₄ O ₂
12	37.14	443.097	-2	Catechin gallate [CG]	C ₂₂ H ₁₈ O ₁₀
13	31.28	473.105	-6	Epigallocatechin methylgallate [EGCGMe]	C ₂₃ H ₂₀ O ₁₁
14	2.02	175.109	6	Theanine	C ₇ H ₁₄ N ₂ O ₃

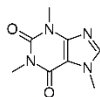
Figure 1: Extracted Ion Chromatograms for each constituent in Green Tea from Table 1



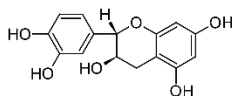


Structures of compounds in Table 1

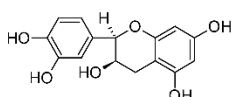
Caffeine



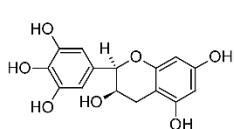
Catechin



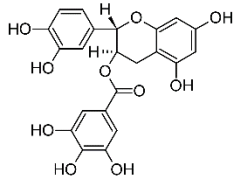
Epicatechin



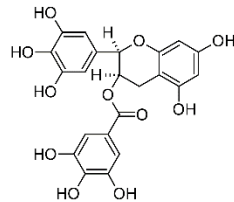
Epigallocatechin



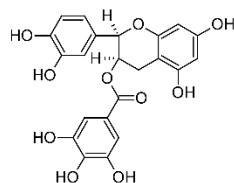
Catechin Gallate



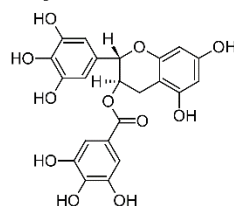
Epigallocatechin Gallate



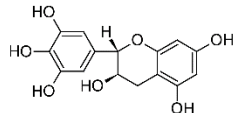
Epicatechin Gallate



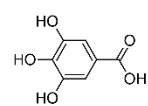
Gallocatechin gallate



Gallocatechin



Gallic Acid



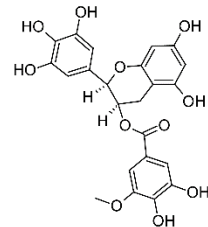
Theobromine



Theophylline



Epigallocatechin Methylgallate



Theanine

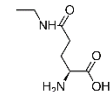


Table 2: MS/MS product ions for the peaks of the botanical extract

Peak No.	RT (min)	Precursor m/z^1	Proposed Compounds (Confirmed with Std in green) (Probable ID is yellow) CAS Number	Product Ions	
				m/z	Rel. Int
1	14.22	195.1	Caffeine 58-08-2	42.036	3.11
				56.056	1.17
				69.047	2.33
				83.061	2.72
				84.960	5.84
				110.072	6.61
				123.043	2.33
				130.965	3.11
				135.924	1.17
				138.066	31.13
				195.088	100.00
2	10.98	291.1	Catechin [C] 18829-70-4	119.050	8.47
				123.042	54.03
				139.039	100.00
				147.042	20.97
				165.057	20.97
				179.075	8.47
				207.061	16.53
				291.084	83.06
3	18.56	291.1	Epicatechin [EC] 490-46-0	111.043	3.63
				119.053	2.75
				123.043	51.95
				127.038	2.75
				135.042	2.75
				138.616	1.95
				139.039	100.00
				140.044	1.95
				143.046	3.63
				147.042	22.87
				151.039	3.63
				161.059	10.99
				165.051	12.77
				179.071	4.61
				181.049	4.61
				189.057	5.50
4	10.48	307.1	Epigallocatechin [EGC] 970-74-1	207.065	15.51
				249.072	3.63
				273.077	5.50
				291.088	39.72
				135.044	2.70
				139.039	100.00
				151.039	7.57
				163.037	12.92
				169.050	4.64
				177.055	4.64
				181.051	9.78
				195.064	7.08
				205.046	1.70
				223.058	3.67
				247.064	1.23
				289.070	6.34
				307.081	30.49

5	20.87	459.1	Epigallocatechin gallate [EGCG] 989-51-5	139.040	100.00
				151.037	8.49
				153.018	6.31
				163.040	2.65
				181.049	2.90
				205.050	1.81
				289.069	20.68
				307.083	2.29
				333.063	1.34
				459.089	16.50
6	33.60	443.1	Epicatechin gallate [ECG] 1257-08-5	123.044	72.44
				139.039	100.00
				151.037	8.90
				153.017	9.40
				165.055	4.09
				273.074	43.11
				291.085	7.67
				317.064	2.26
				443.097	42.28
7	26.02	459.1	Galocatechin gallate [GCG] 4233-96-9	139.037	100.00
				151.036	6.43
				153.020	11.07
				163.039	4.29
				181.047	7.86
				205.050	2.14
				289.070	26.79
				307.086	3.21
				333.061	2.14
				376.985	1.07
				399.799	1.43
				459.090	21.43
8	5.07	307.1	Galocatechin [GC] 3371-27-5	133.027	2.01
				139.039	100.00
				151.037	5.76
				163.039	11.65
				169.050	7.14
				177.055	3.88
				181.049	15.54
				195.066	6.52
				205.050	1.88
				229.885	1.88
				239.879	1.88
				247.057	3.26
				247.894	2.63
				265.861	1.88
				289.068	7.77
				307.081	22.68
9	2.95	171.0	Gallic acid [GA] 149-91-7	51.023	2.94
				53.042	17.65
				79.029	8.82
				81.033	55.88
				88.954	20.59
				109.028	82.35
				116.932	17.65
				125.024	35.29
				127.041	52.94
				134.943	17.65

				135.012	8.82
				153.015	94.12
				171.028	100.00
10	5.69	181.1	Theobromine 83-67-0	42.040	3.31
				54.950	1.99
				67.031	3.97
				69.045	2.65
				83.061	2.65
				84.960	6.62
				95.974	4.64
				108.061	2.65
				110.072	5.30
				113.037	1.99
				116.932	1.99
				122.059	3.31
				123.044	2.65
				134.944	2.65
				135.065	3.97
				137.084	2.65
				138.066	19.21
				139.923	2.65
				162.934	2.65
				163.060	9.27
				181.070	100.00
11	8.06	181.1	Theophylline 58-55-9	42.042	16.00
				95.975	20.00
				116.930	12.00
				124.051	56.00
				134.944	16.00
				139.923	12.00
				162.935	16.00
				181.071	100.00
12	37.06	443.1	Catechin gallate [CG] 130405-40-2	123.045	46.88
				139.037	100.00
				151.043	6.25
				153.019	9.38
				165.050	6.25
				273.077	34.38
				291.086	9.38
				317.072	6.25
				396.787	6.25
				401.728	6.25
				443.095	34.38
13	31.22	473.1	Epigallocatechin methylgallate [EGCGMe] 83104-87-4	139.039	100.00
				151.039	8.11
				163.033	2.70
				167.035	17.57
				205.048	2.70
				289.070	29.73
				347.079	2.70
				413.771	2.70
				454.787	2.70
				473.109	20.27

1 The mass resolution of the quadrupole in the QTOF is 0.1 Da