**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 ℃ |

**Quantitative Method:**

|  |
| --- |
| **LC/UV Method** |
| LC Conditions | UV Conditions |
| System: **Agilent 1260**Column: **Thermo Scientific Accucore aQ****(4.6 x 150 mm, 2.6 um)**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 ℃**Gradient

|  |  |
| --- | --- |
| Time (min) | Mobile phase B (%) |
| **0** | **0** |
| **40** | **10** |
| **50** | **30** |
| **60** | **30** |
| **61** | **0** |
| **70** | **0** |

 | System: **Agilent 1260**Wavelength: **274 nm** |

**Quantitative Resultsa**

|  |  |  |
| --- | --- | --- |
| **Compoundb** | **Concentration in extract****mg/g****Average ± s** | **Concentration in extract****% wt/wt****Average** |
| Caffeine | **1.12 ± 0.02** | **0.112** |
| Catechin [C] | **6.72 ± 0.68** | **0.672** |
| Epicatechin [EC] | **85.1 ± 0.7** | **8.51** |
| Epigallocatechin [EGC] | **147 ± 2** | **14.7** |
| Epigallocatechin gallate [EGCG] | **440 ± 4** | **44.0** |
| Epicatechin gallate [ECG] | **84.5 ± 0.7** | **8.45** |
| Gallocatechin gallate [GCG] | **23.8 ± 0.4** | **2.38** |
| Gallocatechin [GC] | **14.8 ± 0.2** | **1.48** |
| Gallic acid [GA] | **6.21 ± 0.11** | **0.621** |
| Theobromine | **1.43 ± 0.05** | **0.143** |
| Theophylline | **0.148 ± 0.043** | **0.0148** |

1. **Quantitated using UV Chromatograms**
2. **The standards used were a specific enantiomer for the chiral compounds, but the analytical method is not enantioselective so the common name is used in the results**

**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**

****

**Chromatograms**

****

****

****

**Calibration**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compound** | **Lower Limit of Quantitation (LLOQ) ug/mL** | **Calibration Range (ug/mL)** | **Number of Calibration Points** | **R2** |
| **Caffeine** | 0.20 | 0.20 – 10 | 3 | 1.0000 |
| **Catechin [C]** | 0.20 | 0.20 – 2.0 | 3 | 0.9992 |
| **Epicatechin [EC]** | 1.4 | 1.4 – 20 | 3 | 0.9998 |
| **Epigallocatechin [EGC]** | 2.0 | 2.0 – 40 | 3 | 0.9999 |
| **Epigallocatechin gallate [EGCG]** | 8.0 | 8.0 – 120 | 3 | 0.9993 |
| **Epicatechin gallate [ECG]** | 1.0 | 1.0 – 20 | 3 | 0.9999 |
| **Gallocatechin gallate [GCG]** | 2.0 | 2.0 – 16 | 3 | 0.9928 |
| **Gallocatechin [GC]** | 1.0 | 1.0 – 10 | 3 | 0.9997 |
| **Gallic acid [GA]** | 0.80 | 0.80 – 3.0 | 3 | 0.9999 |
| **Theobromine** | 0.10 | 0.10 – 1.0 | 3 | 0.9709 |
| **Theophylline** | 0.10 | 0.10 –1.0 | 3 | 1.0000 |

**Targeted and Untargeted Identification Method:**

**UPLC/MS/MS Method**

|  |  |
| --- | --- |
| UPLC Conditions | MS Conditions |
| System: **Waters Acquity I-Class**Column: **Thermo Scientific Accucore aQ (4.6 x 150 mm, 2.6 um)**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 ℃**Gradient

|  |  |
| --- | --- |
| Time (min) | Mobile phase B (%) |
| **0** | **0** |
| **40** | **10** |
| **50** | **30** |
| **60** | **30** |
| **61** | **0** |
| **70** | **0** |

 | System: **Sciex Triple TOF 5600**Ionization: **Electrospray (ESI)**Polarity: **Positive**Source Settings: · Nebulizer Gas: **30 psi** · Heater Gas: **50 psi** · Curtain Gas: **35 psi** · Ion Source Voltage: **5500 V** · Source Temperature: **450** **℃**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** |

|  |
| --- |
| Table 1. Targeted and Untargeted Identification |
| **ID** | **Retention****Timea****(minutes)** | **Expected M+H****Exact Mass (Da)** | **Found M+H Exact Mass (Da)** | **Mass Error****(Da)** | **CAS****Number** | **Molecular****Formula** | **Structure** | **ID****Confidence****Levelb** |
| Caffeine | 14.18 | 195.088 | 195.087 | 0.001 | 58-08-2 | C8H10N4O2 |  | 1 |
| Catechin [C] | 10.95 | 291.087 | 291.087 | 0.000 | 18829-70-4 | C15H14O6 |  | 1 |
| Epicatechin [EC] | 18.62 | 291.087 | 291.087 | 0.000 | 490-46-0 | C15H14O6 |  | 1 |
| Epigallocatechin [EGC] | 10.60 | 307.082 | 307.083 | 0.001 | 970-74-1 | C15H14O7 |  | 1 |
| Epigallocatechin gallate [EGCG] | 20.94 | 459.093 | 459.092 | 0.001 | 989-51-5 | C22H18O11 |  | 1 |
| Epicatechin gallate [ECG] | 33.62 | 443.098 | 443.094 | 0.004 | 1257-08-5 | C22H18O10 |  | 1 |
| Gallocatechin gallate [GCG] | 25.99 | 459.093 | 459.089 | 0.004 | 4233-96-9 | C22H18O11 |  | 1 |
| Gallocatechin [GC] | 5.09 | 307.082 | 307.083 | 0.001 | 3371-27-5 | C15H14O7 |  | 1 |
| Gallic acid [GA] | 2.97 | 171.029 | 171.030 | 0.001 | 149-91-7 | C7H6O5 |  | 1 |
| Theobromine | 5.65 | 181.073 | 181.071 | 0.002 | 83-67-0 | C7H8N4O2 |  | 1 |
| Theophylline | 8.09 | 181.073 | 181.073 | 0.000 | 58-55-9 | C7H8N4O2 |  | 1 |
| Catechin gallate [CG] | 37.14 | 443.098 | 443.097 | 0.001 | 130405-40-2 | C22H18O10 |  | 3 |
| Epigallocatechin methylgallate[EGCGMe] | 31.28 | 473.108 | 473.105 | 0.003 | 83104-87-4 | C23H20O11 |  | 5c |
| Theanine | 2.02 | 175.108 | 175.109 | 0.001 | 3081-61-6 | C7H14N2O3 |  | 5 |

1. Retention time of the molecular ion peak in the MS for the sample.
2. Level 1: Retention time, exact mass, and MS/MS match with a reference standard

Level 2: MS/MS Match with a library/literature spectrum obtained under similar conditions or only one possible structure consistent with MS/MS

Level 3: MS/MS consistent with the structure of a literature compound or class of compounds or a library spectrum obtained under different conditions; relative retention consistent with literature if available

Level 4: Only one molecular formula fits the exact mass and isotope ratio

Level 5: Exact mass matches a literature compound

1. In addition to the exact mass, the retention time is consistent with literature relative retention

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



















|  |
| --- |
| **Table 2: MS/MS product ions for the peaks of the botanical extract** |
| Peak No. | RT (min) | *Precursor**m/z1* | Proposed Compounds (Confirmed with Std in **green**)(Probable ID is **yellow**)CAS Number | Product Ions *m/z* Rel. Int |
| 1 | 14.22 | 195.1 | Caffeine58-08-2 | 42.036 3.1156.056 1.1769.047 2.3383.061 2.7284.960 5.84110.072 6.61123.043 2.33130.965 3.11135.924 1.17138.066 31.13195.088 100.00 |
| 2 | 10.98 | 291.1 | Catechin [C]18829-70-4 | 119.050 8.47123.042 54.03139.039 100.00147.042 20.97165.057 20.97179.075 8.47207.061 16.53291.084 83.06 |
| 3 | 18.56 | 291.1 | Epicatechin [EC]490-46-0 | 111.043 3.63119.053 2.75123.043 51.95127.038 2.75135.042 2.75138.616 1.95139.039 100.00140.044 1.95143.046 3.63147.042 22.87151.039 3.63161.059 10.99165.051 12.77179.071 4.61181.049 4.61189.057 5.50207.065 15.51249.072 3.63273.077 5.50291.088 39.72 |
| 4 | 10.48 | 307.1 | Epigallocatechin [EGC]970-74-1 | 135.044 2.70139.039 100.00151.039 7.57163.037 12.92169.050 4.64177.055 4.64181.051 9.78195.064 7.08205.046 1.70223.058 3.67247.064 1.23289.070 6.34307.081 30.49 |
| 5 | 20.87 | 459.1 | Epigallocatechin gallate [EGCG]989-51-5 | 139.040 100.00151.037 8.49153.018 6.31163.040 2.65181.049 2.90205.050 1.81289.069 20.68307.083 2.29333.063 1.34459.089 16.50 |
| 6 | 33.60 | 443.1 | Epicatechin gallate [ECG]1257-08-5 | 123.044 72.44139.039 100.00151.037 8.90153.017 9.40165.055 4.09273.074 43.11291.085 7.67317.064 2.26443.097 42.28 |
| 7 | 26.02 | 459.1 | Gallocatechin gallate [GCG]4233-96-9 | 139.037 100.00151.036 6.43153.020 11.07163.039 4.29181.047 7.86205.050 2.14289.070 26.79307.086 3.21333.061 2.14376.985 1.07399.799 1.43459.090 21.43 |
| 8 | 5.07 | 307.1 | Gallocatechin [GC]3371-27-5 | 133.027 2.01139.039 100.00151.037 5.76163.039 11.65169.050 7.14177.055 3.88181.049 15.54195.066 6.52205.050 1.88229.885 1.88239.879 1.88247.057 3.26247.894 2.63265.861 1.88289.068 7.77307.081 22.68 |
| 9 | 2.95 | 171.0 | Gallic acid [GA]149-91-7 | 51.023 2.9453.042 17.6579.029 8.8281.033 55.8888.954 20.59109.028 82.35116.932 17.65125.024 35.29127.041 52.94134.943 17.65135.012 8.82153.015 94.12171.028 100.00 |
| 10 | 5.69 | 181.1 | Theobromine83-67-0 | 42.040 3.3154.950 1.9967.031 3.9769.045 2.6583.061 2.6584.960 6.6295.974 4.64108.061 2.65110.072 5.30113.037 1.99116.932 1.99122.059 3.31123.044 2.65134.944 2.65135.065 3.97137.084 2.65138.066 19.21139.923 2.65162.934 2.65163.060 9.27181.070 100.00 |
| 11 | 8.06 | 181.1 | Theophylline58-55-9 | 42.042 16.0095.975 20.00116.930 12.00124.051 56.00134.944 16.00139.923 12.00162.935 16.00181.071 100.00 |
| 12 | 37.06 | 443.1 | Catechin gallate [CG]130405-40-2 | 123.045 46.88139.037 100.00151.043 6.25153.019 9.38165.050 6.25273.077 34.38291.086 9.38317.072 6.25396.787 6.25401.728 6.25443.095 34.38 |
| 13 | 31.22 | 473.1 | Epigallocatechin methylgallate [EGCGMe]83104-87-4 | 139.039 100.00151.039 8.11163.033 2.70167.035 17.57205.048 2.70289.070 29.73347.079 2.70413.771 2.70454.787 2.70473.109 20.27 |

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