**Table S1** Validation parameters values determined for the polyphenols.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Compounds** | **Linear range**  **(ppm)** | **Regression equation** | **R2** | **LOD (ppm)** | **LOQ (ppm)** | **Repertability (%)** | **Riproducibility (%)** |
| Coumaric acid | 0.5-100 | y = 101.919.0398x - 19.710.9815 | 0.9998 | 0.033 | 0.097 | 1.81 | 1.49 |
| 4-Hydroxybenzoic acid | 0.5-100 | y = 66.976.5781x + 8.811.2215 | 0.9996 | 0.047 | 0.156 | 3.46 | 3.42 |
| Caffeic acid | 0.5-100 | y = 80.403.9809x – 5.618.2263 | 0.9998 | 0.050 | 0.167 | 3.892 | 2.89 |
| Ethylgallate | 0.5-100 | y = 43.307.1523x + 2.133.0116 | 0.9997 | 0.075 | 0.250 | 5.68 | 4.30 |
| Ferulic acid | 0.5-100 | y = 76.190.7484x – 15.485.2230 | 0.9999 | 0.062 | 0.205 | 4.72 | 3.50 |
| Gallic acid | 0.5-100 | y = 58.838.4809x – 34.304.6944 | 0.9996 | 0.065 | 0.218 | 5.16 | 5.19 |
| Kampferol | 0.5-100 | y = 35.708.4046x – 8.738.4609 | 0.9997 | 0.051 | 0.171 | 5.41 | 3.82 |
| Naringin | 0.7-110 | y = 23.180.1541x – 18.258.1645 | 0.9994 | 0.034 | 0.081 | 1.71 | 6.00 |
| Protocatechuic acid | 0.5-100 | y = 56.968.3550x + 10.041.8594 | 0.9996 | 0.079 | 0.264 | 5.57 | 4.19 |
| Rutin | 0.7-110 | y = 26.417.8491x – 27.143.8067 | 0.9993 | 0.048 | 0.159 | 3.40 | 3.79 |
| Syrengin acid | 0.5-100 | y = 45.154.2555x – 19.290.0310 | 0.9998 | 0.081 | 0.270 | 5.81 | 5.01 |
| Vanillic acid | 0.5-100 | y = 39.046.0740x – 17.589.1592 | 0.9998 | 0.076 | 0.254 | 5.69 | 6.00 |
| Hesperidin | 0.5-100 | y = 25.158.0962x – 8.615.2595 | 0.9999 | 0.040 | 0.107 | 1.22 | 2.39 |
| Sinensetin | 0.5-100 | y = 52.911.3069x – 17.330.2806 | 0.9995 | 0.029 | 0.076 | 1.01 | 0.99 |
| Neodiosmin | 0.5-100 | y = 22.188.5733x – 11.478.9917 | 0.9996 | 0.039 | 0.083 | 1.45 | 4.38 |
| Neoeriocitrin | 0.5-100 | y = 21.193.8726x – 8.292.7441 | 0.9998 | 0.043 | 0.086 | 1.66 | 2.13 |
| Hesperetin | 0.5-100 | y = 61.439.7309x – 25.498.1206 | 0.9998 | 0.053 | 0.175 | 1.24 | 2.56 |
| (-)Epicatechin | 0.7-110 | y = 8.995.0663x – 8.543.3500 | 0.9993 | 0.075 | 0.251 | 4.90 | 4.05 |
| Eriocetrin | 0.5-100 | y = 22.104.7983x – 5.509.6099 | 0.9997 | 0.040 | 0.096 | 1.39 | 4.19 |
| Isorhamnetin | 0.5-100 | y = 44.346.4593x – 26.794.1428 | 0.9997 | 0.028 | 0.093 | 2.45 | 3.98 |
| Myricetin | 0.5-100 | y = 44.789.0025x – 37.079.9968 | 0.9997 | 0.057 | 0.190 | 6.38 | 5.41 |
| Neohesperidin | 0.5-100 | y = 10.709.8118x + 3.447.8446 | 0.9993 | 0.032 | 0.106 | 1.36 | 1.65 |
| Diosmin | 0.5-100 | y = 4.214.8056x + 1.093.5404 | 0.9987 | 0.077 | 0.255 | 1.19 | 1.24 |
| Narirutin | 0.5-100 | y = 22293x + 2686.4 | 0.9995 | 0.106 | 0.355 | 4.78 | 5.53 |
| Rhamnetin | 0.5-100 | y = 41523x – 22496 | 0.9995 | 0.048 | 0.159 | 5.10 | 4.15 |
| Tangeretin | 0.5-100 | y = 63271x – 8409.3 | 0.9995 | 0.050 | 0.110 | 0.67 | 2.02 |
| Apigenin | 0.5-100 | y = 68.168.9349x – 21.240.6742 | 0.9994 | 0.036 | 0.120 | 2.81 | 3.23 |
| Chlorogenic acid | 0.75-120 | y = 37088x – 53084 | 0.9992 | 0.084 | 0.281 | 5.08 | 5.34 |
| Nobiletin | 0.5-100 | y = 48.288.0608x – 24.771.5556 | 0.9995 | 0.038 | 0.078 | 1.21 | 2.52 |
| Naringenin | 0.5-100 | y = 36.851.3110x – 17.639.1841 | 0.9997 | 0.033 | 0.077 | 1.69 | 3.69 |

**Table S2** Retention time, instrumental recovery and percentage relative standard deviation of polyphenols

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Compounds** | **Rt** | **±SD** | **RSD%** | **Recovery (%)** | **Compounds** | **Rt** | **±SD** | **RSD%** | **Recovery (%)** |
| Coumaric acid | 12.396 | 0.008 | 0.496 | 102.1 | Neoeriocitrin | 12.986 | 0.011 | 0.772 | 95.1 |
| 4-Hydroxybenzoic acid | 4.060 | 0.014 | 1.310 | 103.8 | Hesperetin | 18.558 | 0.018 | 0.246 | 96.0 |
| Caffeic acid | 7.751 | 0.011 | 0.601 | 102.0 | (-)Epicatechin | 9.880 | 0.013 | 0.868 | 93.9 |
| Ethylgallate | 9.836 | 0.020 | 1.396 | 102.7 | Eriocetrin | 12.597 | 0.015 | 0.657 | 97.0 |
| Ferulic acid | 11.323 | 0.007 | 0.258 | 101.9 | Isorhamnetin | 19.087 | 0.019 | 1.357 | 95.6 |
| Gallic acid | 1.07 | 0.012 | 0.208 | 94.6 | Myricetin | 14.332 | 0.019 | 1.066 | 95.0 |
| Kampferol | 18.551 | 0.014 | 0.767 | 100.8 | Neohesperidin | 14.925 | 0.042 | 0.604 | 100.8 |
| Naringin | 14.125 | 0.010 | 0.358 | 93.2 | Diosmin | 14.864 | 0.011 | 2.077 | 86.9 |
| Protocatechuic acid | 2.157 | 0.009 | 0.769 | 102.3 | Narirutin | 13.721 | 0.016 | 0.931 | 105.1 |
| Rutin | 13.01 | 0.012 | 0.668 | 93.2 | Rhamnetin | 20.826 | 0.008 | 0.368 | 102.3 |
| Syrengin acid | 8.996 | 0.008 | 0.283 | 97.3 | Tangeretin | 23.290 | 0.019 | 0.964 | 104.4 |
| Vanillic acid | 7.344 | 0.015 | 1.172 | 95.9 | Apigenin | 18.489 | 0.009 | 0.655 | 102.8 |
| Hesperidin | 14.531 | 0.007 | 0.321 | 95.8 | Chlorogenic acid | 8.637 | 0.015 | 0.837 | 95.9 |
| Sinensetin | 21.488 | 0.006 | 0.385 | 108.2 | Nobiletin | 22.432 | 0.006 | 0.077 | 99.7 |
| Neodiosmin | 15.191 | 0.014 | 0.356 | 95.5 | Naringenin | 17.644 | 0.011 | 0.101 | 99.6 |

**Table S3** Separation scheme of the bitter acids contained in the analyzed standards

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Peak** | **ICE-4** | **ICS-T3** | **ICS-H2** | **ICS-I4** | **ICS-R3** | **Rt** |
| 1 |  |  |  | trans-Isohumulone |  | 25.044 |
| 2 |  | trans-Tetrahydroiso, Cohumulone | cis-Hexahydroiso cohumulone (1) | trans-Isocohumulone | cis-ρ-Isocohumulone | 25.119 |
| 3 |  |  |  |  | cis-ρ-Isohumulone | 25.715 |
| 4 | Adlupulone, Lupulone |  |  |  |  | 25.853 |
| 5 |  | cis-Tetrahydroiso, Cohumulone |  |  |  | 26.175 |
| 6 |  |  |  | trans-Isoadhumulone | cis-ρ-Isoadhumulone | 26.380 |
| 7 | Cohumulone |  | cis-Hexahydroiso cohumulone (2) |  |  | 26.489 |
| 8 |  |  | cis-Hexahydroiso Humulone |  |  | 26.752 |
| 9 |  | trans-Tetrahydroiso, Humulone |  |  |  | 27.205 |
| 10 |  |  | cis-Hexahydroiso adhumulone |  |  | 27.491 |
| 11 |  | cis-Tetrahydroiso, Humulone |  |  |  | 27.596 |
| 12 | Adhumulone, Humulone |  |  |  |  | 27.625 |
| 13 | Colupulone |  |  |  |  | 27.813 |
| 14 |  | cis-trans-Tetrahydro isoadhumulone |  |  |  | 28.302 |

**Table S4** Equation of calibration curves in solvent and in matrix and matrix effect value (ME%)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Equation Standard** | **Blond beer equation** | **Dark beer equation** | **ME% Blond** | **ME% Dark** |
| **4-Hydroxybenzoic acid** | y = 66.976.58x + 8.897.48 | y = 60344.72x + 122.895.95 | y = 56794.11x + 2.832.079.45 | 90.1% | 84.8% |
| **Tangeretin** | y = 63.271.42x - 8.409.31 | y = 57862.28x - 81.582.57 | y = 52817.02x - 45.756.09 | 91.5% | 83.5% |