METHYLXANTHINES RELEASE FROM VARIOUS TEAS DURING EXTRACTION WITH WATER

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Abstract: Caffeine which is present in plants has a protective function acting as their natural pesticide, among other things. It is accompanied by theophylline and theobromine at various proportions. The effect of caffeine on the human body depends on the dose taken. Moderate doses of caffeine have a beneficial effect on the human body, stimulating the central nervous system. Theophylline and theobromine have stronger diuretic properties but their effect on the central nervous system is less pronounced in comparison with caffeine. The objective of this research work was to find the content of caffeine, theobromine, and theophylline in black, white, red, and green teas by UPLC during extraction with water. The tea test samples were analyzed using the liquid chromatography apparatus ACQUITY UPLC from Waters with PDA detector and ACQUITY UPLC HSS column (2.1 x 100 mm; 1.8 µm). The mobile phase was 0.1% solution of formic acid in water and methanol (90: 10). The content of the methylxanthines is observed to increase with brewing time: after brewing for 6 minutes, the content of theobromine, theophylline, and caffeine in the infusion reaches the state of saturation. The results show that the average content of the compounds of interest in the tea infusions depends on the tea type and that it is increasingly high for longer brewing times. However, the average contents of caffeine, theobromine, and theophylline differ between manufacturers. This seems to suggest it would be reasonable to test certified teas.

Keywords: methylxanthines, teas, UPLC, extraction

Identification of the structure of caffeine in the 19th century was attributed to Hermann Emil Fischer (1852-1919). In 1897, he demonstrated that caffeine and uric acid have similar structures and proved that the compound of interest was trimethylxanthine. In fact, Fischer merely confirmed the structure proposed earlier by Ludwig Medicus (1847-1915) (1).

The pharmacological effect of caffeine is observed in the cerebral cortex, in the autonomous central nervous system, and in cardiac muscles (2). However, many studies on the effect of caffeine fail to provide an unambiguous answer to the question of the nature and intensity of such an effect. This seems to result from individual and environmental differences affecting the metabolism of caffeine (3). Caffeine doses from 100 to 300 mg/day are observed to stimulate the activity of the cerebral cortex enhancing one’s intellectual and physical performance and helping fight drowsiness and fatigue. Opinions on the effect of caffeine on one’s ability to concentrate vary between researchers (2). Among purine group alkaloids, in comparison with theobromine and theophylline, caffeine is the strongest stimulant of the central nervous system (4).

In Europe, the daily consumption of caffeine is in the range of 280-490 mg (5). Caffeine is quickly and almost completely (99% of dosage) absorbed after oral administration and its maximum blood level is obtained after 50 to 75 min. Its pharmacokinetics after intravenous and oral administration is similar (6). From 25 to 40% of the drug is bound with plasma protein and its distribution volume is 0.52-1.05 L/kg. Caffeine readily passes through the placenta barrier, the blood-brain barrier and into the milk of breastfeeding mothers (7-12).

Caffeine is the most frequently used psychoactive substance. It is addictive and sports associations consider it to be a dopant. The substance is used even though it is known to have adverse effects and be harmful to the body (13). Caffeine stimulates the cardiovascular system and respiratory system, boosts metabolism, helps enhance one’s mood, fight fatigue...
and improve concentration. On the other hand, too high doses of caffeine have undesirable effects, such as disturbances of the nervous, respiratory, and circulatory systems and loss of motor control (3, 14, 15).

**EXPERIMENTAL**

**Materials**

Tea infusions for the tests were prepared using 4 types of tea (green, white, red, and black) in the form of tea bags or leaves, from leading manufacturers. A total of 19 various teas were tested, including 8 green teas, 2 white teas, 2 red teas, and 7 black teas. The test teas are listed in Table 1.

**Preparation of the test samples**

1 g of tea was brewed by pouring on it 100 mL of deionized water at a temperature of 95°C (for black or red teas), 80°C (for white teas), and 75°C (for green teas). Due to the specificity of individual teas, the optimal temperature for brewing them was determined on the basis of the analysis of available literature data (16-18). Two weighed amounts were prepared for each tea. The samples were mixed using a magnetic stirrer. Tea samples of 2 mL were drawn after brewing times of 1, 2, 6, and 10 min. Deionized water was added to the beaker after each sample was drawn. The resulting infusions were filtered using a PTFE (polytetrafluoroethylene) injection filter from Waters (0.45 µm; 13 mm).

**Analysis of methylxanthines content by UPLC method**

**Equipment**

The levels of caffeine, theobromine, and theophylline in the infusions of selected teas were measured using the liquid chromatograph ACQUITY UPLC from Waters, equipped with the ACQUITY UPLC HSS column (2.1 x 100 mm; 1.8 µm) with PDA (Photodiode Array Detector), wavelength 210-400 nm. The chromatographic separation was recorded at a wavelength of 273 nm. The mobile phase was a 0.1% solution of formic acid (HCOOH) in water and methanol (CH$_3$OH) at a ratio of 90: 10. The mobile-phase flow rate was 0.4 mL/min., the column temperature was 50°C.

**Standard solutions**

The weighed amounts of methylxanthines (caffeine, theobromine, and theophylline were purchased from Sigma Aldrich) were dissolved in 50% methanol in water to obtain the following concentrations: standard solution of caffeine at a concentration of 400 µg/mL, standard solution of theobromine at

| No. | Type of tea | Tea character | The name of the tea | The origin of the tea according to the producer |
|-----|-------------|---------------|---------------------|-----------------------------------------------|
| 1   | Green       | Express       | Irving              | China                                         |
| 2   | Green       | Leafy         | Dilmah with jasmine | Sri Lanka                                     |
| 3   | Green       | Leafy         | Malwa with orange fruit | Mixture                                      |
| 4   | Green       | Leafy         | Big-active with raspberry fruit | China                                        |
| 5   | Green       | Leafy         | Oskar with jasmine (Chinese) | China                                      |
| 6   | Green       | Leafy         | Bastek with lemon flavor | Mixture                                      |
| 7   | Green       | Leafy         | Premier’s Green Ted | No data                                      |
| 8   | White       | Express       | Dom Herbaty Aromat, Japanese cherry | China                                         |
| 9   | White       | Leafy         | Lipton with pomegranate | Kenya                                         |
| 10  | Red Pu-Erh  | Leafy         | Big-active with raspberry fruit | China                                      |
| 11  | Red Pu-Erh  | Leafy         | Big-active with lemon flavor | China                                      |
| 12  | Red Pu-Erh  | Express       | Savannah with lemon and lime flavor | No data                                      |
| 13  | Black       | Express       | Tetley Intensive | Mixture                                      |
| 14  | Black       | Express       | Lipton Yellow Label Tea | Kenya                                         |
| 15  | Black       | Leafy         | Ceylon Tea Gardens | Sri Lanka                                     |
| 16  | Black       | Leafy         | Posti (Chinese) | China                                         |
| 17  | Black       | Leafy         | Twinings (Classics English Breakfast Tea) | Mixture                                      |
| 18  | Black       | Leafy         | Puerh Tea (Chinese) | China                                         |
| 19  | Black       | Leafy         | Åkta Guteblandning Kränku | No data                                      |
a concentration of 200 µg/mL, standard solution of theophylline at a concentration of 200 µg/mL.

**Calibration curves for theobromine, theophylline, and caffeine**

Calibration curves were obtained from the results of chromatographic analysis of standard solutions of theobromine, theophylline, and caffeine dissolved in the mobile phase at concentrations ranging from 0.1 to 20 µg/mL for theobromine and theophylline and from 0.1 to 100 µg/mL for caffeine. The calibration solutions were obtained by serial dilutions of the standard solutions at a concentration of 200 µg/mL for theobromine and theophylline and 400 µg/mL for caffeine. The obtained correlation coefficients and standard curve equations for theophylline (TPh) is $R^2 = 0.9998$, $y = 18514x – 1205.3$; theobromine (TBr) is $R^2 = 0.9999$, $y = 17441x – 38.051$ and caffeine (Cf) is $R^2 = 0.9999$, $y = 15479x + 1025.4$.

**Precision of measurement**

Six injections of theobromine, theophylline, and caffeine at a concentration of 0.2, 2, and 20 µg/mL into the column were made.

Average peak surface areas for theobromine at the concentrations 0.2, 2, and 20 µg/mL were: 4306.3 ± 136.9; 37188.3 ± 47.1; 366905.5 ± 3225.7, respectively. Coefficients of variation were: 3.18%, 0.13% and 0.88%, respectively.

Average surface peak areas for theophylline for the concentrations 0.2, 2, and 20 µg/mL were: 3652.2 ± 171.2; 38384.0 ± 66.1; 389415.0 ± 1203.9, respectively. Coefficients of variation were: 4.69%, 0.17% and 0.31%, respectively.

Average surface peak areas for caffeine for the concentrations 0.2, 2, and 20 µg/mL were: 3706.3 ± 147.3; 33663.3 ± 59.5; 338652.8 ± 1853.0, respectively, and coefficients of variation were: 3.97%, 0.18% and 0.55%, respectively.

**Precision of a 3-day measurement**

Six injections of theobromine, theophylline, and caffeine at a concentration of 0.2, 2, and 20 µg/mL into the column were made during 3 days. Average retention times and average peak surface areas for the compounds tested, standard deviations, and coefficients of variation are shown in Tables 2 and 3.

**Statistical analysis**

All data obtained were obtained and evaluated using basic descriptive statistics (mean of results, standard deviation, and relative standard deviation).

| Day | Sample | Concentration µg/mL | Mean $t_R$ min. | ±SD | RSD % |
|-----|--------|---------------------|----------------|-----|-------|
| 1   | TBr    | 0.2                 | 1.091          | 0.007 | 0.62 |
|     |        | 2                   | 1.090          | 0.005 | 0.41 |
|     |        | 20                  | 1.087          | 0.005 | 0.49 |
|     | TPh    | 0.2                 | 1.616          | 0.010 | 0.63 |
|     |        | 2                   | 1.612          | 0.008 | 0.51 |
|     |        | 20                  | 1.608          | 0.009 | 0.56 |
|     | Cf     | 0.2                 | 2.474          | 0.017 | 0.71 |
|     |        | 2                   | 2.467          | 0.015 | 0.63 |
|     |        | 20                  | 2.456          | 0.019 | 0.76 |
| 2   | TBr    | 0.2                 | 1.014          | 0.002 | 0.17 |
|     |        | 2                   | 1.015          | 0.001 | 0.12 |
|     |        | 20                  | 1.012          | 0.002 | 0.21 |
|     | TPh    | 0.2                 | 1.467          | 0.004 | 0.24 |
|     |        | 2                   | 1.467          | 0.002 | 0.14 |
|     |        | 20                  | 1.461          | 0.003 | 0.23 |
|     | Cf     | 0.2                 | 2.159          | 0.005 | 0.23 |
|     |        | 2                   | 2.158          | 0.004 | 0.19 |
|     |        | 20                  | 2.145          | 0.006 | 0.26 |
Table 2. Precision of a 3-day measurement of theobromine, theophylline, and caffeine - average retention times (cont.).

| Day | Sample | Concentration µg/mL | Mean t_R min. | ±SD | RSD % |
|-----|--------|---------------------|---------------|-----|-------|
| 3   | TBr    | 0.2                 | 0.962         | 0.001 | 0.12  |
|     |        | 2                   | 0.961         | 0.001 | 0.15  |
|     |        | 20                  | 0.954         | 0.003 | 0.32  |
|     | TPh    | 0.2                 | 1.365         | 0.002 | 0.17  |
|     |        | 2                   | 1.361         | 0.002 | 0.15  |
|     |        | 20                  | 1.349         | 0.005 | 0.40  |
|     | Cf     | 0.2                 | 1.953         | 0.006 | 0.32  |
|     |        | 2                   | 1.939         | 0.004 | 0.19  |
|     |        | 20                  | 1.918         | 0.010 | 0.51  |

Table 3. Precision of a 3-day measurement of theobromine, theophylline, and caffeine – average peak surface areas.

| Day | Sample | Concentration µg/mL | Mean A | ±SD | RSD % |
|-----|--------|---------------------|--------|-----|-------|
| 1   | TBr    | 0.2                 | 4007   | 58  | 1.45  |
|     |        | 2                   | 37403  | 129 | 0.35  |
|     |        | 20                  | 365807 | 582 | 0.16  |
|     | TPh    | 0.2                 | 3224   | 88  | 2.71  |
|     |        | 2                   | 38107  | 74  | 0.19  |
|     |        | 20                  | 387015 | 527 | 0.14  |
|     | Cf     | 0.2                 | 3451   | 135 | 3.91  |
|     |        | 2                   | 34256  | 1527| 4.46  |
|     |        | 20                  | 331631 | 1977| 0.60  |
| 2   | TBr    | 0.2                 | 4306   | 137 | 3.18  |
|     |        | 2                   | 37188  | 47  | 0.13  |
|     |        | 20                  | 366906 | 3236| 0.88  |
|     | TPh    | 0.2                 | 3652   | 171 | 4.69  |
|     |        | 2                   | 38384  | 66  | 0.17  |
|     |        | 20                  | 389415 | 1204| 0.31  |
|     | Cf     | 0.2                 | 3706   | 147 | 3.97  |
|     |        | 2                   | 33663  | 60  | 0.18  |
|     |        | 20                  | 338653 | 1853| 0.55  |
| 3   | TBr    | 0.2                 | 4096   | 52  | 1.27  |
|     |        | 2                   | 37269  | 88  | 0.24  |
|     |        | 20                  | 367065 | 1529| 0.42  |
|     | TPh    | 0.2                 | 3761   | 16  | 0.42  |
|     |        | 2                   | 38348  | 82  | 0.21  |
|     |        | 20                  | 388005 | 598 | 0.15  |
|     | Cf     | 0.2                 | 3779   | 126 | 3.34  |
|     |        | 2                   | 33805  | 136 | 0.40  |
|     |        | 20                  | 338496 | 204 | 0.06  |
RESULTS AND DISCUSSION

The test results are shown in Table 4 for the green (1-8), white (9-10), red (11-12), and black teas (13-19).

Average contents of the compounds of interest in the teas tend to vary between manufacturers. This may result from the fact that the content of methylxanthines in a tea depends on its species, maturity of the leaves at the time of harvesting, the conditions of fermentation and cultivation (9, 19, 20).

Moreover, regardless of the time of brewing, the average content of theobromine, theophylline, and caffeine is the highest in the infusions of black (fermented) teas and the lowest in the infusions of green (non-fermented) teas.

The average content of caffeine in tea infusions is lower for green teas in comparison with black teas regardless of brewing time. This is in agreement with the results obtained by B. Waszkiewicz-Robak, who reported that the caffeine content in 200 mL of a tea infusion was ab. 30 mg for green tea and ab.

### Table 4. Average content of the compounds in the tea samples.

| No | Mean TBR content [mg/g of tea] | Mean TPh content [mg/g of tea] | Mean Cf content [mg/g of tea] |
|----|--------------------------------|--------------------------------|-------------------------------|
|    | 1 min | 2 min | 6 min | 10 min | 1 min | 2 min | 6 min | 10 min | 1 min | 2 min | 6 min | 10 min |
| 1  | 0.467  | 0.725 | 1.198 | 1.447  | 0.164 | 0.254 | 0.305 | 0.661  | 9.200 | 13.500| 16.100 | 17.900 |
| 2  | 1.221  | 1.676 | 2.198 | 2.539  | 0.412 | 0.608 | 0.687 | 0.694  | 16.700| 22.400| 30.400 | 31.300 |
| 3  | 0.319  | 0.429 | 0.825 | 1.014  | 0.050 | 0.095 | 0.193 | 0.359  | 4.000 | 6.300 | 11.800 | 14.200 |
| 4  | 0.222  | 0.328 | 0.460 | 0.527  | 0.048 | 0.202 | 0.277 | 0.334  | 4.700 | 7.700 | 13.200 | 15.400 |
| 5  | 0.603  | 1.340 | 2.414 | 2.715  | 0.038 | 0.214 | 0.279 | 0.421  | 6.900 | 11.200| 20.000 | 25.000 |
| 6  | 0.407  | 0.725 | 1.332 | 1.510  | 0.030 | 0.092 | 0.139 | 0.185  | 5.600 | 10.000| 15.300 | 18.700 |
| 7  | 0.587  | 1.043 | 1.676 | 1.941  | 0.078 | 0.155 | 0.370 | 0.542  | 5.900 | 9.400 | 16.200 | 18.600 |
| 8  | 0.262  | 0.407 | 0.661 | 0.733  | 0.152 | 0.217 | 0.282 | 0.355  | 5.300 | 8.000 | 12.800 | 14.000 |
| Mean | 0.511 | 0.834 | 1.346 | 1.553  | 0.122 | 0.230 | 0.317 | 0.444  | 7.288 | 11.063| 17.571 | 19.388 |
| SD  | 0.319 | 0.483 | 0.709 | 0.802  | 0.128 | 0.164 | 0.165 | 0.175  | 4.117 | 5.096 | 6.000  | 5.959  |
| 9  | 0.526  | 0.601 | 0.934 | 0.982  | 0.137 | 0.163 | 0.414 | 0.769  | 10.700| 13.700| 21.900 | 22.700 |
| 10 | 0.714  | 1.238 | 2.598 | 2.715  | 0.091 | 0.210 | 0.410 | 0.584  | 7.900 | 12.700| 19.600 | 21.500 |
| Mean | 0.620 | 0.920 | 1.766 | 1.849  | 0.114 | 0.187 | 0.412 | 0.677  | 9.300 | 13.200| 20.750 | 22.100 |
| SD  | 0.133 | 0.450 | 1.177 | 1.225  | 0.033 | 0.033 | 0.003 | 0.131  | 1.980 | 0.707 | 1.626  | 0.849  |
| 11 | 0.478  | 0.830 | 1.440 | 1.644  | 0.061 | 0.101 | 0.152 | 0.233  | 7.900 | 12.100| 19.300 | 21.300 |
| 12 | 1.630  | 1.961 | 2.248 | 2.282  | 0.243 | 0.303 | 0.356 | 0.367  | 21.800| 27.300| 32.000 | 32.500 |
| Mean | 1.054 | 1.396 | 1.844 | 1.963  | 0.152 | 0.216 | 0.254 | 0.300  | 14.850| 19.700| 25.650 | 26.900 |
| SD  | 0.815 | 0.800 | 0.571 | 0.451  | 0.129 | 0.162 | 0.144 | 0.095  | 9.829 | 10.748| 8.980  | 7.920  |
| 13 | 2.726  | 4.208 | 4.332 | 4.563  | 0.999 | 1.688 | 1.912 | 1.976  | 19.000| 28.500| 31.200 | 32.100 |
| 14 | 2.278  | 3.036 | 3.734 | 3.786  | 1.036 | 1.574 | 2.133 | 2.331  | 21.700| 27.800| 32.900 | 33.000 |
| 15 | 2.785  | 3.649 | 4.734 | 4.792  | 0.441 | 0.619 | 0.688 | 0.712  | 19.900| 24.200| 29.500 | 30.000 |
| 16 | 0.341  | 0.549 | 0.796 | 0.830  | 0.041 | 0.074 | 0.081 | 0.087  | 8.900 | 13.300| 18.700 | 19.900 |
| 17 | 1.463  | 1.981 | 2.296 | 2.607  | 0.457 | 0.630 | 0.683 | 0.768  | 18.100| 22.900| 26.200 | 26.500 |
| 18 | 0.558  | 0.899 | 1.348 | 1.741  | 0.037 | 0.074 | 0.078 | 0.109  | 8.600 | 13.000| 21.300 | 23.700 |
| 19 | 0.957  | 1.453 | 2.187 | 2.473  | 0.093 | 0.168 | 0.185 | 0.206  | 13.400| 19.700| 28.000 | 29.300 |
| Mean | 1.587 | 2.254 | 2.775 | 2.97  | 0.443 | 0.69  | 0.823 | 0.884  | 15.657| 21.343| 26.829 | 27.786 |
| SD  | 1.019 | 1.404 | 1.512 | 1.472  | 0.430 | 0.685 | 0.861 | 0.915  | 5.358 | 6.331 | 5.188  | 4.717  |

*average content of methylxanthines corresponds to the extracted amount of the compounds
Researchers seem to vary in their opinions on the matter. D. Klódka et al. and A. Stachelska-Wierczowska and A. Knasiak reported higher caffeine contents in green tea infusions compared with black tea infusions (9, 22). According to M. Jarosz et al., caffeine levels in the infusions obtained from black tea and green tea were comparable (20).

The column charts in Figures 1 a, b, c show average contents of theobromine, theophylline, and caffeine after brewing times of 1, 2, 6, and 10 min. The content of methylxanthines increases with longer brewing times. This is confirmed by the results reported by D. Klódka et al. and A. Stachelska-Wierczowska and A. Knasiak (9, 22).

In tea infusions, the levels of theobromine and theophylline are definitely lower than that of caffeine. Although in similar studies, D. Komes determined the content of theophylline in tea infusions (23), some authors (including D. Klódka and A. Stachelska-Wierczowska and A. Knasiak) did not detect theophylline (9, 22).

The diagrams in Figures 2 a, b show that the content of theobromine, theophylline, and caffeine reaches the state of saturation after a brewing time of 6 minutes: a) Sample 2; b) Sample 14.

![Average theobromine content after 1, 2, 6 and 10 min. brewing](image)

![Average theophylline content after 1, 2, 6 and 10 min. brewing](image)

![Average caffeine content after 1, 2, 6 and 10 min. brewing](image)

Figure 1. Average content of a) theobromine. b) theophylline. c) caffeine after brewing time of 1, 2, 6 and 10 min.

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| Sample number | Average content TBr, mg/g tea | 1 min. | 2 min. | 6 min. | 10 min. |
|---------------|-------------------------------|--------|--------|--------|--------|
| 1             | 0                             |        |        |        |        |
| 2             | 1                             |        |        |        |        |
| 3             | 2                             |        |        |        |        |
| 4             | 3                             |        |        |        |        |
| ...           | ...                           |        |        |        |        |

| Sample number | Average content TPh, mg/g tea | 1 min. | 2 min. | 6 min. | 10 min. |
|---------------|-------------------------------|--------|--------|--------|--------|
| 1             | 0                             |        |        |        |        |
| 2             | 1                             |        |        |        |        |
| 3             | 2                             |        |        |        |        |
| ...           | ...                           |        |        |        |        |

| Sample number | Average content Cf, mg/g tea | 1 min. | 2 min. | 6 min. | 10 min. |
|---------------|-------------------------------|--------|--------|--------|--------|
| 1             | 0                             |        |        |        |        |
| 2             | 5                             |        |        |        |        |
| 3             | 10                            |        |        |        |        |
| ...           | ...                           |        |        |        |        |

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Methylxanthines release from various teas during extraction with water

CONCLUSIONS

The tests were made using 4 types of tea (green, white, red, and black) in the form of tea bags or leaves. All three methylxanthines were determined in the tea infusions. The results show that the average content of the compounds of interest in the tea infusions depends on the tea type and that it is increasingly high for longer brewing times. The average content of the compounds of interest is the highest in the infusions of black (fermented) teas and the lowest in the infusions of green (non-fermented) teas. However, the average contents of caffeine, theobromine, and theophylline differ between manufacturers. This seems to suggest it would be reasonable to test certified teas.

Conflicts of interest

The author declares that he has no conflict of interest.

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