HPLC determination of caffeine in tea, chocolate products and carbonated beverages

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Abstract: Different brands of mate and black tea, chocolate products and carbonated beverages available on the Brazilian market were analysed for caffeine by high-performance liquid chromatography with a UV-vis detector at 254 nm. The column was a reverse phase C18 and the mobile phase consisted of methanol-water (30:70, v/v), acetonitrile-water (10:90, v/v) and methanol-water (25:75, v/v) for tea, chocolate products and soft drinks respectively. Caffeine content ranged from 1.05 to 15.83 mg per cup in mate tea, from 32.21 to 56.23 mg per cup in black tea, from 0.14 to 0.95 mg in chocolate products from 2.72 to 7.49 mg per can in guarana-type soft drinks and from 19.81 to 45.89 mg per can in cola soft drinks. These data indicate that the levels of caffeine in Brazilian teas, chocolates and soft drinks are within the ranges reported for similar products in other countries.

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Keywords: caffeine; tea; carbonated beverages; cocoa; chocolate products; HPLC

INTRODUCTION
Caffeine, an alkaloid structurally identified as 1,3,7-trimethylxanthine, is the most widely consumed psychoactive substance in the world from coffee, tea, chocolate products and carbonated beverages.1-3 The reported caffeine content in these main dietary sources varies significantly: 93.0–103.5 mg per cup in ground coffee, 46.7–67.6 mg per cup in instant coffee, 30.2–67.4 mg per cup in bag tea and 0.32–0.54 mg in dark sweet chocolate.4,5 The differences have been attributed to the variety of coffee bean or tea leaf, method of preparation (e.g., the brewing of coffee and tea), volume of a cup and analytical methods utilised for caffeine determination.6 Although the level of caffeine in chocolate is less variable, it still depends on the origin of the beans. In the case of carbonated beverages the variability occurs among brands, since most of the caffeine content in these products is added from other natural sources, i.e., less than 5% of total caffeine present is from cola nuts.8

In view of the lack of information on the caffeine content of Brazilian foods, the objective of the present study was to determine the level of caffeine in different brands of tea, chocolate products and carbonated beverages commonly consumed in Brazil. Coffee, the main source of caffeine in the Brazilian diet, has already been surveyed for its caffeine content by the same authors.9

The interest in investigating the caffeine content of food and beverages in Brazil is to develop a database to estimate the caffeine intake from these products. It has been recognised that an excessive consumption of caffeine by people from dietary sources may reach levels that induce pharmacological effects.10

MATERIALS AND METHODS
Sample preparation
Different brands and batches of soft drinks, mate and black tea and chocolate products were purchased from supermarkets in the city of Campinas, SP. All samples were analysed in duplicate.

Soft drinks
Caffeine was determined in soft drinks according to Galassio et al.11 Samples were decarbonated in an ultrasonic bath for 10 min and mixed into a liquid chromatograph.

Tea
The extraction procedure for mate and black tea (bags and leaves) was based on the Chinese national standard method described by Guo and Wan.12 Prior to analysis the tea was infused according to package directions. Bag mate and black tea (1.80 g) were extracted with 150 mL of boiling water, and mate leaf tea was extracted using 8.52 g (approximately a spoon) to 11 of water. When the extract reached room
temperature, an aliquot of 20 ml was mixed with 0.1 M hydrochloric acid (10 ml) and saturated basic acetate solution (8 ml). The solution was centrifuged at 1057.5 x g for 5 min. After adding NaHCO₃ (ratio of 0.1 g NaHCO₃ to 10 ml of solution) to the supernatant, the solution was centrifuged again at 1057.5 x g for 5 min. This solution was diluted with water into a 50 ml volumetric flask, mixed and injected into a liquid chromatograph.

**Chocolate products**

The extraction of caffeine from chocolate products was based on the procedure described by Kreiser and Martin. A clean-up step was added before injection into the chromatograph. Samples of chocolate bars (0.60 g) and chocolate powders (1.0 g) were weighed into test tubes equipped with Teflon-lined screw caps. The fat was extracted by shaking twice with 30 ml portions of petroleum ether and centrifuging at 470.0 x g for 10 min. The residual solvent was evaporated by placing the test tubes in a warm water bath. To the residue, water (30 ml) and saturated basic acetate solution (5 ml) were added, and this was followed by centrifugation at 1057.5 x g for 5 min. NaHCO₃ was added to the supernatant and the centrifugation was repeated. To the supernatant, 0.1 M HCl was added. The solution was then diluted to volume with water into a 100 ml volumetric flask, mixed and injected into a liquid chromatograph.

**High-performance liquid chromatography**

Analysis was carried out using an HPLC apparatus equipped with a Waters 6000 A pump, a Waters Model 440 fixed wavelength detector at 254 nm, a C18 reverse phase column (Merck, 15 cm x 4.6 mm id, particle size 5 μm) and a sample injector system (Rhodyne) with a 5 μl sample loop. The mobile phase used was methanol-water (25:75, v/v) for soft drinks, methanol-water (30:70, v/v) for tea and acetonitrile-water (10:90, v/v) for chocolate products at a flow rate of 1.0 ml min⁻¹. The peak of caffeine in the samples was identified by comparing the retention time with that of a standard (Sigma Chemical Co).

**Quantitation**

The external standard plot method was used. TriPLICATE injections of 5 μl caffeine standard solution were used to construct linear regression lines (peak area ratio versus caffeine concentrations). The concentration ranges of the standard curves were 0.001–0.100 mg ml⁻¹ for tea, 0.001–0.150 mg ml⁻¹ for chocolate powders and 0.004–0.080 mg ml⁻¹ for soft drinks. The detection limit, defined as the concentration corresponding to a peak height of three times the baseline noise level, was 0.10 μg ml⁻¹.

**Recovery study**

In order to verify the accuracy and precision of the analytical procedure, recovery studies were carried out by spiking selected samples of mate tea and chocolate bars with caffeine. Samples were spiked, just before the addition of the saturated basic acetate solution, by adding 1 ml of a caffeine standard water solution at five different concentrations (0.05–0.25 mg ml⁻¹). The spiked samples as well as the unspiked controls were analysed in duplicate. Recoveries were calculated from the differences in total amount of caffeine between the spiked and unspiked samples (Table 1).

**RESULTS AND DISCUSSION**

The analytical method chosen for the quantitation of caffeine in chocolate products was based on the method of Kreiser and Martin for the determination of theobromine and caffeine in cocoa and chocolate products. As the purpose of the present study did not include the determination of theobromine, less water than the quantity suggested by Kreiser and Martin was used. Besides, a clean-up procedure saturated basic acetate (a good clarifying agent for removing interfering substances) was introduced before injecting the solution into the liquid chromatograph. These procedures did not result in loss of caffeine, as shown by the recovery results (Table 1). The mobile phase suggested by Kreiser and Martin did not result in effective caffeine separation for all chocolate products. A better resolution was obtained using acetonitrile-water (10:90, v/v) (Fig 1(A)). Average values of caffeine concentration in chocolate products are given in Table 2. Among chocolates, the semisweet ones showed the highest amounts of caffeine (0.03–0.05 mg g⁻¹) as compared with the dark sweets (0.03–0.047 mg g⁻¹) and white ones (0.14–0.28 mg g⁻¹). The caffeine levels reported in the literature for dark sweets range from 0.32 to 0.54 mg g⁻¹, while for the white ones the average is 0.05 mg g⁻¹. Values for caffeine content in tea are shown in Table 3. The extraction of caffeine was done according to the Chinese national standard method except that four times more saturated basic acetate solution (8 ml) was used and HPLC was chosen instead of UV to analyse caffeine. Among the mobile phases tested, methanol-water (30:70, v/v) was the one that gave the best resolution for the three types of tea in a short retention time (Fig 1(B)). According to the results, black tea, irrespective of the brand, showed the highest amounts of caffeine (32.21–36.23 mg per cup) (cup = 150 ml), while the lowest concentrations of this alkaloid (1.05–6.75 mg per cup) were found in mate leaf tea.
Table 3. Caffeine content in tea.

<table>
<thead>
<tr>
<th>Tea (brand)</th>
<th>Caffeine (mg g⁻¹)</th>
<th>Caffeine (mg per cup)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mate (bag)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.70 ± 0.06</td>
<td>1.73 ± 0.23</td>
</tr>
<tr>
<td>B</td>
<td>6.75 ± 1.34</td>
<td>13.23 ± 2.46</td>
</tr>
<tr>
<td>C</td>
<td>7.03 ± 0.04</td>
<td>15.63 ± 1.13</td>
</tr>
<tr>
<td>X = 5.08 ± 2.77</td>
<td>X = 10.26 ± 7.50</td>
<td></td>
</tr>
<tr>
<td>Mate (leaf)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6.68 ± 0.37</td>
<td>1.02 ± 0.97</td>
</tr>
<tr>
<td>B</td>
<td>5.56 ± 0.44</td>
<td>0.75 ± 0.67</td>
</tr>
<tr>
<td>C</td>
<td>5.25 ± 0.26</td>
<td>0.50 ± 0.47</td>
</tr>
<tr>
<td>X = 3.63 ± 2.55</td>
<td>X = 4.00 ± 3.76</td>
<td></td>
</tr>
<tr>
<td>Black (bag)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>3.14 ± 1.34</td>
<td>35.34 ± 2.25</td>
</tr>
<tr>
<td>B</td>
<td>4.44 ± 0.51</td>
<td>22.21 ± 2.73</td>
</tr>
<tr>
<td>C</td>
<td>7.06 ± 0.04</td>
<td>34.59 ± 3.18</td>
</tr>
<tr>
<td>D</td>
<td>7.35 ± 0.33</td>
<td>25.29 ± 6.20</td>
</tr>
<tr>
<td>X = 5.33 ± 4.33</td>
<td>X = 34.29 ± 1.72</td>
<td></td>
</tr>
</tbody>
</table>

*a Average of six determinations

*b C = 150ml.

Differences in caffeine content (mg g⁻¹) between mate bag and mate leaf tea were not significant based on Student’s t-test. The caffeine levels of black tea were within the range reported by other authors for the same product (17.0–46.5mg per cup).2,4,11

Table 4 presents the caffeine levels determined in soft drinks. The results show that the caffeine content in diet cola A (45.89mg per can) (can = 350ml) is approximately 30% higher than in regular cola A (33.08mg per can). Similar results were found by Galasko et al11 for regular cola A (35.0mg per can) and diet cola A (45.5mg per can). For regular cola B the caffeine content determined by both Galasko et al11 and Vergnes and Alary12 was around 24.5mg per can, which is similar to the results of the present study. Fig 1(C) shows a typical chromatogram of a cola drink. The guarana-type soft drinks (typical Brazilian naturally containing caffeine soft drinks),

Table 4. Caffeine content in soft drinks.

<table>
<thead>
<tr>
<th>Soft drink</th>
<th>Caffeine (mg g⁻¹)</th>
<th>Caffeine (mg per can)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cola A</td>
<td>0.085 ± 0.004</td>
<td>38.36 ± 0.17</td>
</tr>
<tr>
<td>Diet Cola A</td>
<td>0.131 ± 0.003</td>
<td>45.62 ± 1.04</td>
</tr>
<tr>
<td>Cola B</td>
<td>0.078 ± 0.008</td>
<td>34.66 ± 0.68</td>
</tr>
<tr>
<td>Diet Cola B</td>
<td>0.050 ± 0.049</td>
<td>19.67 ± 0.97</td>
</tr>
<tr>
<td>X = 0.069 ± 0.031</td>
<td>X = 30.91 ± 11.89</td>
<td></td>
</tr>
<tr>
<td>Guarana C</td>
<td>0.037 ± 0.005</td>
<td>2.66 ± 0.07</td>
</tr>
<tr>
<td>Diet Guarana C</td>
<td>0.030 ± 0.01</td>
<td>3.10 ± 0.15</td>
</tr>
<tr>
<td>Guarana D</td>
<td>0.086 ± 0.001</td>
<td>2.73 ± 0.49</td>
</tr>
<tr>
<td>Diet Guarana D</td>
<td>0.11 ± 0.003</td>
<td>3.77 ± 0.61</td>
</tr>
<tr>
<td>Guarana E</td>
<td>0.029 ± 0.000</td>
<td>3.15 ± 0.11</td>
</tr>
<tr>
<td>Diet Guarana E</td>
<td>0.021 ± 0.002</td>
<td>7.49 ± 0.63</td>
</tr>
<tr>
<td>X = 0.011 ± 0.005</td>
<td>X = 3.64 ± 1.62</td>
<td></td>
</tr>
</tbody>
</table>

*a Average of six determinations

*b C = 350ml.
although formulated with guaraná seeds, one of the highest natural sources of caffeine (guaraná seeds
contain around 4% caffeine according to Tocchini et al. [13]), showed the lowest amounts of this alkaloid.
While the caffeine range in cola-type soft drinks was
19.81–45.89 mg per can, the guaraná beverage
concentrations of caffeine were much lower (2.73–
7.49 mg per can). This is probably due to differences
between the formulations of the soft drinks, as in
garaná-type soft drinks caffeine is not added inten-
tionally as it is in cola drinks.

Although different factors may affect the amount of
caffeine in dietary sources, values for caffeine obtained
from Brazilian teas, soft drinks and chocolate products
are within the ranges reported in the literature. The
results also show that the caffeine contents in the
analysed products are much lower than those found in
coffee (64.5–123.0 mg per cup) [3].

ACKNOWLEDGEMENT

Financial support from FAPESP grant 94/5443-8 is
gratefully acknowledged.

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