The Determination of Caffeine in Coffee: Sense or Nonsense?

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In current educational practice in The Netherlands there exists a strong tendency to move from formal lectures to ‘self-teaching’ and ‘self-discovering learning’. At the Eindhoven University of Technology, problem-based learning and project-based learning are also encouraged. In the first and second year, the undergraduates of the Department of Chemical Engineering amass knowledge of diverse analytical tools by introductory presentations on GC, high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and UV–IR spectrophotometry. Over the course of four afternoons, students learn the basic theory of the methods, learn to operate the apparatuses, and carry out some basic experiments. Because we give the students samples of known composition, we can check whether their results are accurate or not. In the second year the theory is extended by lectures on analytical chemistry; ultimately the students complete a final assignment over four afternoons. As part of a problem-posing approach to teaching we often give the students a ‘real’ sample with an unknown composition. The disadvantage of this approach is that it is difficult to check whether the students work accurately or not. To solve this problem we give an analytical problem to a group of four students and they have to solve the problem with at least two different analytical methods. In this way, the students can compare the results of different analytical tools and gain better insights into the possibilities and the accuracies of these methods.

Various articles on the determination of caffeine in coffee indicate that this is a well-known and popular subject in educational analytical chemistry. In 1979 Van Atta (1) described the UV spectrophotometric determination of caffeine in cola drinks as a rapid and accurate spectrophotometric method for the quantitative estimation of the alkaloid in cola drinks (real sample), including a quantitative liquid–liquid extraction of caffeine. DiNunzio (2), Strohl (3), and Ferguson (4) applied HPLC to the determination of caffeine in beverages. CE was also used for the determination of caffeine in beverages (5) and analgesic formulations (6). Finally, Vogt et al. (7) applied micellar electrokinetic capillary chromatography (MECC) for the determination of caffeine. In the studies the results obtained by the students were compared with labeled or reported values. Samples were not analyzed with different analytical methods and compared. Our approach is unique in that it uses multiple methods to determine the caffeine in coffee.

Another motivation to consider the determination of caffeine is the required project for students in the pre-university education in The Netherlands. The students make a report on a project that should be multidisciplinary and contain social and environmental aspects within the framework of self-teaching. The practical component is often a chemical analytical determination. Unfortunately a disadvantage of self-teaching is that often a detailed knowledge of the subject matter is not acquired. The student is often not able to reach a great depth by him or herself. Recently, in a student’s report concerning the determination of caffeine in coffee, I read “prior to the determination of caffeine in coffee with UV spectrophotometry, the coffee was diluted until the color of the coffee was not visible anymore”. I got the impression that the student believed that the influence of the coloring substance of the coffee was negligible. All students should know that the total quantity of all UV absorbing components is measured with UV spectrophotometry according to Beer’s law. However, this example shows that the student did not realize that by diluting the sample solution, the error introduced by the coloring substance is constant and that for the determination of a component in a complex matrix, separation methods have to be applied. To demonstrate the error in the determination of caffeine in coffee applying a UV spectrophotometer, the results obtained are compared with results obtained using separation methods. Thus, the analysis of caffeine in coffee will be considered applying UV spectrophotometry, HPLC, and CE.

Experimental Procedures

Reagents, Standards, and Materials

All chemicals were analytical grade. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA). A standard stock solution of 0.0100 M caffeine in water was prepared and the dilutions were made for the calibration curves from this solution. All dilutions were made using Eppendorf or Finn pipets.

HPLC Equipment

The HPLC equipment (Pharmacia-LKB, Bromma, Sweden) consisted of a model 2150 pump, a model 2152 controller, a low-pressure mixer, a model 2156 solvent conditioner, and a VWM 2141 dual-wavelength UV detector. Chromatographic separation was obtained with a LiChrospher 100 RP-18 end-capped column (125 × 4 mm, 5 µm) from E Merck (Darmstadt, Germany). Injections were made with a model 7125 universal loop injector of 20 µL (Rhodyne, Berkeley, CA). Reversed-phase HPLC was performed at ambient temperature applying an isocratic mobile phase consisting of water/methanol (50:50 v/v). Flow rate was 0.8 mL/min. The mobile phase was degassed by vacuum filtration through a 0.22 µm filter and sparging with helium. Data analysis was performed using the data analysis program DAX.

CE Equipment

For all CE experiments the P/ACE System 5000 HPCE (Beckman Coulter, Fullerton, CA) was used, applying a Beckman eCap capillary tubing (75 µm) with total length of...
57.0 cm and a distance between injection and detection of 50.0 cm. The wavelength of the UV detector was 214 nm. All experiments were carried out applying a constant voltage of 10 kV; the operating temperature was 25 °C. Sample introduction was performed applying 5 s pressure injections at 0.5 psi, whereby a 1 s pressure injection corresponds to an injection volume of ca. 5 nL. Data analysis was performed using the laboratory-written data analysis program, CAESAR. All experiments were carried out in the MECC mode with a background electrolyte (BGE) consisting of 0.01 M tris(hydroxymethyl)aminomethane (Tris) and 0.04 M sodium dodecyl-sulfate (SDS), adjusted to pH 8 by adding acetic acid. The anode was placed at the inlet and the cathode at the outlet.

**UV–vis Spectrophotometer**

An LKB UV-vis spectrophotometer (Ultraspec II, model 4040, LKB, Bromma, Sweden), with 1-cm quartz cuvettes, was used to make the UV determinations.

**Hazards**

Use gloves and safety glasses during the preparation of the background electrolytes and standard solutions. Methanolic solutions should be prepared in a fume hood. Concentrated acetic acid should be handled with care. The hazards of the prepared solutions are minimal at the low concentrations used.

**Results and Discussion**

To demonstrate the advantages of the combined approach, the results from a group of four students are discussed in detail. The results of other groups of students analyzing diverse samples of coffee are also summarized for comparison.

**Table 1. Data for the Calibration Curves Used To Determine the Caffeine in Coffee**

<table>
<thead>
<tr>
<th>[Caffeine]/(10^-3 M)</th>
<th>Peak Area/[10^-3 V min]</th>
<th>[Caffeine]/(10^-4 M)</th>
<th>Peak Area/[10^-3 AU s]</th>
<th>[Caffeine]/(10^-5 M)</th>
<th>Absorbance/AU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPLC1</td>
<td>HPLC2</td>
<td>CE1</td>
<td>CE2</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>74.43</td>
<td>76.54</td>
<td>10.0</td>
<td>556.2</td>
<td>1.72</td>
</tr>
<tr>
<td>8.0</td>
<td>59.20</td>
<td>61.31</td>
<td>8.0</td>
<td>438.8</td>
<td>0.895</td>
</tr>
<tr>
<td>6.0</td>
<td>44.97</td>
<td>46.29</td>
<td>6.0</td>
<td>336.5</td>
<td>0.582</td>
</tr>
<tr>
<td>4.0</td>
<td>29.80</td>
<td>30.88</td>
<td>4.0</td>
<td>202.5</td>
<td>0.301</td>
</tr>
<tr>
<td>2.0</td>
<td>15.19</td>
<td>15.22</td>
<td>2.0</td>
<td>105.1</td>
<td>0.157</td>
</tr>
</tbody>
</table>

**Table 2. Results for the Determination of the Caffeine in Coffee from Student Group 1 Using Various Methods**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HPLC1</th>
<th>HPLC2</th>
<th>CE1</th>
<th>CE2</th>
<th>UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression coefficienta</td>
<td>0.99996</td>
<td>0.99977</td>
<td>0.99907</td>
<td>0.99956</td>
<td>0.99982</td>
</tr>
<tr>
<td>Diluted coffee sampleb</td>
<td>36.37</td>
<td>37.83</td>
<td>339.34</td>
<td>326.5</td>
<td>1.000</td>
</tr>
<tr>
<td>Dilution factor</td>
<td>50</td>
<td>50</td>
<td>4</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>[Caffeine]_{dl, in sample}(mg/L)</td>
<td>473</td>
<td>478</td>
<td>482</td>
<td>490</td>
<td>1982</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.6</td>
<td>0.5</td>
<td>2.1</td>
<td>1.4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*aThe regression coefficients were calculated for the data given in Table 1.
bUnits for the diluted coffee samples are the same units given in Table 1 for each method.*

Figure 1. UV spectrum for caffeine. Measuring conditions: 1-cm quartz cuvettes; 10^-4 M solution of caffeine in water; ambient temperature.
deviation (RSD) of 0.9%. For the determination of all coffee caffeine concentrations a calibration curve was measured, the sample solutions were measured twice, and the average value was used.

**CE**

The concentration of caffeine in the same instant coffee sample as above was determined by applying CE, a separation method (8, 9) in which all components are separated before they are identified and quantified. Because caffeine is a neutral component, MECC (10, 11) was applied with SDS as surfactant. Figure 2 shows examples of the electropherograms for 5 s pressure injections of a 2-fold diluted instant coffee sample and a $6 \times 10^{-4}$ M standard solution of caffeine.

In the coffee sample some extra components are visible in addition to the caffeine peak. These components are also UV absorbing showing that the UV absorbance in UV spectrophotometry is not due to only the presence of caffeine.

The determination is carried out two times, on two different days, and the measured values for the peak area, for the calibration curves, and results of the caffeine determination are given in Tables 1 and 2.

**HPLC**

We applied HPLC (12) to the determination of caffeine in coffee, at a wavelength of 272 nm. The concentrations of the standard solutions, the measured peak areas, the determined caffeine concentrations, and the RSD values are given for the HPLC experiments in Tables 1 and 2. The chromatograms for a 50-fold diluted instant coffee sample and a standard solution of $6 \times 10^{-5}$ M caffeine are given in Figure 3. Just as in the electropherograms of Figure 2, additional UV absorbing components are present in the instant coffee in addition to caffeine. A good agreement between CE and HPLC values is obtained, whereas the UV experiment shows significantly larger values (Table 2).

![Figure 2. Electropherograms for 5 s pressure injections of (A) 2-fold diluted instant coffee sample and (B) $6 \times 10^{-4}$ M standard solution of caffeine: $t_{EOF}$ indicates the migration time of the electroosmotic flow.](image)

![Figure 3. Chromatograms for (A) 50-fold diluted instant coffee sample and (B) standard solution of $6 \times 10^{-5}$ M caffeine. Measuring conditions: flow rate 0.8 mL/min; wavelength detector 272 nm; $t_{M}$ indicates the retention time of the mobile phase.](image)

### Table 3. Concentration of Caffeine in Coffee Determined by Various Student Groups Using Different Methods

<table>
<thead>
<tr>
<th>Student Group</th>
<th>Coffee Sample</th>
<th>[Caffeine]$^a$/mg L$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPLC1</td>
<td>HPLC2</td>
</tr>
<tr>
<td>1</td>
<td>Instant (normal)</td>
<td>473(0.6)</td>
</tr>
<tr>
<td>2</td>
<td>Instant (normal)</td>
<td>481(1.6)</td>
</tr>
<tr>
<td>2</td>
<td>Railway shop</td>
<td>320(2.7)</td>
</tr>
<tr>
<td>3</td>
<td>Instant (mocca)</td>
<td>582(4.5)$^b$</td>
</tr>
<tr>
<td>3</td>
<td>Instant (strong)</td>
<td>533(4.0)$^b$</td>
</tr>
<tr>
<td>4</td>
<td>Canteen</td>
<td>493(3.9)</td>
</tr>
</tbody>
</table>

$a$RSD values (%) are in brackets.

$b$These groups used only one calibration curve.
Comparisons between Groups

The results from four groups of students using the different analytical methods and different coffee samples are shown in Table 3. The results between groups were found to be in good agreement as shown by the results from groups 1 and 2 for the instant coffee sample of normal strength. The expected caffeine variation from different types of coffee was also distinguished by these methods, shown by the different caffeine concentrations in strong versus normal instant coffee.

Conclusions

If students in undergraduate analytical chemistry education analyze samples of known composition, the results can be verified. If the students analyze real samples with unknown compositions, it is difficult to check whether the students work accurately or not. In this case, it is advisable to apply at least two analytical methods. In this article the results for the determination of caffeine in coffee are discussed applying HPLC, CE, and UV–vis spectrophotometry. By this combined approach the students observe that larger values for the concentration of caffeine in coffee are obtained with UV spectrophotometry compared with the values obtained with CE and HPLC. Using this approach the students gain an understanding of the differences between separation methods and UV–vis spectrophotometry. The presence of other components, such as UV-absorbing coloring matter, are observed in the electropherograms and chromatograms. The results for HPLC and CE are similar and no internal standard is needed although different wavelengths are used in HPLC and CE. Interestingly higher concentrations are needed for the standards and the sample for CE because of the short optical path length in CE. Application of large injection volumes combined with sample stacking (13) can improve the minimal detectable concentration in CE.

An important facet in this approach is that students mutually compare their results and they stimulate themselves to repeat experiments if different values are obtained with different analytical methods. Moreover, they obtain a better insight into the possibilities of the different methods and accuracies of the analytical methods. In this way the impact of even such a simple determination is greater. In practice the self-motivation increases and the students learn to look critically at the results. Separation methods are especially suitable for a combined approach in analytical chemistry for many determinations.

Supplemental Material

Notes for the instructor are available in this issue of JCE Online.

Literature Cited