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Analysis of Tea and Soft Drinks for Caffeine Content – A Teaching Laboratory Exercise

Background

In this example experiment, the caffeine content in tea and soft drinks is determined using UV-Vis spectroscopy. Caffeine, a natural stimulant found in coffee, tea, and soft drinks is renowned for its ability to increase alertness and concentration. However, excessive consumption can lead to negative effects such as insomnia, restlessness, and increased heart rate. Therefore, it is crucial to accurately measure caffeine levels in beverages for both quality control purposes and consumer safety. UV-Vis spectroscopy offers a rapid and reliable method for quantifying caffeine content, enabling manufacturers to ensure product consistency and regulatory compliance. Additionally, such analysis provides consumers with valuable information regarding the caffeine content of their beverages, aiding in informed consumption choices.

Instrument and Measurement Conditions

In this experiment, UV-1900i UV-Vis Spectrophotometer (shown in Fig. 1) is recommended for analysis. The measurement conditions are listed in Table 1.

Fig. 1 UV-1900i UV-Vis Spectrophotometer

Table 1 Measurement Conditions

Instrument	: UV-1900i UV-Vis Spectrophotometer
Software	: LabSolutions UV-Vis
Measurement wavelength	: 274 nm
Accumulation Time	: 0.1 sec.
Light source switch wavelength	: 340 nm
S/R Switch	: Standard
Slit width	: 1 nm (fixed)

A) Preparation of Standard Samples

Please see Appendix 1

B) Sample Preparation

Please see Appendix 2

C) Configuring Measurement Parameters

Please see Appendix 3

D) Quantitation Procedure

Specifying Parameter File

1) Launch *LabSolutions UV-Vis,* then select [Quantitation]
2) Click the [Inst. Control] icon from the toolbar to display the instrument control window
3) Click [Parameter] – [Read] and select the file generated in *Step C.*

Specifying File Names

4) In the instrument control window, click [File name] – [Edit]
5) Enter the details for the [Filename] (where the data is to be stored), [Analyst] and [Comment] and click [Ok]. Leave the instrument control window open.

Measuring Standard Samples

6) In the Standard Table enter the calibration standard solutions and their concentrations.
7) Click in the standard sample table to activate it (table becomes enclosed in a red frame)
8) Set the blank sample (chloroform) in the instrument's sample compartment.
9) In the instrument control window, click 

(Baseline).
10) Place the first standard sample in the instrument's sample compartment and close the lid.
11) In the instrument control window, click 

(Standard). The measurement value is displayed in the wavelength column.
12) Repeat the same operations for the remaining prepared standard samples.
13) Once measurement of all standard samples is complete, click [Save] on the toolbar. With all standards scanned, a calibration curve will be generated on the right of the screen.

Measuring Unknown Samples

14) Click in the unknown sample table to activate it (table becomes enclosed in a red frame)
15) Enter the sample information.
16) Place the first sample in the instrument's sample compartment and close the lid.
17) In the instrument control window, click 

(Unknown Sample).
18) Repeat the same operations for the remaining prepared samples.
19) The concentration calculated using the calibration curve is displayed in the concentration column. Multiplication of the concentration by the dilution factor (in this case 25) will yield the caffeine content of the beverage.

Appendix 1 – Preparation of Standard Samples

Standard samples may be prepared following the method detailed in Vuletić, N., Bardić, L. and Odžak, R., 2021. Spectrophotometric determining of caffeine content in the selection of teas, soft and energy drinks available on the Croatian market. *Food research (Kuala Lumpur)*, *5*(2), pp.325-330.
https://doi.org/10.26656/fr.2017.5(2).482

The method is as follows:

1) Prepare a stock solution (100 mg/L) by dissolving 0.01 g recrystallised caffeine in 100 mL chloroform in a volumetric flask.
2) From the stock solution, prepare the following dilutions (quantities outlined in Table 2): 1, 5, 10, 15, 20 and 25 mg/L.
3) Place each sample in a quartz cuvette, ready for analysis.

Table 2 Quantities required for preparation of 10 mL standard samples

Standard sample concentration (mg/L)	Volume of 100 mg/L stock solution (mL)	Volume of chloroform (mL)
1	0.1	9.9
5	0.5	9.5
10	1.0	9.0
15	1.5	8.5
20	2.0	8.0
25	2.5	7.5

Appendix 2 – Sample Preparation

A) Extraction of caffeine from tea and preparation of sample solutions

Extraction and preparation of sample solutions may be prepared following the method detailed in Vuletić, N., Bardić, L. and Odžak, R., 2021. Spectrophotometric determining of caffeine content in the selection of teas, soft and energy drinks available on the Croatian market. *Food research (Kuala Lumpur)*, *5*(2), pp.325-330. https://doi.org/10.26656/fr.2017.5(2).482

The method is as follows:

1. Add 2 g of tea sample to 20 mL of distilled water, then boil the contents for 10 mins.
2. Add 2 g of sodium carbonate to each sample to precipitate the tannins.
3. Filter the sample, then concentrate the filtrate to 5 mL by heating.
4. Extract the caffeine from the filtrate by adding 5 mL of chloroform in the separatory funnel, then stir the contents for a few minutes.
5. Separate the caffeine-containing layer, then add 0.1 mL of the extract to a volumetric flask and make up to 10 mL with chloroform. Place the sample in a quartz cuvette, ready for analysis.

Following this method, the total dilution factor of the tea is **25**

B) Extraction of caffeine from soft and energy drinks and preparation of sample solutions

Extraction and preparation of sample solutions may be prepared following the method detailed in Vuletić, N., Bardić, L. and Odžak, R., 2021. Spectrophotometric determining of caffeine content in the selection of teas, soft and energy drinks available on the Croatian market. *Food research (Kuala Lumpur)*, *5*(2), pp.325-330. https://doi.org/10.26656/fr.2017.5(2).482

The method is as follows:

1. Heat the soft and energy drink samples, then boil for 10 minutes to remove CO_2.
2. Leave the samples to cool to room temperature.
3. Take 10 mL of each sample and place in the separatory funnel. Add 1 mL of 20% (w/v) sodium carbonate solution and 5 mL of chloroform to the sample.
4. Extract the caffeine by stirring the funnel for a few minutes and then separate the lower layer.
6. Add 0.1 mL of each extract to a volumetric flask and make up to 5 mL with chloroform. Place the sample in a quartz cuvette, ready for analysis.

Following this method, the total dilution factor of the soft drink was **25**

Appendix 3 – Configuring Measurement Parameters

Configuration of the measurement parameters may be performed by a laboratory technician in advance of students commencing the experiments. The method is as follows:

1) Launch *LabSolutions UV-Vis,* then select [Quantitation]
2) Select the [Inst. Control] icon from the toolbar to display the instrument control window
3) Click [Parameter] – [Edit] to display the [Set Parameters] window.
4) Click [Registration of Wavelength] to display the [Register Wavelengths] window, then select '*Point'* for [Measuring Method] and '*274'* for [Wavelength 1 (nm)]. Finally select [Add], then click [Ok].
5) Click [Calibration Curve] to display the [Calibration Curve] window and select: '*Measure a standard sample',* '*Fixed Wavelength'* for [Calculation Method] and '*WL274.0'* for [Wavelength 1]. Then, click [Ok].
6) Click [Standard Sample], then select '*Acquire a measuring mode by measurement'.* Then, click [Ok].
7) Click [Unknown Sample], then select '*Acquire a measuring mode by measurement'.* Then, click [Ok].
8) Click [Close after creating new parameter file] in the [Set Parameters] window. Enter a file name and click [Save].