

Spectrophotometric determination of caffeine in beverages

The exercise proposed a spectrophotometric method for determination of caffeine in coffee, tea and various refreshing drinks (coca-cola, energy drinks, etc.)

Caffeine is a bitter white crystalline xanthine' alkaloid that acts as a mild psychoactive stimulant drug. It also possesses a weak diuretic action. It is found in varying quantities in the seeds, leaves, or fruits of many plants species. The most common sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves. In humans, caffeine acts as a central nervous system stimulant, hence it is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs. It is metabolized in the liver into three primary metabolites, paraxanthine (84%), theobromine (12%) and theophylline (4%).

Equipment/Material:

- Spectrophotometer
- quartz cuvetts
- caffeine standards
- degassed coca-cola samples or tea or coffee
- chloroform
- separatory funnels 50 mL
- graduated cylinders
- beakers
- automatic pipets
- 10 mL, 25 mL, volumetric flasks
- waste container
- SPE cartridge
- vacuum system
- methanol,
- ammonia water to remove impurities (2 mL 25% NH_3 aq, + 8 mL H_2O + 1 mL MeOH) -SPE
- eluent: mixture:methanol/ water/acetic acid (75:25:1) (7.5 mL MeOH + 2.5 mL H_2O + 0.1 mL CH_3COOH) - SPE

Procedure:

1. Preparation of caffeine standard solutions:

A stock standard of caffeine prepare by dissolving 25 mg of caffeine in 25 mL CHCl_3 in a volumetric flask (25 mL). Working standards prepare by pipetting the right amount of the stock standard solution into separate volumetric flasks (10 mL) and dilut to volume with purified CHCl_3 to produce concentrations of 40, 80, 120, 160 and 200 $\mu\text{g}/10$ mL, respectively standard solution. The absorbance of each solution measure at absorption maximum of 276 nm using 10 mm quartz cuvette. The absorbance values were then plotted against concentrations to generate a standard calibration curve.

Note: For SPE extraction, standard solutions should be prepared in methanol. The absorbance of each solution was measured at absorption maximum of 269 nm using 10 mm quartz cuvette.

2. Caffeine liquid-liquid extraction procedurę from coca cola:

An aliquot (10 mL) of the drink sample drawn with a 10 mL pipette and place into a 125 mL separating funnel followed by the add of distilled water (10 mL), then 1M NaOH (1 mL) and analytical grade CHCl_3 (10 mL). The caffeine extract by inverting the funnel at least three times, venting the funnel after each inversion. The non-aqueous CHCl_3 layer remove to a clean 25 mL volumetric flask. Another 5 mL (the last portion is 5 mL too) portion of CHCl_3 add to the aqueous solution in the separating funnel and the extraction procedure repeat twice more and the CHCl_3 solvent layers combined. This volume was made up to 25 mL with the solvent. This procedure repeat for all the drink samples. The absorbance of the resulting solutions then measure on UV/Vis spectrophotometer at 276 nm using 10 mm quartz cuvette.

3. Caffeine liquid-liquid extraction procedure from tea (coffee):

100 mL of boiling water add to 250 mL beaker containing 0.2 g of instant coffee, or coffee or 0.1 g tea respectively. The coffee or tea preparations stir and let it brew for 20 minutes. Then filter through a paper filter.

An aliquot (10 mL) of the drink sample drawn with a 10 mL pipette and place into a 125 mL separating funnel followed by the add of distilled water (10 mL), then 1M NaOH (1 mL) and analytical grade CHCl_3 (10 mL). The caffeine extract by inverting the funnel at least three times, venting the funnel after each inversion. The non-aqueous CHCl_3 layer remove to a clean 25 mL volumetric flask. Another 5 mL (the last portion is 5 mL too) portion of CHCl_3 add to the aqueous solution in the separating funnel and the extraction procedure repeat twice more and the CHCl_3 solvent layers combined. This volume was made up to 25 mL with the solvent. This procedure repeat for all the drink samples. The absorbance of the resulting solutions then measure on UV/Vis spectrophotometer at 276 nm using 10 mm quartz cuvette.

4. Caffeine solid phase extraction (SPE) procedure from coffee or tea:

100 mL of boiling water add to 250 mL beaker containing 0.2 g of instant coffee, or coffee or 0.1 g tea respectively. The coffee or tea preparations stir and let it brew for 20 minutes. Then filter through a paper filter. An aliquot (2 mL) of the drink sample drawn with a pipette and place into a baker followed by the add of distilled water (5 mL), then 1M NaOH (1 mL).

Use for isolation of caffeine Octadecyl-modified silica C18 cartridge . Firstly, a SPE cartridge conditione with 5 mL of methanol followed by 5 mL of deionized water, at a flow rate of 1 mL/min. After the conditioning step, a sample tea or coffee percolate through the cartridge at a flow rate of 10 mL/min. The cartridge then rins with 5 mL of ammonia water and the cartridge dry under vacuum for 20 min, to remove excess of water. For elution of a sample use 5 mL of eluent (mixture:methanol/ water/acetic acid (75:25:1)). The absorbance of the resulting solution then measure on UV/Vis spectrophotometer at 269 nm using 10 mm quartz cuvette.

5. Calculations:

Using the graph, determine the concentration of caffeine in beverages in g/L or g/100 g respectively.