Determination of caffeine content in tea beverages

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Abstract. Caffeine is the main component and characteristic indicator of tea beverages, and it is also a stimulant of the central nervous system. In this paper, the content of caffeine in 19 tea beverage samples in the market were determined by the ultraviolet spectrophotometry method, which was convenient, rapid, effective and low cost. The results showed that caffeine was detected in all samples as an inherent component of tea itself. The content of caffeine in green tea beverages, milk-flavored tea beverages and scented tea beverages was higher than that of black tea beverages and oolong tea beverages due to the different types of raw tea. Because of the difference in the source of raw tea and the difference in production process and formula, the caffeine content of beverages from different manufacturers of the same tea varieties was also significantly different, and there was great difference both in green tea beverages and in black tea beverages. The caffeine content in all samples met the requirements of Chinese national standard and did not exceed the prescribed limits set by the United States, Canada, Japan and other countries.

1. Introduction
Tea is a traditional Chinese beverage and is praised as the “national drink” of China. The traditional way of drinking tea is boiling water brewing, but brewed tea is difficult to carry and store. With the industrialization and socialization of food production, tea beverages, which have both the pleasure of drinking tea and the refreshing feeling of drinking beverages, are widely accepted as a new variety of beverages because of their convenience and unique flavor.

A tea beverage is prepared from tea or its water extract or its concentrated solution, tea powder (including instant tea powder, ground tea powder), or fresh leaves of tea as raw material, with or without added food raw materials and/or food additive, and processed into liquid beverage. Such as raw tea juice (tea soup)/pure tea beverage, tea concentrate, juice tea beverage, milk tea beverage, compound (mixed) tea beverage and other tea beverage[1].

Tea beverage is also an important way to the intake of caffeine for modern people. Caffeine is the natural component of the tea itself, and is the main component of the tea taste. It is also the important quality indicator of the tea beverage. Appropriate consumption of caffeine has the effects of driving away drowsiness, relieving fatigue, and stimulating the nerves, so that tea beverages with the caffeine ingredient are very popular. However, the intake of high levels of caffeine also has many side effects that can damage human health.

The content of caffeine varies depending on the type of tea used in the tea beverage and the proportion of tea in the ingredients. Therefore, the amount of caffeine contained in the tea beverages of different raw materials and different brands is different, and the taste is also different, hence it is necessary to determine the content of caffeine in the tea beverages. Various tea beverages have become an important source of high frequency of caffeine intake in people's diets. Therefore, it is very...
important to develop a simple, rapid, reliable, and low-cost analysis method to determine the caffeine content in tea beverages.

At present, the quantitative analysis methods of caffeine include liquid chromatography[2-11], spectrophotometry[12, 13], chromatography-mass spectrometry[14], etc. Analytical methods such as liquid chromatography and chromatography-mass spectrometry often have shortcomings such as long analysis time, high consumption of organic solvents, and relatively high analysis cost. In this paper, ultraviolet (UV) spectrometry is used to determine the content of caffeine in several common tea beverages sold on the market at present. Compared with chromatography, it has the advantages of simple pre-treatment, simple operation, rapid operation and low cost. It is hoped that through these analyses and research, consumers will be able to understand the quality of tea beverages more clearly, also providing a reasonable reference for their consumption, and providing basic data support for the future development of tea beverages.

2. Experimental

2.1. Instruments and reagents
UV-2550 PC UV-visible spectrophotometer (Shimadzu, Japan). Synergy UV ultra-pure water meter (Millipore, USA).
Caffeine standard (98%, Sigma, USA). Anhydrous sodium sulfate, chloroform, zinc acetate, potassium ferrocyanide, potassium permanganate, sodium sulfite, potassium thiocyanate, phosphoric acid and sodium hydroxide were all analytical pure.

2.2. Sample source
Commercially available tea beverages, purchased from supermarkets, include 19 different samples of five types, including black tea beverages, green tea beverages, oolong tea beverages, scented tea beverages and milk-flavored tea beverages.

2.3. Determination method
The method used in this paper for determining caffeine content is the ultraviolet spectrophotometry method in the GB/T 5009.139-2003 standard[15]. The chloroform solution of caffeine has a maximum absorption at a wavelength of 276.5 nm, and the absorption value is proportional to the concentration of caffeine, so that quantitative analysis can be performed.

2.3.1. Standard curve determination.
Using the standard reserve solution of 0.5 mg/mL caffeine, a standard series with concentrations of 0 μg/mL, 5 μg/mL, 10 μg/mL, 15 μg/mL, and 20 μg/mL were prepared by re-distilled chloroform. The absorbance was measured at 276.5 nm by 1 cm colorimetric ware, and the standard curve of absorbance-caffeine concentration was obtained.

2.3.2. Sample processing.
Accurately transfer 10.0-20.0 mL uniform tea beverage sample into a 100 mL volumetric flask, add 2 mL 200 g/L zinc acetate solution, shake well. Add 2 mL 100 g/L potassium ferrocyanide solution, shake well. Dilute to 100 mL with water, shake well, settle for precipitation, and filter.
Take 5.0-20.0 mL of filtrate, add 5 mL 15 g/L potassium permanganate solution, shake well, stand for 5 min. Add 10 mL mixed solution of 100 g/L sodium sulfite and 100g/L potassium thiocyanate, shake well. Add 15% phosphoric acid solution 1 mL, shake well. Add 1 mL 200 g/L sodium hydroxide solution, shake well.
Add 50 mL re-distilled chloroform, shake it 100 times, stand for stratification and collect chloroform. Further add 40 mL re-distilled chloroform into the aqueous layer, shake it 100 times, stand for stratification. The two chloroform extracts were combined and made up to 100 mL with re-distilled chloroform.
2.3.3. Sample determination.
Add 5 g anhydrous sodium sulfate to a 25 mL colorimetric tube, pour into 20 mL sample solution of chloroform, shake well. The absorbance of the clarified chloroform was measured by 1 cm colorimetric ware in 276.5 nm. According to the standard curve, the concentration of caffeine $c$ ($\mu$g/mL) was calculated to be equivalent to the absorbance of the sample. At the same time, the re-distilled chloroform was used as the reagent blank.

2.3.4. Results calculation.
The content of caffeine in tea beverages was calculated as follows:

$$X = \frac{(c-c_0)\times 100 \times 100 \times 1000}{V_1 \times V \times 1000}$$

Scheme 1. Calculation of caffeine content in tea beverage.

Among them:
$X$- caffeine content in tea beverage, mg/L
$c$- caffeine concentration equivalent to the sample absorbance, $\mu$g/mL
$c_0$- caffeine concentration equivalent to reagent blank absorbance, $\mu$g/mL
$V$- the volume of sample taken, mL
$V_1$- the volume of aqueous solution after sample taken, mL

3. Results and discussion

3.1. Structure and nature of caffeine
Caffeine, a white crystal, is a xanthine alkaloid compound. It is mainly used as a central nervous excitation medicine in clinical practice, and its structural formula is shown in figure 1. The system name (IUPAC) was 1,3,7-trimethylpurine-2,6-dione, chemical formula $C_8H_{10}N_4O_2$, molar mass 194.19 g/mol, density 1.23 g/cm$^3$, melting point 235 to 238 °C[16].

Caffeine can form a complex with catechins and theaflavins in tea, which provides a certain stimulating and harmonious refreshing taste. It is the main component that determines the taste of tea and an important quality indicator for tea beverages.

![Figure 1. 2D and 3D structure of caffeine[16].](image)

3.2. Results of caffeine standard curve determination
The standard curve was plotted with the concentration of caffeine standard solution as abscissa and the absorbance $A$ as ordinate, as shown in figure 2. The regression equation was $y = -0.00836 + 0.04936x$, and the correlation coefficient $R^2=0.99976$, indicating that the absorbance $A$ has a good linear relationship with the caffeine concentration $c$ in the range of 0-20 $\mu$g/mL.
3.3. Results of sample determination
According to the different types of raw tea, the 19 different tea beverage samples were divided into five categories: black tea beverage (6 samples), green tea beverage (7 samples), oolong tea beverage (2 samples), scented tea beverage (2 samples) and milk-flavored tea beverage (2 samples). The sample was shaken and sampled, the data absorbance $A$ was taken as the average value of the three measurements. The caffeine content was calculated according to scheme 1, wherein $c_0$ was measured as 0.2 μg/mL, the volume of the sample taken $V$ was 20 mL, and the volume of aqueous solution after sample taken $V_1$ was 10 mL.

Table 1 shows the caffeine content measured in 19 tea beverage samples.

| No | Sample | Type                  | A: Absorbance | $c$: Caffeine content equivalent to absorbance (μg/mL) | $X$: Caffeine content in tea beverage (mg/L) |
|----|--------|-----------------------|---------------|------------------------------------------------------|--------------------------------------------|
| 1  | A-1    | Black tea beverage    | 0.0890        | 2                                                    | 90                                         |
| 2  | A-2    | Black tea beverage    | 0.0932        | 2.1                                                  | 95                                         |
| 3  | A-3    | Black tea beverage    | 0.0906        | 2                                                    | 90                                         |
| 4  | A-4    | Black tea beverage    | 0.0463        | 1.1                                                  | 40                                         |
| 5  | A-5    | Black tea beverage    | 0.0594        | 1.4                                                  | 60                                         |
| 6  | A-6    | Black tea beverage    | 0.0929        | 2.1                                                  | 95                                         |
| 7  | B-1    | Green tea beverage    | 0.1061        | 2.3                                                  | 105                                        |
| 8  | B-2    | Green tea beverage    | 0.0503        | 1.2                                                  | 50                                         |
| 9  | B-3    | Green tea beverage    | 0.0519        | 1.2                                                  | 50                                         |
| 10 | B-4    | Green tea beverage    | 0.1714        | 3.6                                                  | 170                                        |
| 11 | B-5    | Green tea beverage    | 0.1315        | 2.8                                                  | 150                                        |
| 12 | B-6    | Green tea beverage    | 0.1064        | 2.3                                                  | 105                                        |
| 13 | B-7    | Green tea beverage    | 0.1601        | 3.4                                                  | 160                                        |
| 14 | C-1    | Oolong tea beverage   | 0.1018        | 2.2                                                  | 100                                        |
| 15 | C-2    | Oolong tea beverage   | 0.0873        | 1.9                                                  | 85                                         |
| 16 | D-1    | Scented tea beverage  | 0.0856        | 1.9                                                  | 85                                         |
| 17 | D-2    | Scented tea beverage  | 0.1229        | 2.7                                                  | 125                                        |
| 18 | E-1    | Milk-flavored tea beverage | 0.1585 | 3.4                                                  | 160                                        |
| 19 | E-2    | Milk-flavored tea beverage | 0.1022 | 2.2                                                  | 100                                        |

It can be seen that caffeine was detected in all samples. The caffeine content of the six black tea beverages was between 40-95 mg/L, and the value was 50-170 mg/L, 85-100 mg/L, 85-125 mg/L and 100-160 mg/L of the seven green tea beverages, the two oolong tea beverages, the two scented tea beverages and the two milk-flavored tea beverages respectively.
The content of caffeine varied depending on the types of raw tea. The caffeine content in green tea beverage, milk-flavored tea beverage and scented tea beverage was higher than that in black tea beverage and oolong tea beverage. The caffeine content of beverages from different manufacturers of same tea varieties was also significantly different, especially in green tea beverages and black tea beverages. The main reason for these differences may be due to differences in raw tea sources and differences in production processes and formula.

From the caffeine content of 19 different tea beverages, according to the national standard GB/T 21733-2008 of tea beverages[17], the caffeine content of all tea beverages used in this paper met the standard requirements, and the caffeine content in most tea beverages was 1.5-3 times the national standard. According to the regulations of the prescribed limits of the United States, Canada, Japan and other countries, the content of caffeine in beverages should be less than 200 mg/kg, so the caffeine content of tea beverages in this experiment did not exceed the standard.

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