Extraction and Quantitative Analysis of Caffeine in Milk Tea: A Case Study of CHAGEE

Yuxi Wang

Suzhou Foreign Language School, Suzhou, Jiangsu, China

994366593@qq.com

Abstract. This research aimed to extract and quantify caffeine, a natural alkaloid, in CHAGEE, a Chinese milk tea brand, and propose its reasonable intake guidelines. While caffeine is known to enhance alertness and mental refreshment, excessive consumption may cause adverse effects such as increased heart rate, insomnia, and anxiety, posing particular risks to vulnerable groups, including pregnant women and adolescents. To accurately determine the caffeine content for safety guidance, it was first isolated from a deproteinized tea infusion via ethyl acetate and then quantified by iodometric back titration. The core principle relies on the quantitative complexation reaction between caffeine and excess iodine under acidic conditions, with unreacted iodine titrated against standard sodium thiosulfate. The method is simple, low-cost, and suitable for routine analysis in basic labs. Based on the measured caffeine content and its pharmacological profile, intake recommendations are provided for healthy adults, pregnant women, slow metabolizers, and adolescents. These findings offer a scientific basis for formulating consumption guidelines and contribute to food safety monitoring in the beverage industry.

Keywords: Caffeine; Milk Tea; CHAGEE; Iodometric Back Titration; Food Safety.

1. Introduction

Nowadays, milk tea has become an extremely popular beverage, enjoying great favor among young people. Regarding the 2025 survey on the purchase frequency of new-style tea drinks among Chinese consumers, the data indicated that 69.76% of them made purchases 2 to 3 times weekly [1]. Caffeine, as a natural alkaloid, exists in the tea leaves used for milk tea, which not only endows milk tea with a unique flavor but also brings positive effects, such as enhancing attention and alertness. The caffeine content in milk tea can directly affect the tasting experience, consumers' feelings, and even health. In terms of food safety and impact on human health, moderate caffeine intake is beneficial to the human body, with the effect of refreshing the mind [2]. However, excessive intake may cause a series of side effects such as rapid heart rate, insomnia, and anxiety [3]. In view of this, the accurate determination of caffeine content in milk tea is crucial for food safety and other related scientific research, and it helps to formulate reasonable drinking suggestions to avoid adverse symptoms and safeguard consumers' health. The experiment mentioned in this paper will select CHAGEE, a well-known Chinese milk tea brand, as the main research object, considering its prominent presence in the market, evidenced by over 6,000 stores globally, and its unique practice of disclosing caffeine content on each beverage cup, which can serve as a benchmark for the measurements [4]. Therefore, this study aims to extract and quantify caffeine in CHAGEE milk tea using iodometric back titration, and to evaluate reasonable intake levels for consumers.

2. Literature Review

2.1 Properties of Caffeine (See Table 1)

2.2 Pharmacological Effect

2.2.1 Central Nervous System (CNS) Stimulation

Caffeine acts as a competitive antagonist of adenosine receptors, primarily A_1 and A_2A subtypes. By blocking adenosine binding to these receptors, caffeine inhibits its sedative effects. Adenosine

binding normally reduces neuronal excitability through inhibition of adenylate cyclase and subsequent reduction of intracellular cAMP levels. Through structural mimicry, caffeine occupies the receptor sites, reversing this inhibitory process and transiently alleviating drowsiness. The removal of adenosine-mediated inhibition elevates cAMP levels, which in turn triggers the release of excitatory neurotransmitters such as dopamine and glutamate. This neurochemical cascade enhances cognitive functions, including improved alertness, attention, and information processing capacity [3].

Table 1. Properties of Caffeine.

Property	Description/Value
Systematic Name	1,3,7-trimethyl-1H-purine- 2,6 (3H,7H) -Dione
Other Names	1,3,7-trimethylxanthine; 1,3,7-trimethyl-2,6-dioxopurine
Appearance	White, crystalline powder
Smell and Taste	Odorless, and a distinctly bitter taste
Molecular Formula	$C_8H_{10}N_4O_2$
Molecular Mass	194.19 g/mol
Melting Point	238°C
Boiling Point/Sublimation Point	178°C
Solubility in Water	Slightly soluble
Solubility in Ethyl Acetate	Soluble

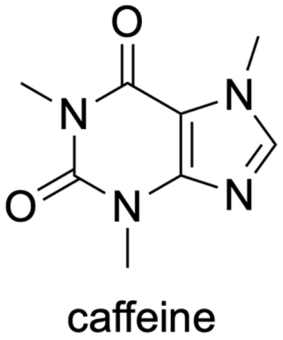

Figure 1. Chemical Structure of Caffeine.

2.2.2 Dual Cardiovascular Effects

Excessive caffeine intake may elicit dual cardiovascular effects: acute hypertensive responses and paradoxical vascular reactivity. Caffeine potentiates catecholamine release, inducing positive chronotropic effects and enhanced myocardial contractility, concomitant with peripheral vasoconstriction. This cascade directly stimulates the sympathetic nervous system, precipitating rapid blood pressure elevation and manifesting as acute hypertension. Furthermore, catecholamine-driven mechanisms provoke systemic arteriolar constriction, while simultaneously promoting endothelium-dependent vasodilation in specific vascular beds via nitric oxide (NO)-mediated pathways. This concomitant induction of vasoconstrictive and vasodilatory responses constitutes the paradoxical vascular effect of caffeine [3]. Thus, excessive caffeine may exacerbate cardiovascular strain in sensitive individuals.

2.2.3 Dual Effects on Bone and Cartilage

High caffeine intake is adversely associated with intestinal calcium absorption, which can disrupt calcium balance and exert a potential negative impact on calcium retention. Moreover, long-term coffee consumption has been linked to a slight reduction in bone mineral density (BMD), and this association is particularly pronounced in postmenopausal women. A decline in BMD increases the risk of osteoporosis and osteoporotic fractures, and this risk is even higher in the elderly population [3]. Additionally, substantial evidence indicates that caffeine intake exerts adverse effects on hyaline cartilage, a type of cartilage that exists within the growth plate and is crucial for longitudinal bone

growth, potentially leading to problems such as joint dysfunction, pain, and disability [5]. Therefore, high caffeine consumption may compromise skeletal and joint health over time.

2.2.4 Prenatal Toxic Effects

Maternally ingested caffeine can easily cross the placental barrier and reach an equilibrium between the fetal and maternal plasma. However, neither the placenta nor the fetus can metabolize caffeine, as they lack the required enzymes. Moreover, the reduced activity of the CYP1A2 enzyme in pregnant women slows down the caffeine clearance rate, leading to the accumulation of caffeine in the systemic circulation of both the mother and the fetus [6,7]. Excessive caffeine intake exerts severe adverse effects on fetal development. It may interfere with fetal development by inhibiting phosphodiesterase, elevating the intracellular cAMP level, and disrupting cell development. A high cAMP level is associated with fetal asphyxia. Additionally, it can promote the release of maternal catecholamines, trigger vasoconstriction, reduce uteroplacental blood flow, and cause an insufficient supply of oxygen and nutrients to the fetus, thus hindering fetal growth. Furthermore, caffeine has a structure similar to that of adenine and guanine, and may be mistakenly incorporated during DNA synthesis, resulting in chromosomal abnormalities and affecting fetal growth [8]. Not only does fetal development get hindered, but the risk of miscarriage also increases significantly with high caffeine intake during pregnancy, such as 400 mg per week. Meanwhile, as caffeine affects adrenal development, inhibits glucocorticoid synthesis, and disrupts growth programming, the risk of offspring developing metabolic syndrome may also increase. Overall, daily caffeine intake of ≥ 300 mg may lead to adverse pregnancy outcomes. When the daily caffeine intake during pregnancy is ≥ 200 mg, the brain development and behavior of the offspring are affected [9]. Given the widespread use of caffeine, it is necessary to re-evaluate the recommended safe dosage, examine whether there is a need to refine the dosage guidelines, and avoid prenatal risks.

2.2.5 Drug Interactions

Caffeine exhibits notable interactions with a variety of medications, and special attention is warranted. Firstly, when co-administered with stimulants such as amphetamines, cocaine, ephedrine, or medications for attention-deficit/hyperactivity disorder (ADHD), it can superimpose stimulant effects, exacerbating side effects like jitteriness, tachycardia, and anxiety [10,11]. Secondly, caffeine can antagonize the actions of antihypertensive drugs such as beta-blockers and diuretics, impairing their blood-pressure-lowering efficacy [12]. Thirdly, certain antibiotics, including ciprofloxacin and norfloxacin, can inhibit the metabolism of caffeine, resulting in prolonged retention and increased concentrations in the bloodstream [13]. This may trigger severe reactions or even fatal risks. Therefore, during the administration of such antibiotics, caffeine intake should be avoided or strictly restricted; if caffeine intake is necessary, an interval of several hours is recommended. However, it should be noted that some antibiotics, such as gentamycin and amoxicillin, may have a synergistic effect with caffeine. Fourthly, caffeine can reduce the sedative and anxiolytic effects of benzodiazepines, such as diazepam and lorazepam, diminishing their sleep-promoting efficacy. Fifthly, concurrent use with thyroid medications may increase the risk of toxicosis and reduce drug absorption, and an interval of at least 4 hours should be maintained between their administrations [14]. In view of the aforementioned interactions, for special populations such as those with anxiety disorders, heart diseases, or sleep disorders, caffeine may significantly exacerbate existing symptoms. During the administration of relevant medications, it is particularly important to consult a physician and strictly follow medical advice to control or avoid caffeine intake.

2.3 Caffeine's Effects on Sleep

The administration of caffeine can have both short-term and long-term effects on sleep. In terms of the acute effects, when caffeine is taken within 3-6 hours before bedtime, sleep latency will be prolonged and sleeping latency will be reduced [15]. Several studies also show that caffeine intake may cause decreased slow-wave activity(SWA), which is a marker of deep sleep, and increased sigma activity, normally 12-15Hz [5]. Besides, adenosine A_2A receptors may be blocked by caffeine as well,

promoting alertness, especially under high sleep pressure, such as sleep deprivation. However, long-term consumption of caffeine can gradually lead the human body to develop tolerance, failing to achieve the expected effects. For instance, sleep latency and efficiency normalize over time despite daily intake, suggesting tolerance. In addition, chronic use may upregulate adenosine receptors A_1 and increase adenosine levels, counteracting caffeine's effects [5]. What's more, compared to EEG changes caused by acute administration, there is no further sustained reduction in SWA while other frequency bands like sigma may continue to be altered.

2.4 Clinical Usage Guidelines for Caffeinated Products

By retrieving and analyzing research data on the toxicity of caffeine, the safe caffeine intake for healthy adults has been determined to be 5.7 mg/kg body weight (400 mg/day). For minors under 18 years old, the safe caffeine intake is 2.5 mg/kg body weight. Minors should minimize their consumption of caffeinated beverages such as milk tea and tea-based drinks. It is recommended to conduct risk communication and public education to enhance consumers' awareness of the potential health risks associated with caffeine intake [16]. Most authoritative organizations, such as the European Food Safety Authority, recommend that pregnant women limit their daily caffeine intake to no more than 200 mg. This threshold is based on research evidence indicating that exceeding this amount may increase the risks of miscarriage, fetal growth restriction, or low birth weight [17]. Based on the safe intake level, it can be estimated that a 60 kg adult can consume up to 969 g of liquid coffee or 1524 g of liquid milk tea per day without exceeding the acceptable caffeine intake, assuming no other caffeine-containing foods are consumed. Since the calculation is based on the average caffeine content of various coffee types, the daily consumption of espresso should be further reduced [16].

2.5 Application of Ethyl Acetate in Caffeine Extraction

Ethyl acetate demonstrates high efficacy in the liquid-liquid extraction purification of caffeine from aqueous solutions, achieving selective recovery of 93.4% caffeine. While dichloromethane exhibits marginally superior extraction efficiency, ethyl acetate is recommended as a more sustainable alternative solvent owing to its enhanced environmental compatibility, low toxicity, and biodegradability [18].

3. Research Method

3.1 Caffeine Extraction from CHAGEE Tea

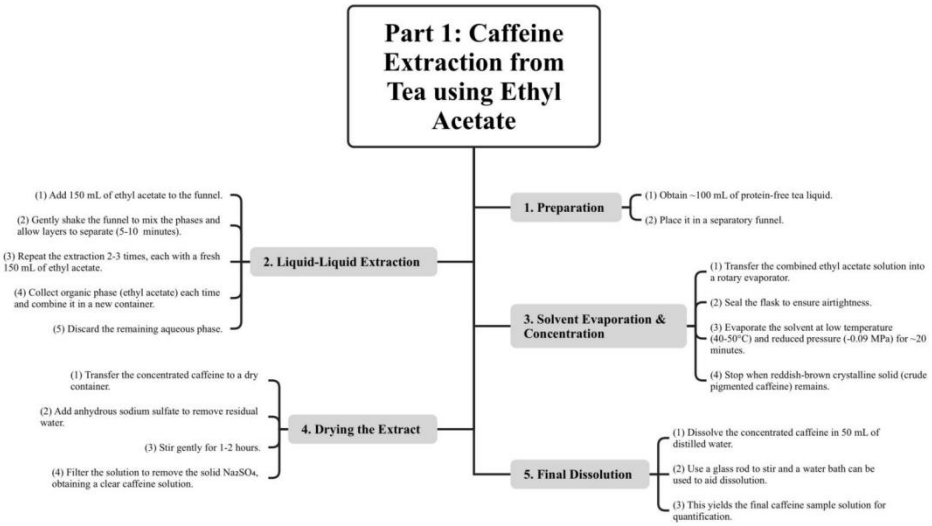

Figure 2. Caffeine Extraction Process from Tea using Ethyl Acetate.

Caffeine was extracted from CHAGEE tea liquid using liquid-liquid extraction with ethyl acetate as the solvent. The overall extraction process, summarized in Figure 2, includes preparation, liquid-liquid extraction, solvent evaporation, drying, and final dissolution.

Approximately 100 mL of protein-free tea liquid was placed in a separatory funnel and subjected to 3 sequential extractions, each with 150 mL of ethyl acetate. The combined organic phases were then concentrated using a rotavapor evaporator under reduced pressure (-0.09MPa) at 40-50℃. Next, the resulting crude caffeine was dried over anhydrous sodium thiosulfate, filtered, and dissolved in 50 mL of distilled water to obtain a sample solution for quantification.

3.2 Iodometric Back Titration for Caffeine Quantification

The caffeine content in the sample solution was determined via iodometric back titration. The experimental procedure, illustrated in Figure 3, consists of four main stages: sample preparation, reaction with iodine, titration process, and calculation.

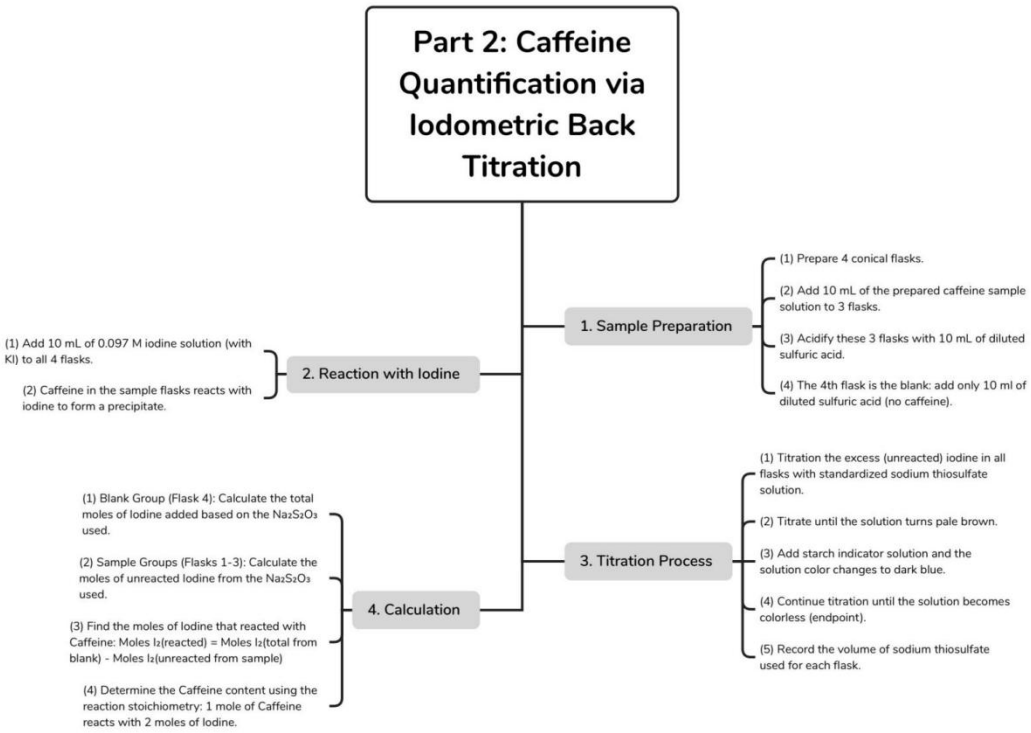

Figure 3. Caffeine Quantification via Iodometric Back Titration.

A sample solution of 10 mL was acidified with 10 mL of diluted sulfuric acid. Then, 10 mL of 0.097 M iodine solution (with KI) was added to form a caffeine-iodine precipitate. The unreacted iodine was titrated with a standardized sodium thiosulfate solution, using starch as an indicator. The titration endpoint was marked by the solution turning from dark blue to colorless.

3.3 Data Processing and Statistical Analysis

The entire extraction and titration procedure was independently repeated three times (n=3) to ensure reproducibility. The caffeine content was calculated based on the stoichiometry of the reaction (1 mole of caffeine reacts with 2 moles of iodine). The moles of iodine consumed by caffeine were determined by subtracting the moles of unreacted iodine (from sample titrations) from the total moles of iodine added (from blank titration).

Key reaction:

$$C_8H_{10}N_4O_2 + 2I_2 + KI + H_2SO_4 \rightarrow C_8H_{10}N_4O_2 \bullet HI \bullet I_4 + KHSO_4$$

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

All results are presented as the mean±standard deviation (SD). The caffeine concentration in the original tea sample was then calculated and expressed in mg/L.

4. Results

In the first experimental group (blank control), titration was performed using the caffeine-free mixed solution. In this group, iodine reacted directly with sodium thiosulfate, consuming a total of 39.85 mL of titrant ($Na_2S_2O_3$ solution). For the subsequent four groups, titrations were conducted using the remaining four flasks containing caffeine-spiked mixed solutions. Due to the presence of caffeine, a portion of iodine was consumed in the caffeine-iodine reaction, leaving only the residual iodine to react with sodium thiosulfate. Consequently, compared to the blank group, the thiosulfate consumption in these four groups was reduced to 38.86 mL, 38.20 mL, 37.70 mL, and 37.24 mL, respectively. The amount of iodine consumed by caffeine was calculated from the difference in thiosulfate volume between each test group and the blank. Based on the reaction stoichiometry (1 mol caffeine: 2 mol I_2), the molar quantity of caffeine was then determined.

Table 2. Titration Results.

Group	Reaction time (min)	$Na_2S_2O_3$ used (mL)	Caffeine mass (mg)
Blank control	-	39.85	0
1	0	38.86	56.5
2	5	38.20	94.1
3	10	37.70	122.6
4	15	37.24	148.9

The data clearly show a trend: as reaction time increased, the volume of sodium thiosulfate consumed decreased, and the calculated caffeine mass increased progressively from 56.5 mg to 148.9 mg. This indicates that the addition reaction between caffeine and iodine is not instantaneous under the experimental conditions and requires sufficient time to approach completion. According to the literature, the extraction efficiency of caffeine using ethyl acetate was determined to be 93.4% [18]. Since prolonged reaction time promotes a more complete reaction between caffeine and iodine, the calculated caffeine content at 15 min more accurately reflects the actual value. Therefore, the data obtained at 15 min reaction time were selected for the final calculation, yielding a caffeine mass of approximately 182.9 mg. A slight deviation was observed between this result (182.9 mg) and the official caffeine content (202.6 mg) provided by CHAGEE. After carefully evaluating the experimental procedure, we speculate that this variation may arise from unavoidable experimental errors, such as sample loss during liquid transfer, instrumental reading deviations, and the inherent limitations of the chosen extraction method.

5. Discussion

In this experiment, the iodometric back-titration method was employed to determine the caffeine content in CHAGEE milk tea samples. The core principle is based on the quantitative addition reaction between caffeine and excess iodine under acidic conditions, with a molar ratio of 1:2 (1 mol caffeine:2 mol I_2). Subsequently, sodium thiosulfate ($Na_2S_2O_3$) standard solution was used to back-titrate the remaining iodine in the system. By calculating the difference between the total amount of iodine added and the remaining iodine, the amount of iodine reacted with caffeine could be determined, and further, the caffeine content in the sample was deduced according to the stoichiometric relationship of the reaction. According to the brand labeling, the expected caffeine content of the sample is 202.6 mg. However, there is a certain deviation between the experimental result, 182.9 mg, and the labeled value, 202.6 mg, which is approximately 9.7%. Through in-depth

analysis of the experimental process and literature research, we believe that the following key factors have a significant impact on the experimental results.

5.1 Reaction Time

As shown in Table 1, we systematically investigated the effect of reaction time on the titration results. The experimental data clearly demonstrate that with the extension of reaction time, the volume of $Na_2S_2O_3$ consumed in titration gradually decreases from 38.86 mL in Group 1 to 37.24 mL in Group 4. This directly indicates that a longer reaction time leads to a greater amount of iodine reacting with caffeine, resulting in a reduction in the residual iodine and thus a decrease in the amount of $Na_2S_2O_3$ consumed. Correspondingly, the mass of caffeine calculated according to the reaction stoichiometry increases from 56.5 mg to 148.9 mg. This confirms that the addition reaction between caffeine and iodine is not instantaneous under the experimental conditions, and a certain reaction time is required to reach equilibrium or near-completion. Therefore, prolonging the reaction time is crucial for obtaining data closer to the true caffeine content. Choosing a group with insufficient reaction time will lead to significantly low results.

5.2 Extraction Efficiency of Ethyl Acetate

In this experiment, ethyl acetate was used as the solvent for liquid-liquid extraction to separate caffeine from the aqueous phase of milk tea. According to the literature [18], the extraction efficiency of ethyl acetate for caffeine can reach 93.4%, indicating that its selective extraction capacity is quite high. However, the extraction process involves multiple transfers, which inevitably introduce operational losses. Even with an efficiency of 93.4%, it means that 6.6% of the target analyte is not effectively recovered. In addition, during the solvent removal by rotary evaporation and the subsequent drying process, trace amounts of caffeine may be lost due to volatilization or adsorption on the container walls. These accumulated losses constitute an important reason why the finally determined caffeine content is lower than the theoretical value and the brand-labeled value. Therefore, although the extraction efficiency is acceptable, the losses during the operational process cannot be ignored and represent one of the key factors contributing to the low results.

5.3 Analysis of Main Sources of Error

Based on a comprehensive analysis of the experimental results and operational processes, the main error sources in this experiment can be summarized as follows. Iodine volatilization loss constitutes one significant source, as iodine is volatile; trace amounts of iodine vapor may escape during the standing period before titration and the titration process itself, leading to an underestimation of residual iodine content, which in turn overestimates the amount of iodine reacted with caffeine and results in a higher calculated caffeine content. Although the overall experimental results are relatively low, this error source still exists and may partially offset the effects of other factors causing low results. Another source is endpoint judgment deviation, since the determination of the titration endpoint, where the solution changes from dark blue to colorless, relies on the human eye's observation of the starch indicator's color change; the inherent color of the solution itself and the operator's subjective factors can introduce small yet cumulative errors in endpoint judgment. Sample matrix interference also plays a role, given that milk tea samples have complex compositions containing various substances such as tea polyphenols, pigments, carbohydrates, and so on. These matrix components may interfere in several ways, including consuming small amounts of iodine, affecting the formation or stability of the iodine-caffeine complex, and interfering with the observation of the endpoint color as pigments may mask the blue color, all of which directly impact the accurate determination of residual iodine content. Additionally, extraction efficiency and operational losses contribute significantly: as mentioned earlier, ethyl acetate does not achieve 100% extraction efficiency, and subsequent processing steps such as evaporation, transfer, drying, filtration, and redissolution can cause irreversible sample losses, making this the primary reason for the systematic underestimation of the final caffeine content. Finally, instrumental and reading errors are

involved, including calibration errors of glass instruments like pipettes, burettes, and volumetric flasks, as well as parallax errors that occur when reading liquid volumes.

5.4 Comparison with Literature and Health Context

The experimental value (182.9 mg/cup) aligns with the understanding that a single serving of milk tea contains a significant dose of caffeine. According to pharmacological data, caffeine intake of 100-200 mg within 8-10 hours before bedtime can delay sleep onset [15]. For healthy adults, the recommended maximum daily intake is 400 mg [16], equivalent to about 2 cups of CHAGEE. However, for sensitive populations like pregnant women (recommended<=200 mg/day [17]), slow metabolizers, and adolescents (recommended<=100 mg/day [16]), even one cup approaches or exceeds daily limits, potentially impairing sleep [15] and posing other health risks such as fetal developmental issues [6,7] or cardiovascular effects [3].

5.5 Advantages and Disadvantages of Experimental Methods

This experimental method presents several notable advantages. It features simple operation, as the experimental procedures are relatively clear, mainly involving basic chemical experimental operations such as extraction and titration, which are easy to master. It also has low instrument requirements, as it does not rely on expensive and sophisticated instruments like high-performance liquid chromatography (HPLC) or gas chromatography (GC). Instead, it only requires conventional laboratory glassware, separatory funnels, rotary evaporators, titration devices, and the like. Additionally, it is cost-effective, with the main reagents, such as iodine, potassium iodide, sodium thiosulfate, ethyl acetate, and sulfuric acid, resulting in operating costs far lower than those of instrumental analysis methods. Moreover, it has wide applicability, being particularly suitable for routine screening and quantitative analysis of caffeine in grassroots laboratories, such as quality inspection stations, school laboratories, and small food enterprises, thus possessing high practical value and economic efficiency [19]. However, the method also has certain disadvantages. Its anti-interference ability is limited; as discussed in 5.3, complex sample matrices, including pigments and other reducing substances, can easily cause significant interference with the titration reaction and endpoint judgment, affecting the accuracy and precision of the results. It also involves multiple operational steps with accumulated errors, as it includes several stages, such as sample pretreatment and titration, making the operational procedures cumbersome and increasing the chances of introducing errors, such as losses, contamination, and reading errors.

5.6 Experimental Method Optimization

To enhance the accuracy and reliability of the experimental results, several optimization strategies can be implemented. First, optimizing the extraction solvent could be beneficial. As indicated in the literature [19], dichloromethane generally exhibits a slightly higher extraction efficiency for caffeine than ethyl acetate. Switching to dichloromethane may further improve the recovery rate of the target analyte and reduce the negative deviation caused by incomplete extraction. It should be noted that dichloromethane is toxic and environmentally hazardous, so operations must be conducted under well-ventilated conditions. Second, refining the reaction kinetics study is advisable. Building on the existing data, additional longer reaction time points, such as 20, 30, and 60 min, should be set for titration. By plotting a curve of reaction time versus caffeine content, the minimum time required for the reaction to reach a plateau can be determined, ensuring that each measurement is performed under the condition of complete reaction and eliminating errors caused by insufficient reaction time. Third, increasing parallel experiments and controlling precision are essential. More parallel experimental groups should be set for each reaction time point or key step. Evaluating the precision of the method by calculating the average value and relative standard deviation (RSD%) can effectively reduce the impact of random errors. Fourth, improving endpoint judgment, if conditions permit, is recommended. Exploring the use of potentiometric titration instead of visual endpoint judgment can effectively overcome sample color interference and subjective errors, thereby enhancing the objectivity and

accuracy of endpoint determination. Fifth, strengthening operational standards to reduce losses is crucial. Transfer steps should be optimized by adopting quantitative transfer techniques and ensuring thorough rinsing of containers. During processes like rotary evaporation, attention should be paid to controlling temperature and vacuum to avoid bumping, and careful operation is required in drying and filtration steps.

6. Conclusion

In conclusion, based on prior literature reviews, for healthy adults, the consumption of 100–200 mg of caffeine should be avoided within 8–10 hours before bedtime, as it may significantly delay sleep onset. Taking the caffeine content of CHAGEE (202.6 mg per serving) as an example, a single cup (≥200 mg) may reduce deep sleep duration by approximately 11.4 minutes and delay sleep onset by 14 minutes. Furthermore, consuming two cups of CHAGEE, even when ingested 12 hours before bedtime, could still result in a 45-minute reduction in total sleep time. In caffeine-sensitive populations, including pregnant individuals, slow metabolizers, and adolescents, even moderate intake, normally 50 to 100 mg, may impair sleep. For these groups, consuming half a cup of CHAGEE within 6 hours of bedtime may lead to insomnia or diminished sleep quality. Therefore, it is recommended that daily caffeine intake should not exceed 200 mg, equivalent to no more than one serving of CHAGEE.

Table 3. Caffeine intake recommendations.

Population	Maximum daily caffeine intake (mg)	CHAGEE intake (cup)	Optimal consumption window
Healthy adults	≤400 mg	≤2 cups (avoid PM)	Before 2 PM
Pregnant women	≤200 mg	≤1 cup (AM only)	Before 12 PM
Slow metabolizers	≤100–200 mg	≤0.5–1 cup	Before 10 AM
Adolescents	≤100 mg	Avoid or ≤0.5 cup	Not recommended

Furthermore, considering the potential health risks associated with the pharmacological properties of caffeine, the following suggestions are proposed. For patients with hypertension or arrhythmias, blood pressure and heart rate should be monitored after caffeine consumption to promptly prevent symptom exacerbation. Individuals with gastric ulcers or irritable bowel syndrome (IBS) should reduce intake to avoid stimulation of gastric acid secretion or diarrhea. Since long-term, high-dose caffeine consumption may accelerate calcium loss, concomitant calcium supplementation is advised. Additionally, given potential drug interactions, concurrent use with quinolone antibiotics, such as ciprofloxacin, should be avoided, as these agents may delay caffeine metabolism. Patients taking benzodiazepines, such as diazepam, should refrain from excessive or prolonged caffeine intake, as it may diminish their sedative effects. Overall, this study provides a reference for public caffeine intake awareness and demonstrates a viable, cost-effective method for caffeine quantification. Future studies could expand to include more brand comparisons, investigate long-term health impacts, and further refine the analytical method for complex matrices.

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