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Quantitative Analysis of Caffeine in Carbonated Beverages from the Bangladeshi Market Using Spectroscopy Methods

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Abstract
This study explores the caffeine content in popular beverages using UV-visible spectroscopy as a cost-effective and accessible analytical method. Caffeine, a naturally occurring alkaloid, is widely consumed for its central nervous system stimulant properties, with sources including coffee, tea, and energy drinks. The analysis involved caffeine extraction using dichloromethane, followed by UV spectroscopy measurement at 260 nm, demonstrating a strong linear relationship ($R^2 > 0.99$) between absorbance and concentration. Among the tested beverages, Red Bull exhibited the highest caffeine content per serving (52.5 mg), followed by Nescafe (45 mg) and Tiger (25.5 mg), while Coca-Cola and Mojo had the lowest levels (21 mg each). Comparative data from published sources confirmed variations in caffeine content, with Stings (160 mg) and Red Bull (80 mg) leading in published caffeine data. Statistical analysis revealed weak negative correlations between total sample volume and caffeine content per serving (-0.456, $p = 0.185$) and per milliliter (-0.426, $p = 0.220$), suggesting inconclusive relationships. The findings underscore the significance of consumer awareness and regulatory measures for caffeine labeling, particularly for high-caffeine products like energy drinks. This study highlights UV-visible spectroscopy as an effective alternative to more resource-intensive methods for caffeine quantification in beverages.

Keywords: Caffeine, Beverage, Spectroscopy, carbonated, labeling

Introduction

Caffeine, a naturally occurring alkaloid, is found in the leaves, seeds, or fruits of over 63 plant species worldwide. Common sources include soft drinks and tea leaves. Its widespread consumption makes caffeine one of the most popular and commonly consumed drugs globally. Caffeine's popularity stems from its pharmacological activity as a mild central nervous system stimulant. Consumption below 300 mg per day poses minimal risk; however, during pregnancy or periods of stress, the Food Standards Agency (FSA) recommends limiting intake to less than 300 mg daily. While there are no mandatory regulations to label caffeine content in food products, several studies have determined the caffeine levels in common beverages. High-Pressure Liquid Chromatography (HPLC) is often the preferred analytical method due to less interference. However, HPLC is expensive and resource-intensive, limiting its availability in many educational laboratories across Bangladesh. This study explores an alternative analytical method using UV spectroscopy to analyze and quantify caffeine content in popular beverages and coffee. Caffeine is extracted using chlorinated solvents like dichloromethane or chloroform, and the extracted solution's absorbance is measured at 260 nm.

Literature Review

Caffeine, a naturally occurring alkaloid, is widely consumed due to its stimulatory effects on the central nervous system and is present in beverages such as coffee, tea, and carbonated drinks. The increasing consumption of caffeinated products necessitates accurate determination of caffeine levels to assess health implications. Several studies have explored different methods for caffeine quantification, including High-Performance Liquid Chromatography (HPLC), UV-visible spectroscopy, and Fourier Transform Infrared (FT-IR) spectroscopy.

HPLC has been the preferred method for caffeine analysis due to its precision and ability to minimize interferences from other compounds. Srdjenovic et al. (2008) demonstrated that HPLC could simultaneously determine caffeine, theobromine, and theophylline in beverages and herbal products with high accuracy and reliability 【1]. However, the high cost and resource-intensive nature of HPLC limit its application in resource-constrained settings like Bangladesh.

Alternatively, UV-visible spectroscopy has gained popularity as a simpler and more cost-effective method for caffeine quantification. Belay et al. (2008) measured caffeine in coffee beans using UV-visible spectroscopy, emphasizing its feasibility for routine laboratory analysis, especially in educational institutions lacking advanced equipment like HPLC 【2】. This method relies on the strong absorbance of caffeine in the UV region, particularly at 260 nm, allowing its quantification in beverages.

Moreover, Paradkar and Irudayaraj (2014) highlighted the use of FT-IR spectroscopy for rapid caffeine determination in soft drinks. While FT-IR spectroscopy offers advantages like minimal sample preparation and faster analysis times, its adoption in Bangladesh is still limited due to the unavailability of the required instruments 【3】.

Caffeine analysis in beverages has been a significant focus of research due to its physiological and psychological effects. Various spectroscopic and chromatographic methods have been employed to determine caffeine content in beverages, each with its own advantages and limitations.

Alpdogan, Karbina, and Sungur (2002) employed derivative spectrophotometry to determine caffeine levels in beverages. This method is cost-effective and provides precise quantification without requiring extensive sample preparation. It is especially suitable for routine analyses in laboratories with limited resources 【4】.

Bolton and Null (1981) explored the psychological effects of caffeine, emphasizing its widespread use and potential for abuse. Their study highlighted caffeine's impact on cognitive function, alertness, and dependence, thereby stressing the importance of monitoring its consumption through accurate analytical methods 【5】.

Bousain et al. (1999) utilized flow injection Fourier Transform Infrared (FT-IR) spectroscopy for caffeine analysis in coffee. This method is notable for its rapid analysis and ability to handle multiple samples simultaneously. However, its application in developing regions is limited by the high cost of equipment 【6】.

Brannstrom and Edenteg (2002) investigated caffeine content in Vietnamese coffee using High-Performance Liquid Chromatography (HPLC). Their findings demonstrated HPLC's precision and reliability, though its high cost and complexity restrict its accessibility for routine analyses in resource-limited settings【7】.

Chen et al. (2006) introduced near-infrared reflectance spectroscopy (NIRS) to simultaneously determine caffeine and polyphenol content in green tea. This non-destructive technique offers quick analysis and is particularly suitable for green tea due to its high polyphenol content【8】.

Edwards, Munish, and Anstis (2005) applied Raman spectroscopy to characterize caffeine and its analogs. Raman spectroscopy is effective for distinguishing caffeine from structurally similar compounds, enhancing its utility in pharmaceutical and food industries【9】.

Najafi, Hamid, and Afshin (2003) demonstrated the use of Fourier Transformation Infrared (FT-IR) spectroscopy with multiple linear regressions for analyzing caffeine in black tea. This method requires minimal sample preparation and provides accurate quantification, making it an efficient tool for caffeine determination【10】.

Ortega-Burrales et al. (2002) developed a flow injection solid-phase spectrometry method using C18 silica gel for the simultaneous determination of paracetamol and caffeine. This approach effectively combines simplicity and high throughput, making it valuable for routine quality control in beverage and pharmaceutical industries【11】. The choice of method depends on the availability of resources, the complexity of the matrix, and the desired accuracy of results. This study leverages UV-visible spectroscopy as a cost-effective and accessible alternative for caffeine determination in beverages, particularly in resource-constrained settings. Despite the availability of these advanced methods, UV-visible spectroscopy remains a practical choice in the Bangladeshi context due to its simplicity, affordability, and effectiveness.

This study aims to leverage UV-visible spectroscopy to provide a comprehensive analysis of caffeine content in beverages sold in the Bangladeshi market, filling a gap in the local literature on caffeine quantification methods.

Materials and Methods

Chemicals and Reagents: Caffeine (1, 3, 7-trimethylxanthine), dichloromethane, and chloroform were obtained from Sigma-Aldrich Co., USA, through a local vendor.

Standard Preparation: A 1000 ppm caffeine stock solution was prepared by dissolving 198 mg of caffeine in 200 ml of purified water. Working standards were diluted from this stock solution at concentrations of 100, 50, 40, 30, 20, 10, and 5 ppm.

Sample Preparation: Samples included instant coffee, tea bags, Coca-Cola, Pepsi-Cola, Mojo, Gear, Tango, Tiger, Sting, and Red Bull. Boiling water (200 ml) was added to 2g of instant coffee or a single tea bag. Soft drink samples were directly sourced from the local market in Dhaka.

Extraction Procedure: A 50 ml aliquot of the sample or standard solution was extracted with 25 ml of dichloromethane using a separating funnel. The process was repeated three times, combining the solvent layers.

Sample Measurement: The extracted samples were analyzed using a SHIMADZU UV-2100 spectrophotometer. Absorbance values were measured, and data was analyzed using Microsoft Excel for linear regression and caffeine quantification.

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Results and Discussion

The caffeine content of the extracted sample solutions is shown in table 2.

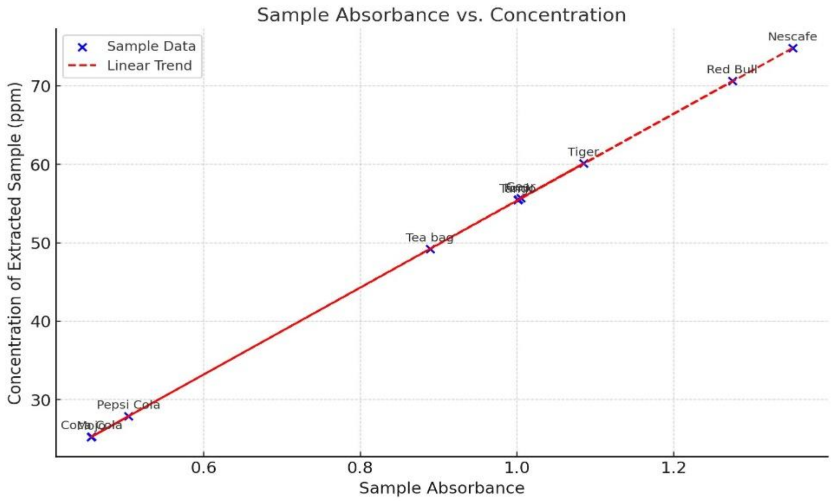

Figure 1 : The graph illustrates the relationship between the sample absorbance and the concentration of extracted caffeine

The graph 1 illustrates the relationship between the sample absorbance and the concentration of extracted caffeine, showing a clear linear trend with a high correlation coefficient ($R^2 > 0.99$). As the absorbance values increase, the concentration of caffeine rises proportionally, following the regression equation $y=55.358 \cdot x y=55.358 \cdot x$, where $y y$ represents the concentration (ppm) and $x x$ the absorbance. Beverages like Nescafe and Red Bull exhibit the highest absorbance values (1.352 and 1.275, respectively) and corresponding caffeine concentrations (74.8 ppm and 70.6 ppm). In contrast, Coca-Cola and Pepsi-Cola show the lowest absorbance values (0.457 and 0.504) with concentrations of 25.3 ppm and 27.9 ppm. This strong statistical relationship confirms the reliability of UV spectroscopy for quantifying caffeine levels, demonstrating its accuracy in establishing a direct proportionality between absorbance and concentration.

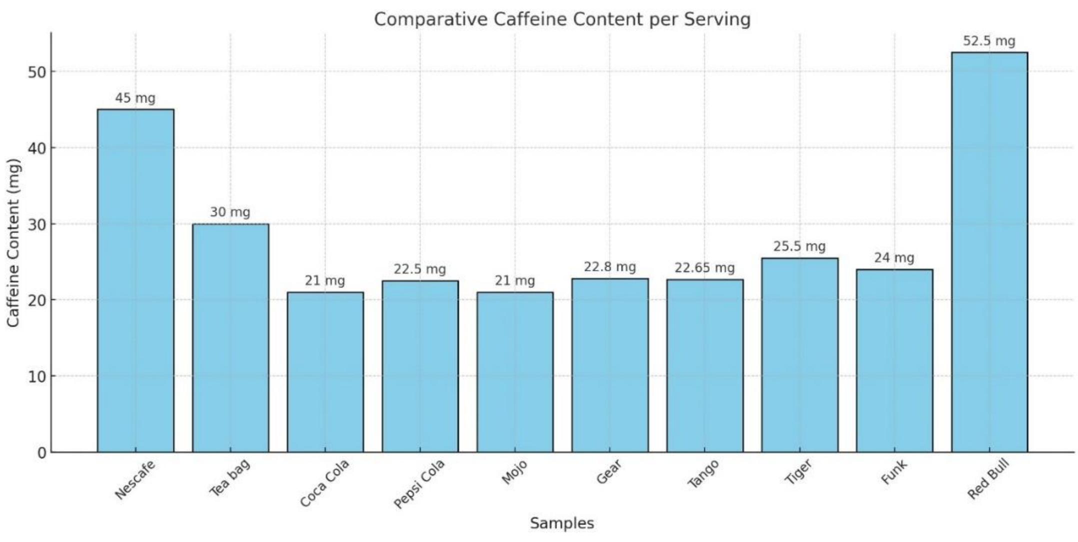

Figure 2 : The chart highlights significant variations in caffeine content

The chart 2 highlights significant variations in caffeine content across different beverages, with Red Bull leading at 52.5 mg per serving, followed by Nescafe at 45 mg, both catering to consumers seeking high energy boosts. In contrast, beverages like Coca-Cola and Mojo have the lowest caffeine levels at 21 mg, appealing to those preferring milder stimulation. The moderate caffeine levels in drinks like Tea Bag, Tiger, and Funk suggest they provide a balance between energy and casual consumption. This variability underscores the importance of consumer awareness about caffeine intake, as excessive consumption of high-caffeine products like Red Bull may pose health risks. The data emphasizes the need for clear labeling and public education to ensure informed choices, particularly for vulnerable groups like adolescents and individuals sensitive to caffeine.

Table 1. The Calculated caffeine content of Beverage products

Sample	Total sample Vol(ml)	Measured sample Vol (ml)	Caffeine content per serving (mg)	Caffeine content (mg/ml)
Nescafe	200	50	45	0.3
Tea bag	200	50	30	0.2
Coca Cola	330	50	21	0.14
Pepsi Cola	330	50	22.5	0.15
Mojo	250	50	21	0.35
Gear	250	50	22.8	0.152
Tango	250	50	22.65	0.151
Tiger	250	50	25.5	0.17
Sting	250	50	24	0.16
Red Bull	250	50	52.5	0.35
Total Volume vs Caffeine per Serving			-0.456	0.185
Total Volume vs Caffeine per mL			-0.426	0.22

In this analysis, the p-value serves as a critical measure to determine the statistical significance of the correlations between total sample volume and caffeine content (both per serving and per milliliter). A p-value greater than the standard threshold of 0.05 indicates that the observed relationships are not statistically significant and may be due to random chance. For the correlation between total sample volume and caffeine content per serving, the p-value of 0.185 suggests no significant relationship despite a weak negative correlation (-0.456). Similarly, the p-value of 0.220 for the correlation with caffeine content per milliliter confirms the lack of statistical significance for the observed correlation (-0.426). These results imply that the relationships are inconclusive and highlight the need for a larger dataset or additional factors to better understand the potential influence of total sample volume on caffeine content.

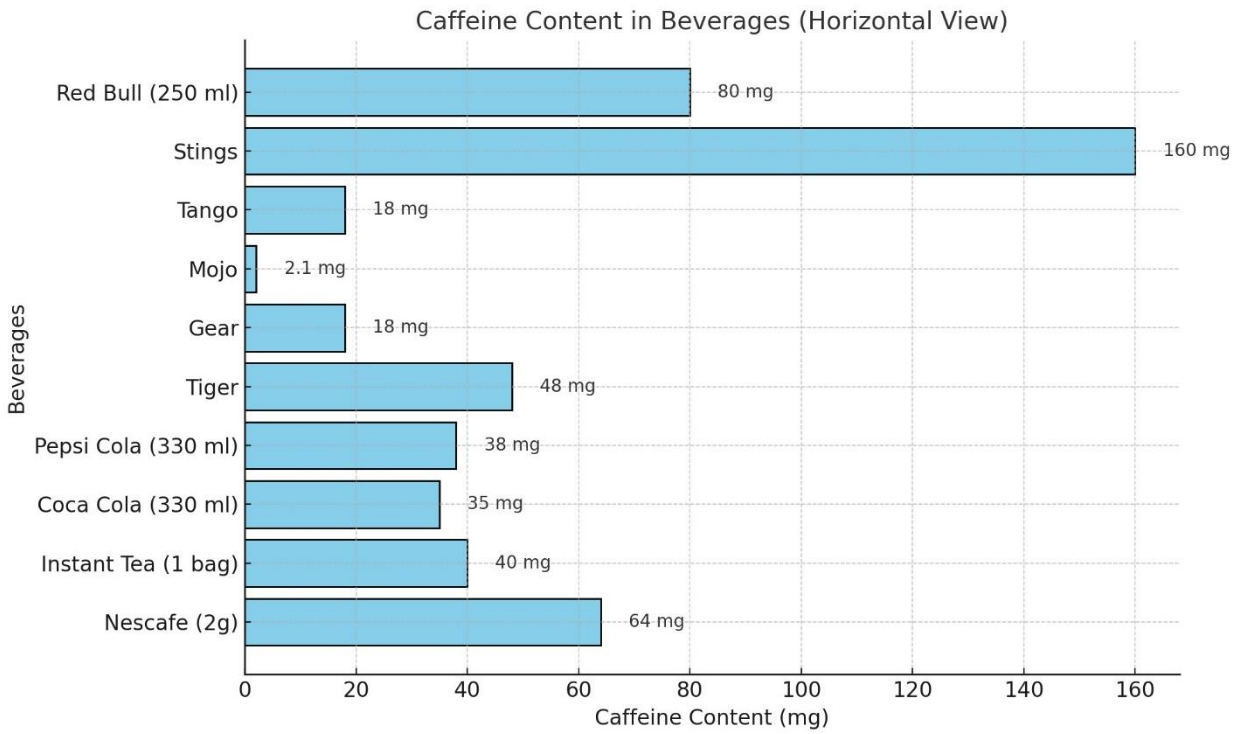

Figure 3 : Published Caffeine Content of Beverage Products

In the figure 3, the outcomes were determined and the findings are Stings, with 160 mg per serving, has the highest caffeine content, making it the most potent choice for those seeking a strong stimulant effect, followed by Red Bull at 80 mg per serving, which is a popular energy drink option. Among beverages with moderate caffeine content, Nescafe (64 mg per serving) provides a strong yet manageable dose, suitable for daily consumption, while Tiger (48 mg per serving) offers caffeine levels typical of energy drinks but lower than Stings and Red Bull. Beverages like Instant Tea (40 mg per serving), Coca Cola (35 mg per serving), and Pepsi Cola (38 mg per serving) deliver mild caffeine doses, making them suitable for those preferring less stimulation. Mojo, at just 2.1 mg per serving, has the least caffeine and is ideal for individuals sensitive to stimulants. Overall, energy drinks (Stings, Red Bull, Tiger) dominate the higher caffeine category, while coffee and tea products (Nescafe, Instant Tea) provide moderate levels, and soft drinks (Coca Cola, Pepsi Cola) cater to a broad audience with mild stimulation. Mojo stands out as a caffeine-light option, offering minimal stimulant effects.

Discussions

The analysis highlights significant findings regarding caffeine content in various beverages, showcasing the utility of UV-visible spectroscopy as a reliable, cost-effective alternative for caffeine quantification. The strong linear relationship ($R^2 > 0.99$) observed between absorbance and caffeine concentration underscores the method's accuracy, as evidenced by regression values linking absorbance to concentration. Beverages like Nescafe and Red Bull exhibited the highest absorbance values (1.352 and 1.275, respectively), correlating to caffeine concentrations of 74.8 ppm and 70.6 ppm, demonstrating their strong stimulant properties. Conversely, Coca-Cola and Pepsi-Cola showed lower absorbance values (0.457 and 0.504), with caffeine concentrations of 25.3 ppm and 27.9 ppm, positioning them as milder caffeine sources.

The caffeine content per serving revealed stark variability among beverages, with Red Bull (52.5 mg) leading, followed by Nescafe (45 mg), while products like Coca-Cola and Mojo displayed the lowest levels (21 mg). This variability points to the diverse formulation of beverages targeting different consumer needs. High-caffeine beverages cater to individuals seeking an energy boost, while lower-caffeine options suit those preferring mild stimulation.

Statistical analysis further emphasized the complexities of caffeine distribution across beverages. The weak negative correlations between total sample volume and caffeine content (per serving: -0.456, per milliliter: -0.426) and their non-significant p-values (0.185 and 0.220, respectively) suggest that volume does not strongly predict caffeine content. This lack of significance calls for more comprehensive studies with larger datasets and consideration of other influencing factors like brand formulation or ingredient variations.

The findings underscore the need for public awareness regarding caffeine consumption, as excessive intake of high-caffeine products like Red Bull may pose health risks, especially for sensitive individuals. Clear labeling of caffeine content on beverage packaging is essential to help consumers make informed choices. Overall, UV-visible spectroscopy proved to be an effective tool for caffeine analysis, reinforcing its practicality in resource-constrained settings such as Bangladesh. However, further research involving advanced techniques like HPLC could complement these findings and provide deeper insights into caffeine variability across beverages.

Conclusion

This study demonstrates the effectiveness of UV-visible spectroscopy as a reliable and cost-efficient method for determining caffeine content in beverages. By leveraging this technique, a clear linear relationship was observed between absorbance and caffeine concentration, validated by a high correlation coefficient ($R^2 > 0.99$). The results reveal significant variations in caffeine content across different beverages. Red Bull exhibited the highest caffeine concentration (52.5 mg per serving), followed by Nescafe (45 mg per serving), making them suitable for individuals seeking strong stimulant effects. Conversely, Coca-Cola and Mojo showed the lowest caffeine content (21 mg per serving), appealing to those preferring milder options.

Despite a weak negative correlation between total sample volume and caffeine content (per serving and per milliliter), the p-values (0.185 and 0.220, respectively) indicated no statistically significant relationship. This suggests that variations in caffeine levels are influenced by factors beyond volume alone, emphasizing the need for further studies with larger datasets.

The findings underscore the importance of consumer awareness regarding caffeine intake, particularly for high-caffeine beverages like Red Bull, which may pose health risks if consumed excessively. Clear labeling and public education are crucial to enabling informed choices, especially for vulnerable populations like adolescents and individuals with caffeine sensitivity. Overall, UV spectroscopy offers a practical alternative to more complex and resource-intensive methods like HPLC, providing an accessible solution for caffeine analysis in resource-constrained settings like Bangladesh.

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Novelty of the Article

This study presents a novel application of UV-visible spectroscopy as an accessible and cost-effective alternative to traditional methods like High-Performance Liquid Chromatography (HPLC) for determining caffeine content in beverages commonly consumed in Bangladesh. While HPLC is recognized for its precision, its high cost and resource-intensive nature make it impractical for many educational and low-resource settings. By leveraging the unique absorbance characteristics of caffeine at 260 nm, this research demonstrates the efficacy of UV-visible spectroscopy in providing reliable results with minimal equipment and preparation.
Additionally, this article fills a critical gap in local literature by quantifying caffeine levels in popular beverages from the Bangladeshi market, a region where such data is scarce. The method employed is tailored for use in resource-constrained environments, making it highly practical for routine analyses. Furthermore, the study's findings highlight significant variations in caffeine content across beverages, emphasizing the need for consumer awareness and regulatory measures, which are particularly relevant for health-conscious markets. This dual focus on methodological innovation and public health implications underscores the originality and impact of the research.

Conflict of Interest

The authors declare no conflict of interest regarding the publication of this article. All experimental procedures, data collection, and analyses were conducted independently and without any influence from commercial or financial entities. The authors have no affiliations, financial interests, or personal relationships that could be perceived as influencing the research outcomes or interpretations presented in this study. This work was carried out solely for academic and scientific advancement in the field of analytical chemistry and public health.

Ethical Considerations

This study adhered to ethical research principles, ensuring transparency, accuracy, and integrity in data collection and analysis. No human or animal subjects were involved, and all samples were commercially available beverages, eliminating the need for ethical approval. The research was conducted solely for scientific and academic purposes, with no conflict of interest or influence from external entities.

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References

1. Srdjenovic, B., Djordjevic-Milic, V., Grujic, N., Injac, R., & Lepojevic, Z. (2008). Simultaneous HPLC determination of caffeine, theobromine, and theophylline in food, drinks, and herbal products. *Journal of Chromatographic Science*, 46(3), 144–149.
2. Belay, A., Ture, K., Redi, M., & Asfaw, A. (2008). Measurement of caffeine in coffee beans with UV/Vis spectrometer. *Food Chemistry*, 108(1), 310–315.
3. Paradkar, M. M., & Irudayaraj, J. (2014). Rapid determination of caffeine content in soft drinks using FT-IR-ATR spectroscopy. *Food Chemistry*, 78(2), 261–266.
4. Alpdogan, G., Karbina, K., & Sungur, S. (2002). Derivative spectrophotometer determination of caffeine in some beverages. Turkish Journal of Chemistry, 26, 295–302.
5. Bolton, S., & Null, G. (1981). Caffeine, psychological effect, use and abuse. Journal of Orthomolecular Psychiatry, 10(3), 202–211.
6. Bousain, Z., Garriques, J. M., Garriges, S., & Guardia, M. (1999). Flow injection Fourier transform infrared determination of caffeine in coffee. Vibrational Spectroscopy, 21, 143–150.
7. Brannstrom, M., & Edenteg, K. (2002). Determination of caffeine in Vietnamese coffee using HPLC. Umea, Sweden: Department of Analytical Chemistry.
8. Chen, Q., Zhao, J., Huang, X., Zhang, H., & Liu, M. (2006). Simultaneous determination of total polyphenols and caffeine contents of green tea by near-infrared reflectance spectroscopy. Microchemical Journal, 83, 42–47.
9. Edwards, H. G. M., Munish, T., & Anstis, M. (2005). Raman spectroscopic characterization and analytical discrimination between caffeine and demethylated analogs of pharmaceutical relevance. Journal of Spectrochimica Acta, Part A, 61, 1453–1459.
10. Najafi, N. M., Hamid, A. S., & Afshin, R. K. (2003). Determination of caffeine in black tea leaves by Fourier transformation IR spectrometry, using multiple linear regressions. Microchemical Journal, 75, 151–158.
11. Ortega-Burrales, P., Padilla-Weigand, R., & Molina-Diaz, A. (2002). Simultaneous determination of paracetamol and caffeine by flow injection solid-phase spectrometry using C18 silica gel as a sensing support. Journal of Analytical Science, 18, 1241–1246.