



## Review Article

# Review Study on Determination of Caffeine Beverages

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Caffeine is one of the most widely consumed psychoactive substances and is commonly present in beverages such as coffee, tea, soft drinks, and energy drinks. The increasing consumption of caffeinated beverages has raised concerns regarding excessive caffeine intake and its potential adverse effects on human health, including nervousness, insomnia, cardiovascular disturbances, and gastrointestinal disorders. Therefore, accurate determination of caffeine content in beverages is essential to ensure consumer safety and regulatory compliance. Various analytical techniques are employed for the quantitative estimation of caffeine, including ultraviolet–visible (UV–Vis) spectrophotometry, high-performance liquid chromatography (HPLC), gas chromatography, and mass spectrometry. Among these, chromatographic methods are preferred due to their high sensitivity, specificity, and reliability. Regulatory authorities mandate regular monitoring of caffeine levels in commercial beverages to ensure they remain within permissible limits. This study emphasizes the importance of analytical determination of caffeine in beverages for quality control, public health protection, and compliance with food safety regulations.

**Keywords:** Caffeine, Beverage analysis, Quantitative estimation, UV–Visible spectrophotometry, HPLC (High-Performance Liquid Chromatography).

## INTRODUCTION

Energy drinks are functional, non-alcoholic beverages formulated to enhance psychophysiological performance. They typically contain ingredients such as caffeine, D-glucuronolactone, B vitamins, taurine, carbohydrates, and herbal extracts like ginseng and guarana [1]. Caffeine, a naturally occurring stimulant belonging to the methylxanthine class of molecules, is found in the leaves, seeds, or fruits of more than 63 plant species worldwide [2,3]. Common dietary sources of caffeine include coffee, tea, guarana, cola nuts, and cocoa. These products vary in caffeine concentration, with guarana containing the highest levels (4–7%), followed by tea leaves (3.5%), coffee beans (1.1–2.2%), cola nuts (1.5%), cocoa beans (0.03%), and mate tea leaves (0.89–1.73%). To maintain product quality and ensure food safety, various substances such as preservatives, antioxidants, and other food additives are added to energy drinks. These additives are also used to extend

shelf life and improve the organoleptic properties of the beverages. Caffeine itself is widely used, both recreationally and medicinally, as a central nervous system and metabolic stimulant to reduce physical fatigue and restore mental alertness during episodes of unexpected tiredness or drowsiness. It is generally eliminated from the body within a few hours of consumption and does not accumulate over time. Different energy drinks contain varying amounts of caffeine, typically ranging from 50 to 300 mg. Most individuals do not experience noticeable behavioural effects when consuming less than 300 mg of caffeine. However, sleep is particularly sensitive to caffeine and can be disrupted by an intake as low as 200 mg. An average cup of coffee contains around 100 mg of caffeine, while decaffeinated coffee still contains up to 12 mg. A typical cup of tea provides about 85 mg of caffeine. A single can of commercially available energy drinks may contain anywhere from 80 to 280 mg of caffeine, depending on the size of the can. Green tea contains approximately 60 mg of caffeine,

followed by white tea with about 55 mg. Slim Fast chocolate drinks contain around 20 mg of caffeine per serving. Caffeine is primarily metabolized in the liver into three main metabolites: paraxanthine (84%), theobromine (12%), and theophylline (4%). This metabolism is carried out by the cytochrome P450 enzyme system, specifically the 1A2 isoenzyme, producing dimethylxanthines each of which has distinct physiological effects.

### **Harmful effects of the caffeine content in cool beverages**

Excessive consumption of caffeine in cold beverages can lead to several adverse health effects, including insomnia, anxiety, and dependence. It may also cause an increased heart rate and blood pressure, dehydration, digestive discomfort, and frequent headaches. For certain groups particularly children, pregnant women, and individuals with pre-existing medical conditions the risks can be more serious, potentially worsening heart problems and negatively affecting bone health or fetal development.

### **Short-term effects**

#### **Sleep disruption:**

Caffeine can cause difficulty falling asleep, reduce total sleep duration, and contribute to sleep debt, which impairs alertness the following day.

#### **Anxiety and mood changes:**

It may lead to nervousness, restlessness, irritability, or worsen existing anxiety disorders.

#### **Cardiovascular issues:**

High intake can result in rapid heartbeat, palpitations, and elevated blood pressure.

#### **Digestive problems:**

Some individuals may experience stomach upset, nausea, heartburn, or diarrhoea.

#### **Dehydration:**

Due to its mild diuretic effect, caffeine can increase urination and contribute to dehydration.

### **Other symptoms:**

Headaches, dizziness, and muscle tremors may also occur with excessive intake.

### **Long-term effects:**

#### **Dependence:**

Prolonged high caffeine consumption can lead to dependence, with withdrawal symptoms such as headaches, fatigue, and irritability.

#### **Bone health:**

Excessive caffeine intake has been associated with reduced bone mineral density and a higher risk of fractures, especially in post-menopausal women.

#### **Heart condition:**

In individuals with existing cardiovascular problems, high caffeine consumption can worsen symptoms by increasing the hearts workload.

### **Special considerations**

#### **Children:**

Their developing cardiovascular and nervous systems make them more sensitive to caffeine's effects.

#### **Pregnancy:**

High caffeine intake during pregnancy is linked to an increased risk of miscarriage and other complications, as caffeine easily crosses the placenta.

**Medication interactions:** Caffeine may interact with certain medications, such as antidepressants and muscle relaxants, altering their effectiveness.

#### **Combination with alcohol:**

Mixing energy drinks with alcohol can mask signs of intoxication, increasing the risk of accidents and unsafe behaviour.

#### **Example:**

Red Bull can temporarily boost energy levels and enhance performance, but some individuals may also

experience side effects such as an increased heart rate, impulsive behaviour, and a higher risk of certain health conditions. It is one of the best-selling energy drinks globally. However, despite its widespread popularity, concerns remain about its potential side effects, particularly when consumed in combination with alcohol. Red Bull is also high in sugar and low in essential nutrients. For many people, beverages like coffee or tea may serve as healthier alternatives. This article discusses the possible side effects of Red Bull and examines whether excessive consumption could be life-threatening. While the exact formulation varies by country, Red Bull and other energy drinks typically contain sugar, artificial colours and flavours, carbonated water, and legal stimulants such as guarana and L-carnitine.

#### **A 12-ounce (355-ml) can of Red Bull provides:**

- Calories: 160
- Protein: 0.99 g
- Fat: 0 g
- Carbohydrates: 40.1 g
- Sugar: 38 g
- Sodium: 99.4 mg

It also contains several B vitamins, including niacin (B3), vitamin B6, and vitamin B12. Red Bull offers sugar-free varieties, such as Red Bull Zero and Red Bull Sugarfree, which use artificial sweeteners like sucralose and acesulfame-K instead of sugar. Although these ingredients may deliver a temporary energy boost, they can also lead to short- and long-term side effects, particularly when consumed in large amounts. The caffeine content in energy drinks such as Red Bull can be measured using High-Performance Liquid Chromatography (HPLC), which is preferred for its high accuracy and reliability, or by UV-Visible (UV/VIS) spectroscopy, which typically requires a prior extraction step. HPLC utilizes a reversed-phase column along with a suitable mobile phase to achieve separation of caffeine from other components. In contrast, UV/VIS' S spectroscopy determines caffeine concentration by measuring its absorbance at a specific wavelength after the compound has been extracted from the beverage.

#### **Determination of caffeine in cold beverage**

The caffeine content in energy drinks such as Red Bull can be measured using High-Performance Liquid Chromatography (HPLC), which is preferred for its high accuracy and reliability, or by UV-Visible (UV/VIS) spectroscopy, which typically requires a prior extraction step. HPLC utilizes a reversed-phase column along with a suitable mobile phase to achieve separation of caffeine from other components. In contrast, UV/VIS' S spectroscopy determines caffeine concentration by measuring its absorbance at a specific wavelength after the compound has been extracted from the beverage.

#### **High-Performance Liquid Chromatography (HPLC)**

##### **Principle:**

HPLC separates caffeine from other components in the drink based on their chemical properties, using a mobile phase and a stationary phase such as a C18 column.

##### **Procedure:**

1. The sample is injected into the HPLC system.
2. As it passes through the chromatographic column, caffeine is separated from other compounds.
3. A detector, typically a UV detector, measures the caffeine as it elutes from the column.
4. The caffeine content is quantified by comparing the detector response to a calibration curve prepared using caffeine standards.

##### **Advantages:**

Highly sensitive, fast, and provides excellent separation, making it the preferred method for accurate caffeine determination.

#### **UV-Visible (UV/VIS) Spectroscopy**

##### **Principle:**

This method determines caffeine concentration by measuring the amount of light absorbed at a specific wavelength.

**Procedure:**

1. Extraction: Caffeine is first extracted from the drink, usually through liquid extraction using a solvent such as carbon tetrachloride (CCl<sub>4</sub>).
2. The solvent layer containing the extracted caffeine is collected.
3. Measurement: The absorbance of the extracted solution is measured at a specific wavelength, typically around 270 nm, using a UV/VIS spectrophotometer.

4. A calibration curve prepared with known caffeine concentrations is used to determine the caffeine content of the sample.

**Advantages:**

Relatively simple and uses commonly available laboratory instruments.

**Disadvantage:**

Requires an additional extraction step, which may be less efficient and more time-consuming.

**Academic literature Example**

Sl.no	Title	Journal	Publication date	Summary
1	<b>Red bull energy drinks: A Comprehensive Analysis of Physiological Effects, Behavioral Manifestations, and Neurobiological Implications Through the Lens of Multiple Theoretical Frameworks</b>	Journall for Research in Applied Sciences and Biotechnology	April 30, 2025	This multidisciplinary paper explores how key ingredients caffeine, taurine, B-vitamins, and sugars influence neurotransmitter systems. It reports that a standard 250 mL can of Red Bull contains approximately 80 mg of caffeine.
2	Analysis of Caffeine in Energy Drinks by Ultra-Fast Liquid Chromatography	Conference Series	November 22, 2025	This study measured the caffeine content in Red Bull, finding an average concentration of 237.1 mg/L, which is lower than the manufacturer-reported level of 320 mg/L but still within legal limits in some regions.
3	Caffeinated Energy Drinks—A Growing Problem	Drug and Alcohol Dependence	January 1, 2009	This review discusses the rapid global expansion of the energy drinks market since the introduction of Red bull and the increasing reports of caffeine intoxicated due to inconsistent caffeine levels and weak regulatory oversight in certain countries.
4	The Review on Adverse Effects of Energy Drinks and Their Potential Drug Interactions	Nutrients	July 25,2025	The authors describe common ingredients in energy drinks and their mechanisms of action, noting the typical 80 mg of caffeine per 250 mL can. The review emphasizes the synergistic effects of caffeine, taurine, and other additives.
5	Caffeine Content of Energy Drinks	Centre for Science and Environment (PML Report)	June 21,2017	This report analyzed several commercial energy drinks and found that Red Bull samples contained approximately 310.08 ppm of caffeine.

## CONCLUSION

The determination of caffeine content in non-alcoholic beverages and energy drinks is an important analytical process that helps safeguard consumers who may be unaware of the potential adverse effects of excessive caffeine intake. In soft drinks, Brand 5 showed the highest caffeine concentration at 42.17 ppm, whereas Brand 3 had the lowest at 10.69 ppm. Among the energy drinks tested, Brand 2 exhibited the highest caffeine concentration at 101.705 ppm, while Brand 9 contained the lowest at 32.05 ppm. Although several analytical methods can be used to determine caffeine levels in beverages, this study employed UV/VIS' S spectrophotometry because it is relatively simple, rapid, cost-effective, highly sensitive, and capable of providing accurate caffeine measurements.

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**Cite:** Dr. B. Thangabalan, Balukrishna Muthyala, Diya Kojam\*, Yakkanti. Pushpalatha, Nandeibam Ledia Devi, Krishnendu Adhikary, Ikramul Hussain, Chandu Chandu, Review Study on Determination of Caffeine Beverages, *Int. J. Med. Pharm. Sci.*, 2026, 2 (1), 266-273. <https://doi.org/10.5281/zenodo.18402542>