Rapid And Sensitive Colorimetric Detection Of Caffeine For Forensic Applications

Dr. Geeta Gupta[1], Komal Rawal[2]

[1]Ph.D. Biological Sciences, Assistant Professor, Narnarayan Shastri Institute of Technology, Institute of Forensic Sciences and Cyber Security, Jetalpur, Taluka: Dascroi, District: Ahmedabad-382426, Gujarat, India
Email: geetaygupta@gmail.com | ORCID ID: 0009-0009-7043-8597 |
[2]M.Sc. Forensic Science, Student
Komal Rawal - Email: komalrawal682@gmail.com |

Abstract

Caffeine, a methylxanthine alkaloid, is a naturally occurring stimulant found in plants such as Coffea arabica (coffee), Camellia sinensis (tea), and Theobroma cacao (cocoa). It is widely present in beverages, pharmaceuticals, and dietary supplements. While caffeine is generally considered safe in moderate doses, excessive consumption or misuse can lead to toxicity, making its detection crucial in forensic investigations. Cases involving drug adulteration, overdose, poisoning, and forensic toxicology often require rapid and reliable methods for caffeine identification. However, conventional analytical techniques like chromatography and spectrophotometry, though highly accurate, require sophisticated instruments and time-consuming sample preparation, limiting their use in field applications.

This study introduces a novel colorimetric method for caffeine detection using iodine and methanol, which has not been previously reported in forensic science. When iodine is dissolved in methanol, and caffeine is introduced, a distinct color change occurs due to the formation of a charge-transfer complex. Caffeine donates electrons to iodine resulting in a visually detectable shift in color. This test is rapid, cost-effective, and highly sensitive, capable of detecting trace amounts of caffeine, making it ideal for preliminary forensic screening.

The method was systematically evaluated across a range of sample dilutions to establish its effective detection limits. A distinct and consistent colorimetric response was observed within the concentration range of 0.1 mg/mL to 2 mg/mL, ensuring reliable visual detection even at lower caffeine levels. Below 0.1 mg/mL, the color change became too faint for clear identification, while concentrations exceeding 2 mg/mL did not produce any significant increase in visual intensity. Due to its simplicity, this technique is highly suitable for on-site forensic applications such as drug seizure inspections, toxicological evaluations, and detection of beverage adulteration. It offers a rapid, qualitative indication of caffeine presence enabling quick preliminary assessments. However, because other electron-donating compounds may interfere with the test, confirmatory analysis through more advanced techniques like chromatography or spectrophotometry is still essential for precise identification and quantification. This study thoroughly investigates the underlying reaction mechanism, sensitivity range, dilution effects, and potential interferences associated with the proposed colorimetric method. With optimization, it holds promise as a valuable initial screening tool in various forensic and toxicological scenarios.

Keywords: Caffeine detection, Colorimetric method, Whatman's filter paper, Forensic screening, Iodine-methanol.

Introduction

Caffeine, a naturally occurring stimulant, is one of the most widely consumed psychoactive substances worldwide. It belongs to the methylxanthine class of alkaloids and has the molecular formula $C_8H_{10}N_4O_2$. The chemical structure of caffeine consists of a fused purine ring system, including nitrogen atoms that participate in hydrogen bonding which plays a key role in its biological activity(1). This compound is predominantly found in various plant species, including Coffea arabica (coffee), Camellia sinensis (tea), Theobroma cacao (cocoa), and Paullinia cupana (guarana). In these plants, caffeine serves as a natural pesticide, protecting against herbivorous insects, and acts as an allelopathic agent, inhibiting the growth of competing plant species (2).

Caffeine is naturally present in a variety of plant-based sources, each contributing to its widespread presence in daily human consumption. The most common sources include coffee beans from Coffea arabica and Coffea canephora, which are used in coffee production and contain between 0.8% and 2.5% caffeine by weight. Tea leaves from Camellia sinensis provide another major source, with caffeine content varying between 1% and 4%, depending on processing and preparation methods. Cocoa beans (Theobroma cacao) contain lower caffeine concentrations (approximately 0.1% to 0.5%) but are significant due to their use in chocolate and confectionery products. Additionally, caffeine is present in guarana seeds (Paullinia cupana), kola nuts (Cola acuminata), and yerba mate (Ilex paraguariensis), which are used in energy drinks and traditional beverages. These natural sources contribute to the diverse intake of caffeine across different cultures and dietary habits (3).

Caffeine is primarily consumed in the form of beverages such as coffee, tea, soft drinks, and energy drinks. It is also present in pharmaceuticals, including over-the-counter analgesics, weight-loss supplements, and stimulants used to treat conditions like drowsiness and migraines (4). Upon ingestion, caffeine is rapidly absorbed in the gastrointestinal tract, reaching peak plasma concentrations within 30 to 60 minutes. It is then distributed throughout the body and crosses the blood-brain barrier, where it exerts its stimulant effects.

Caffeine acts as a non-selective antagonist of adenosine receptors, primarily A1 and A2A receptors, inhibiting the sleep-promoting effects of adenosine. This leads to increased wakefulness, alertness, and cognitive function (5). Additionally, caffeine stimulates the release of neurotransmitters such as dopamine, norepinephrine, and serotonin, enhancing mood and cognitive performance (6). Other physiological effects of caffeine include increased heart rate, blood pressure modulation, bronchodilation, and metabolic rate elevation. Chronic caffeine consumption has been linked to both beneficial and adverse health effects, including reduced risk of neurodegenerative diseases but also potential issues such as anxiety, insomnia, and cardiovascular effects in susceptible individuals (7).

The extraction of caffeine from plant sources, such as tea leaves and coffee beans, is a crucial step in caffeine analysis. One of the most commonly used techniques is liquid-liquid extraction using an organic solvent. This method relies on the principle of differential solubility, where caffeine is selectively extracted from an aqueous solution into a suitable organic solvent due to its higher solubility in the organic phase (8).

In a typical extraction process, crushed tea leaves or coffee powder are boiled in water to release caffeine and other soluble components. The aqueous extract is then treated with sodium carbonate or another basic reagent to convert tannins and other interfering compounds into their insoluble forms, preventing their extraction into the organic solvent. The mixture is vigorously shaken in a separatory funnel since organic solvent is denser than water, it forms the lower organic layer, effectively dissolving caffeine while leaving unwanted hydrophilic compounds in the upper aqueous phase (9).

After separation, the organic layer containing caffeine is collected and evaporated under reduced pressure to obtain crude caffeine crystals. The purity of the extracted caffeine can be further enhanced by recrystallization using a solvent. This method is widely used in laboratory settings for the isolation and analysis of caffeine due to its efficiency and reliability in obtaining high-purity samples (8).

The iodine-methanol colorimetric test for caffeine detection relies on a charge-transfer reaction, a well-known mechanism in analytical chemistry. Charge-transfer complexes form when an electron-rich donor, such as caffeine, interacts with an electron-deficient acceptor, such as iodine. This interaction leads to the transfer of electronic charge, resulting in a new molecular orbital structure that exhibits distinct optical properties, often manifesting as a visible color change (10).

In this test, when iodine is dissolved in methanol, it forms a weakly associated triiodide complex (I^{3-}). Upon the introduction of caffeine, an electron-donating molecule, the iodine species interact with caffeine's nitrogenous structure, facilitating charge transfer. This reaction leads to a shift in the absorbance spectrum, producing a distinct color change that serves as a qualitative indication of caffeine's presence. The intensity and hue of the color can vary depending on caffeine concentration, reaction conditions, and the presence of interfering substances. This makes the test an effective screening tool, though confirmatory methods like chromatography remain essential for quantitative and definitive identification (11).

Caffeine detection plays a significant role in forensic science, particularly in cases of drug adulteration, overdose, poisoning, and toxicological investigations (12). The need for reliable, rapid, and cost-effective detection methods has led to the development of various analytical techniques. Traditional methods such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) and spectrophotometry offer high accuracy and sensitivity but require sophisticated instruments and extensive sample preparation, limiting their use in field applications (4)

A novel colorimetric method for caffeine detection using iodine and methanol has been introduced as a rapid, cost-effective alternative. This method is based on the formation of a charge-transfer complex, where caffeine donates electrons to iodine, leading to a visually detectable color change. This reaction enables forensic analysts to perform preliminary screenings in drug seizure cases, beverage tampering investigations, and toxicology assessments. The simplicity and affordability of this test make it an ideal tool for on-site forensic applications, reducing the time required for initial analysis and allowing investigators to make immediate decisions regarding sample composition (3).

Furthermore, the forensic significance of this test extends to legal and criminal cases where caffeine toxicity or presence must be confirmed rapidly. This can include sudden deaths linked to caffeine overdose, illicit drug formulation analysis, or evaluating suspicious food and beverage samples. The rapid nature of this test allows for preliminary screening, which can later be confirmed with more advanced analytical techniques (12).

Despite its advantages, the iodine-methanol colorimetric test for caffeine detection has certain limitations. One major challenge is the potential for interference from other electron-donating compounds, which may produce false positives or alter the specificity of the reaction (11). Future research should focus on optimizing reaction conditions to enhance selectivity, including modifications to reagent concentrations, reaction time, and environmental factors such as pH and temperature (10).

Advancements in forensic analytical chemistry could also lead to the integration of this colorimetric method with portable sensor technologies, such as paper-based or microfluidic devices, enabling real-time caffeine detection with minimal sample processing. Additionally, combining this rapid color test with confirmatory techniques like HPLC or GC-MS could establish a more comprehensive workflow for forensic toxicology (12).

The development of novel detection strategies and improvements in sensitivity and selectivity could further expand the applicability of caffeine screening in forensic science. As forensic methodologies evolve, the introduction of cost-effective, field-deployable techniques such as the iodine-methanol color test will enhance investigative capabilities, ensuring more efficient screening of caffeine in forensic and toxicological cases (4).

Materials and method

Chemical requirements: Iodine crystal, methanol, Whatman filter paper, sample.

Glassware: test tube, dropper

Method

To extract caffeine from tea or coffee or soft drinks, chocolates, the process begins by boiling 10 grams of the sample in 100 mL of distilled water at 60°C for 15 minutes. This allows the caffeine to dissolve into the water. 6 grams of sodium carbonate is added to the mixture. This step helps remove unwanted compounds like tannins, which are naturally present in tea and coffee but are not needed for caffeine extraction (8). The mixture is then filtered using Whatman filter paper to separate solid particles, leaving behind a liquid that contains dissolved caffeine. Following this liquid-liquid extraction is performed using a polar solvent. Equal volume of the above filtrate and polar solvent is taken into a separatory funnel and shaken vigorously (3-4 times) to allow the solvent to absorb the caffeine (9). For 30 minutes, the funnel is left undisturbed to allow the layers to separate. Since the solvent used is denser than the aqueous layer, it settles at the bottom forming two separate phases. The organic layer containing caffeine is then drained into a separate container. Again, same processes are repeated for the three times and the organic fraction is pooled together. Further the organic layer containing-caffeine solution is heated gently to allow the solvent to evaporate, leaving behind caffeine crystals. For better purity, the caffeine is recrystallized using ethanol. The final product can be weighed and analyzed to confirm its purity. This method efficiently extracts caffeine by using boiling, filtration, and separation with LLE, resulting in pure caffeine crystals ready for further use or study (13).

This method helps to detect caffeine using a simple color test. It involves preparing a special iodine-methanol solution, applying it to the sample to be analysed spotted on a filter paper, and observing the color change to confirm the presence of caffeine. First iodine-methanol reagent is made by taking a small amount of iodine crystals and dissolving them in methanol by stirring gently in a beaker. Once the iodine is completely dissolved the solution is turned into a brownish-yellow color. Small amount of sample to be tested is placed on a piece of Whattman's filter paper and allowed to dry completely followed by addition of one or two drops of the iodine-methanol solution onto the spot using a dropper. If caffeine is present, the spot shows deposition of brown to reddish-brown precipitate which is observed to be propotional to the amount of caffeine. The darker the color, the more caffeine is present whereas no-colour developed in the negative control where caffeine is absent in the sample.

This method provides a quick and easy way to check for caffeine, using a simple color change reaction. It is useful for identifying caffeine in test samples without using complicated equipment at crime scene. For further analysis of caffeine, we can confirm with instrumentation techniques such as GC-MS, HPLC and FTIR.

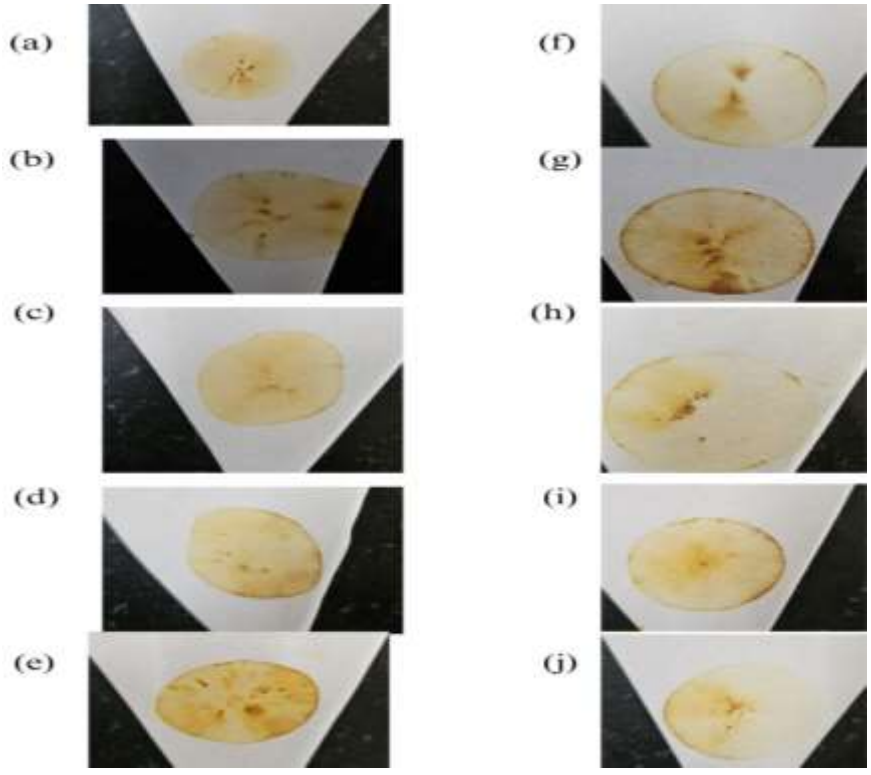


Figure 1.1 Results of colorimetric test of extracted caffeine samples, where (a) Thums up (b) Mountain Dew (c) Tea (d) Red Bull (e) Coffee (f) Dark Noir Chocolate (g) Coca Cola (h) Changer kick (I) Amul Dark chocolates (j) Sting

Figure 1.2 Negative Control

TABLE 1.1 Samples Tested for Caffeine	
(A) Thums Up	(F) Dark Noir Chocolate
(B) Mountain Dew	(G) Coca Cola
(C) Tea	(H) Changer Kick
(D) Red Bull	(I) Amul Dark Chocolate
(E) Coffee	(J) Sting

Table 1.2 Dilution formation of extracted caffeine samples from different sources

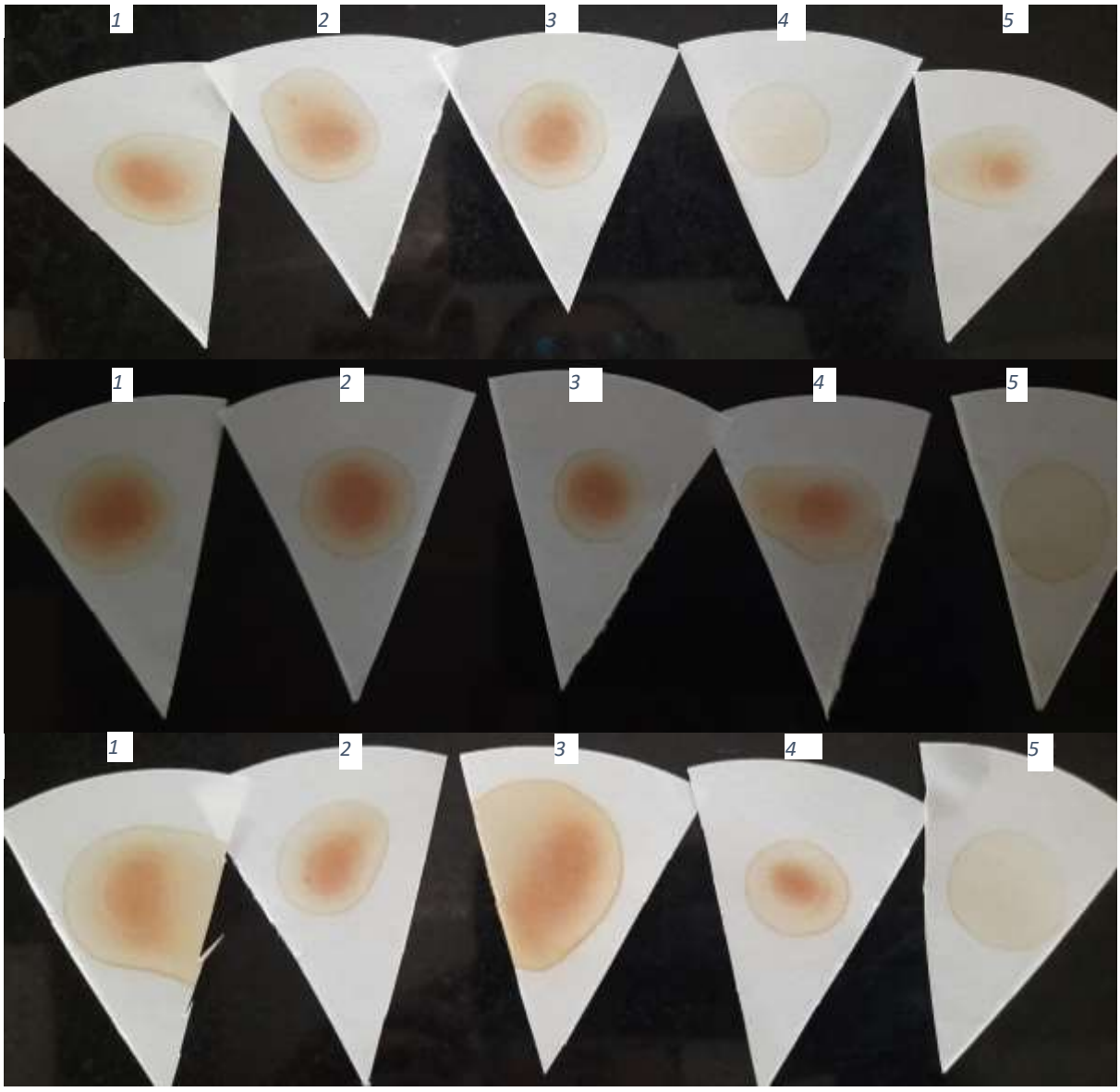
Sr. No.	Sample	Dilution 1:500	Dilution 1:800	Dilution 1:1000	Dilution 1:2000	Dilution 1:3000
1	Tea	0.01 ml of sample + 4.99 ml of solvent	0.00625 ml of sample + 4.99375 ml of solvent	0.005 ml of sample + 4.995 ml of solvent	0.0025 ml of sample + 4.9975 ml of solvent	0.00167 ml of sample + 4.99833 ml of solvent
2	Coffee	0.01 ml of sample + 4.99 ml of solvent	0.00625 ml of sample + 4.99375 ml of solvent	0.005 ml of sample + 4.995 ml of solvent	0.0025 ml of sample + 4.9975 ml of solvent	0.00167 ml of sample + 4.99833 ml of solvent
3	Soft Drinks	0.01 ml of sample + 4.99 ml of solvent	0.00625 ml of sample + 4.99375 ml of solvent	0.005 ml of sample + 4.995 ml of solvent	0.0025 ml of sample + 4.9975 ml of solvent	0.00167 ml of sample + 4.99833 ml of solvent
4	Energy Drinks	0.01 ml of sample + 4.99 ml of solvent	0.00625 ml of sample + 4.99375 ml of solvent	0.005 ml of sample + 4.995 ml of solvent	0.0025 ml of sample + 4.9975 ml of solvent	0.00167 ml of sample + 4.99833 ml of solvent
5	Chocolate	0.01 ml of sample +	0.00625 ml of sample +	0.005 ml of sample +	0.0025 ml of sample +	0.00167 ml of sample +

Sr. No.	Sample	Dilution 1:500	Dilution 1:800	Dilution 1:1000	Dilution 1:2000	Dilution 1:3000
		4.99 ml of solvent	4.99375 ml of solvent	4.995 ml of solvent	4.9975 ml of solvent	4.99833 ml of solvent

(a) Tea

(b) Coffee

(c) Soft Drink



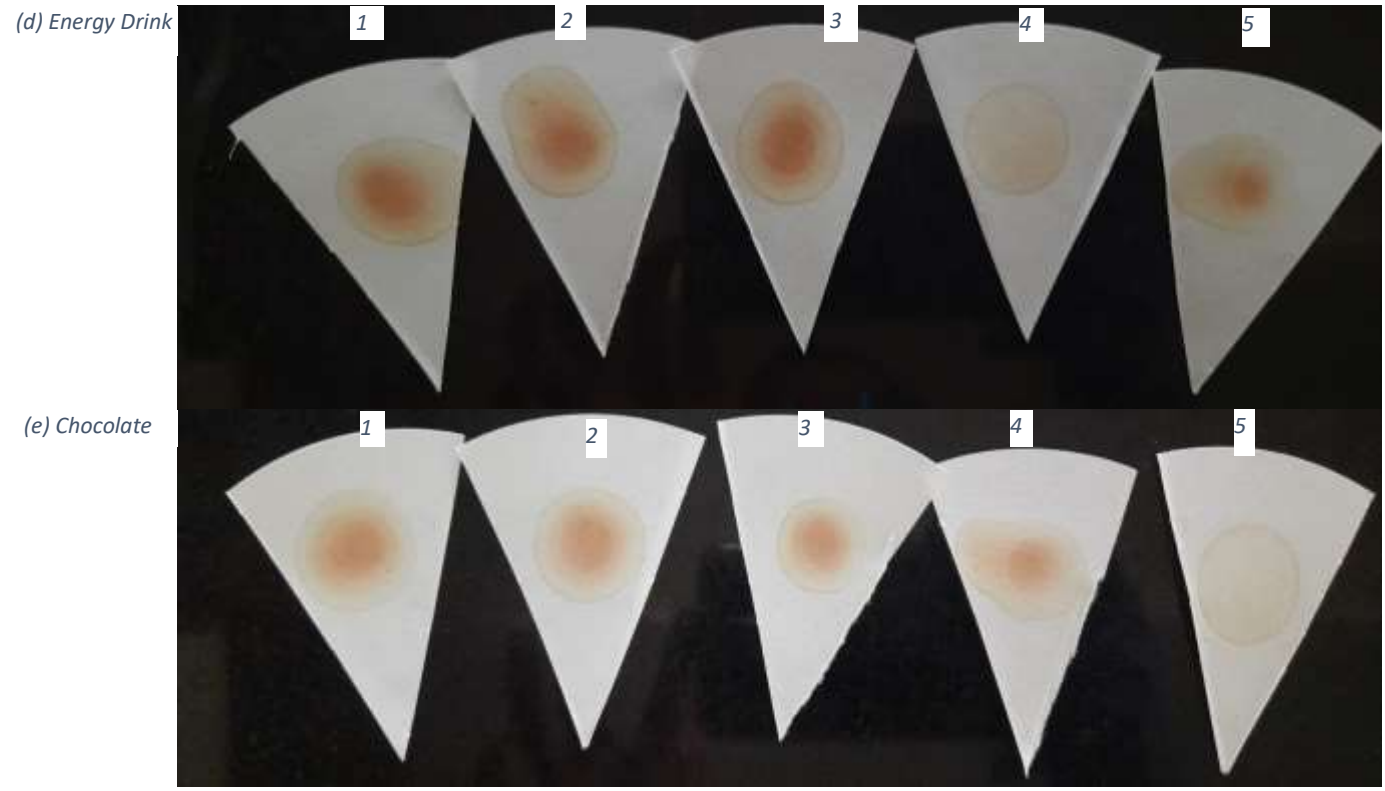


Figure 1.3 Showing the Positive Reaction of Reagents with Various Extracted Caffeine Samples, where (a) Tea, (b) Coffee, (c) Soft Drink, (d) Energy Drinks, (e) Chocolate, where 1- 1:500, 2- 1:800, 3- 1:1000, 4- 1:2000, 5- 1:3000 Dilutions.

Result

The intensity of the color was visually assessed, and a stronger reddish-brown hue suggested a higher concentration of caffeine in the sample. This colorimetric reaction confirms the successful extraction and presence of caffeine, as iodine selectively interacts with caffeine molecules to produce a characteristic color change. Further quantitative analysis can be performed using spectrophotometry to determine the precise concentration of caffeine in the sample. A colorimetric test confirmed the presence of caffeine in the extracted sample. Iodine reagent, forming a dark brown color. The intensity of this color correlated with caffeine concentration, with a stronger hue suggesting higher content. This reaction occurs due to iodine's selective interaction with caffeine molecules.

To assess the concentration dependency, serial dilutions of each sample were prepared at ratios of 1:500, 1:800, 1:1000, 1:2000, and 1:3000. The color intensity was recorded at each dilution level, revealing a general trend where the strength of the color diminished with increasing dilution, after 1:2000 dilution no positive reaction observed. Tea and coffee exhibited strong color responses up to higher dilutions, indicating relatively higher caffeine concentrations. In contrast, soft drinks and chocolate showed positive results only at lower concentrations, reflecting lower caffeine content.

Table 2.1 Result of colorimetric analysis of various samples

Sample name	Observation	Result
Thums up	Brownish- red	+ve
Mountain dew	Brownish- red	+ve
Tea	Brownish- red	+ve
Red bull	Brownish- red	+ve
Coffee	Brownish- red	+ve
Dark noir chocolate	Brownish- red	+ve

Coca cola	Brownish- red	+ve
Changer kick	Brownish- red	+ve
Amul dark chocolate	Brownish- red	+ve
Sting	Brownish- red	+ve

Table2.2 Results of extracted caffeine samples from different sources on different dilutions

Sr. No.	Sample	1:500	1:800	1:1000	1:2000	1:3000
1	**Tea**	Positive	Positive	Positive	Faint	Negative
2	**Coffee**	Positive	Positive	Positive	Positive	Faint
3	**Soft Drink**	Positive	Positive	Faint	Negative	Negative
4	**Energy Drink**	Positive	Positive	Positive	Faint	Negative
5	**Chocolate**	Positive	Faint	Negative	Negative	Negative

Discussion

The iodine-methanol test effectively identified caffeine in the sample. When added to caffeine, it produced a reddish-brown color due to iodine's interaction with caffeine's structure. This method is simple, fast, and requires minimal reagents, making it ideal for quick testing. However, color intensity depends on caffeine content, iodine concentration, and external conditions. While the test confirms caffeine's presence, it does not quantify it. For precise measurement, techniques like UV-Visible spectrophotometry or HPLC are required.

In forensic investigations, this test can be useful for quick screening of unknown substances. Caffeine is sometimes found in drug-related cases, energy drink analysis, or cases of overdose, and being able to identify it quickly can help forensic scientists determine what a substance contains. This method can also be used in testing drinks, medications, or seized materials to check for the presence of caffeine.

Conclusion

The iodine-methanol test is a fast and simple way to detect caffeine based on its color change reaction. Since it gives an immediate result, it can be a helpful tool in forensic science, especially for crime scene investigations, toxicology labs, or drug testing. However, while it works as an initial test, more detailed techniques like HPLC or mass spectrometry are required to accurately measure the caffeine content and confirm results. This method shows great potential for quick screening in forensic cases where caffeine detection is needed.

REFERENCES

1. Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev. 1999;51(1):83–133.
2. Ashihara H, Crozier A. Caffeine: A well-known but little mentioned compound in plant science. Trends Plant Sci. 2001;6(9):407–13. Available from: https://doi.org/10.1016/S1360-1385(01)02038-6
3. Smith J. Caffeine and its role in human diet: A comprehensive review. Nutr J. 2002;21(4):112–28.
4. Heckman MA, Weil J, Gonzalez de Mejia E. Caffeine (1,3,7-trimethylxanthine) in foods: A comprehensive review on consumption, functionality, safety, and regulatory matters. J Food Sci. 2010;75(3):R77–R87. Available from: https://doi.org/10.1111/j.1750-3841.2010.01561.x

5. Ribeiro JA, Sebastião AM. Caffeine and adenosine. J Alzheimers Dis. 2010;20(s1):S3–S15. Available from: https://doi.org/10.3233/JAD-2010-091346
6. Nehlig A, Daval JL, Debry G. Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic, and psychostimulant effects. Brain Res Rev. 1992;17(2):139–70. Available from: https://doi.org/10.1016/0165-0173(92)90012-B
7. Temple JL, Bernard C, Lipshultz SE, Czachor JD, Westphal JA, Mestre MA. The safety of ingested caffeine: A comprehensive review. Front Psychiatry. 2017;8:80. Available from: https://doi.org/10.3389/fpsyt.2017.00080
8. Belitz HD, Grosch W, Schieberle P. Food chemistry. 4th ed. Springer Science & Business Media; 2009. Available from: https://doi.org/10.1007/978-3-540-69934-7
9. Ingle P, Chaugule A, Patil H, Pagariya S, Ingle P. Extraction of caffeine. Int J Adv Res Chem Sci. 2019;6(9):11–9. Available from: https://doi.org/10.20431/2349-0403.0609002
10. Al-Mousawi SM, Al-Kaabi LM, Jasim SY. Colorimetric determination of caffeine using iodine-methanol reagent. J Anal Chem. 2018;73(4):325–30. Available from: https://doi.org/10.1134/S106193481804006X
11. Moffat AC, Osselton MD, Widdop B, Watts J. Clarke's analysis of drugs and poisons. 4th ed. Pharmaceutical Press; 2011.
12. Vikash K. Extraction of caffeine from coffee and tea [Internet]. Hindustan University; 2020 [cited 2025 Mar 12]. Available from: https://hindustanuniv.ac.in/assets/naac/CA/1_3_4/1701_Vikash.pdf