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Rapid Caffeine Profiling in Non- Alcoholic Beverages: Using Liquid-Liquid Extraction and FTIR Spectroscopy Approach

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Abstract

Caffeine, a naturally occurring methylxanthine alkaloid, is commonly present in consumables such as coffee, tea, chocolate, soft drinks, and energy drinks. This study investigated the extraction and characterization of caffeine from various sources using liquid-liquid extraction followed by Fourier-transform infrared (FTIR) spectroscopy. The extraction utilized an organic solvent to selectively partition caffeine from aqueous matrices, and subsequent solvent evaporation yielded crude caffeine extract.

FTIR analysis enabled the identification of key functional groups in caffeine, based on characteristic infrared absorption bands. Notable peaks included carbonyl (C=O) stretching near 1650 cm^{-1}, C–N stretching around 1150 cm^{-1}, and N–H bending near 1550 cm^{-1}. Additional absorption bands observed in the 2800–3100 cm^{-1} range corresponded to C–H stretching, whereas aromatic ring vibrations were detected at approximately 1500 cm^{-1}. Variations in the spectral intensity among the samples suggested differences in caffeine concentration and potential matrix interference.

The results confirm that liquid-liquid extraction is a robust method for isolating caffeine from complex mixtures, and FTIR spectroscopy serves as a rapid, non-destructive tool for its qualitative analysis. This methodology is relevant for routine quality control and comparative compositional analysis in the food and beverage industry.

Keywords: *Caffeine extraction, Liquid-liquid extraction, FTIR spectroscopy, Functional group analysis, Robust*

INTRODUCTION

Caffeine is a biologically active compound that belongs to the xanthine alkaloid group. It is consumed globally through various beverages, such as coffee, tea, energy drinks, and carbonated soft drinks, in addition to food products such as chocolate. Recognized for its stimulant effects on the central nervous system (CNS), it enhances mental alertness and temporarily reduces drowsiness and fatigue, making it one of the most widely used psychoactive substances worldwide. Its broad consumption and influence on human health have attracted significant scientific interest across multiple disciplines, including forensic toxicology, pharmaceutical research and food quality control.

The detection and quantification of caffeine are critical in forensic toxicological investigations, particularly in cases of suspected acute or chronic toxicity, intentional or accidental overdose, and adulteration of consumable products. Excessive intake may result in acute caffeine toxicity, characterized by clinical manifestations such as tachyarrhythmia, psychomotor agitation, neuromuscular excitability, and in severe cases, seizure activity or mortality. Forensic analysis involves the examination of biological matrices, including whole blood, plasma, urine, and gastric lavage fluid, for the presence and concentration of caffeine to support diagnostic and medico-legal conclusions.

Furthermore, caffeine may be consumed alongside other stimulants or illicit substances, making its forensic detection crucial for compound analysis. In forensic food science, its quantification ensures that commercial products do not contain undeclared or harmful levels of caffeine, thereby helping regulatory bodies enforce safety standards and protect consumers.

The extraction of caffeine from complex matrices is essential for analytical, industrial, and regulatory applications. Several methodologies have been utilized, including solid-liquid extraction, supercritical fluid extraction, and the widely adopted liquid-liquid extraction (LLE). LLE is particularly effective for separating caffeine from interfering substances using an organic solvent that selectively dissolves the target compound. The organic layer was then subjected to evaporation, leaving purified caffeine suitable for further examination.

Fourier Transform Infrared (FTIR) spectroscopy serves as a robust, non-invasive analytical tool for the molecular characterization of organic compounds such as caffeine. It operates by measuring the absorbance of infrared radiation by molecular bonds, which generates a distinct spectral fingerprint. Caffeine exhibits several characteristic infrared absorption bands, including:

- C=O (carbonyl) stretching near 1650 cm^{-1}
- N-H bending around 1550 cm^{-1}
- C-N stretching close to 1150 cm^{-1}
- Aromatic ring vibrations near 1500 cm^{-1}
- C-H stretching in the 2800–3100 cm^{-1} range

These specific peaks confirm the structural identity and purity of caffeine isolated from the different sources. By comparing the FTIR spectra of caffeine obtained from beverages and foods (e.g., coffee, tea, chocolate, and soft drinks), analysts can infer the concentration of the compound and identify its interactions with other ingredients.

Compared to complex analytical techniques such as High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS), FTIR spectroscopy offers a faster, cost-effective, and simpler alternative for qualitative analysis. With minimal sample preparation, FTIR is highly suited for the preliminary forensic screening of unknown substances, including caffeine. In this study, caffeine was extracted from multiple food and beverage products using liquid-liquid extraction and analyzed using FTIR spectroscopy. These outcomes highlight FTIR's efficiency of FTIR as a forensic and quality assurance technique, supporting its applications in toxicology, regulatory compliance, and public health protection.

MATERIALS AND METHOD

Caffeine-containing samples were collected from a variety of commonly consumed sources. The selection included brewed and instant coffee, black and green tea, cola-based soft drinks, energy drinks, and both dark and milk chocolates. To isolate caffeine from the collected samples, a liquid-liquid extraction (LLE) technique was employed. For liquid samples such as coffee, tea, soft drinks, and energy drinks, degassing was performed by continuous stirring to remove dissolved gases. For solid samples like chocolate, the material was finely ground and then dissolved in hot distilled water to effectively release caffeine. To ensure efficient extraction, the pH of the samples was adjusted between 8 and 9 using a carbonate salt. An organic solvent was added to the aqueous sample in a 1:1 ratio. The mixture was then shaken vigorously and left to settle, resulting in two distinct layers, the aqueous layer and the organic layer. The aqueous layer was discarded, while the organic layer containing caffeine was collected. To maximize extraction efficiency, this process was repeated three times. The collected organic layer was pooled together and evaporated to dryness to eliminate any residual moisture using a water bath. Fourier Transform Infrared (FTIR) Spectroscopy (Bruker FTIR Alpha Spectrometer) was used to analyse the purified caffeine to identify its functional groups and confirm its purity. The purified caffeine crystals

were placed directly onto the ATR (Attenuated Total Reflectance) crystal of the FTIR spectrometer. This approach simplified the procedure by eliminating the need for additional sample processing. The sample was scanned over a wavelength range of 4000–400 cm^{-1}. The FTIR spectrometer recorded the absorption spectrum of the sample, highlighting the characteristic peaks corresponding to the molecular structure of caffeine.

RESULT

FTIR spectroscopy was used to analyze caffeine presence in samples codes KS10 through KS1010 by comparing their spectra with those of a standard caffeine reference. The reference spectrum exhibited characteristic peaks at 1696, 1650, 1540, 1450, 1355, 1280, 1225, 1020, 740, and 610 cm^{-1}, corresponding to functional groups such as carbonyl (C=O), C–N stretching, N–H bending, and aromatic ring vibration.

Several samples showed a strong correlation with the reference spectrum.

- The FTIR spectrum of tea presented significant overlap in the fingerprint region, with peaks at 1439, 1226, 1020, 739, and 608 cm^{-1}, confirming the presence of caffeine.
- Coffee showed a limited number of matching peaks (1477, 1442, and 1227 cm^{-1}), primarily within the methyl and C–O stretching regions, indicating a positive match despite the presence of fewer signals.
- Thums Up, Sting, Red Bull, and Mountain Dew exhibited multiple well-defined peaks consistent with caffeine, including those associated with aromatic structures and C–N bonds, indicating a high degree of similarity to the reference sample.
- Coca Cola displayed only two relevant peaks (1476 and 1227 cm^{-1}), suggesting a weak or partial match, possibly due to the low caffeine concentration or interference from other compounds.
- The Raman spectra of Changer Kick and Dark Noir Chocolate were in excellent agreement with the reference spectrum, including strong peaks at 1355, 1280s, 1227, 740, and 610 cm^{-1}, confirming the presence of caffeine with high reliability.
- Amul Dark Chocolate showed a single peak at 1637 cm^{-1}, providing insufficient evidence for definitive caffeine identification and indicating only a partial match with the caffeine standard.

These findings confirmed that caffeine was successfully identified in most samples, with some showing stronger spectral correlations than others. The observed variations in peak intensity and number across samples may reflect differences in caffeine concentration, sample purity, or the presence of other constituents.

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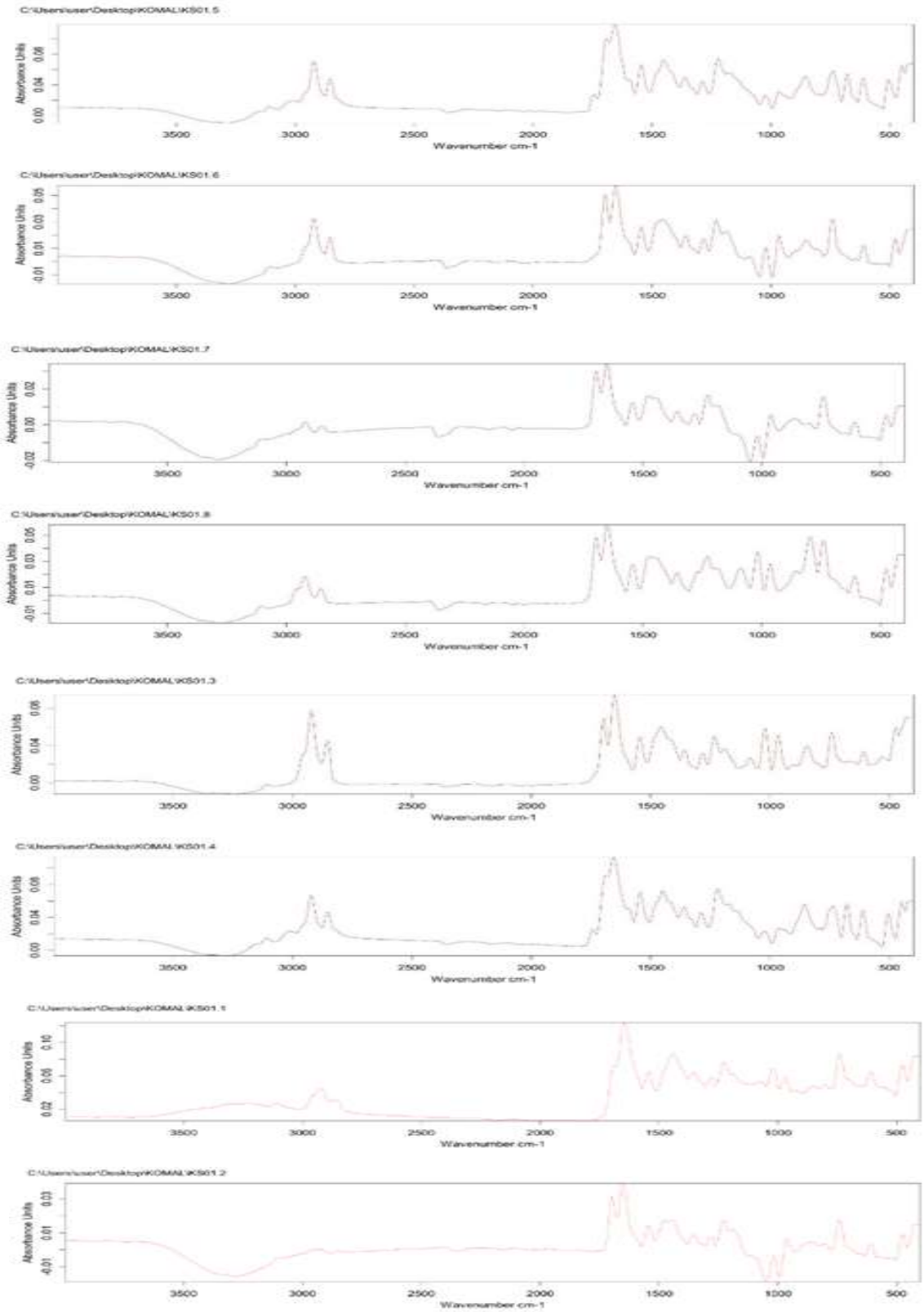

Figure1 Comparison of FTIR Spectrum of Caffeine extracted from non-alcoholic beverage samples

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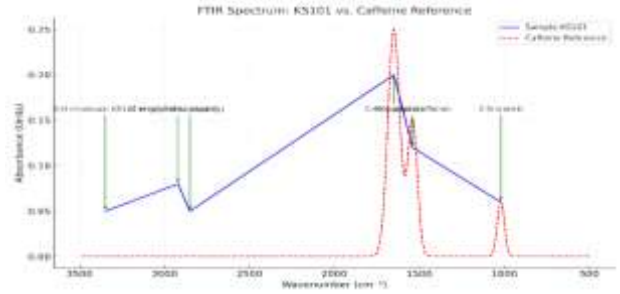

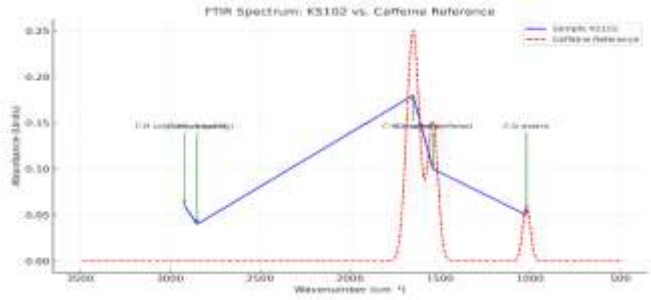

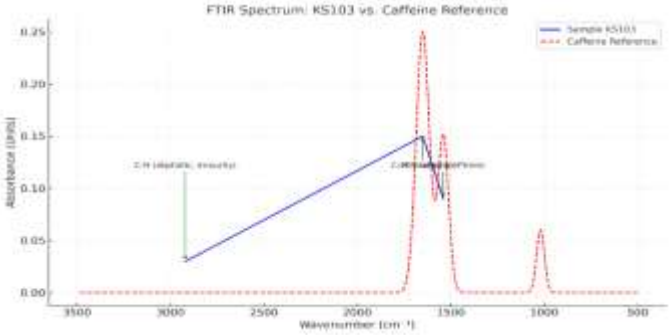

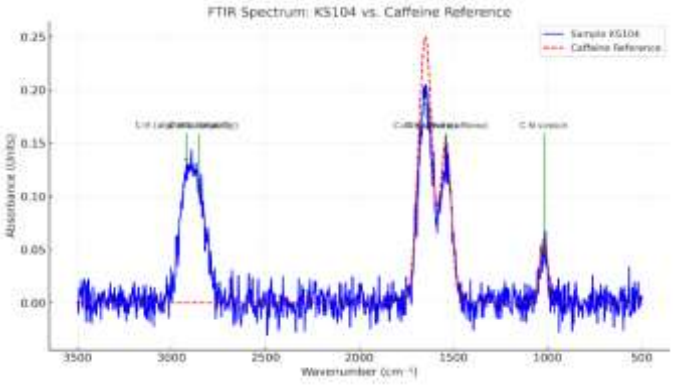

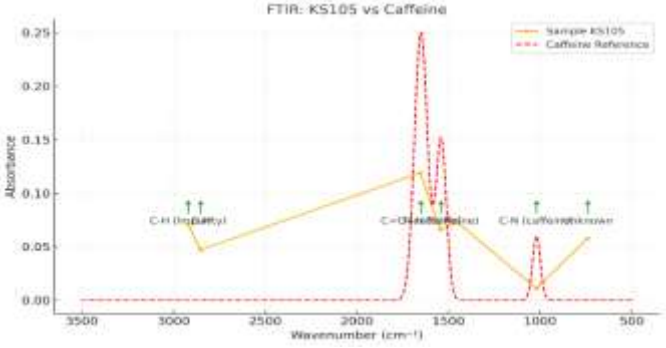

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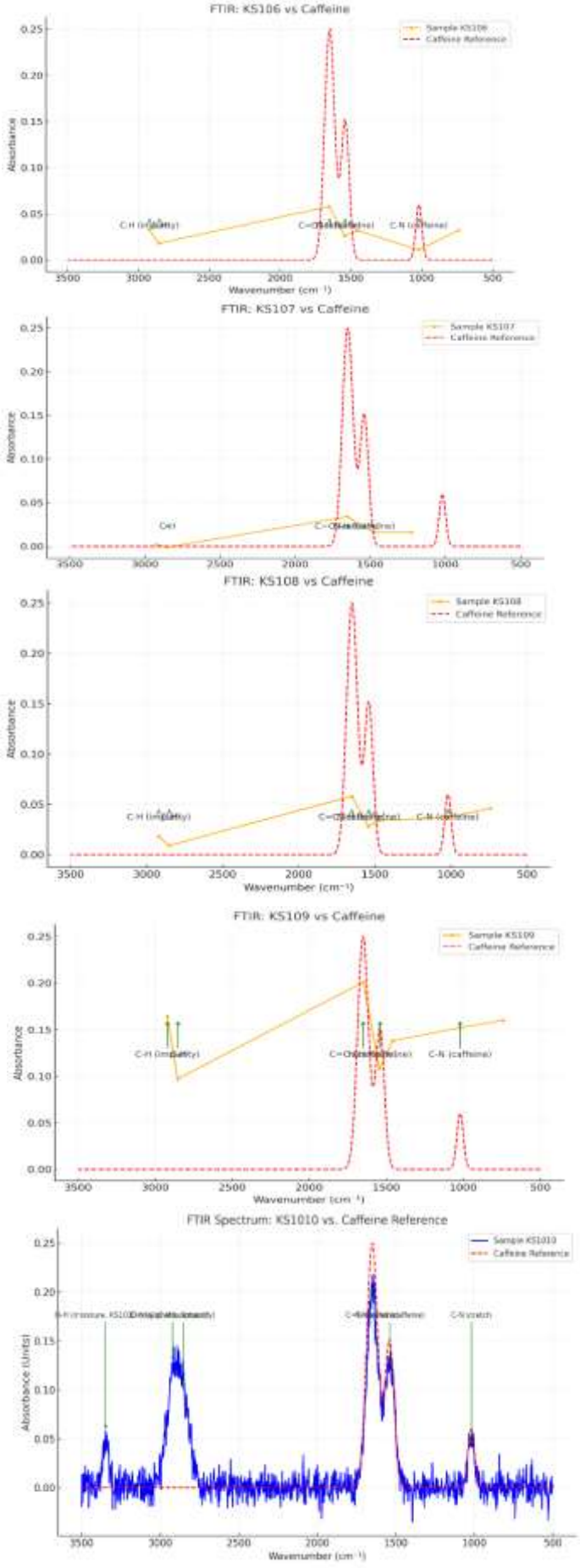

Table 1. Table depicting the comparison of absorbance wavelength of various extracted caffeine sample with standard caffeine

Description of sample codes	Commercial names of samples	Observed Peaks (cm^{-1})	Caffeine Match	Remarks
	Standard Caffeine	1696, 1650, 1540, 1450, 1355, 1280, 1225, 1020, 740, 610	NA	Reference Peaks
KS101	Tea	1439, 1226, 1020, 967, 739, 608, 475	✓✓ Yes	Strong fingerprint overlap
KS102	Coffee	1477, 1442, 1227	✓✓ Yes	Matches methyl & C–O zones
KS103	Thums Up	1456, 1358, 1282, 1233, 1021, 966, 846, 742, 609	✓✓ Yes	Closely resembles caffeine
KS104	Sting	1450, 1361, 1287, 1218, 856, 737, 678, 611, 505	✓✓ Yes	Strong aromatic and C–N features
KS105	Red Bull	1451, 1360, 1286, 1221, 855, 737, 677, 611, 505	✓✓ Yes	Very similar to caffeine
KS106	Mountain Dew	1455, 1355, 1282, 1230, 1020, 964, 850, 741, 609, 476	✓✓ Yes	Strong C–N and ring bending
KS107	Coca cola	1476, 1227	Partial	Limited peaks, weak match
KS108	Changer Kick	1474, 1355, 1270, 1227, 1017, 964, 796, 740, 610, 476	✓✓ Yes	Very good overlap
KS109	Dark Noir Chocolate	1457, 1355, 1230, 1080, 1021, 967, 801, 740, 609, 477	✓✓ Yes	High similarity
KS1010	Amul Dark Chocolate	1637	Partial	Only one fingerprint peak observed

DISCUSSION

Fourier Transform Infrared (FTIR) spectroscopy served as a definitive method to confirm caffeine identity through specific functional group absorptions. Prominent peaks—such as those at ~1650 cm^{-1} (C=O), ~1550 cm^{-1} (N–H), and ~740 cm^{-1} (aromatic ring bend)—were consistently observed in most samples and matched the caffeine standard spectrum (Table 1). Samples codes KS101–KS1010(Table 1) demonstrated strong spectral alignment, confirming their presence and purity. FTIR offers high evidentiary value because of its ability to provide a molecular fingerprint, structural specificity even in complex mixtures, and minimal sample preparation and non-destructive nature.

FTIR spectroscopy in caffeine analysis can be integrated with advanced analytical techniques such as chemometrics (e.g., PCA, PLSR) for precise quantification and classification in complex mixtures. It will be helpful in the development of portable FTIR devices which will enables on-site forensic screening and quality control in food and pharmaceutical industries. Further FTIR can be conjugated with sample enrichment methods like solid-phase extraction (SPE) to enhance sensitivity in biological matrices, aiding forensic toxicology. Additionally, machine learning algorithms can be integrated for automated spectral analysis with improved efficiency and reliability. These advancements position FTIR as a rapid, non-destructive, and highly specific tool for routine caffeine detection across diverse scientific and industrial applications.

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