

Quantitative Validation of FTIR UATR Method for Determining Caffeine Content in Green Tea

Validasi Metode Penentuan Kandungan Kafein dalam Teh Hijau dengan FTIR

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ABSTRACT

The FTIR UATR method for quantifying caffeine in green tea samples was validated. ATR (Attenuated Total Reflectance), a technique for direct analysis without sample preparation, was employed. Caffeine was determined at peak absorption between 1600 and 1700 cm^{-1} . Calibration using standard concentrations from 0 to 5% yielded excellent linearity with a correlation coefficient value of 0.9978. Precision, measured as % RSD (2.8369%), met the acceptable limit of 2/3 the Horwitz CV (4.5323). Accuracy was confirmed through spiking, resulting in a recovery of 100.3%, within the acceptable range of 95-105%. These findings demonstrate the suitability of this method for caffeine analysis in green tea samples.

Keywords: FTIR UATR, Caffein, Precision, Accuracy, %RSD, %Recovery

ABSTRAK

Validasi metode penentuan kafein dalam sampel teh hijau telah dilakukan dengan menggunakan FTIR UATR. ATR (Attenuated Total Reflectance), merupakan teknik analisis *non-destruktif*, tanpa preparasi sampel terlebih dahulu. Kafein ditentukan pada serapan yang muncul pada bilangan gelombang 1600 dan 1700 cm^{-1} . Pengukuran kurva kalibrasi menggunakan larutan standar kafein konsentrasi 0-5% menghasilkan linearitas yang baik yaitu dengan nilai koefisien korelasi (R^2) sebesar 0.9978. Presisi diukur sebagai % RSD (2.8369%), memenuhi syarat keberterimaan yaitu sebesar 2/3 CV Horwitz (4.5323). Akurasi dilakukan dengan metode *spiking*, menghasilkan persentase *recovery* sebesar 100,3%, memenuhi syarat keberterimaan 95-105%. Penelitian ini menunjukkan kesesuaian metode untuk analisis kafein dalam sampel teh hijau.

Kata kunci: FTIR UATR, Kafein, Presisi, Akurasi, %RSD, %Recovery

INTRODUCTION

Caffeine, a white, slightly bitter powder with the chemical name 1,3,7-trimethylxanthine, is an alkaloid compound

found in plants like coffee, tea, chocolate, and energy drinks (Fernandi, 2019; Zduńska et al., 2023). While moderate caffeine

consumption can offer benefits, but excessive intake may lead to negative consequences such as heart problems, high blood pressure, and complications during pregnancy (Meredith et al., 2013). Its neurostimulant effects make it valuable in the healthcare industry, emphasizing the importance of accurate caffeine determination for quality control. Therefore, validation of the caffeine determination method is very important.

Fourier Transform Infrared Spectroscopy (FTIR) is a technique that analyzes molecular vibrations in a sample. The resulting spectrum indicates the presence of specific molecular groups based on their absorption at characteristic wavenumbers (Meredith et al., 2013). FTIR with Universal Attenuated Total Reflectance (UATR) is a non-destructive method that enables direct analysis of samples without extensive preparation (Khofar et al., 2022). This technique offers rapid analysis and is compatible with a variety of sample states, including solids, liquids, and pastes. It's an attractive method for the quantification of caffeine in green tea samples, FTIR-UATR can significantly reduce sample consumption and processing time, especially for complex matrices. While HPLC is a common method, it's requires high costs and time-consuming.

This study aims to validate the accuracy and reliability of this method by

assessing its linearity, detection limit, precision, and accuracy. Linearity is determined by verifying the direct proportional relationship between caffeine concentration and instrument response, as expected by the Lambert-Beer law. The detection limit measures the smallest concentration detectable by the instrument. Precision is assessed by the consistency of repeated measurements, while accuracy is determined by comparing results to known values (Riyanto, 2014)

Previous research has analyzed caffeine levels in black tea and coffee (Singh et al., 1998; Sundalian & Nugrahani, 2018). This study extracts caffeine from green tea by combining techniques from those studies, using hot water with adjusted heating times. FTIR UATR validation aims to establish a precise and dependable caffeine determination method, offering a rapid, non-destructive, and economical approach.

EXPERIMENTAL METHODS

Materials

Chloroform (Merck KGaA Darmstadt Germany), Caffeine (Merck KGaA Darmstadt Germany) and Distilled water (Chemistry Department Laboratory). The sample is green tea leaves from commercial market.

Preparation of Caffeine Stock Solution and Standard Series

A 10% caffeine stock solution was made by weighing 2.5 grams of caffeine, then dissolved using chloroform and diluted to 25 mL in a measuring flask. Standard caffeine solutions were made with concentrations of 0; 0.5; 1; 2; 3; 4 and 5%. A total of 0; 0.25; 0.5; 1; 1.5; 2 and 2.5 mL of 10% caffeine stock solution were put into a 5 mL measuring flask, then the volume was adjusted with chloroform.

Determination of Precision and Accuracy

Precision

Five grams of tea sample was mixed with 50 mL of boiling water (Sundalian & Nugrahani, 2018), then heated for 20 minutes and then filtered. The filtrate obtained was extracted with 5 mL of chloroform. It was left in a separating funnel until 2 phases were formed, the organic phase was separated. The precision of an analysis is determined by calculating the percentage relative standard deviation (%RSD) using the standard deviation (SD) as indicated in equation 1.

$$SD = \sqrt{\frac{\sum_{i=1}^n (X_i - \bar{X})^2}{n-1}}$$
$$\%RSD = \frac{SD}{\bar{X}} \times 100\% \quad (1)$$

where:

SD = standard deviation

RSD = relative standard deviation

X_i = the value of each measurement

\bar{X} = average value of measurement

n = number of measurements

Accuracy

Five grams of tea sample was mixed with 50 mL of boiling water (Sundalian & Nugrahani, 2018), then heated for 20 minutes and then filtered. The filtrate obtained was extracted with 5 mL of chloroform. It was left in a separating funnel until 2 phases were formed, the organic phase was separated. A total of 75 μ L of 4% caffeine standard solution was added to the organic phase to obtain a volume of 2 mL. Accuracy is determined by the percentage recovery (% recovery) using equation 2.

$$\% \text{ Recovery} = \frac{(C_1 - C_2)}{C_3} \times 100 \quad (2)$$

where :

C_1 = spike concentration (analyte in sample + standard)

C_2 = concentration of analyte in sample

C_3 = concentration of added analyte (target concentration)

Caffeine Analysis with FTIR

Caffeine analysis was performed using FTIR UATR Spectrum Two Perkin Elmer. A total of 25 μ L of sample was dropped into the sample compartment and

read at a wave number of 4000-400 cm^{-1} , a resolution of 16 cm^{-1} and 4 scans.

RESULTS AND DISCUSSIONS

Determination of Linearity and Limit of Detection

The FTIR UATR instrument was used to analyze the caffeine content in the tea sample. A caffeine standard, dissolved in chloroform solvent, was used in this analysis. The concentration of the standard solution used were 0%, 0.5%, 1%, 2%, 3%, 4%, and 5%. Caffeine determination was performed at the peak that absorbed at a wavenumber of 1655 cm^{-1} (Singh et al., 1998). Figure 1 shows the spectrum of the standard solution of caffeine readings. It showed that caffeine have characteristic vibrational peak at wavenumber 1658 cm^{-1} , that was assign to C=O that observed at 1755-1650 cm^{-1} (Mafazi et al., 2024). The C-N (amines) was assign to IR peak appeared at 1254 cm^{-1} , and at 1703 cm^{-1} was assign to C=N. The calibration curve in Figure 2 shows the relationship between the concentration of caffeine in the standard solution and its absorbance. This curve was used to assess the linearity of the standard solution, which is indicated by the coefficient of determination (R^2). A value of R^2 closer to 1 signifies better linearity (Suyono, 2015). In this study, the obtained R^2 value of 0.9978 demonstrates good linearity, and the

detection limit was determined to be 0.29%. The calibration curve yielded a linear regression equation of $y = 0.0183x - 0.0004$. This equation will be used to calculate the concentration of the samples.

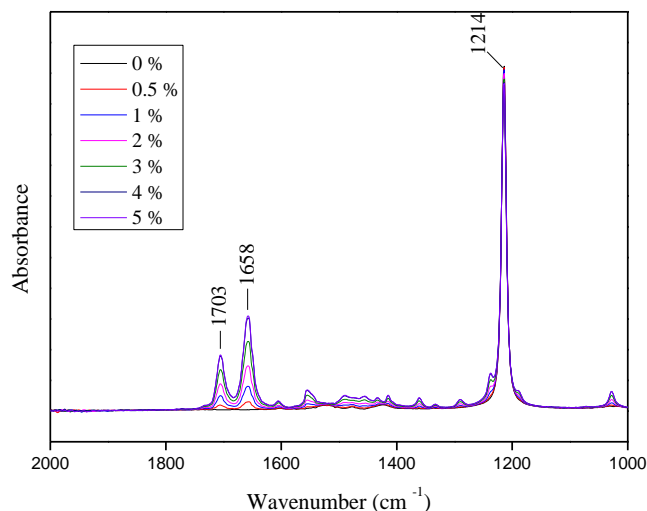


Figure 1. Spectrum for standard solution of caffeine

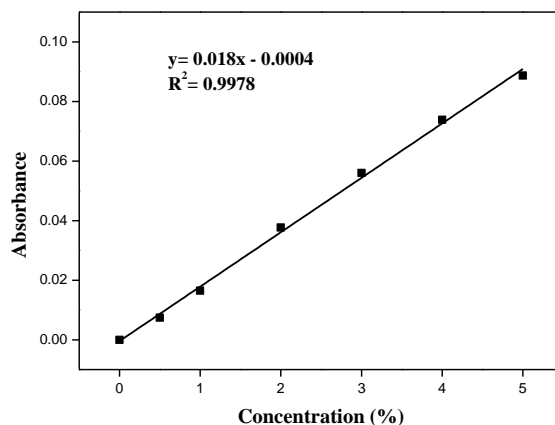


Figure 2. Calibration curve for standard caffeine

Determination of Precision and Accuracy

Caffeine content in green tea samples is presented in Table 1, which were calculated

using a linear regression equation based on a calibration curve. Six replicate measurements yielded an average caffeine concentration of 0.4360%, which falls within the instrument's detection limit of 0.29%. The concentration obtained is then used to calculate precision. Precision is expressed by relative standard deviation or commonly called the coefficient of variation (Riyanto, 2014). Precision is calculated as a percentage of Relative Standard Deviation (%RSD). Precision measurements are presented in Table 2. The %RSD obtained in this measurement is 2.8369% with a Horwitz CV value of 4.5323%. This method is stated to have good precision according to the acceptance requirements, which is less than or equal to 2/3 of the Horwitz CV value (Juliasih et al., 2021).

Table 1. Caffeine content in green tea samples

Sampel	Asorbansi	Konsentrasi (%)
1	0.0075	0.4333
2	0.0078	0.4497
3	0.0073	0.4223
4	0.0076	0.4388
5	0.0078	0.4497
6	0.0073	0.4223
Rata-rata		0.4360

Table 2. Precision measurement results for determining caffeine in green tea samples

Sampel	Konsentrasi (%)
1	0.4333
2	0.4497
3	0.4223
4	0.4388
5	0.4497
6	0.4223
Rata-rata	0.4360
SD	0.0124
% RSD	2.8369
CV Horwitz	4.5323

Accuracy is assessed by comparing the measured value to a known standard. It is often expressed as percent recovery, which should be within a specified range for the method to be considered accurate (Harmono, 2020). In this study, we spiked samples with a known amount of caffeine to evaluate accuracy. With three replicates, we achieved a percent recovery of 100.1%, which falls within the acceptable range of 95-105%. Therefore, our method demonstrates good accuracy, as summarized in Table 3.

Table 3. Accuration measurement results for determining caffeine in green tea samples

Sampel	Konsentrasi awal (%)	Konsentrasi Spike (%)
1	0.4388	1.9013
2	0.4497	1.9615
3	0.4552	1.9944
Rata-rata	0.4479	1.9524
% Recovery	100.3	

Comparison of wave numbers used to determine caffeine in green tea samples was carried out by statistical calculations, namely the t-test. This t-test was carried out on two samples that had the same substance with the same treatment. The wave number used was on the absorption of C = O, which was around 1755-1650 cm⁻¹. The hypothesis taken was Ho, there was no significant difference in the wave numbers obtained. While Hi, there was a significant difference in the wave numbers obtained. The results of the statistical calculation of the T test in this study can be seen in Table 4. The results obtained showed that the calculated t value < t table, this means that Ho is accepted, namely there is no significant difference in the wave numbers obtained in the reading of the caffeine standard and green tea samples.

Table 4. Results of the t-test comparing standard wave numbers of caffeine and green tea samples.

	Caffeine standar	Green tea samples
Mean	1657.8	1658.2
Variance	0.2	0.2
Observations	5	5
Pooled Variance	0.2	
Hypothesized Mean Difference	0	
df	8	
t Stat	1.414213562	
P(T<=t) one-tail	0.097507764	
t Critical one-tail	1.859548038	
P(T<=t) two-tail	0.195015528	
t Critical two-tail	2.306004135	

CONCLUSIONS

The FTIR UATR method for measuring caffeine in tea was validated and found to be accurate, precise, and linear. The method is best used for samples containing between 0 and 5% caffeine, with a detection limit of 0.29%. The precision and accuracy of the method were both within acceptable limits, with relative standard deviation (RSD) values less than or equal to 2/3 of the Horwitz coefficient of variation (CV), which is 2.8369%. Additionally, the method's recovery rate was 100.3%, which is between the acceptable range of 95-105%.

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References

- Fernandi, R. (2019). Efek Kafein terhadap Kesehatan Manusia. *Cermin Dunia Kedokteran*, 46(1), 64–69.
<http://cdkjournal.com/index.php/CDK/article/view/545>
- Harmono, H. D. (2020). Validasi Metode Analisis Logam Merkuri (Hg) Terlarutn pada Air Permukaan dengan Automatic Mercury Analyzer. *Indonesian Journal of Laboratory*, 2(3), 11.
<https://doi.org/10.22146/ijl.v2i3.57047>
- Juliasih, N. L. G. R., Hidayat, D., Pirdaus, P., & Rinawati, R. (2021). Verification of the Determination Method of Dissolved Metal Content using ICP-OES and Its Application for River Water in Bandar Lampung City. *Jurnal Kimia Sains Dan Aplikasi*, 24(1), 29–36.
<https://doi.org/10.14710/jksa.24.1.29-36>
- Khofar, P. N. A., Karim, U. K. A., Elias, E., Safian, M. F., & Halim, M. I. A. (2022). Trends of Forensic Analysis of Pen Ink Using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy. *Indonesian Journal of Chemistry*, 22(4), 1144–1154.
<https://doi.org/10.22146/ijc.72282>
- Mafazi, N., Jalil, Z., & Yusibani, E. (2024). Karakterisasi Kopi Unggulan Indonesia Jenis Arabika Sangrai Medium To Dark Menggunakan Fourier Transform Infra Red. *Jurnal Teori Dan Aplikasi Fisika*, 12(02).
<https://doi.org/10.23960/jtaf.v12i02.397>
- Meredith, S. E., Juliano, L. M., Hughes, J. R., & Griffiths, R. R. (2013). Caffeine Use Disorder: A Comprehensive Review and Research Agenda. *Journal of Caffeine Research*, 3(3), 114–130.
<https://doi.org/10.1089/jcr.2013.0016>
- Riyanto, P. D. (2014). *Validasi & Verifikasi Metode Uji Sesuai dengan ISO/IEC 17025 Laboratorium Pengujian dan Kalibrasi*. 1–154.
<https://play.google.com/books/reader?id=c0mlCgAAQBAJ&pg=GBS.PA17>
- Singh, B. R., Wechter, M. a, Hu, Y., & Lafontaine, C. (1998). Infra-Red Spectroscopy in Combination With Attenuated Total Biochemists. *Biochemical Education*, 26, 243–247.
- Sundalian, M.-, & Nugrahani, I.-. (2018). Determinasi Kadar Kafein Produk Teh Hitam Indonesia Dengan Menggunakan Spektrofotometer Fourier Transform Infrared. In *Jurnal Sains dan Teknologi Farmasi Indonesia* (Vol. 7, Issue 1).
<https://doi.org/10.58327/jstfi.v7i1.73>
- Suyono. (2015). *Analisis Regresi untuk Penelitian* (1st ed.). Deepublish.
- Zduńska, A., Cegielska, J., Zduński, S., & Domitrz, I. (2023). Caffeine for Headaches: Helpful or Harmful? A Brief Review of the Literature. *Nutrients*, 15(14), 1–16.
<https://doi.org/10.3390/nu15143170>