Journal of Science and Technology Research for Pharmacy
Volume 6 Issue 1 Year 2026 Pages 26-32
e-ISSN 2776-0685

Development and Validation of a HPLC for the Quantitative Determination of Caffeine in Rat Plasma for In Vivo Bioanalysis

Original Article

Amelia Dyah Permatasari[1]*, Tri Minarsih[1]
[1]Departement of Pharmacy, Faculty of Medicine, Universitas Negeri Semarang. Jl. Kelud Utara III No.15, Kota Semarang, Jawa Tengah, Indonesia, 50237.

Received : 15.07.26
Accepted : 24.08.26
Published : 15.09.26

Keywords:
High-Performance Liquid Chromatography (HPLC)
caffeine
blood plasma
method validation
in vivo

*Corresponding Author:
ameliadyah1016@students.unnes.ac.id

Abstract:
Caffeine is one of the most widely consumed methylxanthine compounds and is rapidly absorbed into the bloodstream following oral administration. This study aimed to develop, validate, and apply a high-performance liquid chromatography (HPLC) method for the quantitative determination of caffeine in rat plasma following in vivo oral administration. The optimized conditions were evaluated through a system suitability test and validated in accordance with the FDA Bioanalytical Method Validation Guidance, including linearity, precision, accuracy, selectivity, limit of detection (LoD), and limit of quantification (LoQ). The validated method was applied to determine plasma concentrations at 1, 2, and 3 hours after oral administration in rats. The optimized mobile phase, consisting of distilled water and methanol (50:50), produced satisfactory chromatographic separation and fulfilled all system suitability criteria. The method demonstrated excellent linearity, with correlation coefficients (r) of 0.9973 based on peak area and 0.9986 based on peak height. The precision test yielded a %RSD of 1.454%, while the accuracy test showed recovery values of 105.67%, 100.19%, and 104.96%. The average chromatographic resolution was 2.17, whereas the LoD and LoQ based on area were 0.44 mg/L and 1,33 mg/L, and based on peak height were 0.30 mg/L and 0.92 mg/L. The mean plasma caffeine concentrations based on peak area and peak height were 2.39 mg/L and 3.39 mg/L at 1 hour, 1.71 mg/L and 1.77 mg/L at 2 hours, 1.36 mg/L and 1,42 mg/L at 3 hours after oral administration.

INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring methylxanthine alkaloid widely found in coffee, tea, cocoa, energy drinks, and numerous pharmaceutical products. As one of the most commonly consumed psychoactive substances worldwide, caffeine exerts stimulatory effects on the central nervous system, enhancing alertness, reducing fatigue, and improving cognitive performance (Pisacha et al., 2023). Despite these beneficial effects, excessive caffeine consumption has been associated with adverse reactions, including anxiety, insomnia, tachycardia, gastrointestinal disturbances, and, in severe cases, toxicity. Consequently, the accurate determination of caffeine concentrations in biological samples is essential for bioanalytical, pharmacokinetic, therapeutic monitoring, and toxicological investigations (Elfariyanti et al., 2020).

Following oral administration, caffeine is rapidly and almost completely absorbed from the gastrointestinal tract into the systemic circulation. Peak plasma concentrations are generally achieved within 15–120 min after administration. Blood plasma is considered the biological matrix of choice for pharmacokinetic studies because it reflects the circulating concentration of the analyte and provides valuable information regarding drug absorption, distribution, metabolism, and elimination (Willson, 2018). Therefore, reliable quantification of caffeine in plasma is crucial for accurately characterizing its pharmacokinetic profile.

High-performance liquid chromatography (HPLC) remains one of the most widely employed analytical techniques for the quantitative determination of pharmaceutical compounds in biological matrices. Owing to its high sensitivity, selectivity, reproducibility, and excellent separation efficiency, HPLC has become the preferred analytical platform for bioanalytical applications. Reverse-phase C18 columns combined with methanol–water mobile phases are frequently used for caffeine analysis because they provide satisfactory chromatographic resolution while maintaining compatibility with ultraviolet (UV) detection (Aprilia et al., 2018; Sarmento et al., 2020).

Previous studies have successfully applied HPLC methods for the determination of caffeine in beverages, coffee, and pharmaceutical formulations. However, extending these methods to biological matrices such as plasma presents additional analytical challenges because endogenous matrix components may interfere with analyte detection and quantification. Consequently, bioanalytical methods intended for plasma analysis require comprehensive validation to demonstrate that they are capable of producing reliable, reproducible, and accurate quantitative results. According to the U.S. Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance, analytical methods should be evaluated using appropriate validation parameters, including system suitability, selectivity, linearity, accuracy, precision, and sensitivity, as reflected by the limits of detection (LoD) and quantification (LoQ) (FDA & CDER, 2018).

Therefore, the present study aimed to develop and validate an HPLC method employing a methanol–water mobile phase for the quantitative determination of caffeine in rat plasma. The validated method was subsequently applied to quantify plasma caffeine concentrations following oral administration in rats. It is expected that this validated analytical method will provide reliable quantitative data and serve as a useful tool for future bioanalytical and pharmacokinetic studies involving caffeine.

METHODS

Materials

The materials used in this study included a certified caffeine reference standard obtained from the Indonesian Food and Drug Authority (BPOM), HPLC-grade methanol (Merck Supelco, Germany), water for injection (Otsuka Pharmaceutical, Indonesia), plasma collected from male Wistar rats in ethylenediaminetetraacetic acid (EDTA) tubes, 0.22 μm syringe membrane filters, and other analytical-grade reagents.

Instrumentation

Chromatographic analysis was performed using a Waters Arc High-Performance Liquid Chromatography (HPLC) system equipped with an ultraviolet (UV) detector and a reversed-phase C18 analytical column (4.6 × 250 mm). The chromatographic conditions consisted of a flow rate of 1.0 mL/min, a detection wavelength of 273 nm, an injection volume of 10 μL, and a column temperature maintained at 24°C.

Study Design

This study is experimental laboratory investigation employing a quantitative approach to develop and validate a HPLC method for the determination of caffeine in rat plasma. Method validation was performed in accordance with the U.S. Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance and included the evaluation of system suitability, linearity, precision, accuracy, selectivity, LoD, and LoQ. The study was conducted between May and June 2026 at the Instrumental Analysis Laboratory, Animal Laboratory, and Organic Chemistry Laboratory, Faculty of Medicine, Universitas Negeri Semarang, Indonesia.

Preparation of Standard Solutions

A primary stock solution of caffeine was prepared using a certified reference standard obtained from the Indonesian Food and Drug Authority (BPOM). A 200 mg/L stock solution was subsequently diluted with the appropriate solvent to prepare working series of standard solutions. Series of standard solutions at concentrations of 1, 2, 3, 4, and 5 mg/L were prepared and analyzed using High-Performance Liquid Chromatography (HPLC).

Mobile Phase Optimization

Method development was initiated by evaluating three methanol–water mobile phase compositions (50:50, 60:40, and 70:30, v/v). The chromatographic performance of each mobile phase was compared based on retention time, peak symmetry, chromatographic resolution, and detector response to identify the optimum mobile phase composition prior to method validation.

System Suitability Test

Following the optimization of the mobile phase composition, a system suitability

test was performed prior to method validation to verify the performance of the chromatographic system. The evaluated parameters included retention time, relative standard deviation (%RSD), theoretical plate number (N), capacity factor (k′), tailing factor (T), and resolution (Rs).

Method Validation

Linearity was evaluated using caffeine standard solutions at concentrations of 1, 2, 3, 4, and 5 mg/L. Calibration curves were constructed by plotting analyte concentration against both peak area and peak height, followed by linear regression analysis.

Precision was assessed by six replicate analyses of a 2 mg/L standard solution under identical analytical conditions. Precision was expressed as the percentage relative standard deviation (%RSD).

Accuracy was evaluated using the spike recovery method by fortifying blank rat plasma at three concentration levels (80% (2 mg/L), 100% (3 mg/L), and 120% (4 mg/L) of the nominal concentration). Accuracy was expressed as percentage recovery (%Recovery).

Selectivity was assessed by comparing chromatograms of blank plasma, caffeine standard solution, caffeine-spiked plasma, and plasma spiked with both caffeine and paracetamol as a potential interfering compound. The absence of interfering peaks at the retention time of caffeine was used to demonstrate the selectivity of the method.

The limit of detection (LoD) and limit of quantification (LoQ) were calculated statistically based on the slope of the calibration curve and the standard deviation of the intercept.

Plasma Sample Preparation

Male Wistar rats received caffeine orally at a dose of 20 mg/kg body weight. Blood samples were collected at 1, 2, and 3 hour after administration and transferred into microcentrifuge tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Plasma was separated from whole blood and prepared using a protein precipitation method by adding methanol at a plasma-to-methanol ratio of 1:10 (v/v). The mixture was vortex-mixed and centrifuged, after which the resulting supernatant was filtered through a 0.22 µm syringe membrane filter before injection into the HPLC system for quantitative analysis.

RESULT AND DISCUSSION

Mobile Phase Optimization

The mobile phase was optimized by evaluating three methanol–water compositions (50:50, 60:40, and 70:30, v/v) to identify the chromatographic conditions that provided optimal separation of caffeine in rat plasma. Optimization of the mobile phase is a critical step in HPLC method development because it directly influences retention time, peak shape, chromatographic resolution, and overall analytical performance (Basmalah et al., 2020). Among the three mobile phase compositions evaluated, the methanol–water mixture (50:50, v/v) exhibited the best chromatographic performance. Under these conditions, caffeine was eluted with a retention time of approximately 3.86 min, producing a symmetrical peak and satisfactory chromatographic resolution. Increasing the proportion of methanol in the mobile phase resulted in shorter retention times but compromised chromatographic separation, as evidenced by reduced peak resolution and less favorable peak characteristics. These findings indicate that a methanol–water composition of 50:50 (v/v) provided an appropriate elution strength while maintaining sufficient interaction between caffeine and the reversed-phase C18 stationary phase, thereby ensuring efficient chromatographic separation (Chen et al., 2017).

System Suitability Test

Prior to method validation, the chromatographic system was evaluated through a system suitability test to ensure consistent and reliable instrument performance. The assessed parameters included chromatographic resolution (Rs), tailing factor (T), capacity factor (k′), theoretical plate number (N), and the relative standard deviation (%RSD) of retention time, peak area,

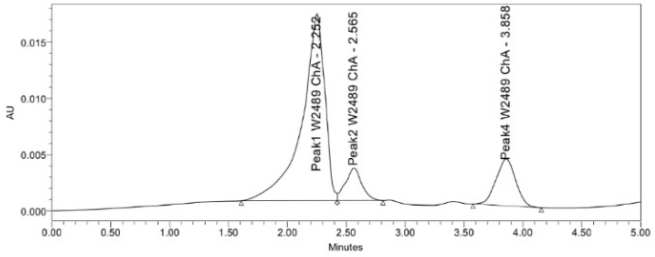

Figure 1. Optimization of the Methanol–Water Mobile Phase (50:50, v/v)

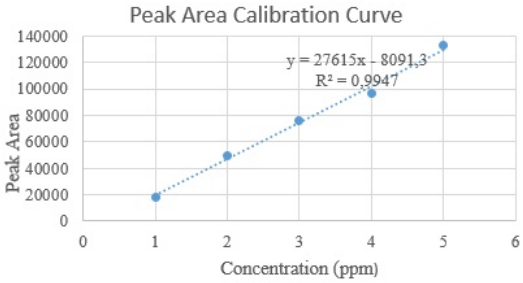

Figure 2. Peak Area Calibration Curve of Caffeine

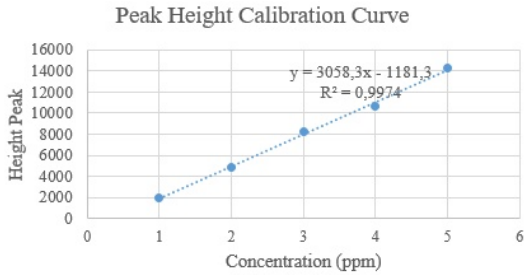

Figure 3. Peak Height Calibration Curve of Caffeine

and peak height (Widyasmara et al., 2025). The resolution values ranged from 4.537 to 4.636, indicating excellent separation between the caffeine peak and adjacent peaks. The tailing factor ranged from 1.0228 to 1.0434, demonstrating symmetrical peak shapes and minimal peak tailing. The capacity factor (k′) ranged from 5.8867 to 5.8899, while the theoretical plate number (N) ranged from 2,481 to 2,616, exceeding the minimum acceptance criterion of 2,000 and indicating satisfactory column efficiency. Furthermore, the %RSD values for retention time (0.0122%), peak area (1.1258%), and peak height (0.8222%) were all below 2%, demonstrating excellent repeatability of the chromatographic system. Overall, these results confirm that the HPLC system fulfilled all system suitability acceptance criteria and was therefore considered appropriate for subsequent method validation.

Method Validation

Linearity

The linearity of the developed HPLC method (Table 1) was evaluated using caffeine standard solutions at concentrations ranging from 1 - 5 mg/L. The calibration curve constructed based on peak area produced the regression equation y = 27,615x − 8,091.3, whereas the calibration curve based on peak height yielded the equation y = 3,058.3x − 1,181.3. The corresponding correlation coefficients were 0.9973 for peak area and 0.9986 for peak height, demonstrating excellent linear relationships between caffeine concentration and detector response. These findings indicate that the developed method provides an analytical response that is directly proportional to analyte concentration within the studied range, thereby satisfying the linearity requirement for quantitative analysis (Supriyono et al., 2020).

Precision

Method precision (Table 2)was evaluated by performing six replicate analyses under identical experimental conditions. The obtained %RSD values were 1.454% for peak area and 1.7608% for peak height, both of which were below the acceptance criterion of 2%. The low %RSD values indicate excellent repeatability and demonstrate that the method provides consistent analytical responses with minimal random variation during repeated measurements (Sulistyani et al., 2021). These results confirm that the developed HPLC method possesses adequate precision for the quantitative determination of caffeine in rat plasma.

Accuracy

Method accuracy was assessed using the spike recovery approach at three concentration levels corresponding to 80%, 100%, and 120% of the nominal concentration. Based on the peak area, the mean recovery values obtained were 104.67%, 100.19%, and 104.96%, respectively. Meanwhile, based on the peak height, the recovery values were 100.28%, 100.15%, and 100.96%, respectively. All recovery values were within the acceptance range of 85–115% recommended by the U.S. Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance (FDA & CDER, 2018). These findings demonstrate that the developed method accurately quantifies caffeine in rat plasma

Table 1. System Suitability Test Results for the Methanol–Water (50:50, v/v) Mobile Phase

Parameter	Replicate			Acceptance Criteria
	1	2	3	
Resolution (Rs)	4.6037	4.5370	4.6356	> 2
Tailing Factor (T)	1.0228	1.0434	1.0428	≤ 2
Capacity Factor (k′)	5.8899	5.8881	5.8867	1 < k′ < 10
Theoretical Plate Number (N)	2481.2992	2615.7237	2607.1890	> 2000
%RSD of Retention Time		0.0122		≤ 2%
%RSD of Peak Area		1.1258		≤ 2%
%RSD of Peak Height		0.8221		≤ 2%

Table 2. Precision Test Results of the Developed HPLC Method

Replication	Concentration Determined from Peak Area (mg/L)	Concentration Determined from Peak Height (mg/L)
1	2.1375	2.0431
2	2.0957	1.9999
3	2.1048	1.9741
4	2.0794	1.9561
5	2.1650	1.9760
6	2.1152	1.9463
%RSD	**1.454**	**1.7608**

Table 3. Accuracy Evaluation of the Developed HPLC Method Using the Spike Recovery Method Based on The Peak Area

Theoretical Concentration		Measured Concentration (mg/L)	Recovery (%)
(%)	ppm		
80	2	2.1374	106.87
	2	2.0957	104.79
	2	2.1048	105.24
Mean Recovery			**105.63**
100	3	3.0867	102.89
	3	2.9571	98.57
	3	2.9731	99.10
Mean Recovery			**100.19**
120	4	4.1753	104.38
	4	4.2855	107.14
	4	4.1344	103.36
Mean Recovery			**104.96**

Table 4. Accuracy Evaluation of the Developed HPLC Method Using the Spike Recovery Method Based on The Peak Height

Theoretical Concentration		Measured Concentration (mg/L)	Recovery (%)
(%)	ppm		
80	2	2.0430	102.15
	2	1.9999	99.99
	2	1.9740	98.70
Mean Recovery			**100.28**
100	3	3.0759	102.53
	3	2.9814	99.38
	3	2.9566	98.55
Mean Recovery			**100.15**
120	4	4.1654	104.13
	4	4.0219	100.54
	4	3.9287	98.21
Mean Recovery			**100.96**

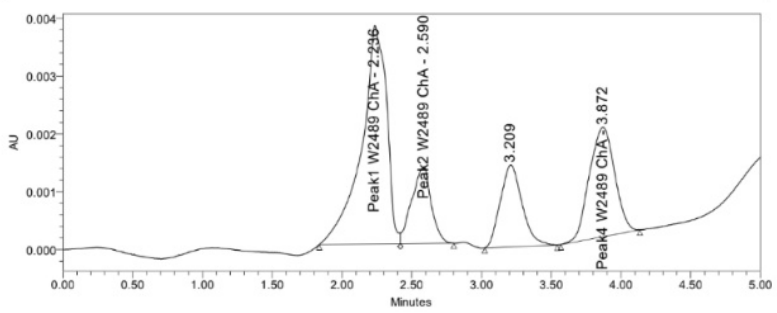

Figure 4. Chromatography Selectivity

Tabel 5. Selectivity Evaluation of the Developed HPLC Method

Parameter	Analyte	Replication		
		1	2	3
Retention Time (min)	Paracetamol	3.209	3.209	3.207
	Caffeine	3.872	3.865	3.863
Resolution (Rs)	Paracetamol	2.2696	2.3332	2.3596
	Caffeine	2.1029	2.1822	2.2320

without significant interference from sample preparation or matrix components, confirming its suitability for quantitative bioanalytical applications.

Selectivity

Method selectivity was evaluated by comparing chromatograms of blank plasma, a caffeine reference standard, caffeine-spiked plasma, and plasma spiked with both caffeine and paracetamol as a potential interfering compound. No interfering peaks were observed at the retention time of caffeine, indicating that endogenous plasma constituents and paracetamol did not interfere with caffeine determination. In addition, the average chromatographic resolution obtained was 2.17, exceeding the minimum acceptance criterion for baseline separation. These findings demonstrate that the developed HPLC method possesses adequate selectivity for the quantitative determination of caffeine in rat plasma.

Tabel 6. LoD and LoQ of the Developed HPLC Method

Replicate	Peak Area	Peak Height
1	18,556	1,938
2	49,331	4,801
3	75,974	8,249
4	97,228	10,700
5	132,684	14,280
6	18,556	1,938
LoD (mg/L)	**0.4415**	**0.3049**
LoQ (mg/L)	**1.3378**	**0.9241**

Tabel 7. Determination of Caffeine Concentrations in Rat Plasma

Sampling Time	Replicate	Caffeine Concentration Determined from Peak Area (mg/L)	Caffeine Concentration Determined from Peak Height (mg/L)
1 h	1	3.1198	3.5242
	2	2.1142	2.8314
	3	1.9652	2.6617
2 h	1	2.0297	2.0437
	2	1.5366	1.6124
	3	1.5935	1.6582
3 h	1	1.6060	1.6356
	2	1.2402	1.3093
	3	1.2479	1.3289

Limit of Detection (LoD) and Limit of Quantification (LoQ)

The sensitivity of the developed method was evaluated by calculating the limit of detection (LoD) and limit of quantification (LoQ) from the slope of the calibration curve and the standard deviation of the intercept. The LoD values were 0.4415 mg/L based on peak area and 0.3049 mg/L based on peak height, whereas the corresponding LoQ values were 1.3378 mg/L and 0.9241 mg/L, respectively. These relatively low LoD and LoQ values indicate that the method possesses sufficient sensitivity for detecting and quantifying caffeine at low concentrations in rat plasma (Sanchez, 2018). The high sensitivity of the method is likely attributable to the optimized chromatographic conditions and the appropriate calibration concentration range employed during method development.

Determination of Caffeine Concentration in Rat Plasma

The validated HPLC method was subsequently applied to determine caffeine concentrations in rat plasma following oral administration. Plasma samples were collected at 1, 2, and 3 h after dosing and analyzed under the validated chromatographic conditions. The chromatographic results demonstrated a gradual decline in both peak area and peak height as the sampling time increased. At 1 h after administration, the peak area ranged from 46,180 to 78,064, corresponding to caffeine concentrations of 1.97–3.12 mg/L. At 2 h, the peak area decreased to 34,324–47,960, with calculated concentrations ranging from 1.54 to 2.03 mg/L. A further reduction was observed at 3 h, where peak areas ranged from 26,159 to 36,260, corresponding to caffeine concentrations of 1.24–1.61 mg/L. A similar trend was observed for peak height. Peak heights ranged from 6,959 to 9,597 at 1 h, corresponding to caffeine concentrations of 2.66–3.52 mg/L. At 2 h, peak heights decreased to 3,750–5,069, with concentrations ranging from 1.65 to 2.04 mg/L, and further declined to 2,823–3,821 at 3 h, corresponding to concentrations of 1.32–1.63 mg/L. In HPLC analysis, both peak area and peak height are directly proportional to the amount of analyte eluted from the chromatographic column. Consequently, decreases in peak area and peak height reflect lower caffeine concentrations in plasma. The progressive decline in plasma caffeine concentration observed over time is consistent with the normal pharmacokinetic profile of caffeine, involving gastrointestinal absorption, systemic distribution, hepatic metabolism, and renal elimination (Novitasari & Alfatika, 2022). Although slight inter-individual variability was observed, the overall decreasing trend remained consistent throughout the sampling period, indicating that the validated HPLC method is capable of reliably monitoring changes in plasma caffeine concentrations during in vivo studies.

The caffeine concentrations calculated based on peak height showed slightly higher values than those calculated based on peak

area at all sampling times. At the 3-hour sampling time, the caffeine concentrations obtained based on peak area in replicates 2 and 3 were below the limit of quantification (LoQ). This condition indicates that the caffeine concentration in the blood plasma had decreased to a level below the quantification limit of the method. Therefore, the analyte could still be detected; however, its quantification results had lower precision and accuracy compared with concentrations above the LoQ.

CONCLUSION

The developed HPLC method employing a methanol–water (50:50, v/v) mobile phase provided optimal chromatographic separation, with a retention time of approximately 3.86 min. The method successfully fulfilled all validation requirements, demonstrating excellent linearity (r = 0.998), satisfactory precision (%RSD = 1.454%), acceptable accuracy (mean recovery ranging from 100.19% to 104.96%), adequate selectivity with an average chromatographic resolution of 2.17, and high sensitivity, whereas the LoD and LoQ based on area were 0.44 mg/L and 1,33 mg/L, and based on peak height were 0.30 mg/L and 0.92 mg/L. The validated method was successfully applied to the quantitative determination of caffeine in rat plasma following oral administration. The mean plasma caffeine concentrations based on peak area and peak height were 2.39 mg/L and 3.39 mg/L at 1 hour, 1.71 mg/L and 1.77 mg/L at 2 hours, 1.36 mg/L and 1,42 mg/L at 3 hours after oral administration. These findings demonstrate that the developed HPLC method is accurate, precise, selective, and reliable for the quantitative determination of caffeine in rat plasma and has the potential to serve as a valuable analytical tool for future bioanalytical and pharmacokinetic studies.

ACKNOWLEDGMENTS

-

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this research.

REFERENCES

Aprilia, F.R., Ayuliansari, Y., Putri, T., Azis, M. Y., Camelina, W. D., & Putra, M. R. (2018). Analisis Kandungan Kafein Dalam Kopi Tradisional Gayo dan Kopi Lombok Menggunakan HPLC dan Spektrofotometri UV/Vis. BIOTIKA Jurnal Ilmiah Biologi, 16(2), 40–44. https://doi.org/10.24198/bjib.v16i2.19829

Basmalah, N., Hariadi, N. N. A., & Mulki, M. A. (2020). Review Artikel: Pengaruh Perbandingan Fase Gerak Dalam Analisis Senyawa Hidrokuinon Pada Sediaan Kosmetik Menggunakan Metode High Performance Liquid Chromatography (HPLC). Journal of Pharmaceutical and Sciences, 2025(2), 1309–1317. https://doi.org/10.36490/journal-jps.com

Chen, F., Hu, Z. Y., Parker, R. B., & Laizure, S. C. (2017). Measurement of Caffeine and Its Three Primary Metabolites in Human Plasma by HPLC-ESI-MS/MS and Clinical Application. Biomedical Chromatography, 31(6). https://doi.org/10.1002/bmc.3900

Elfariyanti, Silviana, E., & Santika, M. (2020). Analisis Kandungan Kafein Pada Kopi Seduhan Warung Kopi di Kota Banda Aceh. In Lantanida Journal (Vol. 8, Number 1). https://pixabay.com

FDA, & CDER. (2018). Bioanalytical Method Validation Guidance for Industry. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htmand/orhttp://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm

Novitasari, A. E., & Alfatika, N. N. (2022). Pengaruh Waktu Ekskresi terhadap Residu Kafein pada Urine Responden Pengkonsumsi Teh Celup Hitam Menggunakan Pereaksi Parry. Indonesian Journal of Pharmaceutical Education (e-Journal), 2(3), 2775–3670. https://doi.org/10.37311/ijpe.v2i3.22162

Pisacha, I. M., Putri, K. D., Ardiansyah, & Miftausakina, T. (2023). Penetapan Panjang Gelombang Maksimum, Kurva Baku, dan Kadar Kafein Pada Teh Menggunakan U-HPLC. Jurnal Farmasi, 12(1), 80–85.

Sanchez, J. M. (2018). Estimating Detection Limits in Chromatography from Calibration Data: Ordinary Least Squares Regression vs. Weighted Least Squares. Separations, 5(4). https://doi.org/10.3390/separations5040049

Sarmento, Z. L. C., Rangdi, O. S. G., & De Sena, B. M. C. (2020). Penetapan Kadar Parasetamol dan Kafein dengan Metode High Performance Liquid Chromatography (HPLC). Indonesian E-Journal of Applied Chemistry, 8, 99–104.

Sulistyani, M., Kusumastuti, E., Huda, N., & Mukhayani, F. (2021). Method Validation on Functional Groups Analysis of Geopolymer with Polyvinyl Chloride (PVC) as Additive Using Fourier Transform Infrared (FT-IR). Indonesian Journal of Chemical Science, 10(3). http://journal.unnes.ac.id/sju/index.php/ijcs

Supriyono, Fitrillah, M., & Putra, A. P. (2020). Validation of High-Performance Liquid Chromatography Method for Determination of Vitamin B1 in Powder Milk. Jurnal Kimia Sains Dan Aplikasi, 23(5), 177–182. https://doi.org/10.14710/jksa.23.5.177-182

Widyasmara, A., Pratiwi, A., & Achmad Nandang Roziafanto. (2025). Uji Kesesuaian Sistem, Spesifitas, Linearitas dan Presisi pada Penetapan Kadar Vitamin E 75 HP dalam Bahan Awal Vitamin E 75 HP secara Ultra High Performance Liquid Chromatography (UHPLC) Ultraviolet Detection. In WARTA AKAB (Vol. 49, Number 2).

Willson, C. (2018). The Clinical Toxicology of Caffeine: A Review and Case Study. In Toxicology Reports (Vol. 5, pp. 1140–1152). Elsevier Inc. https://doi.org/10.1016/j.toxrep.2018.11.002