P-ISSN: 2302-8270
E-ISSN: 2808-1048

Identification of Paracetamol and Caffeine in Jamu Powders Simultaneously using TLC-Densitometry

Identifikasi Parasetamol dan Kafein dalam Serbuk Jamu secara Simultan menggunakan KLT-Densitometri

Etik Wahyuningsih[1)], Isnaeni Isnaeni[1,2)]*, Asri Darmawati[2)], Annisa Kartika Sari[1)]

[1]Study Program of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Surabaya, Surabaya, Indonesia
[2]Study Program of Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
*Corresponding author
E-mail: isnaeni@um-surabaya.ac.id

Article History:
Received: June 29, 2023; Revised: November 27, 2023; Accepted: November 27, 2023; Online: November 28, 2023

ABSTRACT

Paracetamol and caffeine were chemical compounds suspected to be illegally added to traditional herbs claimed as rheumatics drugs. Identification of paracetamol and caffeine was conducted on five samples of jamu powder obtained from the Depot Jamu in Surabaya. This study aimed to simultaneously identify paracetamol and caffeine commonly found in traditional medicine, one of which was jamu powder, using thin-layer chromatography densitometry (TLC-Densitometry). Evaluation of the presence of paracetamol and caffeine in the product of jamu was performed by thin-layer chromatography with silica gel GF_{254} and chloroform-ethyl acetate (1:1) as the stationary and mobile phases, respectively. The spots on the TLC plate were detected using a UV light at 254 nm, and the areas were measured by a Camag TLC scanner. The TLC profile demonstrated good separation of paracetamol, caffeine, and other substances contained in the products. The retardation factors (Rf) of paracetamol and caffeine were 0,42 and 0,26, with detection limits of 0,0125 µg/spot and 0,05 µg/spot, respectively. The simultaneous identification of caffeine and paracetamol using thin-layer chromatography densitometry revealed that none of the five samples were detected to contain paracetamol and caffeine.

Keywords: Simultaneously, Identification, Paracetamol, Caffeine, Jamu Powder, TLC- Densitometry

ABSTRAK

Parasetamol dan kafein merupakan bahan kimia obat yang diduga ditambahkan secara ilegal ke dalam jamu tradisional yang diklaim sebagai obat rematik. Identifikasi parasetamol dan kafein telah dilakukan pada lima sampel jamu serbuk yang diperoleh dari Depot Jamu di Surabaya. Penelitian ini bertujuan untuk mengidentifikasi secara simultan parasetamol dan kafein yang biasa ditemukan dalam obat tradisional salah satunya jamu serbuk dengan menggunakan kromatografi lapis tipis densitometri (KLT-Densitometri). Kromatografi lapis tipis dilakukan dengan menggunakan silika gel GF_{254} dan kloroform-etil asetat (1:1) sebagai fase diam dan fase gerak. Bercak pada pelat KLT dideteksi menggunakan lampu UV pada 254 nm dan luas area diukur dengan pemindai KLT Camag. Eluen kloroform-etil asetat (1:1) memisahkan parasetamol dan kafein dari zat-zat lain dengan resolusi yang baik (≥ 1). Faktor retardasi (Rf) parasetamol dan kafein adalah 0,42 dan 0,26 dengan batas deteksi masing-masing 0,0125 µg/titik dan 0,05 µg/titik. Identifikasi secara simultan parasetamol dan kafein dengan kromatografi lapis tipis densitometri menunjukkan bahwa tidak satu pun dari kelima sampel terdeteksi mengandung parasetamol dan kafein.

Kata kunci: Simultan, Identifikasi, Parasetamol, Kafein, Bubuk Jamu, KLT- Densitometri

INTRODUCTION

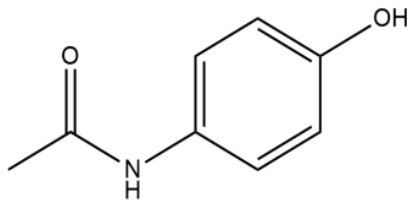
Paracetamol is one of the most widely used analgesics and antypiretics. In the pharmacological formulation, the usage of paracetamol either by alone or combination with others medication including caffeine (Narayanan *et al.*, 2016). Paracetamol and caffeine are medical chemicals that are suspected to be illegally added to traditional medicine, one of which is jamu powder (Gitawati *et al.*, 2013; BPOM *et al.*, 2016). The chemical names of paracetamol and caffeine are N-(4-Hydroxyphenyl) acetamide and 1,3,7 –trimethyl purine – 2,6 dione[1] respectively (Anonim, 2022) (Figure 1). These compounds have been reported as two of several medical chemicals added for rheumatic indication. The addition of chemical substances to traditional medicines is prohibited because it endangers the health of consumers or patients.

Based on the chemical structure, paracetamol, and caffeine contain the UV chromophore, so these analyts can be detected by UV. Previous studies was reported

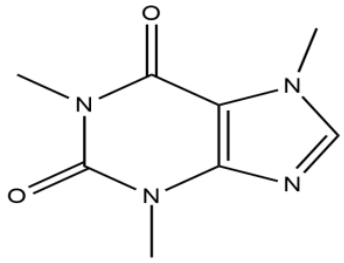
Cite this Wahyuningsih, E., Isnaeni, I., Darmawati, A. and Sari, A.K. (2023) 'Identification of Paracetamol and Caffeine in Jamu Powders Simultaneously using TLC-Densitometry', *Berkala Ilmiah Kimia Farmasi*, 10(1), pp. 17 – 21. DOI: 10.20473/bikfar.v10i1.44242.

P-ISSN: 2302-8270
E-ISSN: 2808-1048

paracetamol in herbs powder spectrophotometry (Sari *et al*., 2021), FT-IR (Wahyuningsih & Dessidianti, 2022), high-performance liquid chromatography (HPLC) (Laksmi & Anoop, 2016; Wisnuwardhani *et al*., 2018; Mamat *et al*., 2021; Sari *et al*., 2021), high-performance thin layer chromatography (HPTC) (Mahesh *et al.*, 2011; Prawez *et al*., 2022), LC/MS/MS (Delahaye *et al*., 2021). However, a simple, fast, selective, and inexpensive method for routine analysis is still preferred. Thin Layer chromatography (TLC) is widely used for the analysis of medical chemicals in herbal medicine (Alina *et al*., 2013; Harimurti *et al*., 2020; Hayun *et al*., 2016). The TLC allows for greater flexibility in the choice of chromatographic system. The method is simpler, more rapid, and lower cost than HPLC and LC/MS/MS (Delahaye *et al*., 2021).



paracetamol



caffein

Figure 1. The Chemical Structure of Paracetamol and Caffeine

Several methods of identification for paracetamol and caffeine in pharmaceutical products generally using thin-layer chromatography densitometry (TLC-Densitometry) have been reported (Alina *et al*., 2013; Harimurti *et al*., 2020; Hayun *et al*., 2016). The analytical method for identification and quantification simultaneous of paracetamol and caffeine in the traditional herbs powder have not been reported in many study. The jamu powder contained a complex matrixe that the presence of different matric can be interference with analyte during analysis. .

Detection limits test in the sample containing the drug include validation category 2. According to ICH guideline Q2 (R2), validation method category 2 refers to the selectivity, detection limit and purity (ICH 2005; USP, 2018). Based on the background, this research this research purposed to optimize the TLC conditions for simultaneous identification of paracetamol and caffeine on jamu powder obtained from the Depot jamu in Surabaya.

EXPERIMENTAL

Instruments

CAMAG TLC-densitometry scanner and winCATs software.

Chemicals

Standards of paracetamol and caffeine (BPFI/Indonesia), methanol (Merck), chloroform (Merck), ethyl acetate (Merck), TLC silica gel GF_{254} plate (Merck). The samples of jamu powder were obtained from the Depot Jamu in Surabaya.

Chromatographic Condition

Chromatography was performed by using silica gel GF_{254} TLC plate (Merck) as stationary phase, and chloroform-ethyl acetate (1:1) as mobile phase. A Camag twin through a chamber containing eluents was saturated to reach a distance of 8 mm. Densitometric scanning was conducted on the CAMAG TLC-densitometry scanner at 254 nm and winCATs software.

Sample Preparation

1 gram of jamu powder was weighed and diluted with 10 ml methanol and sonicated for 15 minutes then filtrated. The filtrate was dried by evaporation in top of the waterbath. The dried filtrate dissolved with 5 ml methanol.

Preparation of Standard Solution

Standards stock solution of paracetamol and caffeine with concentrations of 500 µg/ml and 50 µg/ml were obtained by dissolving 50.0 mg paracetamol and 5.0 mg caffeine standards (BPFI) in 100.0 ml methanol (Merck).

Selectivity

The selectivity test was performed by determining the separation between the paracetamol, caffein, and the matrix, by adding the standards to the sample before chromatographic analysis was done. The chromatogram was measured to determine the characteristic and purity of sample jamu powders. The acceptance criterion of the selectivity value (Rs) is ≥ 1 (AOAC, 2016).

Limit of Detection

The limit of detection (LOD) was determined by establishing the minimum concentration at which the paracetamol and caffeine could be reliably detected (ICH, 2005).

Sample Determination

The identification of analytes in the sample was conducted using optimum conditions. The jamu powder obtained from Surabaya (5 samples) was used as a model. Approximately 1.0 grams of samples were extracted with 10 ml methanol. and sonicated for 15 minutes then filtrated. The filtrate was dried by evaporation in top of the waterbath. The dried filtrate dissolved with 5 ml methanol. Two microliters of the solution containing equivalent to 1% sample were spotted on the chromatographic plate.

RESULTS AND DISCUSSION

Selectivity

The spectrum of paracetamol and caffeine on the chromatogram was depicted in Figure 2. Figure 2 showed that the spotted absorption curve for caffeine (A) and paracetamol (B). To obtain the largest peak area, λ 254 nm is used as λ analysis. The solvents and matrix addition standard did not show the same peaks as the Rf peaks of paracetamol and caffeine (Figure 3). Therefore, peaks of solvents and the matrix did not interfere during analysis. The standards chromatogram and the sample showed the Rs value as a selectivity parameters Rf and Rs (Table 3), the Rf value generally meets the requirement (ICH, 2005).

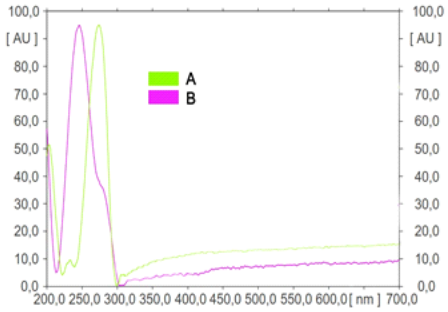


Figure 2. The Spectrum of Caffeine (A) and Paracetamol (B) at 200-700 nm Scanning

The selected wavelength based on the results of the scanning of paracetamol and caffeine spectra (Figure 2) on which the maximum absorption was reached at 254 nm with an absorption value of 95,0 absorbance units (UA).

Good resolution (Rs) of paracetamol, caffeine, and other components in the samples is depicted in Figure 3. Figure 3 showed the chromatogram in which the sample was added to mixture standard paracetamol and caffeine, indicating good separation and the absence of peaks interference between sample and analyte. The acceptance criterion of Rs value is > 1. The Rs values for paracetamol and caffeine standard was 1,23. The tailing factor met the criterion > 0,9 for a symmetric peak (Table 1).

Limit of Detection (LOD)

The limit of detection was determined by establishing the minimum concentration at which paracetamol and caffeine could be reliably detected. The result of scanning standard solution 4 concentration of paracetamol 0,005 µg, 0,0125 µg, 0,020µg 0,025 µg and caffeine 0,020 µg, 0,05 µg, 0,06 µg, 0,10 µg on the Table 1. The LOD of the method was obtained at 0,0125 µg/spot and 0,05 µg/spot for paracetamol and caffeine respectively.

Identification of Paracetamol and Caffeine in Samples

Identification results of paracetamol and caffeine in five samples of jamu powder from Depot Jamu showed that none of the five samples contained both drug chemical substances (Figure 4). Several possibilities can be conveyed that the amount of paracetamol and caffeine added is less than the LOD, so it was not detected. For this reason, the eluent and stationary phase optimization needs to be done. Another possibility was the jamu powders were not really added by the medicinal chemical substances. Furthermore, the results of this study are expected to guide the identification of traditional medicine such as jamu, herbal medicine, simplicial powders, or other dosage forms that are deliberately added by paracetamol with the claim of being an analgesic or anti-inflammatory combined with caffeine from which often found in cold medicine formulas. In the previous study, Alam *et al* (2022) developed simultaneous

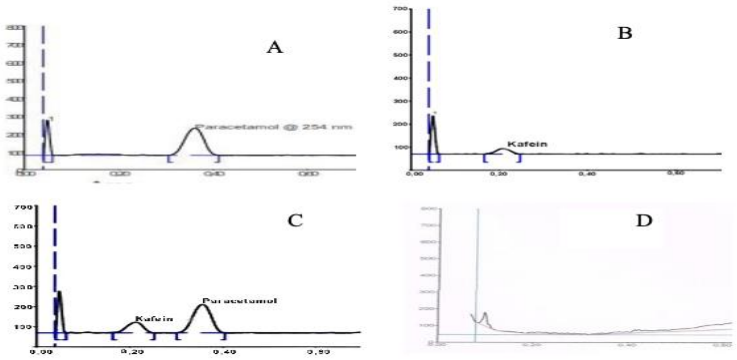


Figure 3. Chromatogram of Paracetamol (A) and Caffeine (B) Standards, Sample Addition Standards (C), and Solvents (chloroform-ethyl acetate) (D) using Optimum Conditions.

Table 1. Limit Detection of Paracetamol and Caffeine on Spott 2

Analyte	Concentration	Start Height	End height	Area	Result
Paracetamol	0,005 µg	-	-	-	Not detection
	0.0125 µg	4,1	8.3	441,7	Detection
	0,020µg	12.1	2.4	1207,0	Detection
	0,025 µg	1.1	7.8	1886,0	Detection
Caffeine	0,020 µg	-	-	-	Not detection
	0,05 µg	3.0	4.0	352.7	Detection
	0,06 µg	2.8	3.8	504.3	Detection
	0,10 µg	5.5	2.7	862.9	Detection

Table 2. Selectivity of Paracetamol and Caffeine

Chemical Name	Concentration	Start Height	Retardation Factor (Rf)	Tailing Factor
Paracetamol	500 ul	2,3	0,42	1,33
Caffeine	500 ul	0,9	0,26	1

Table 3. Identification Paracetamol and Caffeine in Jamu Powder using TLC-Densitometry

Chemical Name	Rf Value	Rs Value	Area
Paracetamol	0,42	1,23	4832,2
Caffeine	0,26		635,2
Jamu A	Negative	Negative	Negative
Jamu B	Negative	Negative	Negative
Jamu C	Negative	Negative	Negative
Jamu D	Negative	Negative	Negative
Jamu E	Negative	Negative	Negative

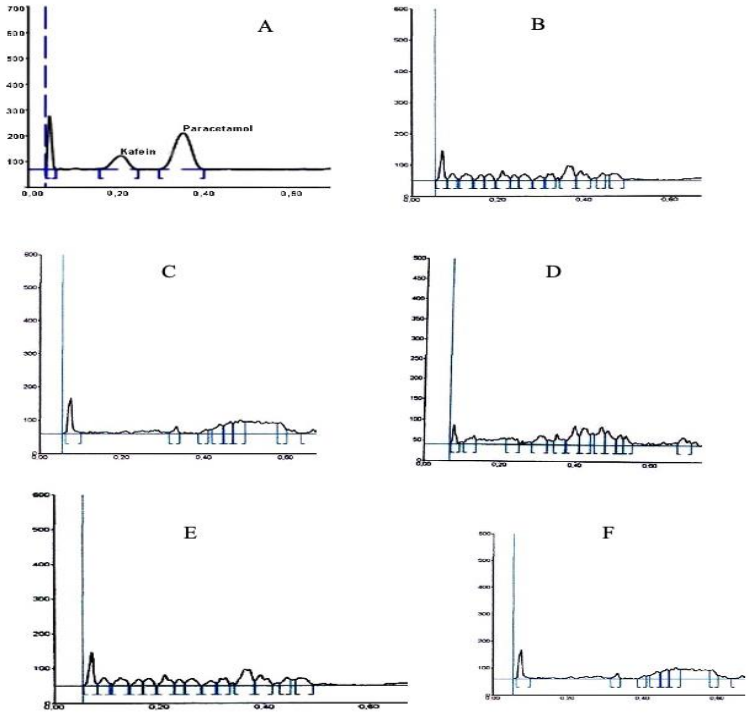


Figure 4. Chromatogram of Paracetamol and Caffeine Standard (A), Sample A, Sample B, Sample C, Sample D, and Sample E on Silica Gel F_{254} using chloroform-ethyl acetate 1:1 as Stationary and Mobile Phase Respectively

determination of caffeine and paracetamol in commercial formulas using Greener Normal-Phase and Reversed-Phase HPTLC Methods. The chosen eluent was ethyl acetate/ethanol (85:15, v/v), which discovered that Rf value = 0,40 ± 0,01 and 0,59 ± 0,02 for caffeine and paracetamol respectively. The resolution value was almost similar to chloroform-ethyl acetate (1:1) used as the mobile phase in this study. This method could be tried on commercial traditional medicine, although a more expensive technique is needed to achieve the nanogram limit of detection.

CONCLUSION

The TLC-Densitometry method with optimized results met acceptance of validation criteria for simultaneous identification of paracetamol and caffeine in jamu powder. None of the five samples were detected to contain paracetamol and caffeine. In future research, application of this method for quantitative analysis is recommended, by which diversity of traditional medicine could be used as samples.

REFERENCES

Alina, P., Marika, B. and Malgorzata, D. (2013) 'Validation thin layer chromatography for the determination acetaminophen in tablet and comparison with a parmacopeial method', Hindawi publishing corporation, 2013, article ID 545703.

AOAC International (2016) Official methods of analysis of AOAC International, 20th edition. Rockville: AOAC International.

BPOM (2021) Public Warning obat tradisional, supplement kesehatan dan kosmetik mengandung bahan kimia obat/bahan dilarang. Available at: https://www.pom.go.id/siaran-pers/siaran-pers-public-warning (cited 2022, September 21).

Delahaye, L., Baerdemaeker, L.D. and Stove, C.P. (2021) 'Determination paracetamol and its metabolites via LC-MS/MS in dried blood volumetric absorptive microsamples: A tool of pharmacokinetic study', Journal of Pharmaceutical and Biomedical Analysis, 206, p. 114361.

Gitawati, R. (2013) 'Analysis of Adulterated Jamu Pegal Linu Obtained From the Market in Jakarta', Buletin Penelitian Sistem Kesehatan, 16(3), pp. 269-274.

Harimurti, S., Ulandari, S., Widada, H. and Lailly, D.V. (2020) 'Identifikasi Parasetamol dan Asam Mefenamat pada Jamu Pegel Linu dan Asam Urat yang Beredar di Daerah Istimewa Yogyakarta', JPSCR, 5(2), pp. 179-188.

Hayun, Baitha, P. and Manggadani, N.A. (2016) 'Determination of sibutramine adulterated in herbal slimming products using TLC Densitometric Method', Indonesian Journal Pharm, 27(1), pp. 15-21.

Hye Suk Lee, Chang Kyun Jeong, Sung-Jin Choi, Sang Beom Kim, Mi Hyun Lee, Geon Il Ko and Dong Hwan Sohn. (2000) 'Simultaneous determination of paracetamol, caffeine, and propyphenazone in ternary mixtures by micellar electrokinetic capillary chromatography', Pharm Biomed Anal, 2, pp. 775-781.

International Conference on Harmonisation, ICH, Q2 (R2), Validation of analytical procedures: Methodology. (2005) Available at: https://www.gmp-compliance.org/files/guidemgr/Q2(R1).pdf (cited September 2022).

Laksmi, N. and Anoop, A. (2016) 'Determination of paracetamol and caffeine using Reverse Phase Liquid (RP-LC) Chromatographic Technique', Journal of Research in Pharmaceutical Sciences, 3(4), pp. 5-10.

Mahesh, A., Mueen, A.K., Bandar, E. and Aldhubaid, S.H. (2011) 'High-Performance Thin Layer Chromatography: A Powerful analytical Technique in pharmaceutical Drug Delivery', Pharmaceutical Method, 2(2), pp. 71-76.

Mamat, P., Dian, H., Lukman, H. and Seniwati, Muammar, F. (2021) 'Determination of Acetaminophen in Jamu Pegel Linu by High Performance Liquid Chromatography', Pharm Prep, 1(1), pp. 12-15.

Merck Index (2022) on https://www.rsc.org/merck-index (cited 2022, September 22).

Prawez, A., Shakeel, F., Abuzer, A., Mohammed, H., Alqarni, A.I., Foudah, T.M., Aljarba, F.K., Alkholifi, S., Alshehri, M.M. and Ghoneim, M.A. (2022) 'Simultaneous determination of caffeine and paracetamol in commercial formulas using Greener Normal-Phase and Reversed-Phase HPTLC Methods: A contrast of validation parameters', Molecules, 27(2), p. 405.

Sari, A.S., Sestry, M. and Ridho, A. (2021) 'Analytical of Medical Chemical Contained on Jamu: A Review', Asian Journal of Pharmaceutical Research and Development, 9(2), pp. 33-46.

The United States Pharmacopeia (2018) Rockville: The United States Pharmacopeia Convention. Available at: http://www.usppf.com/pf/index.html

Wahyuningsih, E. and Dessidianti, R. (2022) 'Aplikasi FT-IR ATR Spektroskopi untuk Identifikasi Parasetamol pada Jamu Sediaan Serbuk', Cammelia, 1(2), pp. 56-60.

Wisnuwardhani, H.A., Rusdi, B. and Yuliawati, K.M. (2018) 'Method Validation for Simultaneous Quantitative Analysis of Acetaminophen and Dexamethasone in Jamu Pegal Linu Using Hplc Method', Journal of Pharmaceutical Sciences and Research, 10(11), pp. 2693-2696.