

Validation of the reagent-based paper test strips method for the detection of paracetamol in herbal medicines

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Abstract:

The widespread use of herbal medicines in Indonesia raises concerns regarding the possible addition of prohibited chemical drugs, such as paracetamol. Therefore, a rapid detection method is needed as an alternative to the standard BPOM methods. This study aims to validate a reagent-based paper test strip for detecting paracetamol in herbal medicines through a specificity test. The test strip prepares with the immersion of Whatman chromatography paper on the FeCl_3 and Folin–Ciocalteu reagents. The response time was determined by observing the color change at intervals of 5 to 60 minutes after immersing the test strip in the paracetamol solution. The specificity test was performed by comparing the RGB values among the standard, sample (herbal medicines for muscle aches, rheumatism, and gout), and spiked solutions. The results showed that the optimal response time occurred between 30 and 45 minutes for both reagents. The specificity test indicated that both reagents could not distinguish paracetamol from other compounds in samples, by the absence of significant differences in the RGB values between the samples and the spiked solutions. Thus, the reagent-based paper test strip may serve as an initial screening tool but lacks sufficient selectivity for confirmatory analysis. Further research is recommended to develop more specific reagents or to integrate the paper-based test with instrumental techniques to improve accuracy.

Keywords:

paper test strips; reagent; paracetamol; herbal medicine; specificity

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INTRODUCTION

Currently, herbal medicines are widely used by the public as alternative therapies, both to support recovery and to maintain health. Due to the high demand, many industries produce herbal medicines with various claimed benefits (BPOM, 2022). According to BPOM Regulation No. 25 of 2023, industries are prohibited from manufacturing, importing, or distributing herbal medicines that contain isolated or synthetic chemical substances, especially Active Pharmaceutical Ingredients (APIs) (BPOM, 2023).

However, BPOM reports that each year, more than 40 herbal medicine products are found to contain APIs. BPOM, as the Indonesian Food and Drug Authority, has supervised over the past three years, also indicates an increasing number of such cases—41 products in 2022, 50 products in 2023, and 68 products in 2025 (BPOM, 2025). Most of these findings involved sildenafil citrate in herbal medicines marketed for male stamina, as well as dexamethasone, phenylbutazone, and paracetamol in herbal medicines intended for muscle aches and pain relief.

Sentat et al. (2019) developed a paper-based test strip for detecting paracetamol in herbal medicines. Their findings demonstrated that two prototype colorimetric paper-based test strips containing 10% iron (III) chloride and Folin–Ciocalteu reagents were capable of detecting paracetamol through a color reaction. However, that study did not include a validation of the reagent-based paper test strip method. Validation is necessary to ensure that the method can be reliably applied to real samples and that its performance is comparable to existing standard methods. Previous research on the use of paper-based test strips for the identification of quinine in beverage samples has validated several analytical parameters, including accuracy, limits of detection and quantitation, precision, selectivity, and stability (Tsaftari et al., 2023).

Therefore, validation of this reagent-based paper test strip method, including a specificity test, is necessary. According to the BPOM (Indonesian Food and Drug Authority) analytical guidelines for testing drugs and food, the identification of dexamethasone as a prohibited APIs is conducted using thin-layer chromatography (TLC) and spectrophotodensitometry (BPOM, 2018). Paper-based analytical devices (PADs) were chosen because they have ideal pores that allow for the even absorption and retention of chemical reagents, resulting in more consistent detection (Malik et al., 2024).

This study aimed to validate the reagent-based paper test strip method through a specificity test. The test was carried out using a standard paracetamol solution, herbal medicine samples, and spiked samples. Three types of herbal medicines were analyzed—those used for muscle aches, gout, and rheumatism.

METHODS

Materials

Iron (III) chloride, Folin–Ciocalteu reagent, and nitric acid were purchased from Merck, and paracetamol BPFI was procured from PPPOMN (*Pusat Pengembangan Pengujian Obat dan Makanan Nasional*).

Reagent Preparation

For the preparation of a 10% iron (III) chloride solution, 10 g of iron (III) chloride hexahydrate was weighed, transferred into a 100 mL volumetric flask, and dissolved in distilled water (Sentat et al., 2019).

Reagent Immobilization on Paper

Immobilization was performed using the entrapment method. Whatman No. 1 chromatography paper was cut into 2 × 3 cm pieces and soaked in 5 mL of each reagent for 60 minutes. The papers were then removed and air-dried for 30 minutes at room temperature.

Preparation of Standard and Sample Solution

A standard and sample solution with a strength of 500 ppm was prepared by dissolving the accurately weighed standard and sample (12.5 mg) with 96% ethanol in a separate 25 mL volumetric flask.

Preparation of Spiked Solution

Approximately 12.5 mg of paracetamol standard and 12.5 mg of the herbal medicine sample were accurately weighed and dissolved together in 96% ethanol in a 25 mL volumetric flask.

Response Time Test

Two drops of the paracetamol reference solution were applied to the test strip, and images were captured under lighting conditions to minimize environmental variation. Images were taken at 5, 15, 30, 45, and 60 minutes after application. The images were then analyzed using a color picker application to determine the optimal response time.

Specificity Test

A 0.05 mL aliquot of each paracetamol reference solution, herbal medicine sample solution, and spiked solution was applied to the test strip. Colour changes were observed at the previously determined optimal response time. Image data were processed using a color picker application to quantify and visualize the colour differences more clearly.

RESULTS

Data processing was performed based on the color changes observed on the test strip after reaction with the samples. The images were analyzed using a color picker application to obtain the color intensity values and to visualize the resulting colors more clearly. The color picker application analyzes the uploaded images and provides numerical values for the red, green, and blue (RGB) color components. These RGB intensity values were used to determine the optimal reaction time for the test strip and to evaluate the specificity of the test.

Response Time Test

The response time refers to the period required for the reagent to react with the analyte and produce a visible color change.

Table 1. Response Time Test Result

Reagent	Replication	Response Time (minutes)	Red, Green, and Blue (RGB) color values		
			R	G	B
Iron (III) chloride	1	5	164	171	122
		15	171	175	125
		30	168	170	79
		45	176	176	90
		60	181	178	75
	2	5	153	156	80
		15	141	145	67
		30	167	167	99
		45	169	166	79
		60	174	168	58
	3	5	129	132	44
		15	120	122	9
		30	141	142	52
		45	128	122	40
		60	148	140	49
	4	5	163	167	107
		15	167	170	85
		30	175	175	95
		45	181	180	103
		60	183	179	97
	5	5	139	141	0
		15	143	145	54
		30	143	143	55
		45	152	147	40
		60	157	149	59
Folin ciocalteu	1	5	102	118	39

Reagent	Replication	Response Time (minutes)	Red, Green, and Blue (RGB) color values		
			R	G	B
		15	95	111	30
		30	98	110	50
		45	92	109	45
		60	105	120	62
		5	122	137	62
	2	15	105	125	40
		30	72	92	25
		45	127	145	89
		60	77	96	47
		5	100	117	43
	3	15	97	113	50
		30	99	115	52
		45	105	120	63
		60	72	93	35
		5	97	117	35
	4	15	88	107	28
		30	95	110	52
		45	109	124	72
		60	88	106	50
		5	91	111	32
	5	15	96	115	31
		30	92	108	47
		45	102	118	60
		60	104	122	65

Specificity Test

The specificity test was performed to confirm that the reagent-based paper test strip selectively responded to paracetamol, without interference from other constituents contained in the herbal medicines.

Table 2. Specificity Test Result

Reagent	The Types of Herbal medicine	Replication	Red, Green, and Blue (RGB) color values		
			Standard	Sample	Spike
Iron(III) chloride	Muscle aches	1	180,179,100	76,65,18	109,98,48
		2	184,186,139	116,103,26	108,97,47
		3	176,168,0	117,102,37	108,97,49
		4	183,180,46	107,95,22	103,89,35
		5	183,178,60	123,108,50	132,121,18
	Gout	1	143,144,64	115,91,41	124,104,63
		2	146,148,65	119,95,52	117,97,57
		3	156,157,88	123,100,61	109,90,53
		4	148,149,31	96,72,34	99,77,25
		5	154,158,67	113,90,41	118,98,41

Reagent	The Types of Herbal medicine	Replication	Red, Green, and Blue (RGB) color values		
			Standard	Sample	Spike
	Rheumatism	1	149,145,53	152,131,25	135,121,59
		2	150,143,36	157,136,21	143,128,19
		3	174,175,110	174,152,78	154,139,96
		4	170,162,59	168,148,56	154,143,63
		5	146,133,9	151,127,62	127,113,47
Folin ciocalteu	Muscle aches	1	126,146,80	102,98,5	107,108,39
		2	111,132,78	83,74,0	93,88,19
		3	123,141,85	83,80,0	81,84,12
		4	112,129,64	83,76,0	89,89,8
		5	106,124,68	73,69,0	82,82,2
	Gout	1	71,88,39	74,77,31	68,70,29
		2	92,109,59	78,84,36	82,86,30
		3	84,100,41	69,72,25	63,68,24
		4	90,108,37	74,75,22	52,46,24
		5	77,91,43	76,80,26	56,55,23
	Rheumatism	1	78,95,27	129,116,0	106,95,0
		2	91,105,36	114,100,0	106,96,0
		3	84,101,37	123,114,0	106,96,0
		4	100,119,46	135,124,0	93,88,1
		5	106,120,50	123,109,0	120,110,0

DISCUSSION

Test strips were prepared using Whatman Chromatography Paper No. 1, which was impregnated with iron (III) chloride and Folin–Ciocalteu reagents through the entrapment method. Whatman No. 1 paper was selected because it has a moderate pore size, a smooth surface, and sufficient thickness. A distinct color change observed on the paper indicates that the reagents were optimally immobilized and effectively absorbed onto the paper. Paper is affordable, lightweight, and simple to dispose of in an environmentally responsible way (Buking et al., 2019).

Reagent selection plays a crucial role in developing test strips for the detection of paracetamol with adequate selectivity. The Folin–Ciocalteu and FeCl_3 reagents were used because they interact with phenolic compounds, including paracetamol, forming a different colored complex. In this study, the entrapment technique was employed for reagent immobilization due to its ability to maintain reagent activity over time and minimize reagent loss during analysis. This technique involves trapping the reagent within the paper surface. The primary advantage of the entrapment method is its chemical stability and the ability to facilitate optimal interaction between the reagent and the analyte (Hermanto et al., 2020).

In preparing the test strips, three types of solutions were used: a paracetamol solution, the herbal medicine sample solutions, and the spiked solution. Dirgantara et al. (2014) demonstrated that paracetamol can be detected in the concentration range of 0.125–5 mg/mL. Ethanol was selected as the solvent due to its ability to dissolve paracetamol (Hakim & Saputri, 2020). Theoretically, paracetamol contains amide and hydroxyl functional groups that can form hydrogen bonds with ethanol, resulting in good solubility (Putra et al., 2023).

Based on the results obtained, the response time test using the iron (III) chloride reagent showed a gradual change in RGB values as the contact time increased. During the initial period (5–15 minutes), the color of the solution remained relatively bright, characterized by high R, G, and B values. However, between 30 and 45 minutes, a decrease in the G and B values was observed, indicating a shift in color toward a more brownish hue. After 60 minutes, the color change tended to stabilize. The consistent pattern observed across replications indicated that the reaction between iron (III) chloride and the compounds in the sample reached equilibrium, with optimal complex formation time occurring within the 30–45-minute range.

Similarly, the response time test using the Folin–Ciocalteu reagent showed a change in RGB values with increasing contact time. In the early phase (5–15 minutes), the color remained light; however, between 30 and 45 minutes, a decrease in the R and G values occurred, resulting in a more intense color. After 60 minutes, the color stabilized, suggesting that the reaction had reached completion. A consistent trend was observed in each replication, confirming that the reaction was stable, and the optimal time for complex formation with the Folin–Ciocalteu reagent fell within the range of 30–45 minutes.

Based on the specificity test results obtained using the two reagents, FeCl_3 and Folin–Ciocalteu, similar patterns were observed. The RGB values between the sample and the spiked solution for both reagents showed no significant differences, and the resulting color changes appeared visually similar. This finding indicates that neither FeCl_3 nor the Folin–Ciocalteu method could selectively distinguish between the sample and the spike, resulting in a more general, non-specific color response.

The lack of specificity can be attributed to the inherent chemical properties of these reagents. Pérez et al. (2023) and Apak et al. (2016) reported that the Folin–Ciocalteu reagent reacts with various reducing agents—including polyphenols, ascorbic acid, sugars, and aromatic amines—through an electron-transfer mechanism, thereby lacking chemical selectivity toward a single analyte. Likewise, FeCl_3 reacts with a broad range of phenolic and polyphenolic compounds to form coloured complexes, without the ability to differentiate a specific target compound from other phenolic constituents in the matrix. The reaction mechanism of FeCl_3 also supports this observation.

As described by Harborne (1998) and Dewi & Purwayantie (2019), FeCl_3 reacts with phenolic hydroxyl groups to form colored ferric–phenolate complexes, a reaction common to many phenolic compounds found in herbal extracts. Consequently, the presence of multiple phenolic and polyphenolic compounds in herbal matrices causes overlapping color responses, leading to the non-specificity observed in this study.

In conclusion, the specificity test demonstrated that neither FeCl_3 nor Folin–Ciocalteu functions as a selective reagent for detecting paracetamol in herbal medicine. Both reagents are more suitable as general indicators of compound groups, such as total phenolics or total reducing substances, rather than as tools for compound-specific identification.

It is recommended that future research be conducted to enhance the analytical performance and selectivity of reagent-based paper test strips for paracetamol detection, which does not rely solely on colour testing as a single method. Based on previous research, paper arrow–mass spectrometry (PA–MS) integration offers a promising method for rapid, sensitive, and non-invasive detection in just minutes (Zhou et al., 2024). In another study, electrochemical paper-based analytical devices (ePADs) were used to separate and quantify steroids in herbal medicines. The ePAD was composed of Whatman SG81 chromatography paper, 3D-printed devices, and a commercial screen-printed electrode (Primpray et al., 2019). Screen-printed technology has significantly contributed to the development of portable electrochemical sensors, as it provides miniaturized yet robust and user-friendly electrodes (Costa-Rama & Fernández-Abedul, 2021). Dual/combined detection systems can overcome the individual limitations of analytical techniques, enable simultaneous determinations, or enhance the devices' sensitivity and/or selectivity (Pradela-Filho et al., 2023).

CONCLUSION

This study demonstrates that the FeCl_3 and Folin–Ciocalteu reagents do not produce specific results in detection tests of herbal preparations. This conclusion is supported by the absence of significant differences in RGB values between the sample and the spiked solution, as well as the visually similar color responses observed. The non-selective behavior of both reagents leads to color development that reflects the cumulative reaction of various compounds within the herbal matrix, particularly phenolics, polyphenols, and other reducing agents.

A limitation of this study is the use of a color-change-based detection approach, which is highly influenced by the complexity of the herbal matrix and thus struggles to clearly distinguish the target compound. Future research is recommended to integrate simple reagent-based detection with more

selective instrumental analytical methods, and to explore or synthesize alternative reagents with greater specificity toward the target analyte.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- Apak, R., Özyürek, M., Güçlü, K., & Çapanoğlu, E. (2016). Antioxidant Activity/Capacity Measurement. 1. Classification, Physicochemical Principles, Mechanisms, and Electron Transfer (ET)-Based Assays. *Journal of Agricultural and Food Chemistry*, 64(5), 997–1027. <https://doi.org/10.1021/ACS.JAFC.5B04739>
- BPOM. (2018). *Metode Analisis untuk Pengujian Obat dan Makanan di Lingkungan Badan Pengawas Obat dan Makanan*. PPOMN
- BPOM. (2022). Retrieved October 5, 2025, from <https://www.pom.go.id/siaran-pers/penjelasan-publik-temuan-obat-tradisional-suplemen-kesehatan-dan-kosmetika-mengandung-bahan-kimia-obat-serta-bahan-dilarang-berbahaya-tahun-2022>
- BPOM. (2023). Retrieved October 5, 2025, from <https://peraturan.bpk.go.id/Details/284994/peraturan-bpom-no-25-tahun-2023>
- BPOM. (2025). Retrieved October 5, 2025, from <https://e-penjelasanpublikotsk.pom.go.id/pw2022/index.php?ls=pwlist#cnt>
- Buking, S., Suedomi, Y., Nacapricha, D., & Kaneta, T. (2019). Characterization of Pieces of Paper That Form Reagent Containers for Use as Portable Analytical Devices. *ACS Omega*, 4(12), 15249. <https://doi.org/10.1021/ACSOMEGA.9B02226>
- Costa-Rama, E., & Fernández-Abedul, M. T. (2021). Paper-Based Screen-Printed Electrodes: A New Generation of Low-Cost Electroanalytical Platforms. *Biosensors 2021*, Vol. 11, Page 51, 11(2), 51. <https://doi.org/10.3390/BIOS11020051>
- Dewi, Y. S. K., & Purwayantie, P. (2019). Phytochemical And Antioxidant Activity From Fruit Of Kulim (Scorodocarpus Borneensis Becc.). *Proceeding of the 1st International Conference on Food and Agriculture*, 2. <https://publikasi.polije.ac.id/ProceedingICOFA/article/view/1876>

- Dirgantara, V. S., Zulfikar, Z., & Andarini, N. (2014). IDENTIFIKASI KUALITATIF BAHAN ANALGESIK PADA JAMU MENGGUNAKAN PROTOTYPE TES STRIP. *BERKALA SAINSTEK*, 2(1), 42–48. <https://bst.jurnal.unej.ac.id/index.php/BST/article/view/1621>
- Hakim, A. R., & Saputri, R. (2020). Narrative Review: Optimasi Etanol sebagai Pelarut Senyawa Flavonoid dan Fenolik. *Jurnal Surya Medika (JSM)*, 6(1), 177–180. <https://doi.org/10.33084/JSM.V6I1.1641>
- Harborne. (1998). *Phytochemical Methods A Guide to Modern Techniques of Plant Analysis*. Retrieved October 5, 2025, from <https://link.springer.com/book/9780412572609>
- Hermanto, D., Sanjaya, R. K., & Ismillayli, N. (2020). A Simple and Sensitive Optode Sensor Glucose Based on Immobilization Benedict Into Nata Cellulose Membranes. *Jurnal Pijar Mipa*, 15(4), 404–407. <https://doi.org/10.29303/JPM.V15I4.1352>
- Malik, S., Singh, J., Saini, K., Chaudhary, V., Umar, A., Ibrahim, A. A., Akbar, S., & Baskoutas, S. (2024). Paper-based sensors: affordable, versatile, and emerging analyte detection platforms. *Analytical Methods*, 16(18), 2777–2809. <https://doi.org/10.1039/D3AY02258G>
- Pérez, M., Dominguez-López, I., & Lamuela-Raventós, R. M. (2023). The Chemistry Behind the Folin–Ciocalteu Method for the Estimation of (Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. *Journal of Agricultural and Food Chemistry*, 71(46), 17543–17553. <https://doi.org/10.1021/ACS.JAFC.3C04022>
- Pradela-Filho, L. A., Veloso, W. B., Arantes, I. V. S., Gongoni, J. L. M., de Farias, D. M., Araujo, D. A. G., & Paixão, T. R. L. C. (2023). Paper-based analytical devices for point-of-need applications. *Microchimica Acta*, 190(5), 1–23. <https://doi.org/10.1007/S00604-023-05764-5/METRICS>
- Primpray, V., Chailapakul, O., Tokeshi, M., Rojanarata, T., & Laiwattanapaisa, W. (2019). A paper-based analytical device coupled with electrochemical detection for the determination of dexamethasone and prednisolone in adulterated traditional medicines. *Analytica Chimica Acta*, 1078, 16–23. <https://doi.org/10.1016/J.ACA.2019.05.072>
- Putra, A. P., Aisyah, R., Sedana, K. P., & Kresnayana, G. I. (2023). ANALISIS KUALITATIF PARASETAMOL PADA JAMU PEGEL LINU DAN ASAM URAT DI KECAMATAN KUBUTAMBAHAN. *JURNAL FARMASI KRYONAUT*, 2(2), 42–45. <https://doi.org/10.59969/JFK.V2I2.27>
- Sentat, T., Nurhasnawati, H., & Dwinand1, Y. R. (2019). DEVELOPMENT OF PAPER-BASED COLOR TEST-STRIP FOR PARACETAMOL DETECTION IN JAMU. *Jurnal Ilmu Kesehatan*, 7(2), 137–142. <https://doi.org/10.30650/JIK.V7I2.1231>
- Tsaftari, V. C., Tarara, M., Tzanavaras, P. D., & Tsogas, G. Z. (2023). A Novel Equipment-Free Paper-Based Fluorometric Method for the Analytical Determination of Quinine in Soft Drink Samples. *Sensors (Basel, Switzerland)*, 23(11), 5153. <https://doi.org/10.3390/S23115153>
- Zhou, Y., Dliso, S., Craske, J., Gill, A., Bracken, L., Landa, K., Arnold, P., Walker, L., Grasim, I., Seddon, G., Chen, T., Davison, A. S., Sham, T. T., Smith, B., Hawcutt, D. B., & Maher, S. (2024). Rapid and non-invasive analysis of paracetamol overdose using paper arrow-mass spectrometry: a prospective observational study. *BMC Medicine*, 22(1), 553. <https://doi.org/10.1186/S12916-024-03776-3>