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

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ABSTRACT

Paracetamol and caffeine are chemical compounds that are suspected to be illegally added to the traditional herbs claimed as rheumatic drugs. Identification of paracetamol and caffeine has been done 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 is jamu powder using thin-layer chromatography densitometry (TLC-Densitometry). Evaluate the presence of paracetamol and caffeine in the product of jamu was performed by thin layer chromatography with silica gel GF254 and chloroform-ethyl acetate (1:1) as the stationary and mobile phase respectively. The spots on the TLC plate were detected using a UV at 254 nm and the areas were measured by a Camag TLC scanner. The TLC profile demonstrated a good separation of paracetamol, caffeine, and others substances that containing in the products. The retardation factor (Rf) of paracetamol and caffeine were 0.42 and 0.26 with a detection limit of 0.0125 µg/spot and 0.05 µg/spot respectively. The simultaneous identification of caffeine and paracetamol by 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.

INTRODUCTION

Paracetamol is one of the most widely used analgesics and antipyretics. 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, 2016). The chemical names of paracetamol and caffeine are N-(4-Hydroxyphenyl) acetamide and 1,3,7−trimethyl purine − 2,6 dione respectively (Merck Index, 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 analytes can be detected by UV. Previous studies were reported paracetamol in herbs powder spectrophotometry (Sari, et al., 2021), FT-IR (Wahyuningsih, et al., 2022), high-performance liquid chromatography (HPLC) (Laksmi, et al., 2016; Wisnuwardani, et al., 2018; Mamat, et al., 2016).
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., 2022). 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).

**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; Lee et al., 2022; Harimurti, et al., 2020; Hayun, et al., 2022). The analytical method for identification and quantification simultaneous of paracetamol and caffeine in the traditional herbs powder has not been reported in many studies. The jamu powder contained a complex matrix that the presence of different matrix 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 proposed to optimize the TLC conditions for simultaneous identification of paracetamol and caffeine on jamu powder obtained from the Depot Jamu in Surabaya.

**EXPERIMENTAL**

**Instrument**

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 GF254 plate (Merck). The samples of jamu powder were obtained from the Depot Jamu in Surabaya.

**Chromatographic Condition**

Chromatography was performed by using a silica gel GF254 TLC plate (Merck) as the stationary phase, and chloroform-ethyl acetate (1:1) as the 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-densitometer 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 on top of the water bath. 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, caffeine, 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 on top of the water bath. The dried filtrate dissolved with 5 ml methanol. Two microilters 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 is depicted in Figure 2. Figure 2 shows 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 parameter Rf and Rs (Table 3), the Rf value generally meets the requirement (ICH, 2005).

The selected wavelength was 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, 0.020 µg, 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

**Table 1. Limit detection of paracetamol and caffeine on spott 2 µL**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Concentration</th>
<th>Start Height</th>
<th>End height</th>
<th>Area</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>0.005 µg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not detection</td>
</tr>
<tr>
<td></td>
<td>0.0125 µg</td>
<td>4.1</td>
<td>8.3</td>
<td>441.7</td>
<td>Detection</td>
</tr>
<tr>
<td></td>
<td>0.020 µg</td>
<td>12.1</td>
<td>2.4</td>
<td>1207.0</td>
<td>Detection</td>
</tr>
<tr>
<td></td>
<td>0.025 µg</td>
<td>1.1</td>
<td>7.8</td>
<td>1886.0</td>
<td>Detection</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.020 µg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not detection</td>
</tr>
<tr>
<td></td>
<td>0.05 µg</td>
<td>3.0</td>
<td>4.0</td>
<td>352.7</td>
<td>Detection</td>
</tr>
<tr>
<td></td>
<td>0.06 µg</td>
<td>2.8</td>
<td>3.8</td>
<td>504.3</td>
<td>Detection</td>
</tr>
<tr>
<td></td>
<td>0.10 µg</td>
<td>5.5</td>
<td>2.7</td>
<td>862.9</td>
<td>Detection</td>
</tr>
</tbody>
</table>
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 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.

Table 2. Selectivity of Paracetamol and Caffeine

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Concentration</th>
<th>Start Height</th>
<th>Retardation Factor (Rf)</th>
<th>Tailing Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paracetamol</td>
<td>500 ul</td>
<td>2.3</td>
<td>0.42</td>
<td>1.33</td>
</tr>
<tr>
<td>Caffeine</td>
<td>500 ul</td>
<td>0.9</td>
<td>0.26</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. Identification paracetamol and caffeine in Jamu Powder using TLC-Densitometry

<table>
<thead>
<tr>
<th>No</th>
<th>Chemical Name</th>
<th>Rf Value</th>
<th>Rs Value</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Paracetamol</td>
<td>0.42</td>
<td>1.23</td>
<td>4832.2</td>
</tr>
<tr>
<td>2</td>
<td>Caffeine</td>
<td>0.26</td>
<td>Negative</td>
<td>635.2</td>
</tr>
<tr>
<td>3</td>
<td>Jamu A</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Jamu B</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Jamu C</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Jamu D</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>Jamu E</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

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.
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, the application of this method for quantitative analysis is recommended, by which a diversity of traditional medicines could be used as samples.

REFERENCES


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


