Identification of Medicinal Chemicals in Herbal Medicine for Shortness of Breath in Cirebon Using Thin-Layer Chromatography Method

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Abstract. Herbal medicine is prohibited from adding Medicinal Chemicals (BKO), both isolated and synthetic, by Permenkes No. 007 of 2012 Article 7. However, several previous studies found herbal medicine containing BKO; the addition of BKO is indicated so that products from the manufacturer feel faster and have instant efficacy. However, the addition of BKO to herbal medicine has side effects on the organs of the consumer. In 2014 – 2022, BPOM released 12 herbal products that were proven to contain BKO. This study aims to identify the existence of BKO in shortness-of-breath herbal medicine and to find out what BKO is contained in the herbal medicine. Using the purposive sampling method, this study tested six herbal medicine samples with shortness of breath circulating in Cirebon. The techniques used in the identification were thin-layer chromatography (KLT) with the silent phase of GF 254 silica gel, the motion phase of Chloroform, Methanol, and Aquabidest with a ratio of 2: 7: 1. Spot detection through 254 nm UV light. The controlled solution is the test solution (A), the positive control solution (B), and the comparator raw solution (C). Based on the study's results, 5 out of 6 herbal samples containing BKO, Samples 1 and 3 contained CTM and Ephedrine Hcl. Samples 2, 5, and 6 contained Ephedrine HCl only. Sample 4 does not contain BKO.

Keywords: Herbal Medicine, Shortness of Breath, Chlorpheniramin Maleat, Ephedrine Hydrochloride, Thin Layer Chromatography

INTRODUCTION

Shortness of breath is one of the symptoms of respiratory tract disorders. The prevalence of respiratory diseases in Cirebon City and Regency is relatively high. Referring to West Java RISKESDAS data in 2018, the prevalence of Acute Respiratory Infections in Cirebon Regency was 2.79%, and in Cirebon City was 3.65%. The prevalence of Pulmonary Tuberculosis in Cirebon Regency is 0.37%, and in Cirebon City is 0.35%. The prevalence of asthma diagnosis in Cirebon Regency is 1.03%, and in Cirebon City is 1.27%. The prevalence of asthma recurrence in Cirebon Regency is 56.66%, and in Cirebon City, it is 61.53% (Ministry of Health RI, 2019). Sarian (galenik), or a mixture of these ingredients has been used for treatment for generations, and can be applied following
the norms that apply in society (regulation the Minister of Health, 2012). In line with the opinion of Parwata, who states that Traditional Medicine is an ingredient or ingredient in the form of plant materials, animal materials, mineral ingredients, galenic preparations or mixtures, and these ingredients, which have traditionally been used for treatment based on experience (Parwata, 2016). Indonesian society is very thick with traditional nuances; this was expressed by the POM Agency, which stated that this tendency to return to nature is based on the general reason that natural medicines are safe to use and easy to obtain (BPOM, 2018). However, with this tendency, there is a negative effect, namely a violation of regulations on the herbal medicine production process, namely the mixing of medicinal chemicals (BKO) in herbal preparations. He referred to the Indonesian Minister of Health Regulation No. 007 of 2012, which states that "medicinal chemicals are prohibited from being added to herbal medicine"(Minister of Health, 2019). This has made various parties interested in researching the existence of BKO in Jammu's shortness of breath. Shortness of breath herbal medicine in Malang contained theophylline. Likewise, the results of Herdini's research stated that his research on three samples of herbal medicine for shortness of breath in Bekasi City that were tested, three samples of herbal medicine were positive for theophylline (Hardini et al., 2021). The latest research related to BKO in shortness-of-breath herbs was carried out by Aziza, stating that of the six samples of shortness-of-breath herbs circulating in the city of Banjarmasin, all positive samples contained theophylline(Aziza, 2022). Findings related to illegal herbal medicine can be seen in the results of BPOM testing from November 2014 to October 2022 and various studies on the existence of BKO in herbal medicine. One published article states that chlorpheniramine maleate is contained in 9 traditional medicine preparations, and Ephedrine HCl is contained in 3 traditional medicine preparations (BPOM, 2018). Chlorpheniramine maleate (CTM) is one of the drugs that belong to the class of antihistamines that work to minimize inflammatory reactions and is the most widely used antihistamine(Saputra et al., 2018). Ephedrine Hydrochloride is a decongestant drug, namely an adrenergic α-1 receptor stimulant (Kurniawati et al., 2023). Ephedrine HCl can be an adrenergic agent agonist, antiasthma, and bronchodilator (Nurcahyanti, 2018).

METHODS

The research conducted is a type of descriptive research, namely by identifying the drug chemicals Chlorpheniramine Maleate and Ephedrine HCl in herbal medicine for
shortness of breath indicated for shortness of breath by interpreting the chromatogram obtained from the Thin Layer Chromatography method. The samples used were six samples of herbal medicine indicated as medicine for shortness of breath with different manufacturers obtained from several herbal medicine shops in Cirebon. The stationary phase in this study is GF 254 silica gel, and the motion phase of the combination of Chloroform, Methanol, and Aquabidest with a ratio of 2: 7: 1 and detection using 254 nm UV light. The research was conducted at the Pharmacognosy Laboratory of the College of Pharmacy Muhammadiyah Cirebon, and the research time was February – May 2023.

**Tools and Materials**

The tools used are Chamber and lid, capillary pipe, glass beaker (pyrex), Erlenmeyer (pyrex), measuring cup (Pyrex), measuring flask (pyrex), 254 nm UV lamp, mortar and stamper, stove, analytical balance, porcelain cup (pyrex), stirring rod, pipette, water bath, vial. The materials used are KLT silica gel GF 25 (Merck) plate, samples of herbal shortness of breath, ethyl acetate (CV. Mustika Lab), chloroform (CV. Mustika Lab), methanol (CV. Mustika Lab), aquabidest (Brataco), CTM tablets (PT. Ciubros Farma), Ephedrine HCl Injection (Mahakam), filter paper.

**Organoleptic and Packaging Observations**

The research began by observing the packaging, including the registration number, composition, type of packaging, production address, and expiration date of the herbal medicine packaging. Meanwhile, organoleptic observations include the shape, color, odor, and homogeneity of the clock preparation (Utami et al., 2019).

**Preparation of Test Solution A (sample)**

Each sample of herbs as a fine powder is put into 150 ml of Erlenmeyer, 50 ml of chloroform-methanol mixture (4:1), and a homogeneous shake is added. The filtrate is evaporated in a bucket of water at approximately 70°C until dry. The remaining evaporation is dissolved in 8 ml of methanol and then filtered. Put in vial (Wulandari et al., 2013).

**Preparation of Bc Solution (positive control of Chlorpheniramine Maleate)**

Each sample of herbs as a fine powder is put into 150 ml of Erlenmeyer. Add chlorpheniramine maleate tablet powder equivalent to 12 mg, as many as three tablets with a dose strength of 4 mg each, add 50 ml of chloroform-methanol mixture (4:1), and shake homogeneously. The filtrate is evaporated in a bucket of water at a temperature of
approximately 70°C until dry. The remaining evaporation is dissolved in 8 ml of methanol and then filtered. Put in vial (Wulandari et al., 2013).

**Preparation of Be Solution (positive control of Ephedrine HCl)**

Make an injection dilution of Ephedrine HCl 50mg/ml ad 5ml with aqua pro injection. The dilution result is equivalent to 10mg/ml. Each sample of herbal medicine as a fine powder is put into 150 ml of Erlenmeyer, 50 ml of chloroform-methanol mixture (4:1) is added, 1 ml of Ephedrine HCl injection dilution is added, and a homogeneous shake is added. The filtrate is evaporated on a bucket of water at a temperature of approximately 70°C until dry. The remaining evaporation is dissolved in 8 ml of methanol, then filtered. Put in vial (Wulandari et al., 2013).

**Preparation of Cc Solution (Chlorpheniramin Maleate Comparative Standard)**

A raw solution of chlorpheniramin maleate was made with 12 mg of chlorpheniramin maleate, then dissolved in 20 ml of methanol. Strain and place in vials (Panjaitan, 1999). 

**Preparation of Ce Solution (Ephedrine HCl Comparative Standard)**

Ephedrine HCl raw solution is made by concentrating 1 ml of Ephedrine HCl injection 50mg/ml in a dish over a water bath until dry, then diluted with 1 ml of methanol. Put it into the vial.

**Motion Phase Orientation**

Cut the GF 254 silica gel plate into 3 x 10 cm plates, then activate the plates at 110°C in the oven for 30 minutes. Make a line of 1.5 cm from the bottom, then a creep distance of 8 cm, and a distance of about 0.5 cm from the top border. Put the filter paper into the chamber, then add the...
motion phase of Chloroform – Methanol – Aquabidest with a ratio of 2:7:1 to the vessel and leave it until saturated. Put four marks on the line made at the bottom of the plate as a place to foul the solution B (Bc and Be) and C (Cc and Ce).

Point solution B (positive control) and solution C (comparator standard) on the plate using a capillary pipe. The plate is inserted into the saturated chamber, closed tightly, and left until the development process is complete. Insert the plate into the saturated chamber, then close it tightly and let it sit until the development process is complete. Lift the plate and then air dry. Observe the plate under 254 nm UV light, record, and mark. Calculate the Rf value of each spot (Wulandari et al., 2013).

Sample Identification by KLT

Cut GF 254 silica gel plates with 6 x 10 cm size. Turn the plate on at 110°C in the oven for 30 minutes. Make a line of 1.5 cm from the bottom, then a creep distance of 8 cm, and a distance of about 0.5 cm from the top border. Put the filter paper into the chamber, then add the motion phase of Chloroform – Methanol – Aquabidest with a ratio of 2:7:1 and leave it until saturated.

Mark the sample nodulations on the line on the plate's bottom at 5 points (A, Bc, Be, Cc, and Ce). Using a capillary pipe, point test solution A (sample), test solution B (positive control), and solution C (comparison standard) according to the point made on the plate.

Insert the plate into the saturated chamber, then close it tightly and let it sit until the development process is complete. Lift the plate and air dry it. Observe the plate under 254 nm UV light, record and mark it, and calculate the Rf value of each spot (Wulandari et al., 2013).

DISCUSSION
Sample Collection

Samples of shortness-of-breath herbal medicine were collected from each herbal medicine depot, which was either in the form of shophouses, tents, or mobile herbal medicine sellers in several areas of Cirebon City and Regency.

Organoleptic and Packaging Observations

As Table 2 shows, not all herbal samples meet the requirements related to the rules set by the Ministry of Health. The registration number is listed on the packaging in samples I, II, and III, as in Table II. However, after checking the BPOM website, the herbal medicine sample with the registration number was not registered.
Table 1. Distribution of Herbal Samples for Shortness of Breath

<table>
<thead>
<tr>
<th>No</th>
<th>Neighborhoods</th>
<th>Depot</th>
<th>Merk Jammu</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adidharma</td>
<td>1</td>
<td>A, B, C, F</td>
</tr>
<tr>
<td>2</td>
<td>Astana</td>
<td>1</td>
<td>A, B, C, D</td>
</tr>
<tr>
<td>3</td>
<td>Kertawinangun</td>
<td>1</td>
<td>B, C</td>
</tr>
<tr>
<td>4</td>
<td>Kesenden</td>
<td>2</td>
<td>C, B, C</td>
</tr>
<tr>
<td>5</td>
<td>Klayan</td>
<td>2</td>
<td>C, E</td>
</tr>
<tr>
<td>6</td>
<td>Lemahwungkuk</td>
<td>3</td>
<td>C</td>
</tr>
<tr>
<td>7</td>
<td>Megu</td>
<td>1</td>
<td>A, B, C, D</td>
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<tr>
<td>8</td>
<td>Panjunan</td>
<td>1</td>
<td>A, B, C, E</td>
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<td>9</td>
<td>Sukapura</td>
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<td>A, E</td>
</tr>
<tr>
<td>10</td>
<td>Sutawinangun</td>
<td>1</td>
<td>A, B, E, F</td>
</tr>
</tbody>
</table>

A: Breath drug (Asma) Head Cobra  
B: Blooming Tanjung Shortness of Breath  
C: Shortness of breath Cough Asthma Stamp Fruit Crown of God  
D: Asmagan Tribe 59 and Sesak Nafas Sabdo Palon  
F: Shortness of the breath of Tjipto's mother

Table 2. Results of Observation of Marking on Packaging, Containers, and Etiquette of Herbal Samples

<table>
<thead>
<tr>
<th>Observation Aspect</th>
<th>Sample</th>
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<tr>
<td>Trade name</td>
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</tr>
<tr>
<td>Dosage of Use</td>
<td>✓ ✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Weight of each package</td>
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<tr>
<td>Khasiat</td>
<td>✓ ✓ ✓ ✓ ✓ ✓</td>
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<tr>
<td>Counter indications</td>
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</tr>
<tr>
<td>Expiry Date</td>
<td>X X X ✓ ✓ ✓</td>
</tr>
<tr>
<td>Batch number</td>
<td>X X X X ✓ ✓</td>
</tr>
<tr>
<td>Registration number</td>
<td>✓ ✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Podusen</td>
<td>✓ ✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Production site</td>
<td>✓ ✓ ✓ ✓ ✓ ✓</td>
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Table 3. Results of Organoleptic Observation of Herbal Samples

<table>
<thead>
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<tr>
<td></td>
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<tr>
<td>Packaging</td>
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<tr>
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<td>SR</td>
</tr>
<tr>
<td>Color</td>
<td>KK</td>
</tr>
<tr>
<td>Construction</td>
<td>KJ</td>
</tr>
<tr>
<td>Homogenitas</td>
<td>HM</td>
</tr>
</tbody>
</table>

SC : Breath drug (Asma) Head Cobra
SR : Blooming Tanjung Shortness of Breath
KK : Shortness of breath Cough Asthma Stamp Fruit Crown of God
KJ : Asmagan Tribe 59 HM Sesak Nafas Sabdo Palon

Motion Phase Orientation

The research began by orienting the motion phase to find the composition of the motion phase that is good for the medicinal chemicals used, namely CTM and Ephedrine HCl.

At the orientation of the first phase of motion, the CTM spots are visible, but the Ephedrine HCl spots are very faint. However, if calculated, the Rf value of the two spots met the chromatogram efficiency parameters, which were 0.25 for CTM spots and 0.625 for Ephedrine HCl spots.

In the orientation of the second phase of motion, orientation is carried out to Ephedrine HCl only. Table 4 shows that Ephedrine HCL patches are still faint, even though a change of motion phase has been made. Even Ephedrine HCl patches are seen to spread. The spread of Ephedrine HCl spots is indicated because the carrier of the injection is water with very high polarity, which causes easy spread.

Then, the orientation of the third phase of motion was carried out with the latest composition, and thickening was carried out for the C Ephedrine HCl solution. In Table IV of the third phase of motion, the CTM and Ephedrine HCL spots are apparent. If calculated, the Rf value of the two spots meets the ideal chromatogram efficiency parameters, which are 0.31 for CTM and 0.71 for Ephedrine HCl. So, the phase of motion suitable for identifying CTM and Ephedrine HCl in this study is the composition of the mixed phase of motion from Chloroform – Methanol – aquabidest with a ratio of 2:7:1.
Table 4. Motion Phase Orientation Chromatogram

<table>
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<th>Sex</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picture</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Cc: CTM Comparator Solution  
Ce: Ephedrine HCl Comparator Solution

**Process Validation**

Process validation is carried out to find an extraction procedure that attracts all the medicinal chemicals to be studied.

![Image](image4.png)

**Figure 1. Process Validation Chromatogram**

B: CTM and Ephedrine HCl Positive Control Solution (Simultaneous)  
Cc: CTM Comparator Solution  
Ce: Ephedrine HCl Comparator Solution

From the results of the orientation, a good developer was obtained, namely chloroform: methanol (9: 1). After the orientation of the motion phase then, validation was carried out by notifying solutions B (positive control) and C (standard comparator). Based on Figure 4.1, the chromatogram produces the same Rf between the spot in solution B and solution C for ephedrin, which is 0.7125. Figure 2 also shows the chromatogram that produces a different Rf between the spots in solution B and solution C for CTM, which is 0.3125 in solution Bc and 0.3 in solution B, but the difference is still less than 10%.
Therefore, it can be concluded that the patch of solution B contains CTM and Ephedrine HCl.

Sample Identification

At the stage of sample identification of these two medicinal chemicals, the results are obtained as in the following table 5:

In identifying this shortness of breath herbal sample, observations were made on the chromatogram produced by comparing the color and hRf value of the test solution (A) on each sample with the positive control hRf (B). At the time of fouling, spots on solution B (positive control) should appear, then spots on solution A (samples) compared to spots on solution B (positive control). The sample is said to be positive if the hRf of solution A and B is the same or the difference must be less than 10%. Based on the description in Table 5 and some of the figures below, it can be concluded that 5 of the asthma herbal samples contain medicinal chemicals.

![Figure 2. Sample Chromatogram 1](image-url)

A1: Sample Test Solution 1  
B1c: CTM Positive Control Solution Sample 1  
B1e: Ephedrine HCl Positive Control Solution Sample 1  
Cc: CTM Comparator Solution  
Ce: Ephedrine HCl Comparator Solution

Based on the study results on sample 1, the A1 solution produced two spots, A1.1 with Rf 0.29 and A1.2 with Rf 0.7. The B1c solution produces two spots, B1c.1 with Rf 0.3 and B1c.2 with Rf 0.71. The B1e solution produces two spots, B1e.1 with Rf 0.31 and B1e.2 with Rf 0.69. The C1c solution produces spots with an Rf of 0.3. The C1e solution produces patches with an Rf of 0.68. There is no difference between the hRf of the B1c.1 spot and the hRf C1c; then the B1c.1 spot is the CTM. The difference between hRf spot B1e.2 and hRf C1e is 1.47%, then the spot B1e.2 is Efedrin HCl. The difference between hRf spot A1.1 and hRf B1c.1 is 3.33%, then spot A1.1 is CTM. The difference between
hRf spot A1.2 and hRf B1e.2 is 1.45%, and spot A1.2 is Ephedrine HCl. So it can be concluded that sample 1 is positive for CTM and Ephedrine HCl.

Figure 3. Sample Chromatogram 2
A2: Sample Test Solution 2
B2C: CTM Positive Control Solution Sample 2
B2E: Ephedrine HCl Positive Control Solution Sample 2
Cc: CTM Comparator Solution
Ce: Ephedrine HCl Comparator Solution

Based on the study results on sample 2, the A2 solution produced one spot, namely A2.1, with Rf 0.69. The B2c solution produces two spots, B2c.1 with Rf 0.3 and B2c.2 with Rf 0.7. The B2e solution produces one spot, B2e.1, with an Rf of 0.7. The C2c solution produces spots with an Rf of 0.3. The C2e solution produces patches with an Rf of 0.69. There is no difference between the hRf of the B2c.1 spot and the hRf C2c, and then the B2c.1 spot is CTM. The difference between hRf spot B2e.1 and hRf C2e is 1.45%, then the spot B2e.1 is Efedrin HCl. The difference between hRf spot A2.1 and hRf B2e.1 is 1.43%, and spot A2.1 is Ephedrine HCl. So, it can be concluded that sample 2 is positive for Ephedrine HCl.

Figure 4. Sample Chromatogram 3
A3: Sample Test Solution 3
B3C: CTM Positive Control Solution Sample 3
B3e: Ephedrine HCl Positive Control Solution Sample 3
Cc: CTM Comparator Solution
Ce: Ephedrine HCl Comparator Solution

Based on the study results on sample 3, A3 solution produced two spots, A3.1 with Rf 0.3 and A3.2 with Rf 0.69. The B3c solution produces two spots, B3c.1 with Rf 0.3 and B3c.2 with Rf 0.69. The B3e solution produces two spots, B3e.1 with Rf 0.29 and B3e.2 with Rf 0.7. The C3c solution produces patches with an Rf of 0.31. The C3e solution produces patches with an Rf of 0.7. The difference between hRf of B3c.1 and hRf C3c is 3.23%, then B3c.1 is CTM. There is no difference between hRf spot B3e.2 and hRf C3e, and spot B3e.2 is Ephedrine HCl. So it can be concluded that sample 3 is positive for CTM and Ephedrine HCl.

Figure 5. Sample Chromatogram 4
A4: Sample Test Solution 4
B4C: CTM Positive Control Solution Sample 4
B4E: Ephedrine HCl Positive Control Solution Sample 4
Cc: CTM Comparator Solution
Ce: Ephedrine HCl Comparator Solution

Based on the study results on sample 4, the A4 solution did not produce spots. The B4c solution produces one spot, namely B4c.1, with an Rf of 0.29. The B4e solution produces one spot, B4e.1, with an Rf of 0.7. The C4c solution produces spots with an Rf of 0.28. The C4e solution produces patches with an Rf of 0.7. The difference between the hRf of B4c.1 and the hRf of C4c is 3.57%, so the B4c.1 is CTM. There is no difference between the hRf of B4e.1 and hRf C4e, so the B4e.1 is Ephedrine HCl. So it can be concluded that the negative sample 4 contains CTM and Ephedrine HCl.
Figure 6. Sample Chromatogram 5

A5: Sample Test Solution 5
B5c: CTM Positive Control Solution Sample 5
B5e: Ephedrine HCl Positive Control Solution Sample 5
Cc: CTM Comparator Solution
Ce: Ephedrine HCl Comparator Solution

Based on the study results on sample 5, the A5 solution produced one spot, namely A5.1, with an Rf of 0.68. The B5c solution produces two spots, B5c.1 with Rf 0.31 and B5c.2 with Rf 0.71. The B5e solution produces one spot, B5e.1, with an Rf of 0.7. The C5c solution produces spots with an Rf of 0.33. The C5e solution produces patches with an Rf of 0.69. The difference between hRf of B5c.1 and hRf C5c is 6.06%, then B5c.1 is CTM. The difference between hRf of B5e.1 and hRf C5e is 1.45%, then the B5e.1 is Ephedrine HCl. The difference between hRf of B5.1 and hRf B5e.1 is 2.86%, then the A5.1 is Ephedrine HCl. So it can be concluded that sample 5 is positive for Ephedrine HCl.

Figure 7. Sample Chromatogram 6

A6: Sample Test Solution 6
B6c: CTM Positive Control Solution Sample 6
B6e: Ephedrine HCl Positive Control Solution Sample 5
Cc: CTM Comparator Solution
Ce: Ephedrine HCl Comparator Solution
Based on the study results on sample 6, the A6 solution produced one spot, namely A6.1 with Rf 0.71. The B6c solution produces two spots, B6c.1 with Rf 0.31 and B6c.2 with Rf 0.71. The B56 solution produces one spot, B6e.1, with an Rf of 0.71. The C6c solution produces patches with an Rf of 0.33. The C6e solution produces spots with an Rf of 0.7. The difference between hRf of B6c.1 and hRf C6c is 6.06%, then B6c.1 is CTM. The difference between hRf spot B6e.1 and hRf C6e is 1.43%, then the spot B6e.1 is Ephedrine HCl. There is no difference between hRf spot A6.1 and hRf B6e.1, so spot A6.1 is Ephedrine HCl. So it can be concluded that sample 6 is positive for Ephedrine HCl.

CONCLUSION
Based on the results of this study, 5 out of 6 samples are indicated to contain Medicinal Chemicals (BKO), namely samples I, II, III, V, and VI. Samples 1 and 3 are positive for CTM and Ephedrine HCl. Samples 2, 5, and 6 are positive for Ephedrine HCl only. Sample 4 does not contain Medicinal Chemicals, either CTM or Ephedrine HCl. The suitable composition of the motion phase is a mixture of Chloroform – Methanol – aquabidest with a ratio of 2:7:1.

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