



## Determination of Dexamethasone in Unregistered Herbal Weight Gain Using HPTLC-Densitometry

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**Abstract.** A method was described for the simultaneous determination of dexamethasone in herbal weight gain. Three unregistered herbal weight gains (sample A, B, and C) were analyzed by using HPTLC-densitometry method. Samples were extracted as bases into methanol, separated by HPTLC silica gel 60 F<sub>254</sub> plate using chloroform: methanol (9:1) as mobile phase followed by densitometry measurement of its spot. The result showed that the detector response was linear for concentrations between 100-500 µg/mL ( $r = 0.998$ ). The limits of detection and quantitation were 9.19 µg/mL and 30.64 µg/mL, respectively. Dexamethasone contents from samples were analyzed. The result showed that two samples (sample A and B) were positively containing dexamethasone and the other one showed a negative result. The average contents of dexamethasone from both samples were 0.23% and 0.25%, respectively.

**Keyword:** Dexamethasone, Herbal, HPTLC-Densitometry, Weight Gain.

**Abstrak.** Sebuah metode telah dideskripsikan untuk penetapan deksametason dalam herbal penambahan berat badan. Terdapat tiga jenis herbal penambah berat badan yang tidak terdaftar (sampel A, B, dan C) dianalisis menggunakan metode HPTLC-densitometri. Sampel diekstraksi sebagai basis ke dalam metanol, dipisahkan menggunakan HPTLC silika gel 60 F<sub>254</sub> dengan kloroform: metanol (9: 1) sebagai fase gerak dan masing-masing noda diukur menggunakan densitometer. Hasil penelitian menunjukkan bahwa respon detektor linier terhadap konsentrasi antara 100-500 µg / mL ( $r = 0,998$ ). Batas deteksi dan kuantisasi masing-masing adalah 9,19 µg / mL dan 30,64 µg / mL. Kadar deksametason dari kedua sampel dianalisis. Hasil penelitian menunjukkan bahwa dua sampel (sampel A dan B) positif mengandung deksametason dan yang lainnya menunjukkan hasil negatif. Kadar rata-rata deksametason dari kedua sampel masing-masing adalah 0,23% dan 0,25%.

**Kata Kunci:** Deksametason, Herbal, HPTLC-Densitometri, Penambah Berat.

Received [9 August 2018] | Revised [24 October 2018] | Accepted [26 November 2018]

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## 1. Introduction

The Indonesian traditional medicine refers to Jamu, a specific Javanese term which is predominantly herbal medicine made from natural materials, such as plant material including roots, bark, flowers, seeds, leaves and fruits. Animal materials are also often used, such as honey, milk, and eggs. It is commonly embraced in Indonesia to both maintain health [1] and treat specific health problems [2,3]. Herbal medicine has long been used for the prevention and treatment of diseases since the beginning of human civilization. To date, they remain as the primary source of healthcare for approximately 80% of the world's population [4] and the demand for these products is increasing in developed countries every year [5]. Herbal is widely used for treatment because it is generally cheaper than synthetic drugs and allegedly did not give unwanted side effects as is the case with synthetic drugs.

Nowadays, the increasing use of herbal medicine from year to year in the community has encouraged more traditional medicine industries and herbs to produce herbal medicines. The large number of traditional medicine industries make a difficulty for the government to supervise the quality and circulation of this product. This phenomenon encourages manufacturers to commit fraud by increasing the sale of their products, one of which is by adding medicinal chemicals in herbal medicine so that the desired properties are easily achieved [6]. According to the regulation of the Minister of Health, traditional medicine should not contain synthetic chemicals, the result of isolation is efficacious as drugs or narcotics, which are allowed as additives are substances that are not efficacious as a medicine [7]. Herbal medicine must be registered with the BPOM, Indonesian Agency for Food and Drug Administrations, special registration number.

The monitoring results in the market, there are many herbs which included indications that these herbs have medicinal properties. One of the drugs commonly added to the herbal weight gain is dexamethasone. Dexamethasone is a strong corticosteroid with immunosuppressant and anti-inflammatory properties used to treat various inflammatory conditions [8]. The therapeutic meaning of corticosteroids lies in antiflogistic (antireumatic), allergic, and immunosuppressive [9]. Dexamethasone is also used to gain weight so that manufacturers often add dexamethasone to herbal preparations, because of the demand of consumers who want to gain weight at a low cost and in a short time. This drug should only be used in medical supervision because it can increase blood pressure and blood sugar levels, decrease the immune response and the risk of bone loss. The FDA states that one of the herbs from China contains dexamethasone, with side effects seen as moonfaced [10].

This has become one of the considerations of this study to determine whether there was a dexamethasone that was added to the herbal medicine sold in the community. Furthermore, the number of unregistered herbal weight gain led to allegations addition of synthetic drugs into it.

## **2. Materials and Methods**

### **2.1. Sample collection and preparation**

The herbal weight gains were collected and purchased from drug stores in Padang city. From ten collected herbal weight gains, three herbals were not registered with BPOM without special registration number and these samples (sample A, B, and C) were used for dexamethasone analysis. Twenty capsules from each sample were carefully removed the cap and very gently emptied the capsule powder contents directly into a suitable container. These powders were grinded and sieved with a mesh width 500  $\mu\text{m}$  and these sieved powders were used for the further analysis.

### **2.2. Chemicals and Reagents**

HPTLC analyses were performed on Merck 20 cm  $\times$  10 cm (0.25 mm) plates. Dexamethasone used as standard material was purchased from Tianjin Tianyao Pharmaceutical Company. All the reagents used in the experiment were of analytical grade and were supplied by Merck, Darmstadt, Germany.

### **2.3. Preparation of Sample**

A weight of 250 grams of each herbal powders was accurately weighed then it was taken in 25 mL volumetric flask and subsequently methanol was adjusted to the mark. The solution was shaken and filtered by using Whatman No. 41. Each filtrate was used for analysis.

### **2.4. Preparation of Standard Solution**

A weight of 25 mg of standard dexamethasone was accurately weighed, quantitatively transferred into a 25 mL volumetric flask, dissolved in methanol and the volume was adjusted with the same solvent.

### **2.5. Calibration curve**

The calibration curves were constructed using standard solution of dexamethasone. The standard solution was applied to the plate corresponding to a concentration of 100, 200, 300, 400, 500  $\mu\text{g/mL}$  for HPTLC, and chromatograms for each concentration were obtained by reading the plate using a densitometric scanning (CAMAG<sup>®</sup> TLC scanner 4 and the WinCATS<sup>®</sup> 4.3 software). The calibration curve was constructed by plotting peak area against concentration.

### **2.6. Chromatographic Procedure**

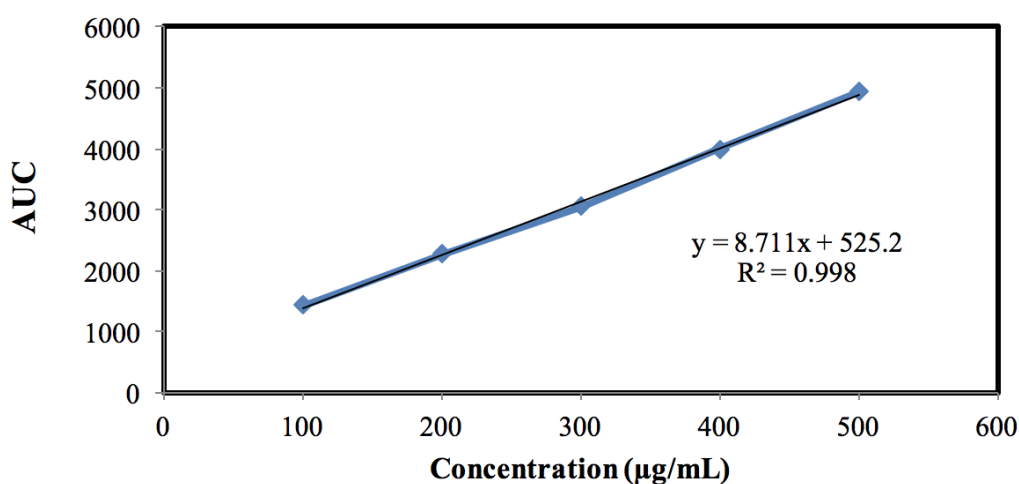
2  $\mu\text{L}$  of samples and standard solutions were applied as bands of 4 mm wide, 0.3 mm high and 6 mm apart in the form of bands on pre-coated HPTLC silica gel plates 60 F<sub>254</sub> (20 cm  $\times$  10 cm with 250  $\mu\text{m}$  thickness) by means of band applicator CAMAG Linomat 5<sup>®</sup> sample applicator equipped with a 100  $\mu\text{L}$  Hamilton<sup>®</sup> syringe. Samples were applied at 15 mm from the bottom

edge of the chromatographic plate. The plates were allowed to dry for 15 minutes before elution using a solution of chloroform: methanol (9:1) as mobile phase. The developing distance was 85 mm, measured from the lower edge of the plates. The plates were allowed to dry for 15 minutes for visualization of the spots using CAMAG® UV Cabinet dual wavelength, 254/366 nm. Migration distances were measured and retention factors ( $R_f$ ) were calculated. Chromatograms were obtained by reading the plate using a densitometric scanning (CAMAG® TLC scanner 4 and the WinCATS® 4.3 software). Samples extracts are spotted in triplicate. Evaluation was done by peak area measurement with linear regression  $y = 8.711x + 525.2$ .

### 3. Results

#### 3.1. Calibration Curve Data of Dexamethasone

The result of calibration curve using standard solution of dexamethasone was shown in figure 1.



**Figure 1.** Standard calibration curves for dexamethasone using HPTLC-densitometry (The plot was linear in the range of 100-500 µg/ml ( $y = 8.711x + 525.2$ ,  $R^2 = 0.998$ )).

#### 3.2. Dexamethasone Content of Herbal Weight Gain

The result of dexamethasone content from herbal weight gain was shown in table 1.

**Table 1.** The Result Analysis

No	Dexamethasone Content	
1.	Standard deviation	26.6940 µg/mL
2.	LOD	9.1932 µg/mL
3.	LOQ	30.6440 µg/mL
4.	Sample A	0.23 % ± 0.032
5.	Sample B	0.25 % ± 0.027
6.	Sample C	not detected

#### 4. Discussions

The analysis of dexamethasone content of herbal weight gain has been studied. The herbal weight gains were collected and purchased from drug stores that distributed in Padang city, West Sumatera, Indonesia. Ten samples of herbal weight gain were collected. From ten collected herbal weight gains, three herbals were not registered with BPOM without their special registration number and these samples (sample A, B, and C) were used for dexamethasone analysis. The objective of present study was to determine whether there was a dexamethasone that was added to the unregistered herbal medicine sold in Padang. The dexamethasone contents of herbal weight gains were evaluated and quantified by using HPTLC-densitometry method. In these studies, the methods were used by three replicate studies. HPTLC has recently been becoming more significant for quality control of some drugs. The availability, sensitivity and low cost are the advantages of this method [11]. In the proposed method pre-coated HPTLC silica gel plates 60 F<sub>254</sub> was used and the optimum conditions for analysis dexamethasone by HPTLC was chosen experimentally by considering the effects of several factors such as the solution concentration, the ratio of solvents in the eluent and the type of HPTLC plates. The solvent system used was system chloroform: methanol (9:1) which gave good resolution and good chromatogram with R<sub>f</sub> value  $0.26 \pm 0.05$ . A satisfactory separation was obtained permitting simple and fast scanning of the components on the plates directly under UV-densitometer. The results showed linear relationship between the peak areas and the concentrations is shown in Figure 1.

Qualitative analysis of samples using the HPTLC method was obtained the average R<sub>f</sub> value in the samples which was  $0.26 \pm 0.05$  for sample A and B. this R<sub>f</sub> value is equal to the R<sub>f</sub> value of the dexamethasone standard which means both samples contain dexamethasone. Whereas in sample C, dexamethasone was not detected as indicated by the absence of spot on the HPTLC plate. Dexamethasone contents were analyzed by fast scanning of the components on the plates directly under UV-densitometer. The result showed that the average contents of dexamethasone from both samples (A and B) were  $0.23\% \pm 0.032$  and  $0.25\% \pm 0.027$ , respectively. From the result showed that the dexamethasone contents that were added to the samples have nearly the same levels of about 0.23-0.25 %. According to the Regulation of the Minister of Health, traditional medicine should not contain synthetic chemicals [12].

The use of herbal is usually recommended 3 times a day with brewed with 250 ml of water each sachet, meaning if a pack of herbal weight gain contains approximately 0.23-0.25% of dexamethasone. This is likely to cause the user or customer to think that she was taking the herbs that are effective with not aware of any additional substances into it. Dexamethasone is drugs known as corticosteroids who has strong anti-inflammatory effects. If used according to the rules, corticosteroid drugs have many uses, especially in inflammatory diseases. However, the side effects are quite extensive, among others, is to increase blood sugar levels (increasing

the risk of diabetes), bone loss (osteoporosis), inhibits the growth of children, cause fat in certain body parts (round face/ moon face, shoulders like humped), causing red streaks in the abdomen (striae), land owered immune system so prone to infection [13]. Uncontrolled usage of unregistered herbal weight gain containing dexamethasone can certainly increase health risks for patients and BPOM, Indonesian Agency for Food and Drug Administrations, must increase supervision of herbal products circulating in the community.

## 5. Conclusions

There was a synthetic drug that was added to the herbal medicine namely jamu sold in Padang. Of 3 herbal weight gains examined, two were found to the addition of dexamethasone. The obtained results suggest that the authorities more intensively to monitor the manufacture and distribution of jamu and herbal medicines especially for unregistered herbal medicines.

## Acknowledgments

We are grateful for the technical assistance of Faculty of Pharmacy, Andalas University, Padang for supporting this project.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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