Identification of glibenclamide in antidiabetic jamu by high performance liquid chromatography method: Study in Purwokerto, Indonesia

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Abstract. Adulteration of synthetic pharmaceutical drugs in jamu is prohibited by law in Indonesia. Glibenclamide is one of the drugs commonly added to antidiabetic jamu. The purpose of this study was to obtain a valid high performance liquid chromatography (HPLC) method for qualitative and quantitative analysis of glibenclamide in antidiabetic jamu. HPLC separation was carried out with a Kromasil 100 C18 column (150 x 4.6 mm i.d, 5 μm particle size) using methanol: water (75:25) v/v as the mobile phase at a flow rate of 0.5 mL/min, UV detection was set at 301 nm. There was no potential interference from other compounds at the glibenclamide retention time (retention time of 5.234 ± 0.056 minutes). The method has good linearity (r = 0.9936) in the range 10–50 µg/mL. The detection limit of the method was 6.21 µg/mL while the quantitation limit was 20.71 µg/mL. The relative standard deviation (RSD) of intraday precision was 0.85%. The average recovery using standard addition method was 100.63 ± 13.71%. The application of the HPLC method for the analysis of five antidiabetic jamu samples obtained from the market in Purwokerto showed that glibenclamide was detected in one sample with a level of 1.88 ± 0.25 µg/g.

1. Introduction
For more than three decades, the use of herbal medicines and supplements has increased very significantly. About 80% of people in the world use it to overcome their health problems [1]. Jamu, traditional herbal medicine is widely used in Indonesia. This traditional herbal medicine was prepared in such a way from many plants.

One problem with herbal products including jamu is that manufacturers illegally add synthetic drugs to their products [2]. Adulteration of synthetic pharmaceutical drugs in jamu is prohibited by law in Indonesia. An examination of distributed jamu thus becomes an important issue to prevent harmful side effects due to adulterated herbal medicine.

Glibenclamide as an adulterant in antidiabetic herbal medicine has been studied. The study in Hong Kong found the use of 29 antidiabetic herbal products that were adulterated [3]. Glibenclamide is a member of the second generation antidiabetic sulfonylurea. The main action of this drug is by increasing insulin secretion [4].

Jamu as herbal products is complex sample matrices. Most methods available for illegal adulteration screening are usually time-consuming and may provide false positives [2]. Adulterant analysis requires a fast, selective and efficient method [5]. HPLC is an analytical method that meets these requirements.
Several HPLC methods have been reported to identify chemical drugs in herbal medicines such as analysis of prednisone in herbs using solid phase extraction (SPE) and HPLC [5], analysis of several analgesics in herbal preparations by HPLC with a UV detector [6], and analysis of sulfonylurea antidiabetic in health food by HPLC with gradient elution and using UV detectors [7]. The SPE-HPLC method requires a sample preparation stage using SPE that may not always be available in many laboratories. HPLC with gradient elution is more complex than isocratic elution. The purpose of this study was to obtain a valid high performance liquid chromatography (HPLC) method using simple isocratic elution for qualitative and quantitative analysis of glibenclamide in antidiabetic jamu.

Figure 1. Chemical structure of glibenclamide.

2. Material and method

2.1. Materials and instruments
The materials used were glibenclamide reference (PT. Mersifarma), HPLC grade methanol (Smart Lab), methanol pro analysis (Merck German), and aquadestilata (PT. Otsuka).

The instruments used in this study were an analytical balance (Shimadzu AY 220), pH meter (Hanna Instrument), ultrasonic bath (Branson 1510), a set of high performance liquid chromatography (ECOM HPLC S2000) with UV-Vis SPD 10.A detector (ECD Detector S2000), HPLC pump (ECP S2000), computer set (HP), 0.25 mL Hamilton injector, filtration unit for HPLC (Whatman), vacuum pump, membrane filter 0.45 μm and vortex.

2.2. Methods

2.2.1. Sampling. Jamu samples were collected from the local market in Purwokerto with a purposive sampling technique. The selected jamu are those which are indicated as antidiabetic. Five samples were obtained. The dosage forms obtained were two powders, two capsules, and two pills.

2.2.2. HPLC system. The HPLC system used was a reversed phase system with column C18 (Kromasil 100 C-18 5 μm 150-4.6 mm). The detector UV detector was used and set at 301 nm. The mobile phase used was a mixture of methanol and water (75:25) with a flow rate of 0.5 mL/minute. An injection volume of each sample was 20 μL.

2.2.3. Preparation of standard and sample solution. Glibenclamide standard stock solutions were made by dissolving 10 mg of glibenclamide standard in 10 mL of the mobile phase. Further dilution was done to obtain a concentration of 100 μg/mL. The sample (jamu) was weighed 0.1 grams and then put in a 10 mL volumetric flask and added a mobile phase. Then centrifuged for 10 minutes. The solution was injected into HPLC.

2.2.4. Mobile phase optimization. Optimization of the mobile phase composition was carried out by varying the ratio of methanol: water in the mobile phase 75: 25; 50: 50; and 25: 75 v/v.

2.2.5. Method validation. Glibenclamide solutions with a concentration series of 10 to 50 μg/mL were injected into HPLC for linearity testing. Detection limits (LOD) and quantification limits (LOQ) were obtained by statistical calculations through a linear regression line from the calibration curve of glibenclamide. Precision was tested by preparing 6 replicates of 30 μg/mL glibenclamide solution.
injected into HPLC. Based on the chromatogram obtained then the relative standard deviation (RSD) value was calculated. The accuracy was determined by the standard addition method. The sample was spiked by a known glibenclamide standard solution, then the percentage of recovery was determined.

3. Results and discussion

3.1. Wavelength selection
Based on the UV spectrum of glibenclamide, the wavelength of 301 nm was used as the detection wavelength. The spectrum is recorded at wavelengths of 200 - 400 nm.

3.2. Mobile phase optimization
The asymmetry factor is a parameter to indicate the tailing peak. Based on the data in Table 1, all the methanol-water comparisons show good result, because of the tailing factor < 2 [8]. The mobile phase mixture to be used in the further study was methanol-water with a ratio of 75:25 v/v.

Table 1. The results of the mobile phase composition optimization.

| Rasio methanol:water | Flow rate (mL/min) | Retention time (minute) | Asymmetry factor |
|----------------------|--------------------|-------------------------|------------------|
| 25:75                | 0.5                | 1.458                   | 1.475            |
| 50:50                | 0.5                | 2.778                   | 1.400            |
| 75:25                | 0.5                | 5.11                    | 0.219            |

3.3. Method validation
The selectivity of the method was evaluated by comparing chromatogram of the blank sample, glibenclamide standard, and sample. The glibenclamide peak has a retention time of around 5.234 ± 0.056 minutes and there is no potential interference from other compounds (figure 2).

Figure 2. Chromatogram for selectivity test. (a) Blank sample (free of glibenclamide), (b) Glibenclamide reference, and (c) sample.
Good linearity was indicated by an increase in peak area which is proportional to the concentration of the injected solution (figure 3). The concentration range used in this study were 10-50 µg/mL. Their value ($r = 0.9936$) indicates good linearity [8].

Limit of Detection (LOD) is the limit of the lowest concentration of an analyses in a sample that can still be detected, although it cannot always be calculated. Limit of Quantitation (LOQ) is the lowest limit of concentration of analyses in a sample that can still be quantitatively determined with acceptable precision and accuracy in operational conditions of the method used [9]. In this study, the LOD and LOQ values were 6.21 and 20.71 µg/mL, respectively.

Based on the results of injection of 6 replicates, the peak area, retention time, and tailing factor were obtained with RSD values <2%. These results indicate that the method used meets the precision test criteria as a valid analysis method due to RSD <2% [8].

The recovery value is used to describe the proximity of the results of the analysis to the true value [10]. Table 2 shows that the average recovery is 100.64% that meets the requirements [8].

| No | Standard added (µg/mL) | Standard found (µg/mL) | Recovery (%) |
|----|------------------------|------------------------|--------------|
| 1  | 20                     | 21.28                  | 106.41       |
| 2  | 20                     | 22.78                  | 113.89       |
| 3  | 20                     | 16.32                  | 81.61        |
| Mean |                      | 20.128                | 100.64       |
| SD  |                        | 2.759                 | 13.80        |
| RSD (%) |                    | 13.708                | 13.71        |

3.4. Method application
From five antidiabetic jamu samples showed that glibenclamide was detected in one sample with a level of $1.88 \pm 0.25$ µg/g. This can be said that 20% of the samples were adulterated with glibenclamide. Table 3 indicates that adulteration of synthetic drugs in traditional medicinal products such as jamu is often found.
Table 3. The results of adulterant analysis in several traditional medicine products.

| Sample                      | Adulterant                                         | Number of samples | Samples confirmed the presence of adulterants | Reference     |
|-----------------------------|----------------------------------------------------|-------------------|-----------------------------------------------|---------------|
| Jamu kuat lelaki            | Sildenafil Citrate                                  | 2                 | 1                                             | [11]          |
| Herbal antidiabetic products| Glibenclamide, phenformin, metformin, rosiglitazone, gliclazide, glimepiride, nateglinide, and repaglinide. | 29                | 29                                            | [3]           |
| Jamu pegal linu             | Paracetamol                                         | 8                 | -                                             | [12]          |
| Herbal slimming product     | Sibutramine                                         | 7                 | 6                                             | [13]          |
| Anti-diabetic jamu          | Glibenclamide                                      | 5                 | 1                                             | This study    |

A study in Hong Kong showed 27 cases in which 29 antidiabetic products were adulterated. Toxic effects and hypoglycemia are shown by patients who consume traditional antidiabetic medicines. Of the 19 antidiabetic herbal products, 8 products contain glibenclamide, phenformin, metformin, rosiglitazone, gliclazide, glimepiride, nateglinide, and repaglinide. Antidiabetic products that are adulterated with synthetic drugs that are not stated on the label are a significant problem [3].

4. Conclusions
The HPLC method was successful in clearly identifying and quantifying glibenclamide present at antidiabetic jamu. From five antidiabetic jamu, showed that one sample confirmed the presence of glibenclamide as an adulterant. This also calls for a thorough focus on making the regulation systems for this jamu stricter. The regulations related to licensing and labeling of jamu should be as strong as to ensure 100 % product integrity.

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