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Analysis of Prednisone in Indonesian Uric Acid Herbs Using High Performance Liquid Chromatography

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Abstract

The problem of adulteration of herbal medicines product with active pharmaceutical ingredients (API) has existed for years in Indonesia. According to the government's rules, it is not allowed to add API to traditional herbal medicine. Uric acid herbs are one of the most popular herbal medicine products. Prednisone is one of the corticosteroids that has been reported to be detected in herbal products. The purpose of this study is to identify prednisone in uric acid herbs using High Performance Liquid Chromatography (HPLC). The stationary phase used in this study was C18 Puroshper®STAR RP-18e LiChroCART® column (250 - 4.6 mm; 5 μm i.d.), while the mobile phase was methanol : water (60 : 40 v/v). The mobile phase flow rate was set at 1 ml/min. Prednisone in the sample was detected at wavelength 243 nm using a UV detector. Validation methods in this study consisted of precision, linearity, the limit of detection (LOD), the limit of quantitation (LOQ), and accuracy. The precision is indicated by the relative standard deviation (RSD) value of 0.33% (<2%). The correlation coefficient value (r) of 0.9955 obtained from the prednisone calibration curve shows the method's linearity. The recovery values of 100.11 ± 0.82 % indicates the accuracy of the method that meets the requirements. The LOD and LOQ values were 2.96 and 9.85 μg/ml, respectively. Method validation parameters have been proven that meet the requirements. The HPLC method can be used to analyze prednisone in uric acid herb samples. The application of the method for analysis of eight herbal products taken from the market shows that prednisone was detected in two products.

Key words : prednisone · uric acid· herbs · HPLC

1. Introduction

Jamu / Herbs is a herbal preparation, an Indonesian traditional medicine. Herbs that produced by the manufacturer has to be given label JAMU and a unique logo of jamu in the package. Based on Indonesia's regulations, traditional medicines are prohibited from containing isolated or synthetic medicinal chemicals. However, the existence of active pharmaceutical in herbal products is still found (1).

Steroid, including prednisone, was one of the most frequent adulterants. An examination of distributed jamu thus becomes an important issue to prevent harmful side effects due to adulterated herbal medicine.

Some methods reported have been published to analyze prednisone in medicinal herbal medicine products such as Thin Layer Chromatography (2); Solid Phase Extraction-High Performance Liquid Chromatography/ HPLC (3);Ultra-Performance Liquid Chromatography-Mass Spectrometry (4), and Gas Chromatography-Mass Spectrometry (5). The purpose of this study is to identify prednisone in uric acid herbs using simple High Performance Liquid Chromatography (HPLC) method with an ultraviolet detector.

2. Materials and Methods

The Separation was carried out on a set of HPLC instruments (Shimadzu Prominence-i LC-2030C) equipped with Puroshper®STAR C18 columns (250 mm x 4.6 mm i.d. 5μm). The absorption spectrum was made using a Shimadzu 1800 UV-VIS Spectrophotometer. The different brands of uric acid herbs were taken from several shops in Purwokerto, Central Java, Indonesia. Sample from eight different companies were coded as A, B, C, D, E, F, G, and H.

Preparation of the mobile phase **:** The mobile phase was made from a mixture of methanol and water at a ratio of 60:40 v/v. The mixture is then filtered and sonicated for 20 minutes.

Preparation of standard solution **:** The prednisone reference standard was carefully weighed as much as 10

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mg and put in a 10 ml volumetric flask, then dissolved with a mobile phase quantitatively to obtain a 1000 µg/ml solution. From there, the solution was pipetted 1.0 ml and put into a 10 ml volumetric flask, and then diluted quantitatively with a mobile phase so that a solution of 100 µg / ml is obtained.

Determination of the maximum wavelength of prednisone :The 10 µg/ml prednisone reference solution was scanned at a wavelength of 200 - 400 nm using a UV-Vis spectrophotometer. The wavelength with maximum absorption was determined from the spectrum obtained.

***Optimization of the composition of the mobile phase*:** Some mobile phase compositions used are: buffer pH 4 and methanol (80:20 v/v); buffer pH 4 and acetonitrile (80:20 v/v); methanol and water (50:50 v/v); and methanol and water (60:40 v/v).

***System suitability test* :** A prednisone standard solution of 25 µg/ml was prepared 6 times and then injected into HPLC with a volume of 20 µl. The mobile phase flow rate was set at 1.0 ml/min. Retention time, peak area, and tailing factor were recorded. Then the average, SD, and RSD were calculated.

***Preparation of prednisone calibration curve* :** Prednisone solution in the mobile phase with a concentration of 10; 15; 20; 25; 30 and 35 µg/ ml were prepared. Each solution was filtered and sonicated for 10 minutes. Then each of them was injected into the HPLC. The peak area shown on the chromatogram was recorded. The plot between prednisone concentration and peak area was made.

***Validation of analytical methods* :**The validation parameters of the tested analytical methods include selectivity, linearity, the limit of detection and limit of quantitation, precision, and accuracy. Validation was done according to ICH guidelines (6).

***Determination of prednisone in the sample* :** Uric acid herbs samples of brands A, B, C, D, E, F, G, and H were carefully weighed 150; 4500; 75; 40; 560; 187.5; 750; and 2500 mg, respectively. Then, put in a 10 mL volumetric flask, dissolved with the mobile phase, then filtered and sonicated for 20 minutes. The solution was injected into the HPLC. The determination was carried out in triplicate.

3. Results and Discussion

***The maximum wavelength of prednisone* :** Based on the UV absorption spectrum of prednisone, a wavelenght of 243 nm was used for the detection of the drug in this HPLC method.

***The optimum composition of the mobile phase* :** In this study, prednisone analysis in uric acid herbs was carried out by HPLC with a stationary phase of RP-18. Based on the results of the optimization of the mobile phase, the most optimal separation is produced by a mixture of the mobile phase of methanol : water (60:40 v/v).

***System suitability* :** System suitability is an integral part of the analysis procedure. The test is based on the concept that equipment, electronics, analysis procedure, and the samples to be analyzed are the whole system so that they can be evaluated (6). Table 1 shows the results of the system suitability test results. From repeated injection of prednisone solution in HPLC, the RSD values for the parameters of retention time, peak area, and tailing factor are <1.0%, respectively. The tailing factor of 1.639 shows the shape of the peak that meets the criteria of asymmetric aspects because its value is less than 2.0 (7). The system suitability test results show that the conditions used to determine prednisone levels have a good system suitability based on the RSD value <2% (7).

Table 1 : System Suitability

Injection No.	Retention time (min)	Area	*Tailing factor*
1	6.567	822,764	1.615
2	6.517	822,175	1.643
3	6.615	827,412	1.626
4	6.610	827,821	1.645
5	6.609	823,670	1.651
6	6.611	828,167	1.655
Average	6.588	825,334.83	1.639
SD	0.039	2,752.53	0.015
RSD (%)	0.594	0.33	0.944

***Prednisone calibration curve* :** Figure 1 shows a prednisone calibration curve made in the range of 10 - 35 μg/mL. The calibration curve obtained is used to calculate prednisone levels in the sample.

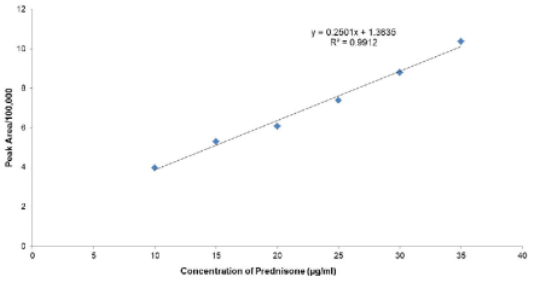

Fig. 1. Prednisone calibration curve

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Table 2 : Method validation result

Parameter	Result
Retention time	6.588 ± 0.039 minute
Linearity	r = 0.9955
Range	10-35 μg/mL
LOD	2.95 μg/mL
LOQ	9.85 μg/mL
Precision	RSD = 0.33 %.

Table 3: Accuracy of Prednisone Quantitative Analysis Method in Herbs

Sample	Standard added (μg/mL)	Standard found (μg/mL)	Recovery (%)	SD	RSD (%)
A	35	32.41	92.26	2.24	2.42
B	35	36.06	100.94	1.70	1.65
C	35	35.01	100.04	0.07	0.07
D	35	33.59	95.96	0.95	0.99
E	35	35.78	102.24	0.18	0.18
F	35	35.82	102.34	0.40	0.39
G	35	35.80	102.27	0.36	0.35
H	35	36.07	103.05	0.66	0.65

Validation of analytical methods **:** Figure 2 shows the selectivity of the HPLC method that meets the criteria.

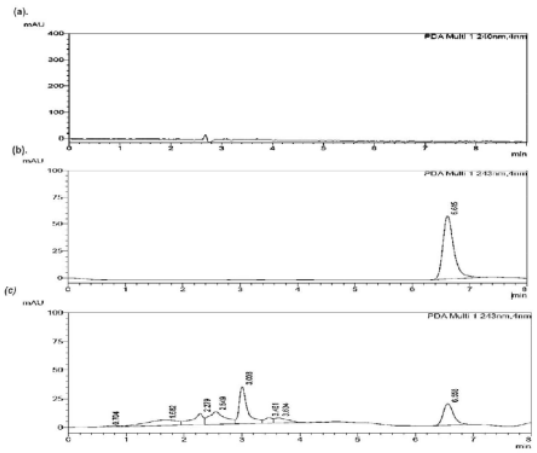

Fig. 2. Chromatogram of (a) Blank; (b). Prednisone reference; and (c) Uric acid herbs product.

Around the prednisone peak, which is retention time around 6, 5 minutes, there is no potential for interference from other components of the mobile phase or sample matrix.Table 2 shows the results of the analysis method validation. The prednisone retention time was 6.588 ± 0.039 minutes. Linearity is indicated by the correlation coefficient close to 1.0 (r=0.9955). The linear relationship between concentration and peak area was proven in the range of 10-35 μg/mL.The detection limit and the quantitation limit were determined by mathematical calculation of the calibration curve. The detection limit and the limit of quantitation are 2.95 μg/mL and 9.85 μg/mL, respectively. Precision is indicated by the RSD value of less than 2.0 %. Table 3 shows the accuracy of the HPLC method for Prednisone analysis in uric acid herbs. The recovery value is from 92.26 to 103.05% (average recovery 100.11 ± 0.82 %). The accuracy criterion for the analysis method is that the average recovery value is 100 ± 2% (7).

Table 4 : The results of the analysis of prednisone in uric acid herb products

Sample	Prednisone content (μg/mg)
A	n.d.
B	n.d.
C	21.76 ± 0.44
D	50.07 ± 0.30
E	n.d.
F	n.d.
G	n.d.
H	n.d

n.d. = not detected

The results of the analysis of prednisone in uric acid herb products **:** The results of the analysis of uric acid herbs samples shown in Table 4 shows that the adulteration of active pharmaceutical ingredients, especially prednisone, is still found in two products taken from the market in Indonesia. The presence of adulteration in this study is an addition to the previous findings. In previous studies it has been reported that in antidiabetic jamu still found the active pharmaceutical ingredients of glibenclamide (8). In "kuat lelaki" jamu found sildenafil as an adulterant (9), whereas in "pegal linu" jamu there was no paracetamol detected (10). Another study also reported that sibutramine as adulterant was detected in herbal slimming products collected from the market in Depok City, West Java, Indonesia (11).

The active pharmaceutical ingredients are also found in various herbal supplement products in several countries

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such as the presence of cyproheptadine and dexamethasone in weight gain product in Iran (12); sildenafil, tadalafil, and vardenafil hydrochloride in herbal medicine and food samples collected in Sultanate of Oman (13).

4. Conclusion

The HPLC method was successful in clearly identifying and quantifying prednisone present in uric acid herbs. From eight herbs, showed that two samples (25%) confirmed the presence of prednisone 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.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

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