ANALYSIS OF BKO CONTENT (ANTALGIN AND DEXAMETHASONE) IN HERBAL MEDICINE USING IODIMETRY TITRATION AND HPLC METHOD

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Abstract : Misuse of adding chemical drugs – BKO – into herbal medicines is often done by irresponsible people. The aim is to increase efficacy instantly, to attract consumer interest. BKO, which are often added to herbal medicine, are antalgin and dexamethasone. Antalgin content analysis was carried out quantitatively using the iodimetry titration method by conducting a preliminary test. The dexamethasone content analysis was carried out with qualitative and quantitative methods. The qualitative method was carried out using TLC. The quantitative method was carried out using the HPLC technique. The results obtained for the analysis of antalgin content of 10 samples were obtained two samples of herbal medicine containing antalgin, respectively 0.0749% and 0.1083%. Analysis of the dexamethasone content from 10 samples obtained 5 herbal samples containing dexamethasone respectively 0.0979%, 0.222%, 0.4521%, 0.5131% and 0.2809%. So based on these results, it is necessary to take action from the relevant institution regarding the discovery of the content of BKO in the herbal medicine on the market in Lombok.
Keywords : Antalgin, Dexamethasone, TLC, HPLC

Abstrak : Penyalahgunaan penambahan bahan kimia obat (BKO) kedalam jamu sering sekali dilakukan oleh oknum yang tidak bertanggung jawab. Hal ini dilakukan dengan tujuan meningkatkan khasiat secara instan, sehingga dapat menarik minat konsumen. BKO yang sering ditambahkan kedalam jamu adalah antalgin dan deksametason. Analisis kandungan antalgin dlakukan dengan cara kuantitatif menggunakan metode titrasi iodimetri dengan melakukan uji pendahuluan. Sedangkan analisis kandungan deksametason dilakukan dengan metode kualitatif dan kuantitatif. Metode kualitatif dilakukan dengan menggunakan KLT. Metode kuantitatif dilakukan dengan menggunakan teknik HPLC. Hasil yang diperoleh untuk analisis kandungan antalgin dari 10 sampel didapat 2 sampel jamu yang mengandung antalgin masing-masing sebesar 0,0749% dan 0,1083%. Analisis kandungan deksametason dari 10 sampel diperoleh 5 sampel jamu yang mengandung deksametason masing masing sebesar 0,0979%; 0,222%; 0,4521%; 0,5131% dan 0,2809%. Sehingga berdasarkan hasil tersebut maka perlu dilakukan adanya tindakan dari instansi terkait perihal masih

ditemukannya kandungan BKO dalam sediaan jamu yang beredar di pasaran pulau Lombok.

Kata kunci : Antalgin, Dexametason, KLT, HPLC

Introduction

Herbal medicine is a legacy that has been used for the treatment and maintenance of health. Herbal medicine is one of the traditional medicine that has developed widely in many countries. Based on research conducted by Vera et al. (2018) shows that there are many types of medicinal plants used by the community. For example, for one area in the Pulo Seunong community, Pidie, Aceh used 79 species of medicinal plants spread into 40 families. So it can be said that Indonesia has a diversity of plants that can be used as plants that have medicinal properties. Using herbal medicine as an alternative to modern medicine in the community is quite high. This is caused by the rapid development of modern medicine and the era of globalization; most of the people still using herbal medicine to maintain health and cure disease (Andriati and Wahjudi, 2016). The use of herbal medicine has an impact on increasing the level of public confidence in consuming rather than using synthetic drugs (Saputra, 2015).

According to the Minister of Health Regulation No. 003 / MENKES / PER / I / 2010 clause one concerning the identification of herbal medicine in research based on health services, traditional medicine is an ingredient or ingredient derived from plants, animals, minerals, galenic preparations, or mixtures of these ingredients which has been traditionally used for treatment based on previous experiences.

With the increased use of traditional medicine, especially herbal additions, the result is often found to abuse Medicinal Chemicals (BKO) into herbal preparations. This was also confirmed by the Republic of Indonesia's Minister of Health (2012) Regulation number 6, which states that all types of traditional medicines are prohibited from containing chemical or synthetic medicinal products. This also contradicts the Regulation of the Minister of Health of the Republic of Indonesia (2012) Number 007 clause 7 Alinea 1 about drug registration, which states that traditional medicines are prohibited from containing chemical drugs resulting from isolation.

Abuse of adding BKO into herbal preparations is often identified for handling diseases such as rheumatism, pain relief, and aphrodisiac (Nurohmah & Mita, 2017). One of the herbs that are commonly used to relieve pain is herbal stiff to reduce pain, relieve muscle pains, fatigue, muscle, and bone pain, accelerate blood circulation, strengthen the immune system, and relieve pain throughout the body. Based on several cases of stiff BKO in herbal medicine that successfully disclosed the Drug and Food Control Agency (BPOM), the most common BKO is antalgin. Antalgin is a methanesulfonate derivative of amidopyrine that works on the central nervous system, which reduces the

sensitivity of pain receptors and affects the body's temperature-regulating center. Three main effects of antalgin are analgesic, antipyretic, and anti-inflammatory (Fatimah, Rahayu, & Indari, 2017).

Besides antalgin, medicinal products that can be obtained easily is dexamethasone. Dexamethasone is usually used to treat pain in the body. These drugs include synthetic corticosteroid drugs, which have very strong anti-inflammatory potential (Budiarti & Faza, 2018). This anti-inflammatory effect can treat pain in the body so that many abused are mixed in herbal preparations.

The existence of the misuse of BKO in herbal preparations is what makes the main reason for analysis BKO in herbal preparations. The herbal medicine samples were taken in several areas on the island of Lombok, West Nusa Tenggara Province.

Research Methods

The method used in this research is experimental. The samples used were herbal medicine samples taken using a purposive sampling method on the island of Lombok.

Materials and Tools

Materials used in this research are herbal stiff, standard antalgin, standard dexamethasone, aquadest, methanol, acetonitrile, ethanol, chloroform, silver nitrate, hydrochloric acid, 1% starch solution, iodine solution. The tools used are HPLC, micropipette, analytical balance, TLC plate, chamber, UV lamps 254 and 366, and burette.

Research Procedure

Antalgin content analysis

The antalgin content analysis procedure was adapted from Banureah (2009). In the standard antalgin preparation, as much as 400 mg of standard antalgin was taken into an Erlenmeyer and then added 50 ml of aquadest, add 5 ml of HCl 0.2 N shake and filtered. At the preparation stage of the herbal sample, 400 mg of sample is taken, added 50 ml of aquadest, and add 5 ml of HCl 0.2 N than shaken and filtered.

The analysis method is divided into two parts, qualitative and quantitative method. The same treatment was carried out for both of the standard and the sample. In qualitative methods, results of each sample preparation and standard were taken as much as three drops, placed inside plate drops, and added $AgNO_3$ as much as five drops.

In quantitative analysis, the results preparation of standard and samples were taken as much as 5 ml, put into Erlenmeyer, 1 ml of 1% starch solution was added as an indicator. Titration with iodine solution and calculate the volume of iodine titration results (1 ml of 0.01 N iodine solution is equivalent to 16.67 mg).

The existence of BKO in the preparation of herbal medicine can be known by calculating the amount of antalgin with the following equation:

$$Antalgin\ level = \frac{V.N.\infty}{B} . 100\%$$ ………………………(1)

Information:
V = sample titration volume
N = iodine normality
∞ = 16.67 mg
B = sample weight

Dexamethasone content analysis

This research used the HPLC method for the analysis of dexamethasone content. The standard dexamethasone standard was diluted from 1000 ppm to 5 ppm, 3 ppm, 2 ppm, 1 ppm, and 0 ppm using a 25 mL ethanol solvent. This standard solution was injected into the HPLC instrument and measured at a wavelength of 254 nm to establish a dexamethasone standard curve.

The sample weighed as much as 25 mg, put in a measuring cup, and added 25 mL of ethanol. The sample was shaken for a few moments. 10.00 μL of the shaken sample was injected into the HPLC instrument. The mobile phase that has been used in the instrument is acetonitrile: water (7 : 3). The column used is a C18 column. The instrument was programmed with a running time of about 5 minutes at a wavelength of 254 nm. Chromatograms obtained were analyzed so that it can be known levels of dexamethasone in the sample.

Results and Discussion

BKO content analysis needs to be determined for the possibility of its content in the preparation of herbal stiffness. This is needed because it is not allowed any BKO content in traditional or herbal medicine preparations. Antalgin and dexamethasone BKO are often misused to be added to aches and pains to accelerate the effects of pain relief or rheumatic pain, thereby increasing the value of herbal medicine sales. Herbal samples were taken in several areas on the island of Lombok. The sample is divided into 2 categories. A total of 10 samples were prepared for antalgin content analysis, and 10 samples were prepared for dexamethasone content analysis.

Antalgin content analysis

In the analysis of antalgin content, an analysis was carried out using qualitative and quantitative methods. Sample preparation was carried out by added 50 ml aquadest and 5 ml HCl 0.2 N, then shaken and filtered. It aims to dissolve antalgin in the samples. Identifying the presence of antalgin is done by the qualitative method. At this stage, the results of sample preparation are taken as many as 3 drops and put into a drip plate, then dripped with $AgNO_3$ and produced

a precipitate and purple color. The reaction mechanism that occurs between Antalgin and $AgNO_3$ is as follows:

$$SO_3^{2-} + Ag^+ \rightarrow AgSO_3 \ldots\ldots\ldots\ldots(2)$$

By adding more reagents, a precipitate form with the reaction:

$$[AgSO_3]^- + Ag^+ \rightarrow Ag_2SO_3 \ldots\ldots\ldots..(3)$$

The presence of purple sediment proves the existence of antalgin content in samples. From a total of 10 samples, 6 samples were thought to contain antalgin. Table 1 illustrates the results of antalgin content identification. Based on that table, there are six positive samples containing antalgin (A, B, F, G, H, and I). Based on the results obtained, the six samples that were suspected of containing antalgin were analyzed quantitatively using the iodimetry method.

Table 1. Results of the qualitative identification antalgin presence in herbal preparations.

Sample	Color of the solution	Sediment color
Standard antalgin	Purple	Purplish white
A	Pink	Pink
B	Purple	Purplish white
C	Pink	Red
D	Purplish pink	Purplish pink
E	Purplish pink	Purplish pink
F	Purple	Purplish white
G	Purple	Purplish white
H	Purple	Purplish white
I	Purple	Purplish white
J	Brown	Brown

The basic principle of the iodimetry titration method is emphasized in the presence of an oxidation reaction between iodine and a reducing agent, which has a weaker oxidation potential than the iodine-iodide system. Based on this, quantitative analysis to find out how much antalgin BKO content in herbal medicine is done using this method.

A total of 5 ml of sample was taken, added 1 ml of 1% starch, and titrated with I_2 0.1 N. The endpoint of the titration was marked by the appearance of a purplish colored solution. Based on the standard method in Pharmacopoeia Indonesia (1995) to analyze antalgin is used iodine 0.1 N. The reason for the choice of iodine 0.1 N is because, in this concentration, iodine has a strong color so that iodine can be an indicator for itself. The determination of Antalgin using iodimetric titration will give a clear color. Detection of the endpoint of this reaction is the appearance of blue colors. The reaction that occurs is as follows:

$$NaSO_3 + I_2 + H_2O \rightarrow NaSO_4 + 2HI \ldots\ldots\ldots..(4)$$

The reaction that occurs at this stage is the reaction of releasing hydrogen from antalgin, which acts as an oxidizer and reduces I_2 as a standard solution. Antalgin undergoes oxidation so that I_2 acts as a reducing agent that captures hydrogen. Based on the results of iodimetric titration, 2 samples containing antalgin were obtained, B and F. The results of the antalgin content test can be seen in Table 2.

Table 2. Quantitative results of antalgin tests using iodimetric titration.

Sample	Repetition	Antalgin levels (%)	Average (%)	Range
Standard Antalgin	1	0,5001	0,5001	$0,5001 \pm 0$
	2	0,5001		
	3	0,5001		
Sample B	1	0,0833	0,0694	$0,0694 \pm 0,024$
	2	0,0833		
	3	0,0416		
Sample F	1	0,0923	0,0968	$0,0968 \pm 0,047$
	2	0,125		
	3	0,123		

Based on table 2, sample B is a sample of herbal medicine that does not have a distribution permit from BPOM, and sample F has a distribution permit from BPOM. Quantitatively, both samples contained a BKO antalgin.

Research using the same method was conducted by Banureah (2009). The results obtained from this research are from 10 samples that used; all of that samples contain antalgin.

Analysis of dexamethasone content

A total of 10 samples from various brands have been analyzed. The dexamethasone content analysis was performed using qualitative (TLC) and quantitative (HPLC) methods. The results of the identification of dexamethasone content using the TLC method obtained 5 samples that were estimated to be positive containing dexamethasone. This is shown in Figure 1 which shows the results of TLC seen in UV lamps 254 and 366.
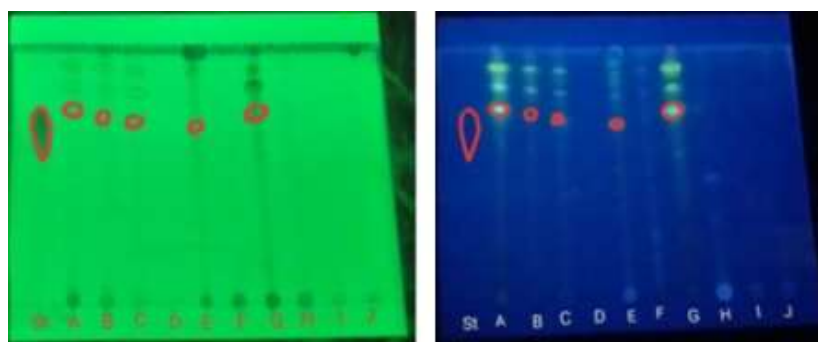

Figure 1. TLC results on UV rays 254 and 366, St = Standard Deksametason

Based on Figure 1, it can be concluded that some of the samples contain dexamethasone. This is since between the sample and the standard shows the location of the spotting position have the same Rf value. The similarity of the Rf values can be seen in Table 3.

Table 3. Rf value of the sample and dexamethasone standard

Sample code	Spot distance (cm)	Rf value
A	5,9	0,73
B	5,8	0,72
C	5,6	0,70
E	5,4	0,67
G	5,8	0,72
St	4,3 – 5,9	0,53 – 0,73

Based on the identification using TLC, samples A, B, C, E, and G were tested using the HPLC instrument to determine how much the content of BKO dexamethasone in herbal medicine samples. The analysis process using HPLC begins by injecting samples and dexamethasone standards into the instrument as much as 10.00 µL. The mobile phase has flowed through the column to the detector. The separation of mixed components in the column occurs because of the strength of the difference in the interaction between solutions to the stationary phase. Solutes that lack strong interaction with the stationary phase will come out of the column first, then be detected by a detector and recorded in the form of a chromatogram. The stationary phase used in this instrument is column C18 µm. This column can be applied for non-polar samples. The mobile phase used is acetonitrile: water (7 : 3), which is polar.

The standard concentration of dexamethasone used was 0 ppm, 1 ppm, 2 ppm, 3 ppm, and 5 ppm. The wavelength used in the measurement is 254 nm. The dexamethasone standard area values can be seen in Table 4.

Table 4. The value of the dexamethasone standard area

Concentration (ppm)	Area	Retention time (minute)
0	0	0
1	33527	1,51 – 2,20
2	76769	1,30 – 2,10
3	103729	1,40 – 2,00
5	219745	1,50 – 2,20

The standard curve of standard dexamethasone can be seen in Figure 2. Based on Figure 2, obtained the equation y = 43425x - 8780 and the value of $R^2 = 0.9815$. The resulting curve has good linearity because the requirement for knowing good linearity is that the value of R^2 must be close to 1 or equal to 1.
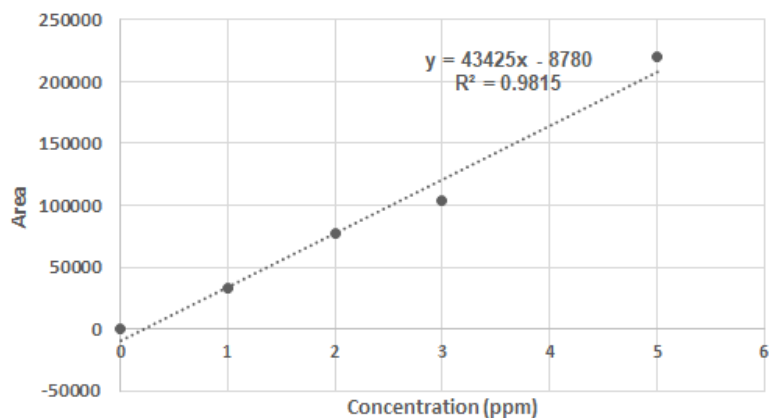
Figure 2. The dexamethasone standard curve.

The alignment of the regression model can be explained using the value of R^2. If the R^2 value is close to 1, it means the regression model is getting better. R^2 value has the characteristics of which are always positive. The maximum R^2 value is 1. The results obtained in this study have an R^2 value of 0.9815; it means that it has a good meaning of conformity.

After obtaining the line equation to determine the dexamethasone content in the sample, then testing is carried out to determine the area of the sample so that the "y" value can be obtained. The results of the analysis of the samples are then interpreted in the form of chromatograms.

Table 5. Data from sample analysis using HPLC

Sample code	Retention time (Minute)	Area
A	1,988	33734
B	1,573	87632
C	1,561	187540
E	1,522	214045
G	1,784	82700

Based on Table 5, to find out the dexamethasone content in the sample, the area value obtained entered as the "y" value in the line equation obtained previously. The dexamethasone level in the sample can be calculated using the line equation obtained based on the previous line equation. Table 6 shows the dexamethasone level in the sample.

Table 6. Data levels of dexamethasone in the sample

Sample code	Deksametason level	% Level
A	0,979	0,0979
B	2,220	0,222
C	4,521	0,4521
E	5,131	0,5131
G	2,107	0,2809

Similar research related to the analysis of dexamethasone content was also conducted by Aulia et al. in 2016. The research also used a more or less similar method and used the HPLC instrument. Based on the research from 3 test samples, 1 positive sample containing dexamethasone (2,220%).

Based on the data obtained in this research, the misuse of the addition of BKO (antalgin and dexamethasone) into herbal medicine preparations has still been found. This is contrary to the Regulation of the Health Minister of the Indonesian Republic Number 006 (2012) that BKO is not permitted to be added to herbal preparations.

Conclusions

Based on the research, it can be concluded that there is still an abuse of the addition of BKO, which is included in the herbal preparations. The results obtained for the analysis of antalgin content from 10 samples obtained 2 samples of herbal medicine containing antalgin, respectively 0.0749% and 0.1083%. The analysis of dexamethasone content from 10 samples obtained 5 samples of herbal medicine containing dexamethasone, respectively 0.0979%, 0.222%, 0.4521%, 0.5131%, and 0.2809%.

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