International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

The Application of UV-VIS Spectrophotometry Method to Identify Phenylbutazone in Herbal Packages Circulating in Big Cities in Indonesia

Dessy Abdullah[1], D. Yadi Heryadi[2], Miftahul Falah[3], Donny Hendriyanto[4], Loso Judijanto[5]*

1. Universitas Baiturrahmah, Indonesia.

2. Universitas Siliwangi, Indonesia.

3. Universitas Muhammadiyah Tasikmalaya, Indonesia.

4. Universitas Andalas Padang, Indonesia.

5. IPOSS Jakarta, Indonesia.

Abstract

The aim of this research is to find out how to analyze the chemical content of the drug phenylbutazone in herbal medicine using thin layer chromatography and UV-Vis's spectrophotometers. The samples in this study were obtained from several drug stores and traditional markets that sell herbal medicine. The sampling technique used was non-probability sampling using purposive sampling. A total of 13 samples of herbal medicine with different brands were taken with the inclusion criteria, namely herbal medicine in powder or capsule form, and herbal medicine indicated as herbal medicine for aches and pains or rheumatism without a BPOM registration number or with a BPOM registration number but not registered on the official BPOM website. Meanwhile, the sample exclusion criteria were herbal medicines in liquid form. Analysis was carried out using TLC. The conclusion drawn from the results of this research is as follows: There is one sample of tested herbal medicine that is suspected to contain BKO phenylbutazone, namely 0.3%. In analysis using TLC, there was one sample containing BKO phenylbutazone with an Rf value in three consecutive mobile phases of 0.5, 0.7, and 0.6. Meanwhile, in the analysis using UV-Vis's spectrophotometry, data was obtained in the form of maximum wavelength, absorbance, calibration curve equation, concentration for calculating precision tests, accuracy, and sample levels. In the quantitative analysis of phenylbutazone, it was found that the limit of detection (LOD) was 0.09 µg⁄mL and the limit of quantitation (LOQ) was 0.3 µg⁄m.

Keywords: Phenylbutazone, Medicinal Chemicals, Herbal Medicine, UV-VIS Spectrophotometry.

Full length article **Corresponding Author*, e-mail: dessyabdullah@fk.unbrah.ac.id

1. Introduction

In traditional medicine (OT), a component or mixture be it plant, animal, mineral, or extract preparations (galenic) is utilized for treatment and has been used for generations. It can be applied in accordance with socially acceptable criteria. Previous people have proven the effectiveness of OT, so OT is still used today. As one of the nation's cultural heritages that has been proven to bring many health benefits, herbal medicine, which is considered authentic Indonesian traditional medicine, must be preserved and developed [1]. In the current era, due to changes in lifestyle that tend to go back to nature and the effectiveness of modern medicines, the demand for medicinal plants is increasing, and the tendency of people to consume traditional medicines is increasing day by day. 80% of WHO member countries, with more than 90% of member countries in the Eastern Mediterranean, Southeast Asia, and Western Pacific regions, reported an increase in the use of traditional medicine among the public [2]. Driving factors for the use of traditional medicine are the increasing prevalence of chronic diseases, the lack of use of modern medicine for some diseases (such as cancer), and widespread access to information about traditional medicine throughout the world [3]. People have motivation to use herbal medicine. This motivation is related to people's trust in conventional medicine due to failure or uncertainty, fear of excessive use of chemical drugs, and surgery for several diseases after consuming these drugs.

This belief encourages people to look for alternative treatments that are considered more appropriate than conventional treatments. Due to this phenomenon, many traditional medicine producers have emerged because they see that the market share for traditional medicine, which is considered quite good and creates competition between business actors, is getting higher, so it cannot be avoided that there are some traditional medicine producers who do not apply CPOTB (good traditional medicine manufacturing methods). CPOTB involves all aspects of traditional medicine production so that the products produced meet quality requirements according to their intended use [4]. Because traditional medicine (especially herbal medicine) heals illnesses more quickly and is therefore more acceptable to the majority of people, irresponsible producers frequently take advantage of this phenomenon to boost sales by adding medicinal chemicals [5]. Medicinal chemicals (BKO) are the result of chemical synthesis from natural materials, which are generally used for modern treatment methods. The use of BKO in modern medicine must be accompanied by dosage, conditions on how to use it, and other provisions that must be followed to ensure the safety and health of the user. The addition of chemicals to traditional medicines is very dangerous because, apart from causing side effects, chemical medicines also have certain doses that must be adhered to so as not to cause toxicity effects [6]. In accordance with Minister of Health Regulation concerning Registration of Traditional Medicines, article (7) explains that traditional medicines are prohibited from containing several ingredients, one of which is medicinal chemicals, which are isolated or synthetic products with medicinal properties. In addition, Article 23 of Minister of Health Regulation Number 246 says that traditional medicines must meet certain criteria. These include having been tested in experiments to make sure they are safe and effective for humans, using raw materials that meet regulatory requirements, not having any synthetic chemicals or drugs in them (like isolates) [7]. The Food and Drug Supervisory Agency (BPOM) issued public warning, concerning traditional medicines containing medicinal chemicals. Based on this data, 50 traditional medicines were found to contain medicinal chemicals (BKO) [8]. The BKO contains phenylbutazone, allopurinol, dexamethasone, and so on. Apart from that, it was also reported that as many as 50 traditional medicines and health supplements contained medicinal chemicals and prohibited ingredients [9]. According to the data above, phenylbutazone is a medicinal chemical that is often found and used in traditional medicine. Phenylbutazone is a strong drug that must be used under a doctor's instructions. When used incorrectly, it can lead to harmful side effects like nephritis, hepatitis, stomatitis, mumps, inflammation of the parotid gland, problems with vision, low white blood cell count, low platelet count, agranulocytosis, aplastic anemia, erythema multiforme (Stevens-Johnson syndrome), epidermal necrolysis toxic (Lyell's syndrome), and pulmonary toxicity [10]. Phenylbutazone itself is an active substance with the physical characteristics of a white or slightly whitish crystalline powder, odorless, slightly soluble in water, easily soluble in acetone and ether, and soluble in ethanol. Phenylbutazone is a pyrazalone derivative nonsteroidal anti-inflammatory drug (NSAID) with strong anti-inflammatory properties and is effective in the treatment of acute gout through inhibiting the cyclooxygenase enzyme and the formation of inflammatory mediators. NSAID-class drugs are one of the treatments used for rheumatism or rheumatoid arthritis [11]. Rheumatism, or rheumatoid arthritis (RA), is a chronic inflammatory autoimmune disease that initially affects the joints and develops into larger joints, spreading to the skin, eyes, heart, kidneys, and lungs. Common symptoms of RA are morning stiffness in the joints affected by RA for >30 minutes, fatigue, fever, weight loss, tender, swollen joints, and rheumatoid nodules under the skin [11]. There is a trend towards an increase in the prevalence of non-communicable diseases, one of which is rheumatism, which shows a figure of 7%. This figure makes rheumatism the third most common disease suffered by Indonesians. Based on prevalence data, it cannot be avoided that traditional medicine for treating rheumatic diseases is becoming easier to find [12]. To carry out an analysis of the substances contained in it, several stages are required to be able to prove whether the herbal medicine being analyzed contains medicinal chemicals or not. The technique for identifying these substances is qualitative analysis using thin layer chromatography with a modified mobile phase mixture until optimal. TLC data will be obtained by calculating the Rf obtained in the experiment. Next, quantitative analysis was carried out using the UV-Vis spectrophotometer method by comparing the wavelengths of the test samples and standard samples, and detection was carried out under ultraviolet (UV) light at a wavelength of 254 nm [13]. There are three mobile phases that can be used to identify the presence of phenylbutazone in herbal medicine, including n-hexane: ethyl acetate with a concentration of 8:2, cyclohexane: chloroform: methanol: glacial acetic acid with a concentration of 60:30:5:5, and ethyl acetate: methanol: ammonia with a concentration of 85:10:5. However, this study did not optimize the mobile phase used and did not validate the assay method [11]. Another study carried out a determination of phenylbutazone levels in herbal medicine for aches and pains using the optimal mobile phase for detection, namely ethyl acetate: chloroform (2:1). However, this research only used one optimal mobile phase and did not use UV-VIS spectrophotometry as quantitative analysis, so it was necessary to optimize other mobile phases and use UV-Vis spectrophotometry as quantitative analysis [13]. In this research, qualitative analysis of phenylbutazone will be carried out using thin-layer chromatography with a mobile phase that has been optimized first, followed by quantitative analysis using UV-VIS's spectrophotometry [10]. The selection of sampling locations in East Jakarta for this study was based on locations that adequately represented the criteria for sampling. In previous research, it was said that people in one of the sub-districts in the city of East Jakarta use a lot and are quite familiar with the existence of traditional medicine. According to data, as many as 98% of people use traditional medicine because they have good knowledge of traditional medicine [12]. Traditional medicines are grouped into three categories, namely Jamu, which is a medicinal preparation made from natural ingredients, the safety status and efficacy of which have been proven empirically [9]. Herbal medicine circulating on the market must meet several requirements, including being safe in accordance with established requirements, making proven effectiveness claims based on experimental data, and meeting applicable requirements.

Standardized herbal medicines are preparations of ingredients that have been standardized for use in finished products that must meet safety and quality requirements in accordance with applicable requirements and scientifically proven efficacy claims. Standardized herbal medicines that are marketed must meet a number of requirements, including safety according to specified requirements, efficacy claims that are scientifically or preclinically proven, existing standards for the raw materials used in finished products, and meeting applicable quality requirements. Phytopharmaceuticals are natural medicinal preparations that have been standardized, the safety status and efficacy of which have been scientifically proven through clinical trials [11]. Phytopharmaceuticals on the market have to meet a number of criteria, such as being safe according to established standards, making claims of scientific or preclinical proof of efficacy, standardizing the raw materials used to make finished products, and being safe according to quality standards. The nonsteroidal anti-inflammatory drug (NSAID) phenylbutazone is a pyrazalone derivative that has strong anti-inflammatory properties [12]. It works to treat acute gout by stopping the cyclooxygenase enzyme and the production of inflammatory mediators. NSAID-class drugs are one of the treatments used for rheumatic diseases or rheumatoid arthritis. This pyrazolidine derivative has a basic formula like phenazone. Its anti-inflammatory properties are stronger than its analgesic properties. Therefore, this drug is specifically used for certain types of arthritis [13]. Phenylbutazone is often used illegally (without being stated on the label) in foreign mini-manufactured products or is often added to tonics (using ginseng) for lethargy and fatigue, muscle aches, and feelings of weakness [14]. Often, this drug is combined with corticosteroids, which are known to be very dangerous because the side effects of both, when combined, damage blood cells and weaken the immune system. The serious side effects caused include the blood and stomach, so many western countries have withdrawn it from distribution since the end of 1980 [15]. Rheumatoid arthritis (RA) is an auto-immune disease in the form of inflammatory arthritis in adult patients. There are three types of arthritis that many suffer from, namely osteoarthritis, gouty arthritis, and rheumatoid arthritis, which causes simultaneous swelling of lumps or inflammation in the joints [14]. Rheumatic diseases can be classified into two parts, namely diseases of the connective tissue that supports the body frame and internal organs. Conditions that can be grouped into this group are osteoarthritis, gout, and fibromyalgia. The second group is called autoimmune diseases because they occur when the immune system, which normally protects the body from infection and disease, begins to destroy the body's healthy tissue. Diseases that can be classified in this group are rheumatoid arthritis, spondylitis, systemic lupus erythematosus, and scleroderma [13]. Thin-layer chromatography is one of the simplest chromatographic methods that is widely used in analysis. The use of equipment and materials needed to carry out sample separation and analysis in TLC is quite simple, using a closed vessel (chamber) containing solvent and TLC plates [11]. Efficient separation and accurate quantification can be achieved by optimizing the method and using commercially available instruments. To obtain pure compounds from a mixture, it is necessary to carry out a process called the separation process. The type of stationary phase is a determinant of the separation process in chromatography because it determines the interaction that occurs between the analyte, the stationary phase, and the mobile phase used [15]. Analysis with a UV-Vis spectrophotometer is known as the main method used for determination, purity checking, and testing. Absorption spectroscopy is the measurement of the absorption of electromagnetic radiation of certain wavelengths, near monochromatic, by a substance. Absorbance measurements can be carried out in the ultraviolet region or in the visible region. The most commonly used visible light source in the visible spectrum is the tungsten lamp [14]. Tungsten, also known as tungsten, is a chemical element with the symbol W and atomic number 74. Tungsten has the highest boiling point (3400°C) compared to other metals. This tool is used as a light source. Samples that can be analyzed using this method are only colored ones [16]. This is a disadvantage of visible spectrophotometry. Therefore, for colorless samples, prior staining with certain reagents will produce colored compounds.

2. Materials and methods

The samples in this study were obtained from several drug stores and traditional markets that sell herbal medicine. The sampling technique used was non-probability sampling using purposive sampling. A total of 13 samples of herbal medicine with different brands were taken with the inclusion criteria, namely herbal medicine in powder or capsule form, and herbal medicine indicated as herbal medicine for aches and pains or rheumatism without a BPOM registration number or with a BPOM registration number but not registered on the official BPOM website. Meanwhile, the sample exclusion criteria were herbal medicines in liquid form. The standard ginger extract herbal medicine was made in duplicate by weighing 25 mg of ginger each, then extracted with 25 mL of ethanol solvent. Homogenized and added 10 ug/L phenylbutazone to one of the standard extracts that had been made, each filtered with filter paper. The known extraction results are that solution (1) is an herbal preparation without phenylbutazone as a control blank, and solution (2) is an herbal preparation with phenylbutazone. Phenylbutazone was weighed as much as 50 mg, then put into a 50-mL volumetric flask and dissolved with ethanol solvent until the limit mark. Each test herbal sample that was obtained was weighed at 5 mg and dissolved in 50 mL of ethanol solvent, homogenized, and then filtered using filter paper. Making the mobile phase with several comparisons of chloroform, ethyl acetate, methanol, n-hexane, and ammonia. Optimization of the mobile phase is carried out by cutting the TLC plate to a height of 6 cm and a width of 2 cm, depending on the amount of sample solution or standard solution to be analyzed. Before the TLC plate is used for sample testing, it is first placed in the oven for ±30–60 minutes at a temperature of 110 °C, then the sample solution or standard solution is applied using a capillary tube with a distance between spots of ±1 cm. Prepare 10 mL of the mobile phase (adjust to the chamber to be used), then the plate is eluted to a height of around 5 cm in a glass chamber that has previously been saturated with filter paper. The mobile phases used were chloroform, ethyl acetate, methanol, n-hexane, and ammonia in various ratios. 5 mobile phases were used, namely ethyl acetate: n-hexane (1:4), chloroform: ethanol (9:1), n-hexane: chloroform: methanol (6:3:1), ethyl acetate: chloroform (2:1), and ethyl acetate: methanol: ammonia (7:2:1).

The results of each standard solution with various mobile phases can be seen in UV light with a wavelength of 254 nm. Rf is calculated and recorded; the R_f value is said to be good if it is in the range of 0.3–0.7. Analysis was carried out using TLC by cutting a TLC plate to a height of 6 cm and a width of 10 cm. Before the TLC plate is used, it is first placed in the oven for ±45 minutes at a temperature of 110°C, then the sample solution or standard solution is applied using a capillary tube with a distance between spots of ±1 cm and a development distance of 5 cm each. We put the test solution and the reference standard, phenylbutazone, into the GF254 silica stationary phase. We then used an optimized mobile phase and a UV light spot detector with a wavelength of 254 nm to get the mixture out of the phase. TLC data will be obtained by calculating the Rf obtained in the experiment. The standard stock solution and standard solution, which is 5.0mg of phenylbutazone BPFI, are carefully weighed out and then put into a 50-mL volumetric flask. Ethanol solvent is then used to dissolve the substance until it reaches the line. A solution with a concentration of 100 ug/mL is obtained, which is hereinafter referred to as the mother solution. Take 5mL of the phenylbutazone stock solution with a concentration of 100 ug/mL, then put it in a 50mL volumetric flask, then add ethanol. Dilute the solution with the same solvent to the limit line, then shake until homogeneous to obtain a phenylbutazone solution with a concentration of 10 µg/mL. The absorption was measured at a wavelength of 300 nm. A standard solution was made in 650mL measuring flasks, each having a concentration of 3; 4; 5; 6; and 7 ug/L, and pipetted at 1.5; 2; 2.5; 3; and 3.5 mL of phenylbutazone stock solution. Put it into 550 mL measuring flasks and fill the volume with ethanol solvent. UV-VIS spectrophotometry at a set maximum wavelength was used to measure the absorbance. A standard solution of phenylbutazone with concentrations of 3, 4, 5, 6, and 7 ug/L was used. From the absorbance data, the standard curve equation can be calculated to obtain the line equation $y = bx \pm a$ with an r value that is close to 1 or >0.9. The standard herbal remedy for ginger extract was made in duplicate, as was done in the preparation of the reference standard for simulated phenylbutazone herbal medicine. UV-VIS's spectrophotometry was used to measure the samples at the longest wavelength of phenylbutazone. Pure ginger extract was used as a control. The levels that can be detected are calculated from the linear equation of the standard phenylbutazone calibration curve, and then the percentage recovery is calculated, which will be compared with the actual levels. When performing precision testing, the same analyst repeats the procedure under the same circumstances and within a short period of time, calculating the measurement results as the standard deviation or relative standard deviation (coefficient of variation). The LOD and LOQ values are determined by using the standard deviation data and slope of the calibration curve that have been obtained. By calculating the k value, the k value is 3 for the detection limit and 10 for the quantitation limit. Analysis of phenylbutazone in herbal medicine samples. The test solution and the reference standard phenylbutazone were put into the GF254 silica stationary phase. Three different mobile phases with a 5cm propagation distance were then used to elute the mixture. The detection was carried out under ultraviolet (UV) light at a wavelength of 254nm. The observation results were documented, and TLC data was obtained by calculating the R_f obtained in the experiment. Next, re-confirmation was carried out of the presence of phenylbutazone in the herbal medicine, which was suspected to be positive using UV-Vis's spectrophotometry. The wavelength of phenylbutazone was measured using UV-VIS's spectrophotometry and pure ginger extract as a blank. Calculated the actual level of absorbance obtained using the calibration curve of simulated herbal medicine containing phenylbutazone.

3. Results and Discussions

Optimization of the mobile phase was carried out by cutting the TLC plate to a height of 6 cm and a width of 2 cm. Before the TLC plate is used for sample testing, the plate is first baked for ±45 minutes at a temperature of 110°C. This heating aims to activate the silica plate to reduce the moisture of water adsorbed on the plate so that it does not interfere with the elution process that will be carried out. After the plate is activated, the standard solution is then applied using a capillary tube. Optimization of the mobile phase in the standard aims to obtain accurate measurement results so as to produce efficient separation. The mixed solvents used are chloroform, ethanol, ethyl acetate, methanol, n-hexane, and ammonia in various ratios. In addition, mixing two or more solvents with various ratios aims to obtain the optimum eluent. To determine the optimal mobile phase in the standard, 5 mobile phases were used in this study with different concentrations, namely ethyl acetate: n-hexane (1:4), as in previous research, chloroform: ethanol (9:1), n-hexane: chloroform: methanol (6:3:1), ethyl acetate: chloroform (2:1), and ethyl acetate: methanol: ammonia (7:2:1). From the results of the eluent optimization (Table 1) that has been carried out, analysis data is obtained, namely the Rf values of various eluent compositions. Based on the optimization results that have been carried out in this research, there are three mobile phases that will be used for the analysis of the BKO phenylbutazone compound, namely ethyl acetate: n-hexane (1:4), n-hexane: chloroform: methanol (6:3:1), and ethyl acetate: chloroform (4:1) with Rf values of 0.6, 0.8, and 0.76. The selection of the three mobile phases is based on a good Rf value, which is in the range of 0.3–0.7. In this research, simulated herbal medicine was made by making it in duplicate, namely simulated herbal medicine containing pure ginger, hereinafter referred to as the negative control, and simulated herbal medicine that was deliberately added with BKO phenylbutazone, hereinafter referred to as the positive control. The purpose of making this simulation of herbal medicine is to see the stain spots that are parallel to the BKO and the spots found in the positive control. This method is used as an indicator to ensure whether the method used is correct or not. The simplicial used to make simulated herbal medicine is the simplicial that is commonly used from several brands of herbal medicine for stiffness or rheumatism in circulation and comes from the simplicial of ginger powder, where the selection refers to previous research that used the simplicial of ginger powder as one of the components of simulated herbal medicine. The standard R_f value can be compared with the sample R_f value. After looking at the R_f value of the standard and herbal medicine samples, if the R_f value of the sample is found to be the same or close to the R_f value of the standard, it can be analyzed further.

The results of TLC identification on 13 brands of tested herbal medicine samples showed that samples F and H showed stains, and only sample H was close to the standard Rf value of phenylbutazone. The sample is declared positive if the Rf value is close to the standard Rf value or the sample Rf value is <0.05. This means that the UV-Vis spectrophotometric method will be used to test H samples that are thought to contain BKO phenylbutazone. Determining the maximum wavelength has the aim of determining the measurement wavelength so as to provide optimum absorbance. Finding this out is a key part of using the spectrophotometric method to analyze chemicals because it helps keep measurement errors to a minimum when doing multiple measurements and replications, which can happen when absorbance changes for each unit level. The measurement results showed that the highest standard lambda of phenylbutazone was found to be 270nm, with an absorbance of 0.6 at 10 ppm. This wavelength does not differ much from the provisions stated in the Indonesian Pharmacopoeia VI, namely that the maximum absorption is approximately 260nm, which differs by no more than 2.0%. Next, wavelength measurements were carried out on the positive control and negative control simulation herbal solutions. Based on the measurement results, the maximum lambda of the positive control simulation herbal medicine was 267 nm with an absorbance of 0.7 for a concentration of 10 ppm. Judging from the results obtained in this study, the maximum lambda has a difference of 0.2 nm. A change in the pH of the solution causes the maximum absorption to shift. The shift to the left is caused by the pH of the solution being slightly acidic, and the shift to the right is caused by the pH of the solution being slightly basic. As a result, the pH of the ginger solution affects the lambda shift that the solution experiences. The determination of a calibration curve from the phenylbutazone standard was carried out to determine the relationship between the solution concentration and the absorbance value. So that the concentration of the solution to be tested can be known. The standard curve will be a straight line if the Lambert-Beer law is fulfilled. In this study, the standard curve was determined at concentrations of 3 ppm, 4 ppm, 5 ppm, 6 ppm, and 7 ppm. This is done so that the resulting absorbance value meets Lambert-Beer requirements, namely an absorbance number between 0.2 and 0.8. After obtaining the absorbance value of each concentration, a standard curve was then created from the phenylbutazone standard. Linearity is measured through a calibration curve by plotting the measured absorbance values with standard solution levels. From the linear regression equation obtained, the correlation coefficient (r) value is calculated, and the resulting value is the calibration curve, which shows the linear relationship between the two variables. The phenylbutazone calibration curve equation in this study with a range of 3–7 ppm was 0.9, and the R2 value obtained was 0.9. This value meets the minimum requirements for the r value, namely being close to 1. The sensitivity of an analytical method can be expressed in terms of the limit of detection (LOD) and the limit of quantitation (LOQ). The detection limit is the smallest analyte concentration in a sample that can be detected, while the quantitation limit is the lowest analyte concentration that can be quantified accurately and thoroughly. Based on the test results, the LOD value was 0.09 µg⁄mL and the LOQ value was 0.3 µg⁄mL. If the standard concentration in the sample is less than or below the LOD value, then the method cannot detect the sample and has a high error result, while the LOQ value can be said to be good because the concentration value of the sample tested is above the LOQ value. Based on the LOD and LOQ values obtained, these values show that this method is capable of detecting levels in the analyte of 0.09 µg⁄mL, and the value of the analyte that can be quantified with precision is above 0.3 µg⁄mL so that it can be accepted for accuracy and precision. The relative standard deviation (RSD) determines whether precision tests are necessary to ascertain whether the instrument response to an analyte is consistent and repeatable over time. In this research, the analytical method of precision testing is expressed in terms of repeatability. From the observed levels obtained, the average value is calculated from the square of the difference between the actual levels and the observed levels. The results of 10 repetitions at a concentration of 5 ppm obtained an average value of 3.908 x 10-4 with an SD value of 0.007 and an RSD of 0.13%. The coefficient of variation value is said to provide good precision if the value is <2%, so that the accuracy of the tool obtained in this research is 99.9%. An accuracy test is a parameter that shows how close the analysis results are to the actual analyte content, usually expressed as a percentage or % recovery. The accuracy method is still considered good if the percentage of recovery is still within the specified range. There are two ways to determine accuracy when determining drug substance levels: the simulation method (spiked-placebo recovery) and the conventional addition method. In the addition method, an herbal solution with a concentration of 100 ppm is made with 5 repetitions. The concentration of the simulated sample is measured first, and then a 10-ppm standard is added to the simulated sample. Accuracy test data shows that the average obtained in this study was 96%. This method provides good accuracy. This study used one sample that was suspected to be positive based on the results of qualitative tests using TLC. To measure sample levels, 1 gram of the test herbal medicine is required and dissolved in 50 mL of ethanol, then filtered to obtain the filtrate. Next, UV-Vis's spectrophotometry is used to measure the filtered test herbs at 267 nm, which is the longest wavelength of phenylbutazone. Level measurements were carried out five times to minimize errors. Based on the test results, the concentration weight in mg was 2.5 mg, and in percent, it was 0.25% with a concentration of 4.9 ppm. The results show that there are still herbal medicines for gout or rheumatism that are suspected to contain phenylbutazone and are circulating in East Jakarta. According to Minister of Health Regulation Number concerning Registration of Traditional Medicines, Article (7) explains that traditional medicines are prohibited from containing many ingredients, one of which is medicinal chemicals from decomposition, compilation, and synthesis processes. medical properties. Apart from that, in the Minister of Health Regulation Number 246, Article 23, it is explained that traditional medicines must fulfill one of the requirements, one of which is that they do not contain synthetic chemicals or isolates with drug-like effects and do not contain ingredients that are dangerous to health and are classified as hard drugs or narcotics.

Table 1: Phenylbutazone Mobile Phase Optimization Results.

Movement Phase	R_f Score
Ethyl Acetate: N-hexane (1:4)	0.6
Chloroform: Ethanol (9:1)	0.9
N-hexane: Chloroform: Methanol (6:3:1)	0.8
Ethyl Acetate: Chloroform (2:1)	0.87
Ethyl Acetate: Methanol: Ammonia (7:2:1)	0.95

4. Conclusions

The conclusion drawn from the results of this research is as follows: There was one sample of the tested herbal medicine that was suspected to contain BKO phenylbutazone, namely sample H, and the sample H concentration was found to be 0.25%. In analysis using TLC, there are 3 mobile phases, which will then be used on the test herbal medicine samples, namely ethyl acetate: n-hexane (1:4), n-hexane: chloroform: methanol (6:3:1), and ethyl acetate: chloroform (4:1). It is suspected that there is one sample containing BKO phenylbutazone with an Rf value in three consecutive mobile phases of 0.5, 0.7, and 0.6. Meanwhile, in the analysis using UV-Vis's spectrophotometry, data was obtained in the form of maximum wavelength, absorbance, calibration curve equation, concentration for calculating precision tests, accuracy, and sample levels. In the quantitative analysis of phenylbutazone, several data were obtained, including the value of the correlation coefficient (r) of 0.9, the standard deviation (SD) of 0.007, the relative standard deviation (RSD) of 0.13%, the test recovery (% recovery) of 96%, the limit of detection (LOD) of 0.09666 μg⁄mL, and the limit of quantitation (LOQ) of 0.3 μg⁄mL. These data have met the requirements according to existing literature. The suggestion in this research is that future researchers need to carry out further sample testing on herbal medicine for stiffness and rheumatism circulating in the community by using more specific analysis techniques or choosing a different sampling location.

References

[1] A. Andriati, & R. T. Wahjudi. (2016). Tingkat penerimaan penggunaan jamu sebagai alternatif penggunaan obat modern pada masyarakat ekonomi rendah-menengah dan atas. Masyarakat, Kebudayaan dan Politik. 29 (3): 133-145.

[2] D. Hendriyanto, S. Rahman, & A. Elliyanti. (2023). Molecular Targets of Thymoquinone Protection Against Cisplatin-Induced Organ Toxicity: A Literature Review. International Journal of Chemical and Biochemical Sciences. 24 (4): 79-86.

[3] H. Fabiani, S. Ndraha, H. T. Tan, F. Wician, & M. Santoso. (2012). A Deterioration of Anemia in Hemoglobin E Disease caused by Cholestatic Hepatitis A. The Indonesian Journal of Gastroenterology, Hepatology, and Digestive Endoscopy. 13 (3): 185-188.

[4] H. Aprilia, & Y. Lukmayani. (2015). Qualitative Analysis of the Medicinal Chemical (BKO) Glibenclamide in Diabetes Herbal Preparations on the Market. Prosiding Farmasi. 649-653.

[5] D. Hendriyanto, & H. Helmi. (2023). Protective role of Nigella sativa oil against cisplatin-induced ototoxicity: a literature review. Journal of the Medical Sciences (Berkala Ilmu Kedokteran). 55 (2): 168-178.

[6] H. D. Harmono. (2020). Validation of the Dissolved Mercury (Hg) Metal Analysis Method in Surface Water with an Automatic Mercury Analyzer. Indonesian Journal of Laboratory. 2 (3): 11-16.

[7] H. Tannady, H. Tannady, & A. Zami. (2019). The Effect of Organizational Culture and Employee Engagement on Job Performance of Healthcare Industry in Province of Jakarta, Indonesia. Quality-Access to Success. 20 (169).

[8] A. W. HS. (2017). Analysis of Sibutramine HCl Medicinal Chemicals in Slimming Herbal Medicine Circulating in Manado City. Pharmacon. 6 (4).

[9] I. P. Ridwan, R. Abdullah, & H. S. Supriati. (2017). Identification of Phenylbutazone in Rheumatic Herbal Medicine Circulating in Manado City Using Thin Layer Chromatography Method. Media Farmasi Indonesia. 12 (1).

[10] H. T. Tan, S. Ndraha, H. Fabiani, F. Wician, M. Santoso, & M. Tendean. (2013). Diabetes Mellitus Due to Liver Cirrhosis in 33-Year-Old Female. The Indonesian Journal of Gastroenterology, Hepatology and Digestive Endoscopy. 14 (2): 117-119.

[11] R. Rollando, E. D. Embang, & E. Monica. (2019). Determination of Phenylbutazone and Paracetamol Levels in Herbal Medicine Circulating in Malang City Using Thin Layer Chromatography Densitometry. Jurnal Insan Farmasi Indonesia. 2 (1): 126-138.

[12] A. Dharmawan, M. J. Istia, H. T. Tan, S. Suparto, M. C. Anastasia, & N. Layanto. (2021). The outcome of patients with sepsis at Tarakan Hospital Central Jakarta in 2018. Muhammadiyah Medical Journal. 2 (2): 49-54.

[13] R. A. M. R. Syamsudin, F. Perdana, & F. S. Mutiaz. (2019). Temulawak Plant (Curcuma Xanthorrhiza Roxb) as Traditional Medicine. Jurnal Ilmiah Farmako Bahari. 10 (1): 51-65.

[14] H. Fabiani, & H. T. Tan. (2021). The Importance of Dietary Fiber on Stress Hormone in Pregnancy: A Literature Review. Journal of Midwifery. 5 (1): 13-19.

[15] J. F. Andry, H. Tannady, G. D. Rembulan, & A. Rianto. (2022). The importance of big data for healthcare and its usage in clinical statistics of cardiovascular disease. Journal of Population Therapeutics and Clinical Pharmacology. 29 (04): 107-115.

[16] E. D. Madyatmadja, A. Rianto, J. F. Andry, H. Tannady, & A. Chakir. (2021). Analysis of big data in healthcare using decision tree algorithm. In 2021 1st International Conference on Computer Science and Artificial Intelligence (ICCSAI) (Vol. 1, pp. 313-317). IEEE.