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INVESTIGATION OF HERBAL MEDICINE IDENTIFIED SIBUTRAMINE HCL THROUGH ANALYSIS OF THIN LAYER CHROMATOGRAPHY AND UV-VIS SPECTROPHOTOMETRY METHODS

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ABSTRACT
The use of chemical drugs in traditional medicine preparations is prohibited. Even so, there are still herbal products on the market that contain chemicals (BKO). This study aims to analyze the presence of sibutramine HCl in various herbal slimming products circulating in Medan City. The Thin Layer Chromatography method was used for qualitative analysis and the UV-Vis spectrophotometric method for quantitative analysis, which was validated beforehand. The validation parameters used are linearity, precision, accuracy, LOD, and LOQ. The results of the research obtained levels of herbal medicine samples A, B, and C, which were 9.47 mcg/ml, 10.42 mcg/ml, 9.37 mcg/ml respectively, and thin layer chromatography (TLC) 0.41, 0.2, and 0.51. Analytical validation, including linearity testing 0.9953, accuracy of 94.91%, precision SD 0.001, 0.0004, 0.0036 RSD 0.3086%, 0.1253%, and 1.180%, LOD = 0.298088 and LOQ = 0.9936627. The results of the study indicate that several herbal slimming products circulating in Medan City contain sibutramine HCl.
Keywords: Adulteration, Herbal Slimming Supplements, Sibutramine, Hcl, Thin Layer Chromatography, UV-Vis Spectrophotometric, Weight Loss.
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INTRODUCTION

The use of herbal medicines and dietary supplements has become increasingly popular in recent years.[1,2] Approximately half of American adults (49%) used dietary supplements from 2007 to 2010.[3] Despite being marketed as natural remedies, herbal medications are thought to be harmless and free of negative effects; yet, several hazardous substances have been found in them. Falsified or subpar herbal remedies can cause harm to patients and not effectively treat the illness or condition they are intended to address. The herbal medicine industry sometimes resorts to extreme measures to stay competitive, including using unmeasured amounts of potentially harmful ingredients to achieve quick results. This can be dangerous for people's health because taking excessive doses can lead to adverse effects, contraindications, and even poisoning.[4] Slimming herbal medicine is a popular herbal remedy that is well-known for its ability to aid in weight loss. Many people think that using herbal remedies to reduce weight significantly reduces adverse consequences.[5,6] Nonetheless, most individuals are unaware that sibutramine HCl is one of the therapeutic compounds present in herbal medicines that promote weight loss. [7] An effective anorexant and anti-obesity medication is sibutramine hydrochloride. Anorexia is a medication used to treat obesity that suppresses appetite and works in conjunction with diet.[8,9] Obesity is the accumulation of abnormally high body fat levels, which causes overweight and obesity at a certain height and muscle mass. Because sibutramine HCl alters the brain chemical dopamine, which influences weight management, it cannot be utilized as an ingredient in herbal medications.[10,11,12,13] This herbal remedy is readily available for purchase on the internet.[14] It was found that 44% of herbal slimming products included sibutramine. Sibutramine

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concentrations in some of these products could reach up to 35 mg per pill. In another investigation, it was discovered that sibutramine was inadvertently taken by 80% of patients who overdosed on weight loss tablets, and that sibutramine was present in 73% of the products.[15] According to some findings, some herbs that promote weight loss may aggravate or even exacerbate mental health problems. [16] Figure 1 is the structure of sibutramine HCl.

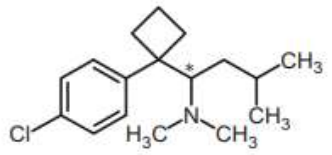

Fig.-1: Structure of Sibutramine

One technique that is commonly used for this investigation is the screening of different weight loss medicines and related drugs in dietary supplement ingredients using spectrometry and chromatography.[17,18] The study's findings showed that sibutramine, phenolphthalein, bumetanide, and phenytoin were among the contaminants found in herbal supplements used to help people lose weight. Other contaminants included caffeine, pseudoephedrine, theobromine, and amphetamine, which were qualitatively identified as other compounds in the supplement.[19] The purpose of this paper is to investigate a popular herbal weight-loss drug in Medan. that is suspected of containing dangerous drugs. The Thin Layer Chromatography (TLC) method was used in this study for qualitative determination.[20] and the UV-Vis spectrophotometry method to quantitatively assess the content of sibutramine HCl and determine it subjectively.[21,22] No previous researchers have ever conducted this kind of investigation with the same object and place.

EXPERIMENTAL

Material and Methods

The ingredients used include three brands of slimming herbal medicine believed to contain sibutramine HCl, which are sold in the city of Medan, Indonesia. The specific ingredients are sibutramine HCl (pa), methanol (pa), aqua bidestilata, ethyl acetate, n-hexane, acetone, and chloroform. The tools used for the experiment include a mortar, stamper, glassware (Pyrex), an analytical balance (KERN ACJ 220-4M), a chamber, a micropipette (eco pipette), silica plate GF254, and a UV-Vis spectrophotometer (Shimadzu 00787).

Making Qualitative Standard Solutions

Precisely measure out fifty milligrams of sibutramine hydrochloride (HCl), transfer it into a 100-milliliter measuring flask, and mix it with methanol to obtain 500 parts per million solution. To produce a concentration of 50 ppm, take 10 ml from a 500 ppm concentration and dilute it into a 100 ml measuring flask.

TLC Sample Preparation

TLC sample preparation is prepared from 100 mg of slimming herbal medicine and placed in an Erlenmeyer flask. Dissolve it in 100 ml of methanol, then stir and let it sit for 30 minutes. After that, filter the mixture and take 2 ml of the filtrate. Next, transfer the 2 ml of filtrate into a 25 ml measuring flask and fill it with methanol up to the mark line. Finally, stain the TLC plate with standards and samples, using the mobile phase Acetone: Chloroform (7:3).

Preparation of Standard Sibutramine HCl Solution

The comparison standard Sibutramine HCl was carefully weighed, 100 mg was put into a 100 ml volumetric flask, dissolved and diluted using methanol, shaken slowly, then added until the marking line obtained a solution with a concentration of 1000 ppm (LIB 1). Then take 10 ml from a concentration of 1000 ppm, put it in a 100 ml measuring flask, dilute it with methanol to the mark line, and get a concentration of 100 ppm (LIB 2).

Determination of Maximum Wavelength

A standard solution of Sibutramine HCl at a concentration of 100 ppm was transferred from a 2 ml pipette to a 25 ml measuring flask. The solution was then diluted with methanol to the mark, shaken well, and the

absorbance was measured in the range of 200-400 nm. The wavelength at which the highest absorption value is observed is considered the maximum wavelength.

Standard Curve
The 100 ppm solution was diluted using 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml, and 3 ml to create concentrations of 2 ppm, 4 ppm, 6 ppm, and 8 ppm, respectively. Additionally, 10 ppm and 12 ppm solutions were each diluted with methanol in a 25 ml volumetric flask and then brought to the mark. Subsequently, the absorption was measured using a UV-Vis spectrophotometry tool with a reference wavelength of 230 nm.

Assessment of the Levels of Active Ingredients in Samples of Herbal Slimming Medicine
First, carefully measure 10 mg of each sample estimated to contain Sibutramine HCl. Then, place the measured sample in a 100 ml volumetric flask and add methanol until the marking line, resulting in a concentration of 100 ppm. After filtering, take 0.5 ml of the solution and put it into a 25 ml measuring flask to obtain a concentration of 2 ppm. Fill the flask with methanol and then use UV-Vis spectrophotometry to read the absorbance.

Linearity
In order to conduct quantitative tests for the presence of Sibutramine Hydrochloride in samples, a series of concentrations (2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm) were used to create standard curves and linear equations. The absorbance value can then be determined by the correlation coefficient for each calibration curve. The required coefficient (r) should be equal to or greater than 0.9990. The range indicates the lowest and highest limits of the analyte that have been confirmed to be accurate, precise, and linear.

Accuracy
To evaluate the accuracy of the sample measurement analysis method, we pipetted 1 ml of the sample and placed it in a 25 ml measuring flask to obtain a concentration of 4 ppm. The absorbance was then read three times for validation. The accuracy is determined by achieving a recovery value between 80% and 120%. This method expresses the obtained levels as a ratio of the actual results. When using a concentration of 4 ppm, please measure the absorbance at the maximum wavelength. This accuracy test should be conducted with 3 repetitions. The repeated absorbance results will be used to calculate the average concentration, standard deviation (SD), coefficient of variation (CV), and instrument accuracy. Accuracy will be measured as standard deviation or relative standard deviation (coefficient of variation), with an acceptance criteria of less than 2%.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)
The concentration series of solutions are 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm, respectively. The Limit of Detection (LOD) and Limit of Quantification (LOQ) are calculated using the calibration curve method with the following equation: The Limit of Detection (LOD) is the smallest amount of analyte contained in a sample that can be detected and still provide a significant response compared to a blank. The limit of quantification (LOQ) is a parameter in analysis. It is defined as the smallest quantity of analyte in a sample that can still meet the criteria for accuracy and precision.

RESULTS AND DISCUSSION

Maximum Wavelength Results
The research was conducted on standard Sibutramine HCl dissolved in methanol with a concentration of 100 ppm in the standard stock solution. Two dilutions were carried out in a solution volume of 1 mL with a concentration of 4 ppm. Maximum wavelength measurements were taken within the standard maximum wavelength range of Sibutramine HCl of 200-400 nm. The results showed a maximum wavelength of 230 nm for Sibutramine HCl. The graph can be seen in Fig.-2.

Standard Calibration Curve Results for Sibutramine HCl
Research was conducted to create a standard curve for Sibutramine HCl using a series of standard solutions. The absorbance values from the measurement data were calculated using a regression equation to compare with the sample. The absorbance of the standard solutions was measured, resulting in a regression value of 0.9953, indicating a linear relationship. The graph can be seen in Fig.-3.

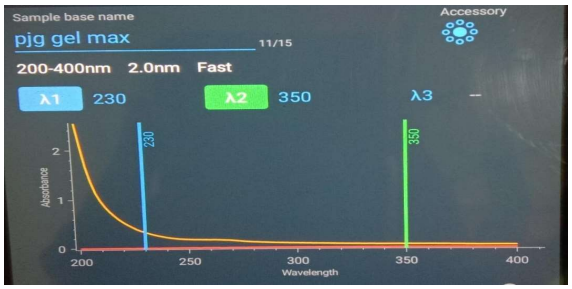

Fig.-2: Maximum Wavelength Graph

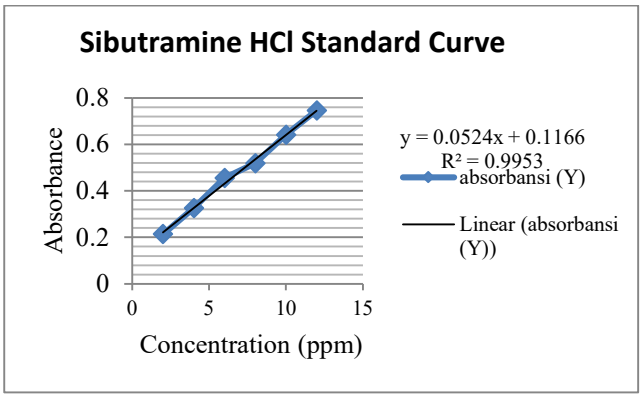

Fig.-3: Standard Brasi Times Curve for Sibutramine HCl

The standard curve equation for Sibutramine HCl expresses the linear relationship between concentration and absorbance. The correlation coefficient (r) is used as a linearity parameter to show the correlation between concentration and absorbance. A higher (r) value closer to 1 indicates a better linearity of the equation. In this study, the linear regression equation obtained from the standard curve is y = 0.0524x + 0.1167, where y represents absorbance and x represents sample concentration. The square correlation coefficient (R) value is found to be 0.9953. Creating a Sibutramine HCl calibration curve helps determine the correlation between the concentration of Sibutramine HCl and its absorbance. It's observed that higher concentrations result in higher absorbance, and lower concentrations lead to lower absorbance.[23] Complete data can be seen in Table-1.

Table-1: Standard Calibration Curve Results for Sibutramine HCl

No.	CONS (X)	ABS (Y)	X^2	Y^2	XY
1	2	0.214	4	0.045796	0,297
2	4	0.326	16	0.106276	1.304
3	6	0.455	36	0.207025	0,134
4	8	0.519	64	0.269361	4.152
5	10	0.641	100	0.410881	06.41
6	12	0.746	144	0.556516	8.952
∑	42	2.901	364	1.595.855	23.976
Average	7	0.4835	60.066	0.265976	3.996

Results of Determination of Sibutramine HCl Levels

The levels of Sibutramine HCl were determined, and the absorbance value was measured at a wavelength of 230 nm. The average absorbance value with the same concentration was found to be 0.219. Using the regression equation y=ax+b, the Sibutramine HCl levels in the sample were calculated, resulting in sample A at 9.47%, sample B at 10.42%, and sample C at 9.37% w/v. Complete data can be seen in Table-2.

The levels of Sibutramine HCl in Slimming Herbal Medicine were determined using an Ultraviolet Spectrophotometer with the calculation of the regression equation y=ax+b. The sample values for brand A, brand B, and brand C were found to be 9.47%, 10.42%, and 9.37% respectively. Previous research reported a Sibutramine content of 18 mg per capsule.[4,24] Complete data can be seen in Table-2.

Table-2: Results of Sibutramine HCl levels

Sample	Absorbance	Concentration mcg/ ml	%
A	0.216	1.89	9.47
B	0.226	2.08	10.42
C	0.215	1.87	9.37

Accuracy Value Results

In the accuracy analysis validation test, absorbance measurements were performed within a specific range of 80% to 120%. The recovery value represented the analyte concentration, resulting in an average recovery value of 94.91%. Complete data can be seen in Table-3.

Table-3: Accuracy value data

No	Concentration	Recovery (%)
1	0,323	96.42
2	0,324	98.9
3	0,325	99.37
4	0,319	96.51
5	0,320	96.99
6	0,319	96.51
7	0,304	89.36
8	0,302	88.4
9	0,309	91.74
$\bar{X}$ Rec	0,316	94.91

Precision Value Results

Precision analysis validation test to demonstrate the compliance of the sample with the average substance content, using standard deviation with an acceptance criterion of <2%. Complete data can be seen in Table-4.

Table-4: Precision value data

Sample	SD	RSD
A	0.001	0.3086
B	0.0004	0.1253
C	0.0036	1.1803

Precision tests were conducted using standard deviation (SD) and relative standard deviation (RSD) parameters. The calculated SD prices for brand A, brand B, and brand C are 0.001, 0.0004, and 0.0036, respectively. The RSD values for brand A, brand B, and brand C are 0.3086%, 0.1253%, and 1.180%, respectively.

LOD and LOQ Value Results

The limit of detection (LOD) analysis validation test involved measuring the lowest concentration of analyte in the sample that was still detected. The standard deviation value obtained for the LOD result was 0.298088. In the limit of quantification (LOQ) analysis validation test, the highest concentration of analyte in the sample that was still detected was measured with a standard deviation value of 0.993627.

T-Count Results

According to the research results, the calculated t-value for each sample is 0.27896 for brand A, 0.65757 for brand B, and 0.37859 for brand C. These values are all smaller than the critical t value, which is 5.94358. As a result, we accept the alternative hypothesis.

Retardation Factor (RF) Value Results

According to the qualitative research conducted using the Thin Layer Chromatography method, the RF values obtained for the three samples are as follows: brand A - RF1 0.25 and RF2 0.41, brand B - RF1 0.23 and RF2 0.2, and brand C - RF1 0.5 and RF2 0.51. These results confirm that all three samples tested positive for containing sibutramine HCl. The RF values were determined using a 365 nm wave as indicated in the image below. It can be concluded that all three slimming drugs tested positive for containing the medicinal chemical sibutramine HCl, meeting the requirement for a Retardation Factor value of 0.2-0.8.25 Complete data can be seen in Table-5 and the fixture can be seen in Fig.-4.

Table-5: Qualitative Results

Sample	Test Result	RF Value
A	+	0.41
B	+	0.2
C	+	0.51

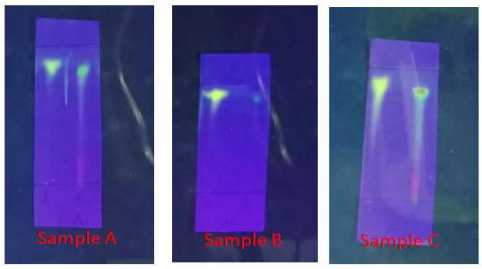

Fig.-4: Image of TLC Results

CONCLUSION

Based on the results of the research, it can be concluded that UV-Vis spectrophotometry and Thin Layer Chromatography methods can effectively analyze Sibutramine HCl. The accuracy, precision, LOD/LOQ, and T results were used to calculate the test results for three samples suspected to contain dangerous chemicals. The research findings indicated that all three samples tested contained different doses of the synthetic chemical Sibutramine HCl. It is hoped that the government, particularly the Food and Drug Monitoring Agency (BPOM), will thoroughly re-examine the content of herbal medicines circulating in Indonesia, especially in Medan City. If these medicines are found to contain dangerous chemicals, they should be withdrawn from circulation.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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