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Identification and Determination of Levels the Drug Chemical Sibutramine Hydrochloride in Body Slimming Herbs Circulating in the City of Salatiga

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Abstract: **Background:** Sibutramine hydrochloride is strictly prohibited from being present in weight loss herbal medicines according to the regulations of the Indonesian National Agency of Drug and Food Control (Badan POM RI) No.KH.00.01.43. However, due to increasing competition, many traditional medicine producers seek greater profits by adding sibutramine hydrochloride (SH) to their products. **Aim:** This study aimed to establish the validity of the UV-VIS spectrophotometer method for analyzing sibutramine hydrochloride and to test the levels and content of sibutramine hydrochloride in weight loss herbal medicine samples sold in Salatiga City. **Materials and Methods:** A total of 8 samples of weight loss capsules obtained from traditional medicine stores in Salatiga City were subjected to qualitative and quantitative tests. Validation parameters included linearity, limit of detection (LoD), limit of quantification (LoQ), precision, and accuracy. **Results:** The study revealed a linear regression equation of y=0.0397x + 0.046 with r=0.9975 and r^2=0.9950, with a maximum wavelength of 223 nm. The LOD was 1.01 mg/L and LOQ was 3.37 mg/L. Precision, expressed as % RSD, was 0.147%, and percent recoveries (% recovery) were 98.263%, 89.337%, and 91.703%. Qualitative tests on each sample compared spectra and maximum wavelengths with standard sibutramine hydrochloride spectra, identifying samples A, B, C, E, and F as negative for sibutramine hydrochloride, while samples D, G, and H tested positive. Quantitative analysis determined sibutramine hydrochloride concentrations in weight loss herbal medicines using linear regression equations. The concentrations in samples D, G, and H were 6.0117 mg/L, 6.7254 mg/L, and 6.0117 mg/L, respectively. The percentage content of sibutramine hydrochloride in samples D, G, and H was 3.00%, 3.36%, and 3.00%, respectively.

Keywords: Sibutramine Hydrochloride, UV-VIS Spectrophotometer, Weight Loss Herbal Medicine, Analytical Method Performance

INTRODUCTION

Overweight or obesity continues to increase among adolescents and adults, showing a sharp rise in prevalence in recent years. In 2013, the prevalence of overweight or obesity was 28.9%, which then increased to 35.4% in 2018 among adults. This imbalance in overweight or obesity in 2018 was indicated by 44.4% among adult women compared to 26.6% among adult men (UNICEF, 2019). According to Riskesdas in 2018, obesity cases in Indonesia include 1 in 5 school-age children (20% or 7.6 million), 1 in 7 adolescents (14.8% or 3.3 million), and 1 in 3 adults (35.5% or 64.4 million) living with overweight or obesity. This research shows that children, adolescents, and adults still experience overweight or obesity.

One of the traditional medicines widely consumed by the community is body slimming herbal medicine (*jamu pelangsing badan*). The increasing use of traditional medicine based on traditional efficacy expands the opportunity for the falsification of herbal ingredients, and some traditional medicines contain banned chemical drugs either intentionally or unintentionally added to these products. BPOM (Indonesia's Food and Drug Administration) found traditional medicines containing chemical drugs during the period from September 2022 to October 2023 (Public Relations and Cooperation Bureau, 2023). The addition of these chemical drugs contradicts Minister of Health Regulation Number 007 Year 2012 concerning Traditional Medicine Registration, Article 7, which states that traditional medicines are prohibited from containing chemical drugs that are isolated or synthetic. Some manufacturers of body slimming traditional medicine products add chemical drugs to their products to achieve faster effects. Sample C had an average content of 6.776%, and sample D had an average content of 8.373%, which positively identified the presence of the chemical drug sibutramine hydrochloride.

Therefore, the researchers are interested in identifying the chemical drug sibutramine hydrochloride in various body slimming herbal medicine products circulating in Salatiga City. With the presence of the chemical drug sibutramine hydrochloride, researchers are interested in studying Salatiga City, which has not been previously researched. This study requires a valid, efficient, inexpensive, easy, and relatively fast method. The method used in this study is UV-VIS spectrophotometry, which will be tested qualitatively and quantitatively.

METHODS

This research was conducted at the Chemistry Laboratory, School of Pharmacy (STIFAR) Semarang. A standard solution of sibutramine hydrochloride at 1000 mg/L was prepared by dissolving 10 mg of sibutramine hydrochloride standard in 10 mL. A standard curve was prepared by pipetting the sibutramine hydrochloride standard solution into concentrations of 6, 9, 12, 15, and 18 mg/L. The maximum wavelength was determined from the 12 mg/L concentration solution within the range of 200 – 400 nm using UV-VIS spectrophotometry1. To validate the analysis method, tests were conducted for linearity, Limit of Detection (LoD), Limit of Quantification (LoQ), precision, and accuracy.

Linearity Test

A standard solution of 1000 ppm was pipetted with volumes of 0.3, 0.45, 0.6, 0.75, and 0.9 mL, and each volume was diluted with distilled water to the 10 mL mark and homogenized. This resulted in solutions with concentrations of 6, 9, 12, 15, and 18 mg/L, which were then read using UV-VIS spectrophotometry at the previously determined maximum wavelength. The relationship between concentration and absorbance values was analyzed to obtain the linear regression equation $y = ax + b$.

Limit of Detection (LoD) & Limit of Quantification (LoQ)

From the data of the linear regression line equation on the standard curve, single absorbance (xi) and average absorbance (xi) were obtained. Thus, LoD and LoQ could be calculated.

Precision Test

A sample solution of 150 µL was pipetted into a 10 mL volumetric flask and diluted with distilled water to the mark. The solution was transferred to a cuvette, and its absorbance was read at the maximum wavelength using UV-VIS spectrophotometry. Precision was tested with 6 replicates. The absorbance results of the replicates were used to calculate the average concentration, standard deviation (SD), coefficient of variation (CV), and instrument precision. The RSD value equaled the CV value, meeting the requirement for good precision, which is ≤ 2%2.

Accuracy Test

200 mg of powdered body slimming herbal medicine sample was weighed and dissolved in 10 mL of distilled water in a beaker. After homogenization, the solution was transferred to a 25 mL volumetric flask, diluted to the mark with distilled water, and filtered using Whatman No.1 filter paper. A 250 µL aliquot was pipetted into a 10 mL volumetric flask, diluted to the mark with distilled water, and transferred to a cuvette. The sample solution was pipetted three times, and standard sibutramine hydrochloride solutions at concentrations of 6, 12, and 18 mg/L were added to the sample in a 1:1 ratio. The same treatment was performed on a sample without the addition of standard sibutramine hydrochloride. The absorbance was read at the maximum wavelength using UV-VIS spectrophotometry, and the % recovery was calculated. Good accuracy requires a range of 80-110%2.

Preparation of Body Slimming Herbal Medicine Capsule Samples

200 mg of powdered body slimming herbal medicine sample was weighed and dissolved in 10 mL of distilled water in a beaker. After homogenization, the solution was transferred to a 25 mL volumetric flask, diluted to the mark with distilled water, and filtered using Whatman No.1 filter paper. A 250 µL aliquot was pipetted into a 10 mL volumetric flask, diluted to the mark with distilled water, and transferred to a cuvette. The sample solution was read using UV-VIS spectrophotometry at the maximum wavelength.

RESULT AND DISCUSSION

The scanning to determine the maximum wavelength of the standard sibutramine hydrochloride is at 223 nm with an absorbance value of 0.539 (Figure 1). This theory is supported by research conducted by Hibatullah (2022) and Novani & Sa (2021), which mentioned that the maximum wavelength of sibutramine hydrochloride is at λ 223 nm with values that are not significantly different from the research conducted. Therefore, this wavelength was chosen for analyzing the concentration of sibutramine hydrochloride in body slimming herbal medicines.

Calibration curve was performed at concentrations of 6, 9, 12, 15, and 18 mg/L. This was done to ensure that the absorbance values obtained meet the Lambert-Beer requirements between 0.2-0.8[3]. Absorbance measurements were carried out using a UV-VIS spectrophotometer at a wavelength of 223 nm, and the absorbance obtained was plotted into a standard curve to obtain the sibutramine hydrochloride calibration curve with the equation of the calibration curve.

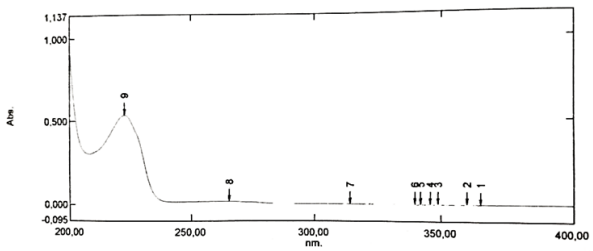

Figure 1. Results of scanning for determination of sibutramine hydrochloride

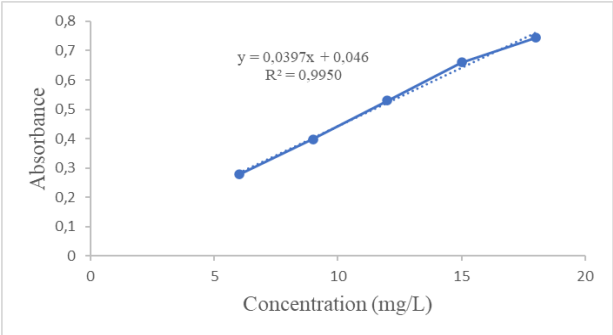

Figure 2. The standard curve of sibutramine hydrochloride

The standard curve of sibutramine hydrochloride in this study showed a linear relationship between absorbance and concentration, indicated by a coefficient of determination (r2) of 0.9950 with the regression equation y = 0.0397x + 0.046. This regression equation serves as the standard for determining the concentration of samples of body slimming herbal medicines.

Linearity Test

The linearity test of the standard curve of sibutramine hydrochloride was determined from 5 concentrations: 6 mg/L, 9 mg/L, 12 mg/L, 15 mg/L, and 18 mg/L in distilled water solvent, then read at the maximum wavelength of 223 nm to create the standard curve of sibutramine hydrochloride. The equation relating concentration (x) and absorbance (y) obtained was y = 0.0397x + 0.046 with a coefficient of determination (r2) of 0.9950 (Figure 4.2). From this equation, the correlation coefficient (r) value was determined to be 0.9975. The values of r and r2 are very good as determined by SNI ($r \geq 0.995$) and Eurachem ($r^2 \geq 0.995$)[4]. Linearity can be achieved if the value of (r) approaches 1, which is indicated by r = >1 or r = <1[5]. Thus, the values of r and r2 in this study have met the requirements. In terms of method performance, the results obtained meet the criteria for good linearity.

Limit of Detection (LoD) & Limit of Quantification (LoQ)

Table 1. Values of LoD and LoQ

Concentration (mg/L)	Absorbance (y)	y'	y- y'	(y- y')²
6	2,704E-05	0,284	-0,0052	2,704E-05
9	1,849E-05	0,403	-0,0043	1,849E-05
12	5,776E-05	0,522	0,0076	5,776E-05
15	0,00034225	0,641	0,0185	0,00034225
18	0,00027556	0,760	-0,0166	0,00027556
			Σ (y- y')²	0,0007211
			Σ (y- y')²/n-2	0,000240367
			SD	0,015503763
			LoD	1,011114977
			LoQ	3,370383257

The data from the concentration and average absorbance were calculated, resulting in a LoD value of 1.01 mg/L and a LoQ value of 3.37 mg/L. LoD represents the limit at which an instrument can detect; in this study, the instrument can detect at a minimum concentration of 1.01 mg/L, meaning measurements can still be made at this concentration that provide the precision of an instrument based on the individual accuracy level of the analysis results. LoQ

represents the smallest limit at which an instrument can quantify; in this study, the smallest quantifiable concentration is 3.37 mg/L, indicating that measurements at this concentration can still provide analytical accuracy.

Precision Test

Table 2. Results of precision testing on body slimming herbal medicine samples

Sample	Concentration (mg/L)	SD	RSD (%)
A	10,2476	0,0004	0,1608
B	14,9160	0,0004	0,0757
C	11,6918	0,0004	0,0992
D	8,5054	0,0005	0,1814
E	14,5843	0,0008	0,1700
F	15,4869	0,0007	0,1339
G	9,2317	0,0005	0,1747
H	8,5054	0,0005	0,1814
Average		0,0005	0,1392

RSD indicates the precision of the testing method: if RSD ≤ 1%, it means the method is very precise; if 1% < RSD ≤ 2%, it is precise; if 2% < RSD ≤ 5%, it indicates moderate precision; and if RSD > 5%, it means the method is not precise[6]. The percentage relative standard deviation (%RSD) values indicate a very precise level of accuracy for all samples of body slimming herbal medicines from samples A, B, C, D, E, F, G, H, I, to J because their %RSD values are ≤ 1%. The average %RSD value across all samples is 0.1392%. A smaller %RSD value indicates more precise analysis and better suitability for analyzing a chemical compound. The average %RSD value meets the requirements and demonstrates that the method provides good precision, which is ≤ 2%. This indicates that the UV-VIS spectrophotometer method used in this study is highly precise as it meets the tested criteria.

Accuracy Test

Table 3. The average percentage recovery (%recovery)

Sample	Measured Concentration			Sample Theoretical Concentration	%Recovery		
	6 mg/L	12 mg/L	18 mg/L		6 mg/L	12 mg/L	18 mg/L
A	10,579	16,070	21,460	5,235	89,070	90,295	90,143
B	17,439	22,762	31,150	12,422	83,613	86,167	104,044
C	15,197	20,008	23,635	9,198	99,986	90,085	80,208
D	12,283	16,842	21,242	6,011	104,534	90,260	84,616
E	17,900	22,636	30,848	12,090	96,837	87,881	104,207
F	19,252	23,811	31,721	12,993	104,324	90,155	104,044
G	13,022	17,481	21,460	6,738	104,743	89,525	81,794
H	12,300	16,859	21,242	6,011	104,813	90,400	84,616
Average					98,263	89,337	91,703

The average percentage recovery (*%recovery*) from concentrations of 6 mg/L, 12 mg/L, and 18 mg/L were 96.263%, 8%, and 90.097%, respectively. These percentage recovery values are acceptable as they fall within the accuracy requirement range of 80-110%[2]. The obtained percentage recovery values indicate that the UV-VIS spectrophotometer method provides good accuracy, making it accurate for analyzing sibutramine hydrochloride in body slimming herbal medicines for this study.

Qualitative and Quantitative Analysis of Sibutramine Hydrochloride

Based on the spectrum shape and maximum wavelength of standard sibutramine hydrochloride, samples of body slimming herbal medicines with codes A, B, C, E, and F were identified as not containing sibutramine hydrochloride. The results showed spectra different from that of the standard sibutramine hydrochloride solution. The absence of sibutramine hydrochloride in the samples of body slimming herbal medicines may be due to either these tested samples genuinely not containing sibutramine hydrochloride or other compounds present in the herbal samples contributing to absorbance at different wavelengths, thereby affecting the analysis results. This theory is supported by research conducted by Maluf[7], which indicated degradation of herbal products containing various compounds that could affect the absorption region of sibutramine hydrochloride. Simaremare[8], also mentioned the presence of

multiple compounds in plants used in herbal formulations, which could potentially cause shifts in spectra and maximum wavelengths.

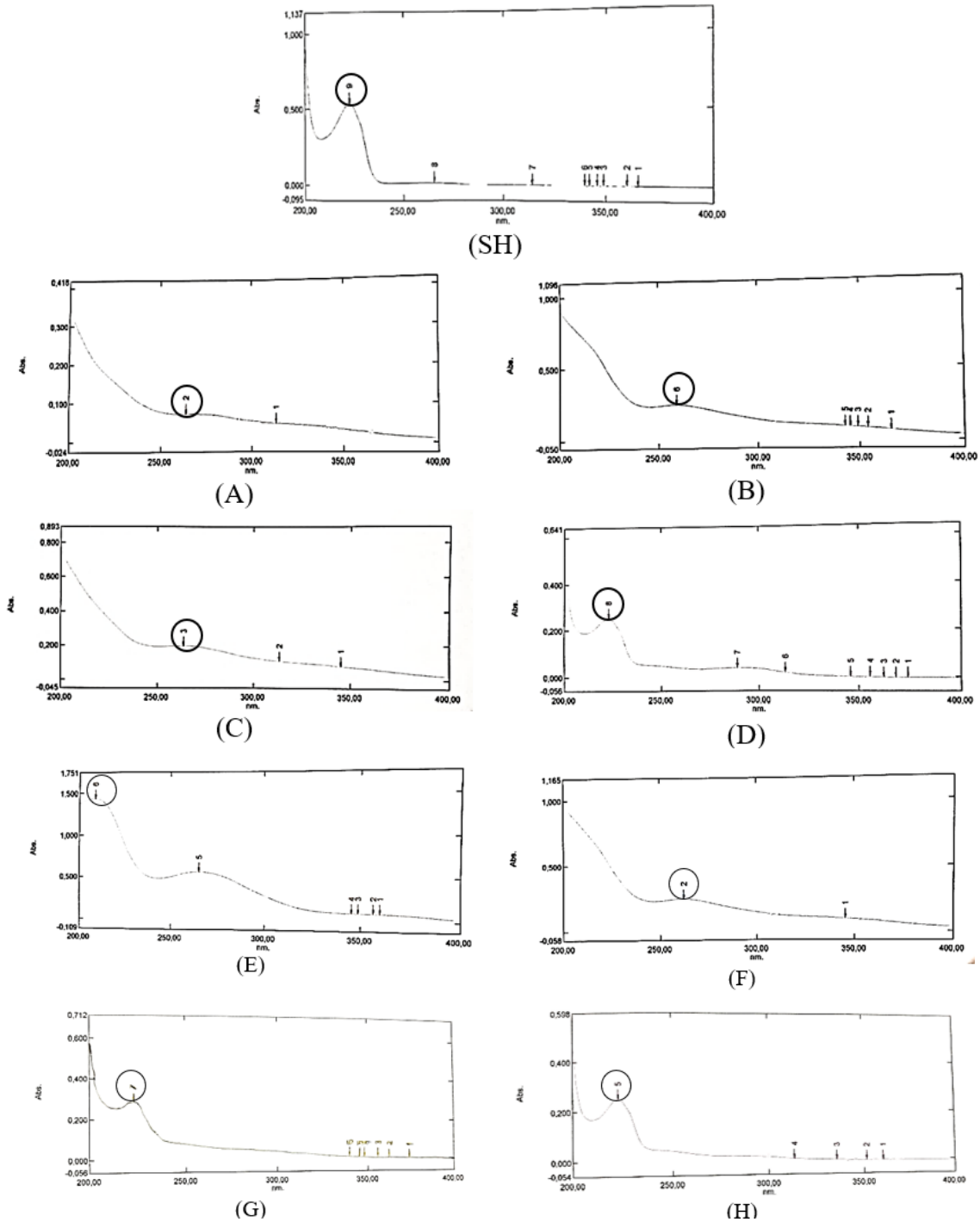

Figure 3. The spectrum of a sample of body slimming herbal medicine and sibutramine hydrochloride (SH) in sample A - H

Table 4. Qualitative and quantitative testing results of sibutramin hydrochloride in body slimming herbal medicine

Sample	BPOM	Qualitative			Quantitative	
		Scanning λ (nm)	LoD	LoQ	Concentration (mg/L)	Concentration (%)
A	√	-	≥	≥	5.235	2.617
B	-	-	≥	≥	12.422	6.211
C	√	-	≥	≥	9.198	4.599
D	-	+	≥	≥	6.011	3.005
E	√	-	≥	≥	12.090	6.045
F	√	-	≥	≥	12.993	6.496
G	-	+	≥	≥	6.725	3.369
H	-	+	≥	≥	6.011	3.005

The concentrations of samples A, B, C, E, and F were 5.235 mg/L, 12.422 mg/L, 9.198 mg/L, 12.090 mg/L, and 12.993 mg/L, respectively. These samples of body slimming herbal medicines with codes A, B, C, E, and F had concentrations above the detection and quantification limits of the UV-VIS spectrophotometer used, thus confirming the determined concentrations of sibutramine hydrochloride in these samples. The scanning of maximum wavelength in codes A, B, C, E, and F showed that these samples were negative for containing sibutramine hydrochloride due to the possibility of other compounds in the herbal samples contributing to absorbance at the wavelength, affecting the concentration determination. Whereas samples D, G, and H had concentrations above the detection limit but below the quantification limit, they can still be considered determined. This is supported by scanning the spectrum and maximum wavelength showing similarity or nearly approaching that of the spectrum and maximum wavelength of standard sibutramine hydrochloride. The concentrations of samples D, G, and H were 6.0117 mg/L, 6.7254 mg/L, and 6.0117 mg/L, respectively. The percentage concentrations of sibutramine hydrochloride in body slimming herbal medicines D, G, and H were 3.00%, 3.36%, and 3.00%, respectively. Sibutramine hydrochloride belongs to the category of prescription-only drugs for obesity treatment, and it is strictly prohibited to be present in body slimming herbal medicines according to the regulations of the Indonesian National Agency of Drug and Food Control (BPOM) No. KH.00.01.43.2773/2008 regarding traditional medicines containing chemical substances (BKO).

CONCLUSION

Based on the results of the study, it can be concluded that the performance of the sibutramine hydrochloride analysis method in slimming herbs meets the parameters of linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. Linear regression equation y = 0.0397x + 0.046 with r = 0.9975 and r2 = 0.9950, limit of detection of 1.01 mg/L, limit of quantification of 3.37 mg/L, precision of 0.147% at 98.263%; 89.337%; and 91.703%. Qualitative test results showed that 3 out of 8 samples of body slimming herbs analyzed were positively identified as containing sibutramine hydrochloride, namely samples D, G and H. Quantitative test results showed the percentage of sibutramine hydrochloride levels in body slimming herbs D, G and H were 3.005%; 3.369%; and 3.005%, respectively, so they did not meet the BPOM regulations which state that sibutramine hydrochloride is absolutely not allowed in slimming herbs in accordance with the regulations of the Indonesian POM Agency No. KH.00.01.43.2773/2007. .01.43.2773/2008 concerning traditional medicine containing medicinal chemicals (BKO).

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CONFLICT OF INTEREST

We declare that we don't have any conflict of interest.

REFERENCES

Bali S, Putri ASN, Aprilia S. Journal of Pharmacy and Science Validation and Determination of Paracetamol Contents in Pegal Linu Jamu Circulated in Pekanbaru by UV-Vis Spectrophotometry Pendahuluan Metode Penelitian Alat dan Bahan. *J Pharm Sci*. 2023;7(1):27-35.

Hibatullah FA, Gatera VA, Sholih MG. Identifikasi Kualitatif dan Kuantitatif Sibutramin Hidroklorida Pada Produk Herbal Pelangsing Yang Beredar di Kabupaten Karawang. *J Bid Ilmu Kesehat*. 2022;12(4):387-393. doi:10.52643/jbik.v12i4.2349

Maluf DF, Farago P V., Barreira SMW, Pedroso CF, Pontarolo R. Validation of an analytical method for determination of sibutramine hydrochloride monohydrate in capsules by Uv-Vis spectrophotometry. *Lat Am J Pharm*. 2007;26(6):909-912.

Novani N, Sa H. Analisis Kandungan Sibutramin Hidroklorida Pada Produk Herbal Pelangsing Dengan Metode Spektrofotometri Uv-Vis. *Med Sains J Ilm Kefarmasian*. 2021;6(1):45-56. doi:10.37874/ms.v6i1.214

Nugraha F, Kurniawan H, Yastiara I. Penetapan Kadar Paracetamol dalam Jamu di Kota Pontianak Menggunakan Instrumen Spektrofotometri UV-Vis. *Indones J Pharm Educ*. 2023;3(1):77-87. doi:10.37311/ijpe.v3i1.18876

Simaremare ES, Susilowati RA, Astuti YD, et al. Analysis of acetaminophen, mefenamic acid, sibutramine hydrochloride, and sildenafil citrate. *J Appl Pharm Sci*. 2018;8(11):48-56. doi:10.7324/JAPS.2018.81107

Suharyanto S, Prima DAN. Penetapan Kadar Flavonoid Total pada Juice Daun Ubi Jalar Ungu (Ipomoea Batatas L.) yang Berpotensi Sebagai Hepatoprotektor dengan Metode Spektrofotometri UV-Vis. *Cendekia J Pharm*. 2020;4(2):110-119. doi:10.31596/cjp.v4i2.89

Sulistyani M, Kusumastuti E, Huda N, Mukhayani F. Method Validation on Functional Groups Analysis of Geopolymer with Polyvinyl Chloride (PVC) as Additive Using Fourier Transform Infrared (FT-IR). *Indones J Chem Sci*. 2021;10(3):198-205. http://journal.unnes.ac.id/sju/index.php/ijcs