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Optimization of The Concentration of Precipitation Reagent in Cyclamate Analysis and Determination of Cyclamate Contents in Jamu

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Abstract: Sodium cyclamate is one of the food additives sweeteners that are often added to food products including jamu. It has a low price and a taste 30 - 50 times sweeter than natural sugar. It is known that excessive & longterm use of sodium cyclamate can cause adverse health effects. Currently, the standard method used to detect the presence of cyclamate refers to the precipitation method of SNI 01-2893. However, the level of sensitivity of the method is unknown. This study aims to determine the sensitivity of the precipitation method of SNI 01-2893, as well as to optimize reagents that have better sensitivity to be used as a reference for cyclamate precipitation reagents and applied to measure cyclamate in jamu. The determination the method's sensitivity is based on the smallest concentration of cyclamate standard that can be detected by the method. Optimization of the precipitation reagent was carried out on 6 variations of precipitation reagent concentration to obtain a new formula that has a better sensitivity than the SNI 01-2893 method. Jamu samples were tested using the new optimized reagent formula. The result showed the detection limit of the of SNI 01-2893 method is 5 ppm. Optimization of the precipitation reagent formula with the composition of HCl 10%, $BaCl_2$ 10%, and $NaNO_2$ 20% showed as the highest sensitivity. It was marked by an increase in the amount of precipitate formed and had a detection limit of up to 0.9 ppm. Based on testing using the new reagent, 9 out of 10 jamu samples were positive for cyclamate. The cyclamate levels of the 9 jamu samples were measured using the spectrophotometric method, the results showed cyclamate levels are in the range of 4.3 - 6.32 mg/kg sample and meeting BPOM requirements.

Keywords: Jamu, Precipitation Method, Sodium Cyclamate

INTRODUCTION

Sodium cyclamate is one of the sweetener-type food additives favored by the public and food manufacturers in Indonesia. One type of processed food product that can be enriched with the addition of artificial sweeteners is Jamu. Jamu is usually made from natural herbs that have certain properties and tend to have a bitter taste, so the use of additional sweeteners is important to reduce the bitter taste. Artificial sweeteners such as sodium cyclamate are often preferred because they are 30-50 times sweeter than natural sugar without leaving a bitter aftertaste, making them effective in masking the bitter taste of herbs. In addition, the low price of artificial sweeteners and their addition in small amounts can reduce production costs incurred by manufacturers.

The use of jamu in daily consumption by Indonesians is closely related to the long-term use of artificial sweeteners. Long-term and uncontrolled utilization of sodium cyclamate is known to increase health risks. In vivo studies conducted by Nugraheni, (2022) tested the effect of cyclamate administration on the number of polymorfonuclear cells (PMN) in rat test samples and showed the short-term effects of sodium cyclamate given in a dose of 19.5 mg / 200 grams of rat BW. It can cause oxidative stress effects and trigger cell damage or infection and leukocytosis. Research by Zhou *et al.*, (2022) showed that the occurrence of acute liver, heart, and kidney injury caused by sodium cyclamate was closely related to the inflammatory response mediated by TNF-α (Tumor Necrosis Factor alpha) and Interleukin-1 beta (IL-1β), where the expression of TNF-α and IL-1β increased gradually within 120 hours with increasing doses of sodium cyclamate. In addition, Zhou also mentioned that the administration of cyclamate at a high dose of 12,000 mg/kg/day can even cause acute diarrhea. Long-term toxicity studies conducted at Covance Laboratories in America on non-human primates (macaques) given doses of cyclamate of 100-500 mg/kg per day for 24 years also found tumors in the treatment group (Takayama *et al.*, 2000). The results of these studies can be a strong scientific basis and a reason to limit the use of sodium cyclamate.

According to the Food and Drug Supervisory Agency (BPOM) Regulation No. 11 of 2019, the maximum limit for the use of sodium cyclamate based on food type categories, such as in traditional medicine products including jamu is 1,250 mg/kg, calculated on products that are ready for consumption (BPOM RI, 2019). Based on BBPOM annual report data for 2021-2022, the use of sodium cyclamate in Indonesia increased from 2,223 to 3,371 positive cyclamate samples with an increase in the number of abuse from 100 cases to 128 cases in the following year. Identification of cyclamate in food, beverage, or medicine samples can be done easily using the SNI 01-2893 precipitation method. This method has been standardized and the detection result is a white precipitate of barium sulfate which clearly reflects the presence of sodium cyclamate in the sample (Sumantiri, 2018). After conducting a qualitative test of

cyclamate in the sample, the next step is to measure the levels using the UV-Visible spectrophotometric method (Rohmah *et al*., 2021).

Several studies on cyclamate test in food, beverage and medicine samples have been conducted by precipitation method, but the sensitivity level of the method is unknown. In addition, an accurate and sensitive analysis method is needed Therefore, this study was conducted to examine the sensitivity of the SNI precipitation method and formulate the optimum reagent for higher sensitivity of the precipitation method. The optimum reagent obtained was used to identify cyclamate in jamu samples marketed in Semarang City.

METHODS

Material and Instrument

The materials used in this study were Jamu beras kencur in bottles marketed in Semarang City, sodium cyclamate (BPL), distilled water, sodium nitrite p.a (Merck), barium chloride p.a (Merck), HCl 37% (Merck), sodium hydroxide p.a (Merck), sulfuric acid p.a (Merck), cyclohexane p.a (Merck), sodium hypochlorite (Merck), ethyl acetate p.a (Merck).

The instrument used in this study include, cuvette, a set of Personal Computer (PC) equipped with Origin 10.5.113.50894 software, laboratory glassware, Mettler Toledo AL204 analytical balance (4 digits), hot plate (Thermo), Whatman no. 42 filter paper, spatula, micro pipette (Dragon Lab).

Sensitivity Test of Precipitation Method SNI 01-2893-1992

The method sensitivity test was carried out by making a standard solution of sodium cyclamate in several concentrations and then adding SNI 01-2893 precipitation reagent. The reagent was 10 ml of 10% HCl solution and 10 ml of 10% $BaCl_2$ solution, then stirred and left for 30 minutes. The resulting solution was filtered using filter paper. Next, 10 ml of 10% $NaNO_2$ solution was added and heated using a hotplate at 125-130 °C for 30 minutes and analyzed the minimum concentration when the white precipitate formed.

Optimization of Precipitation Method SNI 01-2893-1992

The new formulation of precipitation reagent was tested against cyclamate standard with concentrations below the minimum standard that can be detected using the precipitation reagent of SNI 01-2893.

Table 1. Formulation for Optimization of Cyclamate Precipitation

Formulas	Reagent Concentration		
	10 ml HCl	10 ml $BaCl_2$	10 ml $NaNO_2$
F1 (control)	10%	10%	10%
F2	10%	15%	10%
F3	10%	20%	10%
F4	10%	25%	10%
F5	10%	10%	15%
F6	10%	10%	20%

Test of Optimum Reagent Formula on Jamu Samples

The optimum reagent formula was tested on 10 samples of Jamu beras kencur in bottles marketed in Semarang City. From the 10 selected samples, 10 ml each was taken. 10 ml of distilled water was added and then homogenized. The solution was filtered using filter paper. The filtrate solution was then reacted with the optimum reagent formula that had been made. The mixture of solutions was heated on a hotplate at 125- 130 °C, then analyzed whether there was a white precipitate or not. formation of white precipitate means the sample is positive for Cyclamate Sodium.

Cyclamate Analysis Using Spectrometer UV-Visible

Preparation of Calibration Curve

Standard solution of cyclamate at concentrations of 0; 40; 50; 60; 70; 80; 90; and 100 ppm were prepared. Each solution was taken 25 mL and put into the first separatory funnel, added with 0.5 mL of 10 N NaOH, 2.5 mL of cyclohexane and shaken for 1 minute. The water layer (bottom) was separated and put into the second separatory funnel, added with 1.25 mL of H_2SO_4 30%, 2.5 mL of cyclohexane, and 2.5 mL of sodium hypochlorite solution, shaken for 2 minutes. The cyclohexane layer (top layer) will be greenish yellow, if it is colorless, another hypochlorite solution is added approximately 2.5 mL, The water layer is discarded (bottom layer). Then the cyclohexane layer (top layer)

was washed with 10 mL of 0.5 N NaOH and shaken for 1 minute and the bottom layer was discarded, then the top layer (cyclohexane) was rinsed with 10 mL of distilled water, the cyclohexane layer (top) was collected for absorbance measurement using Ultraviolet-Visible Spectrophotometer (Flourence BMG Labtech). The same procedure was done for the jamu samples.

RESULT AND DISCUSSION

Sensitivity Test of the SNI 01-2893 Precipitation Method

The principle of the precipitation method is the formation of a white precipitate in the sample reacted with the test reagent which indicates the presence of artificial sweetener sodium cyclamate in the sample. SNI precipitation method reagents consist of HCl 10%, $BaCl_2$ 10%, and $NaNO_2$ 10%. The addition of hydrochloric acid serves to make the solution more acidic so that sodium cyclamate reacts more easily with $BaCl_2$ The addition of $BaCl_2$, aims to precipitate impurities in the solution. The addition of $NaNO_2$ reagent and heating to break the sulfate bond on the primary aliphatic amine bond, the heating process will trigger a distinctive odor. If the sulfate bond is broken, there will be a reaction between (Ba^{2+}) ions and sulfate ions (SO_4^-) which then produces an insoluble barium sulfate precipitate ($BaSO_4$) (Pratama et al., 2023). The reaction occured can be seen in Figure 1.

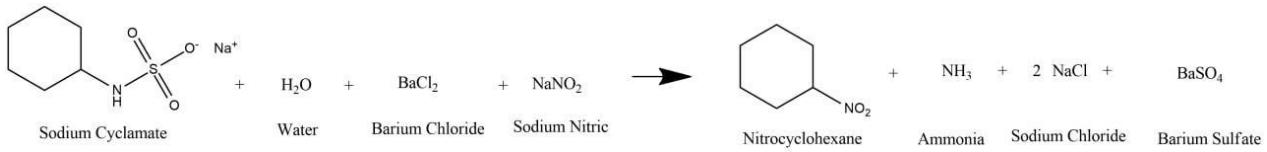

Figure 1. Reaction for Precipitation Method in Cyclamate Analysis

In conducting analysis with the aim of identification or quantification, analytical methods that have high sensitivity are needed (Shrivastava & Gupta, 2011). In this study, the sensitivity test of the method was carried out with the aim of measuring the sensitivity of the method in detecting cyclamate. Cyclamate solution starting from 5% or 50,000 ppm was reacted with the SNI 01-2893 precipitation method reagent resulting a white precipitate (Figure 2). Furthermore, a gradual decrease in the concentration of cyclamate solution was carried out and reacted with the same reagent, so that it can be seen how much the minimum concentration of cyclamate can be detected using the SNI 01-2893 reagent. The analysis results show that the SNI 01-2893 reagent has a detection limit at a concentration of 5 ppm. This was indicated by the formation of a white precipitate. At concentrations below 5 ppm no precipitate was formed.

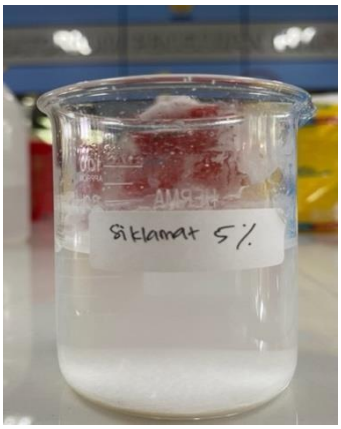

Figure 2 White Precipitate Formed on The Result of The SNI 01-2893 Precipitation Method on Cyclamate 5%

Optimization of Precipitation Reagent Formula

After knowing the sensitivity of the SNI 01-2893 method, optimization was then carried out to increase its sensitivity. Method optimization was carried out by making a new precipitation reagent formula. the SNI 01- 2893 formula was used as a control (Table 2).

Table 2 Data of Reagent Formula Optimization Result Againts 4 ppm Cyclamate

Formulas	Reagent Concentration			Test results*
	10 ml HCl	10 ml $BaCl_2$	10 ml $NaNO_2$	
F1 (control)	**10%**	**10%**	**10%**	**-**
F2	10%	15%	10%	+
F3	10%	20%	10%	+
F4	10%	30%	10%	+
F5	10%	10%	15%	+
F6	10%	10%	20%	+ + + + + +

*The more positive signs (+) the more precipitate is formed

The optimization results in Table 2 showed that formula 6 (F6) produced the most precipitate. F6 consists of HCl 10%, $BaCl_2$, 10%, and $NaNO_2$, 20%. The difference of F6 from the previous reagents lies in the concentration of sodium nitrite ($NaNO_2$). In this study, it was found that changes in the concentration of sodium nitrite ($NaNO_2$) played a role in the formation of precipitation reactions in the sample. The increase in sodium nitrite concentration is directly proportional to the amount of precipitate formed.

After obtaining the optimum precipitation reagent formula, the reagent sensitivity level was measured. The results showed that F 6 is sensitive to cyclamate sample up to a concentration of 0.9 ppm as shown in Table 3 which is characterized by the formation of white precipitate.

Table 3. Sensitivity Test Data of F6

Sample Concentration	Reagent	Precipitation Reaction Results
3 ppm	F6	+
2 ppm	F6	+
1 ppm	F6	+
0.9 ppm	F6	+
0.8 ppm	F6	-

The F6 formula was then used in qualitative testing of 10 samples of jamu beras kencur in ready-to-consume bottles. It was found that of the 10 samples tested, 9 of them showed positive results of sodium cyclamate (Table 4), which was characterized by the formation of a white precipitate.

Table 4. Analysis of Cyclamate In Jamu Using F6 Formula

Sample Code	Reaction (precipitate)*	Information
Sample 1	+	There is sodium cyclamate
Sample 2	-	No sodium cyclamate
Sample 3	+	There is sodium cyclamate
Sample 4	+	There is sodium cyclamate
Sample 5	+	There is sodium cyclamate
Sample 6	+	There is sodium cyclamate
Sample 7	+	There is sodium cyclamate
Sample 8	+	There is sodium cyclamate
Sample 9	+	There is sodium cyclamate
Sample 10	+	There is sodium cyclamate

*Positive sign (+) there is precipitate, negative sign (-) there is no precipitate

Samples that were positive for cyclamate were then tested for sodium cyclamate levels quantitatively using the UV-Vis spectrophotometric method. Determination of the maximum wavelength of cyclamate was carried out and obtained the maximum wavelength for sodium cyclamate was 313 nm, with an absorbance value of 0.943, as seen in Figure 3.

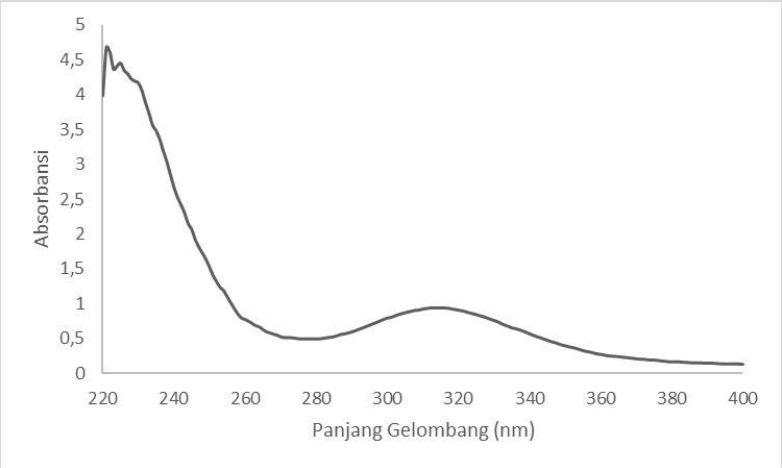

Figure 3. Maximum Wavelength of Sodium Cyclamate 313 nm

The preparation of the calibration curve of the cyclamate solution was carried out by preparing cyclamate standard solutions at various concentrations 40, 50, 60, 70, 80, 90, and 100 ppm. The absorbance of these solutions was measured at a maximum wavelength of 313 nm. The results of the calibration curve calculation are documented in Figure 4.

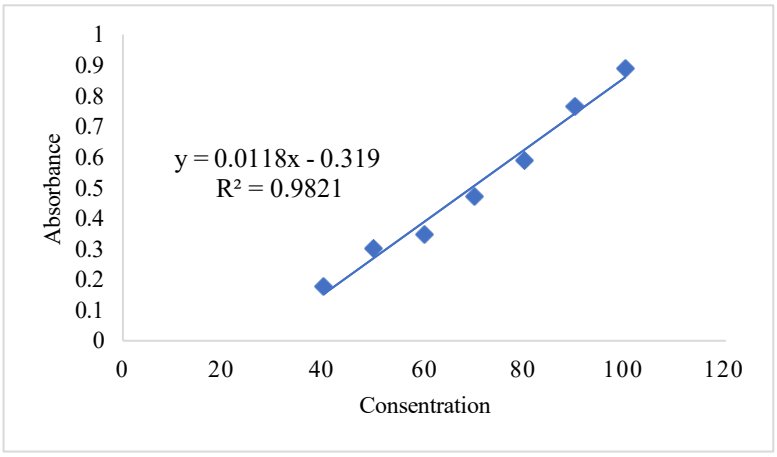

Figure 4. Calibration Curve of Sodium Cyclamate

The calculation resulted in a regression equation y = 0.0118x 0.319 with a correlation coefficient (R) of 0.990962 and a coefficient of determination (R) of 0.982005. From these results, it can be concluded that there is a positive correlation between concentration and uptake. In other words, the results show linearity when the concentration increases, the absorbance value will also increase. In addition, the results of the correlation coefficient meet the requirements of the value (r) in the range of $0.9 \leq r \leq 1$ (Rantung *et al.*, 2021).

Determination of cyclamate content in the sample was carried out by extracting cyclamate from the jamu sample, then measuring the absorbance using a UV-Visible spectrophotometer at the maximum wavelength. The first thing to do was to react the jamu sample with concentrated sulfuric acid to produce a colorless and hot solution. The purpose of adding concentrated sulfuric acid to the sample is to convert sodium cyclamate into cyclamic acid (Azizah *et al.*, 2022).

Then, the cyclamic acid solution was cooled and extracted using ethyl acetate solvent to form cyclamic acid in the organic phase. After that, cyclamic acid extraction is carried out using distilled water. The result of the extraction was a colorless solution which was then added with NaOH and cyclohexane. The function of adding sodium hydroxide was to provide an alkaline solution and to re-form sodium cyclamate (Juniar *et al.*, 2022). Sodium cyclamate is a salt and has good solubility in water, then a colorless water layer was collected. The water layer was added with 30% H_2SO_4, cyclohexane and hypochlorite to form 2 layers. The top layer was cyclohexane and the bottom layer was colorless water layer. The addition of cyclohexane solvent served as a solvent that can extract cyclamate, while the addition of hypochlorite aimed to give color to the extraction results containing cyclamate. In the cyclohexane layer, cyclamate has been extracted in it and the absorbance was measured at a maximum wavelength of 313 nm (Azizah *et al.*, 2022).

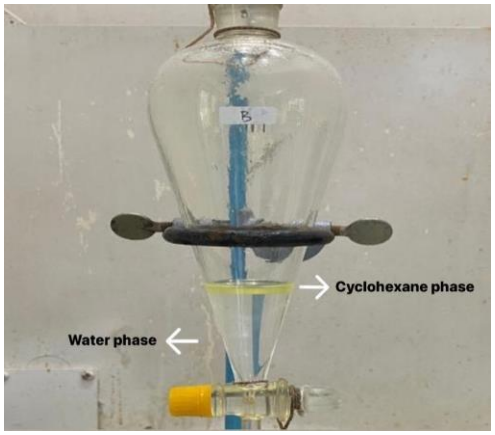

Figure 5. Cyclamate Extraction Results after Addition of Sodium Hypochlorite

The concentration of cyclamate in each sample was calculated using a linear regression equation obtained from the calibration curve. The results of the quantitative analysis of can be seen in Table 5.

Table 5. Sodium Cyclamate Level in Jamu Samples

Sample	Replication 1 (mg/kg)	Replication 2 (mg/kg)	Replication 3 (mg/kg)	Average levels (mg/kg)
Sample 1	3.7473	6.2286	7.7369	5.9043
Sample 3	7.4539	7,265	7.8541	7.5243
Sample 4	4.6036	4.2042	4.8624	4.5567
Sample 5	8.5826	4.7988	4.6186	6
Sample 6	5.4656	7.3405	7.7769	6,861
Sample 7	6.4235	6.5423	7,322	6.7626
Sample 8	7.3293	4.4417	8.9973	6.9227
Sample 9	7.1156	5.6558	6.3380	6.3698
Sample 10	4.2715	6.3689	6.6393	5.7599

According to the regulation, the maximum limit of sodium cyclamate in traditional medicine products including jamu is 1,250 mg/kg of product, calculated on products that are ready for consumption (BPOM RI, 2019). Based on a total of 9 samples tested using the spectrophotometric method, it showed that all samples meet the requirements of BPOM as shown in Table 6.

Several previous studies have determined cyclamate levels in jamu samples, such as research by Jamila *et al*., (2023) determined cyclamate levels in instant jamu marketed in the Pamekasan City using the gravimetric method. The results showed that of the 3 samples tested, 1 sample was positive for cyclamate with a level of 4,804 mg/kg sample. Another study that also used the gravimetric method was conducted by Kesuma, (2021) on samples of tamarind turmeric jamu marketed in several traditional markets in Malang City, it was found that 1 out of 4 samples was positive containing cyclamate with a level of 4.0182 g/L.

Other research was also conducted by Falahudin *et al*. (2016) using the gravimetric method with the results of 5 jamu samples from different traders were contained cyclamate with levels of 3 grams/liter each. the results of previous and current studies showed that the use of artificial sweetener sodium cyclamate in the jamu is exist. Regulations that allow the use of sodium cyclamate in food and its availability at a more affordable price than natural sugar encourage food and beverage manufacturers to choose cyclamate as an alternative sweetener. They should follow recommended limit of sodium cyclamate to avoid adverse effects due to the compound.

Table 6. Cyclamate Level in Jamu samples

Sample	Cyclamate (mg/kg)	Level suitability*
Sample 1	6.98275	M.S
Sample 3	7.55955	M.S
Sample 4	4.5333	M.S
Sample 5	4.7087	M.S
Sample 6	7.5587	M.S
Sample 7	6.93215	M.S
Sample 8	6.7195	M.S
Sample 9	5.9969	M.S
Sample 10	6.5041	M.S

*MS = Qualified; TMS = Not Qualified

CONCLUSION

The SNI 01-2893 precipitation method reagent is sensitive to cyclamate solutions up to a concentration of 5 ppm which is characterized by the formation of a white precipitate. The optimized formula for the precipitation method consists of HCl 10%, $BaCl_2$ 10%, and $NaNO_2$ 20%. The formula can produce a white precipitation at cyclamate concentrations up to 0.9 ppm, below the minimum concentration that can be detected by the SNI precipitation reagent. The optimized formula F6 used to examine 10 samples of jamu sold in the Semarang area and found that 9 samples showed positive results for sodium cyclamate. Analysis of sodium cyclamate levels in the samples based on spectrophotometric method showed that all samples met the requirements of BPOM.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest

REFERENCES

Apriliyani, SA, Martono, Y., Riyanto, CA, Mutmainah, M., & Kusmita, K. (2018). Validation of UV-VIS Spectrophotometric Methods for Determination of Inulin Levels from Lesser Yam (Dioscorea esculenta L.). Journal of Chemical Science and Applications, 21(4), 161–165. https://doi.org/10.14710/jksa.21.4.161-165

Azizah, N., Gatera, VA, & Ratnasari, D. (2022). Analysis of Cyclamate Levels Using the UV-VIS Spectrophotometry Method in Powdered Drinks in East Telukjambe. Journal of Education and Counseling (JPDK), 4(6), 1149–1155.

BPOM RI. (2019). Food and Drug Supervisory Agency Regulation Number 11 of 2019 concerning Food Additives. 1–1156.

Darni, Rahmadani, & Alawiyah, T. (2020). Analysis of SO42- Sulphate Levels in Drinking Water Containing Alum Using the UV-Vis Spectrophotometer Method. Journal of Pharmaceutical Care and Science, 1(1), 1–9.

Falahudin, I., Pane, ER, & Arumsari, A. (2016). Test of Cyclamate Content in Legen Jamu Gendong in Sekip Jaya Village, Palembang. UIN Raden Fatah Biota Journal, 2(2), 138–142.

Harminta. (2006). Quantitative analysis of raw materials and pharmaceutical preparations (Harminta (ed.); 1st ed.). Department of Pharmacy FMIPA-UI. https://lib.ui.ac.id/detail?id=144337&location=lokal

Hernaningsih, M., & Jayadi, L. (2021). Syrup circulating in the Malang Large Market Quantitatively Using the Uv-Vis Spectrophotometry Method. Indonesian Journal of Pharmaceutical Research, 3(3), 199–210.

Hoo, D., & Hu, C. C. (1972). Quantitative Conversion of Cyclamate to N,N-Dichlorocyclohexylamine, and Ultraviolet Spectrophotometric Assay of Cyclamate in Food. Analytical Chemistry, 44(12), 2111–2113. https://doi.org/10.1021/ac60320a045

Irawan, A. (2019). Spectrophotometer Calibration as Quality Guarantee of Measurement Results in Research and Testing Activities. Indonesian Journal of Laboratory, 1(2), 1. https://doi.org/10.22146/ijl.v1i2.44750

Jamila, N., Rahman, A.P., & Humaidi, F. (2023). Analysis of Cyclamate Content in Unbranded Instant Herbal Medicinal Drink on Market in the City of Pamekasan. Scientific Periodicals of Pharmaceutical Chemistry, 10(2), 55–59.

https://doi.org/10.20473/bikfar.v10i2.51955

Juniar, IM, Utami, MR, & Hilmi, IL (2022). Sodium Cyclamate in Candied Fruit Circulating in Karawang Regency. Health Research Shoots, 12(3), 286–293.

Kesuma, S. (2021). Safety of Traditional Medicine Turmeric Asem Herbal Medicine in Several Traditional Markets in Malang City. MEDFARM: Journal of Pharmacy And Health, 10(1), 11–17. https://doi.org/10.48191/medfarm.v10i1.49

Lusiana, U. (2012). Application of Calibration Curve, Accuracy and Precision Chart as Internal Quality Control at COD Testing in Wastewater. Biopropal Industry, 3(1), 1–8.

Nugraheni, RA (2022). Effect Of Administration Of Sodium Cyclate (C6h12nna03s) On The Number Of Polymorphonuclear Cells (PMN) In Rats (Rattus Norvegicus). Graduate Bioscience Journal, 24(1), 50–55. https://doi.org/10.20473/jbp.v24i1.2022.50-55

Padmaningrum, RT, & Marwati, DS (2015). Validation of Cyclamate Analysis Method With Spectrophotometry and Turbidimetry Validation of Cyclamate Analysis Method With Spectrophotometry and Turbidimetry. J. Basic Science, 4(1), 23–29.

Pratama, SI, Suhaenah, A., & Tahir, M. (2023). Analysis of Artificial Sweeteners, Sodium Cyclamate, in Packaged Beverages and Powders by the Gravimetric Method. Pharmaceutical Reports, 2(2), 2–5. https://doi.org/10.33096/pharmrep.v2i2.267

Rantung, O., Korua, AI, & Datau, H. (2021). Comparison of Vitamin C Extraction from 10 Types of Fruit Using Sonication and Homogenization. Indonesian Journal of Laboratory, 4(3), 124–133. https://doi.org/10.22146/ijl.v4i3.69983

Rohmah, SAA, Muadifah, A., & Martha, RD (2021). Validation of the method for determining levels of sodium benzoate preservative in soybean juice in several sub-districts in Tulungagung Regency using a UV-Vis spectrophotometer. Journal of Science and Health, 3(2), 120–127. https://doi.org/10.25026/jsk.v3i2.265

Shrivastava, A., & Gupta, V. (2011). Methods for the determination of limits of detection and limits of quantitation of the analytical methods. Chronicles of Young Scientists, 2(1), 21. https://doi.org/10.4103/2229-5186.79345

Sumantiri, AR (2018). Food Analysis. UGM Press. https://books.google.co.id/books/about/Analysis_makanan.html?id=25RjDwAAQBAJ&redir_esc=y

Takayama, S., Renwick, A.G., Johansson, S.L., Thorgeirsson, U.P., Tsutsumi, M., Dalgard, D.W., & Sieber, S.M. (2000). Long-term toxicity and carcinogenicity study of cyclamate in nonhuman primates. Toxicological Sciences, 53(1), 33–39. https://doi.org/10.1093/toxsci/53.1.33

Zhou, Q., Peng, Z., & Huang, X. (2022). Establishment of a Stable Acute Drug-Induced Liver Injury Mouse Model by Sodium Cyclamate. Journal of Inflammation Research, 15(February), 1599–1615. https://doi.org/10.2147/JIR.S354273