ANALYTICAL METHOD VALIDATION OF CYCLAMATE IN RED SYRUP IN NORTH BANJARMASIN USING UV-VIS SPECTROPHOTOMETER

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ABSTRACT

Cyclamate is an artificial sweetener that is not allowed for public consumption, because it is specifically permitted for diabetics and consumers with low-calorie diets. Syrup usually added as a flavor enhancer (sweetener), color, and aroma. The purpose of adding artificial sweeteners to syrup is to reduce production costs because cyclamates have a higher sweetness and lower price than sugar. The purpose of this study was to determine the content and levels of cyclamate in syrup sold in various stalls and street sellers in the North Banjarmasin area, and to determine the data validation method with linearity, precision, accuracy, LOD, and LOQ. The results of the qualitative test showed that 4 of the 9 positive samples contained cyclamate (samples A, C, E, and I). The results of the method validation parameter test for linearity at 20, 40, 60, 80, 100, and 120 ppm gave a value of the correlation coefficient (r) of 0.9987 with an LOD value of 6.4307 ppm, LOQ of 21.4359 ppm,d precision value of 0.74%, and an accuracy value of 89.5126%. The results of the quantitative tests were carried out on four samples, namely samples A, C, E, and I, with cyclamate levels of 12.9423, 31.9833, 23.4166, and 115.6469 mg/kg, respectively.

Keywords: Cyclamate, Syrup, Precipitation, Validation Method, UV-Vis Spectrophotometry

INTRODUCTION

Humans require food and drinks as energy sources for their daily activities. Food safety is an important requirement for food and beverages consumed by Indonesians (Siregar et al., 2013). Therefore, the food and drink consumed must be nutritious, safe, and healthy but should not pose a risk of causing health problems. Food safety is related to the avoidance of biological, chemical, and other contaminants that can endanger health and are safe for consumption (BPOM, 2016).

Currently, various ingredients, including sweeteners, flavorings, and preservatives, are being added to food and beverages. Materials added to these foods and beverages are known food additives. Food Additives (BTP) are ingredients added to food to influence the nature or form of food (BPOM RI, 2019). One of the materials used in Food Additives (BTP) is a sweetener. In addition to increasing taste and aroma, sweeteners function as preservatives and improve physical properties (Novitasari, Rahma and Puspitasary, 2019). Low prices and stronger sweetness than sugar make artificial sweeteners in great demand by large and home industries (Effendi, Fardian and Maulina, 2017). Although it can be used as a sweetener, this ingredient is known to have no nutritional value (BPOM, 2014).

Sweeteners consist of both natural and artificial sweeteners. Cyclamate is one of the ingredients of artificial sweeteners. The use of cyclamate is not permitted for general consumption because artificial sweeteners such as cyclamate are sweeteners that are

specifically permitted for diabetics and consumers on a low-calorie diet (Musiam, Hamidah and Kumalasari, 2016).

According to the Indonesian National Standard (SNI 01-6993-2004), the permissible content of cyclamate in sugar and other syrups is 500 mg/kg. Short-term use of cyclamate can cause very common symptoms, such as dizziness, nausea, vomiting, diarrhea, and difficulty defecating, whereas if consumed in excess, it can cause dangerous side effects (Effendi, Fardian and Maulina, 2017; Yusuf and Nisma, 2017).

One drink that is often supplemented with artificial sweeteners is syrup. Syrup is a processed liquid product consumed by many people to quench thirst. Syrup is made from a mixture of water and sugar with a sugar solution content of at least 65%, with or without other food ingredients or food additives approved in accordance with applicable regulations (Badan Standardisasi Indonesia, 2013). Syrup is often found in stalls and street seller Most traders add syrup to coconut ice, es campur, es teler and other drinks as a taste enhancer (sweetener), color and aroma. The syrup that they usually use is their own processed syrup whose quality and safety have not been guaranteed. In addition, sugar, which is the basic ingredient for making syrup, sometimes experiences price increases; therefore, traders can add artificial sweeteners to reduce production costs.

Research has been conducted on the cyclamate content of various beverage products. According to Suliati's research (2020) it was found that 2 samples in es *campur* drinks sold in the Kopelma Darussalam area, Syiah Kuala District, Banda Aceh, were positive for cyclamate at levels of 205.65 ppm and 172.708 ppm. Another study conducted by Hidayat (2019) on snack drinks sold at an elementary school on Sunggal Street No. 223 Medan by UV spectrophotometry showed that all three samples tested positive for cyclamate, and the levels in successive samples were 25.8328 mg/kg, 15.0330 mg/kg, and 26.6781 mg/kg. Another study conducted by Hernaningsih and Jayadi (2021) on syrup circulating in the Malang big market using the UV-Vis spectrophotometry method found that all three positive samples contained cyclamate artificial sweeteners with successive levels of 238.78 mg/kg, 239.65 mg/kg and 241.39 mg/kg.

Several traders in North Banjarmasin have found that cyclamate is an artificial sweetener. This is evidenced by research conducted by Musiam et al., (2016) on red syrup sold in North Banjarmasin. This study showed that 6 of the 15 samples were positive for cyclamate, which varied using the gravimetric method.

Analysis of cyclamate compounds can be performed using qualitative precipitation and TLC methods. Meanwhile, quantitative analysis of the cyclamate content can be carried out by measuring the sample absorption and maximum wavelength using UV-visible spectrophotometry UV-Vis spectroscopy because of the presence of its chromophore (from sodium cyclamate) that can be detected by UV-Vis spectroscopy. spectrophotometry. This approach has several advantages, including significant precision, accuracy, low cost, and easy handling, even when only a small concentration or volume is used (Rohmah Muadifah and Martha, 2021).

Our study aimed to analyze the levels of cyclamate in syrup sold in various stalls and street vendors in the North Banjarmasin area using more specific and quantitative methods such as UV-Vis spectrophotometry.

RESEARCH METHODS

Equipment and Materials

The equipment used in this study included a UV-Vis spectrophotometer (PG Instruments). Red syrup was used as the sample, with Sodium Cyclamate 98% pro analysis (Alfa Aesar) as the standard (BPFI) and another material, 10% HCl solution, $BaCl_2$ p.a (Smart-Lab), $NaNO_2$ p.a (EMSURE®), sodium hydroxide (Sodium hydroxide) p. a (Merck), sulfuric acid p.a (Smart-Lab), NaOCl (Smart-Lab), cyclohexane p.a (Loba Chemie), and ethyl acetate p.a (Smart-Lab).

Research Procedure

1. Qualitative Analysis

The analysis used in this study was carried out according to the deposition method of SNI 01-2893-1992, as in another study, with slight modifications (Effendi, Fardian and Maulina, 2017). The negative control solution was prepared by adding 100 ml of distilled water to a volumetric flask. The positive control was prepared by dissolving 1 g of sodium cyclamate (BPFI) in a volumetric flask containing 100 ml of distilled water. The solution was transferred into a 100 ml Erlenmeyer flask, 20 ml mixture of 10% hydrochloric acid and 10% Barium Chloride (1:1), and allowed to stand for a half an hour. Then, 10 ml of 10% $NaNO_2$ solution was added. The solution was heated in a water bath for about 20-30 minutes. The positive solution contained cyclamate if a white precipitate formed.

The red syrup sample (25 mL) was placed in an Erlenmeyer flask, diluted with distilled water at a ratio of 1:1, and filtered. Then, 20 ml mixture of 10% hydrochloric acid and 10% Barium Chloride (1:1) was added to the filtrate and left for a half an hour. Then, 10 ml of 10% $NaNO_2$ solution was added. The solution was heated on a hot plate or water bath for approximately 20-30 minutes. The presence of a white precipitate indicated that the sample was positive for cyclamates.

2. Method Validation

a. Linearity test

A standard solution was prepared from 1000 ppm sodium cyclamate at concentrations of 20, 40, 60, 80, 100, and 120 ppm in a 50 ml volumetric flask. Each of these solutions was placed in a separatory funnel containing 25 ml of distilled water, 98% H2SO4 (2.5 mL) was added, and the mixture was cooled. Subsequently, 50 ml of $CH_3COOC_2H_5$ was added and shaken for 2-3 minutes and the clear part was removed. The clear portion was then placed in the second separatory funnel and extracted with 15 ml of water, which was repeated 3 times. The water layer was collected and transferred to a third separatory funnel, and 1 ml of Sodium Hydroxide (10 N) and 5 ml of C_6H_{12}. The mixture was then shaken for approximately 60 seconds. The aqueous layer (bottom layer) was then placed in a fourth separatory funnel. About 2.5 ml of 30% sulfuric acid, 5 ml of $C_6H_{12,}$ and 5 ml of NaOCl were added and shaken for approximately 2-3 minutes. The top layer (cyclohexane) had a greenish-yellow color. If this layer was colorless, 5 ml NaOCl solution was added and then removed. The top layer (cyclohexane) was washed with 25 ml of 0.5 N NaOH and shaken for 1 minute. While the bottom layer of NaOH was discarded, the top layer (cyclohexane) was washed with 25 ml of distilled water. The mixture was then shaken and the water layer was separated. Cyclohexane was used and the absorbance was read on a UV-Vis spectrophotometer at the maximum wavelength and repeated 3 times (Hadju et al., 2013).

Calibration curves were generated using the standard concentrations (x-axis) and absorbance (y-axis). The linearity test was determined through the linear regression equation Y = bx + a and the value of r resulting from the standard curve. The correlation is considered to be very good if the value of r received is close to 1 or greater than 0.9970 (Riyanto, 2014).

b. Precision test

A standard cyclamate solution with a concentration of 60 ppm was used, and absorbance was measured at the maximum wavelength. The measurements were repeated 6 times. Precision values were expressed as the Relative Standard Deviation (%RSD). The obtained value met the criteria for precision testing requirements, that is, <2% (Rohman, 2014).

c. Accuracy test

Accuracy was determined using the standard addition method at three different concentrations: 20, 60, and 120 ppm. The absorbance of each concentration

was measured and replicated 3 times (the concentration obtained was expressed as Ca). The syrup sample solution that was positive for cyclamate was taken as much as 2 ml and then the absorbance was read with 3 repetitions. (the concentration obtained was expressed as Cu). Then, a standard solution with concentrations of 20, 60, and 120 ppm was added to the cyclamate-positive syrup samples. A number of standard solutions were put into a 10 ml volumetric flask, and then a positive sample solution was added to the volumetric flask up to the mark. The absorbance of the solution was measured using a UV-Vis spectrophotometer with 3 repetitions (the concentration obtained was expressed as Cf) (Manoppo, Sudewi and Wewengkang, 2019). Accuracy is expressed as percent recovery.

d. Test limit of detection and limit of quantitation

The limit of detection (LOD) and Limit Of Quantitation (LOQ) were determined using the results of standard concentration data, whose absorption was measured using a UV-Vis spectrophotometer. The LOD and LOQ values were calculated using the linear equation of the calibration curve (Riyanto, 2014).

3. Quantitative Analysis

a. Preparation of standard solutions and standard curves

A standard solution was prepared at a concentration of 1000 ppm. As much as 250 mg of cyclamate was put into a 250 ml volumetric flask and dissolved in distilled water up to the mark. For the standard curve solution, from a standard cyclamate solution of 1000 ppm, dilutions were made with volume variations: 1; 2; 3; 4; 5; and 6 ml to obtain concentrations of 20; 40; 60; 80; 100, and 120 ppm, diluted in a 50 ml volumetric flask with distilled water up to the mark. Then, each of these solutions was put into a separatory funnel containing 25 ml of distilled water, 98% H2SO4 (2.5 mL) was added, and the mixture was cooled. Subsequently, 50 ml of $CH_3COOC_2H_5$ was added, shaken for approximately 2-3 minutes and the clear part was taken. The clear portion was then placed in the second separatory funnel and extracted with 15 ml of water, which was repeated 3 times. The water layer was collected and inserted into the third separatory funnel, and 1 ml of 10 N sodium hydroxide and 5 ml of C_6H_{12} were shaken for 60 seconds. The aqueous layer (bottom layer) was put into the fourth separatory funnel, 2.5 ml of 30% sulfuric acid, 5 ml of C_6H_{12}, and 5 ml of NaOCl solution were added and shaken for about 2-3 minutes. The top layer (cyclohexane) had a greenish-yellow color. If this layer was colorless, ± 5 ml of NaOCl solution was added. Cyclohexane (top layer) was then washed with 25 ml of 0.5 N NaOH and shaken for 60 seconds. Then, the top layer (cyclohexane) was washed with 25 ml of distilled water, shaken, and removed. Cyclohexane (top layer) was obtained (Hadju *et al*., 2013).

b. Preparation of blank solutions

Approximately 50 ml of water was inserted into a separatory funnel, 98% H2SO4 (2.5 mL) was added, and the mixture was cooled. Subsequently, 50 ml of $CH_3COOC_2H_5$ was added, shaken for approximately 2-3 minutes and ± 40 ml of the clear portion was taken. Then, the clear portion was brought into the second separatory funnel and extracted with 15 ml of water, which was repeated 3 times. The water layer was collected and inserted into the third separatory funnel, and 1 ml of 10 N sodium hydroxide and 5 ml of C_6H_{12} were shaken for 60 seconds. The ethyl acetate was removed, the water layer then brought into the fourth separatory funnel, added 2.5 ml of 30% Sulphuric acid, 5 ml of C_6H_{12} and 5 ml of NaOCl solution, shaken for 2-3 minutes. The top layer (cyclohexane) has a greenish-yellow color; if this layer is colorless, ± 5 ml of NaOCl solution is added. The water layer was removed, the cyclohexane layer was washed with 25 ml of NaOH (0.5 N) and shaken for 1 minute, and the bottom layer was removed. The top layer (cyclohexane) was washed with 25 ml of distilled water and shaken. The water layer was separated and cyclohexane was used as a blank (Hadju *et al.*, 2013).

c. Maximum wavelength scanning

Then, 5 ml of 1000 ppm cyclamate standard solution was diluted in 50 ml distilled water using a volumetric flask to the boundary mark to obtain a concentration of 100 ppm. The solution was placed in a cuvette and the absorbance was read at a specified wavelength range (200-400 nm) (Hidayat, 2019).

d. Calibration curve measurements

The absorbance of the top layer of each calibration curve series solution was measured at the maximum wavelength, and a blank solution was used for comparison. A standard curve is constructed between concentration and absorption to obtain a regression equation, which is used for calculations in the next analysis (Hadju *et al.*, 2013).

e. Determination of cyclamate content

A total of 25 ml of the sample was diluted with 25 ml of distilled water, placed in the first separatory funnel, 98% H2SO4 (2.5 mL) was added, and the mixture was allowed to cool. Subsequently, 50 ml of $CH_3COOC_2H_5$ was added, and the mixture was shaken for approximately 2-3 minutes. The top layer was separated and the clear part was collected and placed in a second separatory funnel. Water (15 ml of water was added and shaken 3 times, and the water layer was collected and inserted into the third separatory funnel, 1 ml of 10 N sodium hydroxide, and 5 ml of $C_6H_{12,}$ and shaken for 60 seconds. The top layer then put into the fourth separatory funnel, added 2.5 ml of 30% sulfuric acid, 5 ml of $C_6H_{12,}$ and 5 ml of NaOCl were shaken for 2-3 minutes. The cyclohexane layer had a greenish-yellow color. If this layer was colorless, approximately 5 ml of NaOCl solution was added. The top layer (cyclohexane) was then added to 25 ml of 0.5 N NaOH, shaken, and separated. Then, the bottom layer was removed. The bottom layer was removed and rinsed with 25 ml of distilled water. The absorbance of cyclohexane (top layer) was measured (Hadju *et al.*, 2013).

Data Analysis

The data analysis used in this study included qualitative, descriptive, and quantitative analyses. A qualitative descriptive analysis was used to describe the research results from the laboratory tests. The results of the data obtained in the form of numbers were analyzed using Microsoft Excel

.

RESULTS AND DISCUSSION

Sodium cyclamate is an artificial sweetener with a low-calorie content and is composed of a sodium salt because it is more soluble in water than free acid. According to its structure **Figure 1**, NH-SO_3 and cyclohexane enable the structure to absorb ultraviolet radiation. Chromophores and auxochromes lead to UV absorption bands in the structure, characterized by the wavelength of the absorption maximum. The Lambert-Beer Law showed that absorbance is proportional to concentration, and quantification of sodium cyclamate could be performed using a UV-Vis Spectrophotometer.

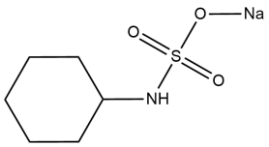

Figure 1. Sodium Cyclamate Structure

1. Sample Identity

The sample used in this study was red syrup sold by street sellers who sell coconut ice and mixed ice, which usually use red syrup as a sweetener. Sampling was conducted in

the North Banjarmasin area with certain considerations so that the sample could represent the red syrup population sold by street sellers in this area. The characteristics of the samples taken in this study can be seen in **Table I**.

Table I. Organoleptic Characteristics of the Sample

No	Sample Code	Organoleptic			
		Taste	Viscosity	Color	Odor
1.	A	Sweet	slightly viscous	Red	Rose
2.	B	Sweet	liquid	Orange red	Vanilla
3.	C	Sweet	liquid	Orange red	Vanilla
4.	D	Sweet	slightly viscous	Orange red	Vanilla
5.	E	Sweet	liquid	Red	Vanilla
6.	F	Sweet	slightly viscous	Red	Vanilla
7.	G	Sweet	liquid	Red	Vanilla
8.	H	Sweet	liquid	Orange red	Vanilla
9.	I	Sweet	liquid	Pink	Vanilla

2. Qualitative Test

A qualitative test of the cyclamate content in red syrup was performed using the precipitation method. Before the samples were analyzed, positive and negative control solutions were prepared for comparison. In the positive control, a white precipitate was formed, whereas in the negative control, no precipitate was observed. The results of the positive and negative control solutions are shown in **Figure 2**.

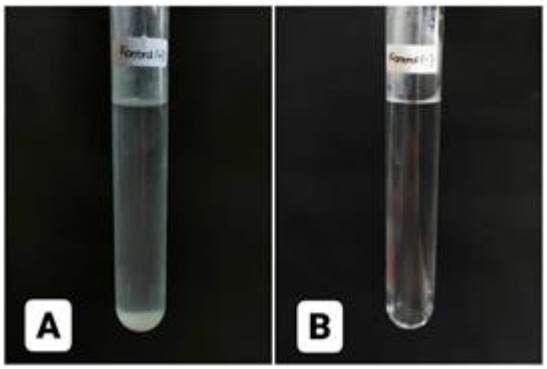

Figure 2. Positive Control = A, Negative Control = B

Sample evaluation was initiated by diluting the sample using an aquadest at a ratio of 1:1. This aims to hydrolyze Na-cyclamate into Na+ ions and cyclamate ions to facilitate the reaction between the sample and the reagent or reagent. Addition of 10% HCl acidified the solution. The solution was prepared in an acidic state to allow the solution to react faster. The addition of $BaCl_2$ was aimed at precipitating impurities in the solution. Barium Sulfate ($BaSO_4$) is an impurity obtained from the addition of $BaCl_2$ and $NaNO_2$ when reacting with cyclamate. $NaNO_2$ is useful for breaking sulfate bonds in cyclamates. When the sulfate bond is broken, Ba^{2+} ions react with the sulfate ions to produce a precipitate of Barium Sulfate ($BaSO_4$) (Qamariah and Rahmadhani, 2017). The reaction that occurred in a sample containing cyclamate is shown in **Figure 3**.

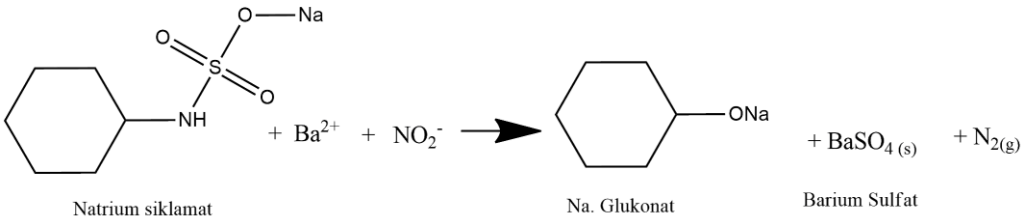

Figure 3. Cyclamate deposition reaction (Musiam, Hamidah and Kumalasari, 2016)

Positive results in the qualitative test for cyclamate content using the deposition method at the time of the study were indicated by the presence of white-to-pink precipitates. These results are in accordance with the study by Musiam et al (2016), where the pink precipitate is considered to be the same as the white precipitate, which indicates the presence of cyclamate in the sample. The pink precipitate is the result of the base color of the syrup sample, which is red. The results of qualitative testing of the cyclamate content using the precipitation method are shown in **Table II**.

Table II. Qualitative test results for red syrup samples

No	Sample Code	Result	Detail information
1	Positive Control	(+)	White Precipitate
2	Negative Control	(-)	No Precipitate
3	A	(+)	White Precipitate
4	B	(-)	No Precipitate
5	C	(+)	White Precipitate
6	D	(-)	No Precipitate
7	E	(+)	White Precipitate
8	F	(-)	No Precipitate
9	G	(-)	No Precipitate
10	H	(-)	No Precipitate
11	I	(+)	Pink Precipitate

Based on the results of the research conducted, it was shown that 4 out of 9 samples of red syrup contained cyclamate, namely samples A, C, E, and I. This was indicated by the presence of white and pink precipitates. Samples B, D, F, G, and H did not contain sediment.

3. Maximum Wavelength

The maximum wavelength (λ max) is used to determine the absorption area where the substance can be read optimally by a UV spectrophotometer (Manoppo, Sudewi, and Wewengkang, 2019). Based on the results of the measurements of the wavelength of the cyclamate standard solution, a maximum wavelength of 292 nm was obtained with an absorbance of 0.698 nm. The wavelengths obtained were still in the range of 200–300 nm and in the range of the optimum absorption region of cyclamate (Rauf, Sudewi and Rotinsulu, 2017). This indicates that the sample met the requirements for the analysis.

4. Method Validation

a. Linearity

Before the linearity test, a standard cyclamate solution was prepared at concentrations of 20; 40; 60; 80; 100; and 120 ppm of 1000 ppm cyclamate. Each concentration was pipetted as much as 1, 2, 3, 4, 5, and 6 ml into a 50 ml volumetric

flask, and distilled water was added up to the mark. The absorbance of each solution was measured at the maximum wavelength using a UV-Vis spectrophotometer. The measurement results for the standard cyclamate solution are listed in **Table III**.

Table III. Results of absorbance measurements of standard cyclamate solutions

Concentration (ppm)	Absorbance			
	R1	R2	R3	Average
20	0,219	0,214	0,209	0,214
40	0,331	0,328	0,329	0,329
60	0,455	0,451	0,440	0,449
80	0,579	0,582	0,581	0,581
100	0,698	0,694	0,691	0,694
120	0,853	0,853	0,853	0,853

From the results of measuring the absorbance of standard cyclamate solutions, a standard curve was constructed, as shown in Figure 3. From this curve, the regression equation for the standard cyclamate was y = 0.0063x + 0.0775, with a coefficient of relation (r) = 0.9987. The acceptance requirement for the linearity test results according to Riyanto (2014) is more than 0.9970, meaning that the results of the linearity test carried out fulfilled these requirements.

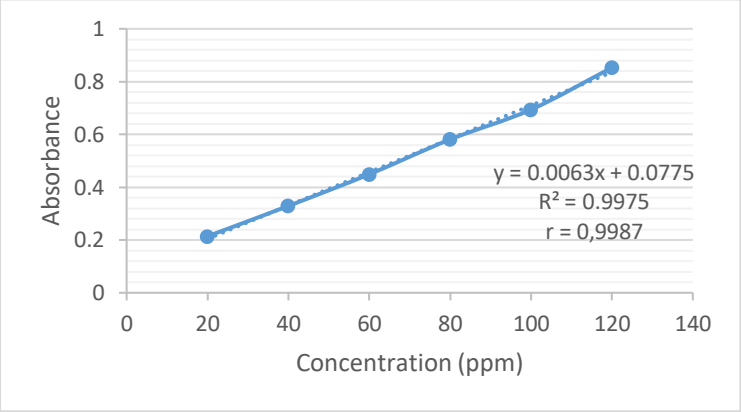

Figure 4. Cyclamate Standard Linearity Curve

Based on the results obtained, the curve shows that the absorbance produced is proportional to the increase in cyclamate concentration, where the higher the cyclamate concentration, the higher the absorbance produced.

b. Precision

Precision aims to determine how closely the results of the analysis of the same sample are repeated. Precision tests were performed to show the degree of correspondence between the results measured under certain conditions (Rohman, 2014). Precision was determined by means of repeatability of a standard solution with a concentration of 100 ppm for 6 repetitions. The data and precision results are listed in **Table IV**.

Precision was expressed as the Relative Standard Deviation (RSD). The more precise the analytical method, the smaller the standard deviation (RSD) value obtained. The resulting RSD value was expected to be ≤ 2% to meet these requirements (Rohman, 2014). The RSD value obtained was 0.74%, indicating that the obtained value met the requirements.

Table IV. Precision Result Data

Replication	Absorbance	X	$\bar{X}$	X-$\bar{X}$	(X-$\bar{X}$)^2
1	0,698	98,49206349	97,4867	1,005291005	1,010610005
2	0,691	97,38095238		0,105820106	0,011197895
3	0,694	97,85714286		0,37037037	0,137174211
4	0,694	97,85714286		0,37037037	0,137174211
5	0,687	96,74603175		0,740740741	0,548696845
6	0,686	96,58730159		0,899470899	0,809047899
		∑			2,653901067
		SD			0,72854
		%RSD			0,74%

Note :
X : Concentration
$\bar{X}$: Average concentration
∑ : Total
SD : Standard Deviation
RSD : *Relative Standart Deviation*

c. Accuracy

Accuracy is the accuracy of the analytical method, or the closeness of the measured value to the actual analyte content. Accuracy is expressed as percent recovery (% recovery) (Rohman, 2014). In this study, the accuracy was determined using the standard addition method.

Table V. Accuracy Result Measurement

Sample Code	(+) Standard	R	Concentration (ppm)			% *Recovery*	Average % *Recovery*
			Cf	Cu	Ca		
A	20	1	0,364	0,195	0,219	77,1689	80,4075
		2	0,368	0,195	0,214	82,7751	
		3	0,373	0,195	0,209	81,2785	
	60	1	0,675	0,195	0,455	105,4945	107,0051667
		2	0,675	0,195	0,451	106,4301	
		3	0,675	0,195	0,44	109,0909	
	120	1	0,887	0,195	0,853	81,1254	81,1254
		2	0,886	0,195	0,853	81,0082	
		3	0,888	0,195	0,853	81,2426	
Total Average % *recovery*							**89,5126 %**

note :
Cf : Concentration of sample added with standard solution
Cu : Sample concentration
Ca : concentration of standard solution

First, the sample was analyzed, and a number of analytes to be tested (pure analytes/standards) were added to the sample, mixed, and re-analyzed (Riyanto, 2014). Accuracy was determined by adding standard solutions of 20, 60, and 120 ppm to syrup samples that positively contained cyclamate. Then, the absorbance of each solution was measured three times using a UV-Vis spectrophotometer 3 repetitions. The accuracy measurement results can be seen in **Table V**. Based on the calculations, the average % recovery was 89.5126%. The accuracy is said to be good

if the % recovery value obtained is 80-110%. In this study, the average value of % recovery obtained met these requirements.

d. Limit of Detection (LOD) and Quantitation Limit (LOQ)

The limit of detection is defined as the minimum concentration of the analyte in a sample that can still be detected, although it is not always quantified. The quantitation limit is defined as the minimum concentration of a sample that can be determined with acceptable precision and accuracy (Rohman, 2014). The LOD and LOQ values were calculated using the standard curve linear regression equation: y = 0.0063x + 0.0775. Data on the test results and the calculation of the LOD and LOQ values are shown in **Table VI**.

Table VI. Calculation results of LOD and LOQ values

	Values
a	0,0775
b	0,0063
S (y/x)	0,01350
LOD	6,4307
LOQ	21,4359

Based on these data, the LOD value obtained was 6.4307 ppm, indicating that this was the lowest concentration of cyclamate obtained without proper quantification. The LOQ value obtained was 21.4359 ppm, which indicates the lowest concentration that can be obtained by correct quantification.

5. Determination of Cyclamate in Samples

In this study, the levels of cyclamate in the syrup samples were determined using UV-Vis spectrophotometry. Prior to determining the sample concentration, a blank solution was prepared. The blank solution did not contain analytes and was used for comparison.

The cyclamate content in the sample was determined by dissolving the sample in distilled water at a 1:1 ratio. It was then reacted with concentrated H_2SO_4 to produce a colorless and hot solution. H_2SO_4 converted sodium cyclamate to cyclamate acid. The solution was cooled, and ethyl acetate was added, which is useful for extracting cyclamate. Then cyclamate acid was extracted with distilled water 3 times with the aim of binding the deepest cyclamate compound in the sample. The resulting extract was colorless. NaOH and cyclohexane were then added to the extract. NaOH aims to provide an alkaline atmosphere and reform Na-cyclamate. Cyclamate in the form of a salt, namely Na-cyclamate, dissolves in water; thus, at this stage, the water layer is taken. H2SO4, cyclohexane, and sodium hypochlorite were then added to the water layer. Cyclohexane aims to extract cyclamate, while hypochlorite is used as a reagent to give a yellowish-green color to solutions containing cyclamate.

In this phase, 2 layers are formed. The top layer is slightly greenish yellow, which is a cyclohexane solution, whereas the bottom layer is colorless, which is the water layer. The cyclohexane layer was rinsed with NaOH and again with distilled water. The cyclohexane layer was washed until a colorless solution was obtained (Hernaningsih and Jayadi, 2021). The top layer, namely, the cyclohexane layer, was used because the cyclamate had been extracted. The absorbance of the layer was measured using a UV-Vis spectrophotometer at a wavelength of 292 nm. The results of measuring the cyclamate levels in the samples are shown in **Table VII.**

Table VII. Results of measurements of cyclamate levels in the sample

Sample Code	Replication	Absorbance			Concentration (ppm)	Content (mg/Kg)	Average Content (mg/Kg)
		Sample	Blank	Sample – Blank			
A	R1	0,195	0,016	0,179	16,1111	12,9423	12,9423
	R2	0,195		0,179	16,1111	12,9423	
	R3	0,195		0,179	16,1111	12,9423	
C	R1	0,307		0,291	33,8888	30,8281	31,9833
	R2	0,313		0,297	34,8412	31,6945	
	R3	0,325		0,309	36,7460	33,4273	
E	R1	0,295		0,279	31,9841	30,6725	23,4166
	R2	0,225		0,209	20,8730	20,0170	
	R3	0,222		0,206	20,3968	19,5603	
I	R1	0,890		0,874	126,4285	116,2796	115,6469
	R2	0,887		0,871	125,9523	115,8416	
	R3	0,880		0,864	124,8412	114,8197	

Based on the results of measurements and calculations in **Table VII**, it is known that 4 samples that positively contained cyclamate were samples A, C, E and I. The average levels of cyclamate obtained respectively were sample A of 12.9423 mg/kg, sample C of 31.9833 mg/kg, sample E was 23.4166 mg/kg, and sample E was 115.6469 mg/kg **Table VII**. Cyclamate is an artificial sweetener that is not allowed for general consumption because artificial sweeteners such as cyclamate are sweeteners that are specifically permitted for diabetics and consumers on a low-calorie diet. According to the Indonesian National Standard (Badan Standardisasi Indonesia, 2013), the allowable cyclamate content in sugar and other syrup products is 500 mg/kg. The cyclamate levels obtained in this study indicated that the levels obtained were below 500 mg/kg/BB. This indicates that the cyclamate content in the sample was still below the permissible limit.

CONCLUSION

Based on the results of the qualitative test using the precipitation method, four out of nine samples contained white and pink precipitates, namely samples A, C, E, and I. The UV-Vis spectrophotometry method showed linearity with a correlation coefficient value (r) of 0.9987, precision test with an RSD value of 0.74%, accuracy test with an average % recovery of 89.5126%, LOD value of 6.4307 ppm, and LOQ value of 21.4359 ppm, indicating that this concentration is the lowest concentration of cyclamate obtained without being quantified precisely. The results of the method validation in this study met the requirements; therefore, it can be concluded that this method can be used to check cyclamate levels in syrup samples. Based on the results of the quantitative tests on 4 samples (samples A, C, E, and I) with cyclamate levels of 12.9423 mg/kg, 31.9833 mg/kg, 23.4166 mg/kg, and 115.6469 mg/kg. The cyclamate content obtained in the samples was below the permitted limit of 500 mg/kg body weight.

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