

Analysis of Sodium Benzoate Levels in Tomato Sauce Sold at Gadingrejo Market Using the U-HPLC (Ultra High Performance Liquid Chromatography) Method

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Abstract:- Food Additives (BTP) are ingredients that are added to food to affect the properties or form of food. One of the types of food additives that are allowed is preservatives. The preservative used for preservatives in food and beverages is sodium benzoate. This study aims to determine the content and content of sodium benzoate in tomato sauce sold at the Gadingrejo market. The sampling technique was in the form of random sampling. This study uses a qualitative method by looking at the color reaction of the reagent and quantitatively with U-HPLC. The motion phase used was methanol:aquabidest (50:50), octadecyl silica (ODS) stationary phase, flow rate 1 ml/min, UV-Vis detector 225 nm and injection volume 20 µL. Based on the results, the four positive samples contained sodium benzoate with each sample, namely the entire tomato sauce had a level of 1.4 mg/g. Based on the results of the study, it can be concluded that the four tomato sauce samples still meet the requirements for the maximum limit of sodium benzoate set by the Regulation of the Head of the Food and Drug Supervisory Agency Number 11 of 2019, which is 1 g/kg of material weight.

Keywords:- Sodium Benzoate, Preservative, Tomato Sauce, U-HPLC

I. INTRODUCTION

Food safety is an important requirement that must exist in food consumed by humans [1]. Food is everything that comes from biological sources, both processed and unprocessed, that is intended as food or beverage ingredients for human consumption, including food additives and food raw materials [2]. Food Additives (BTP) or food additives are ingredients or mixtures of ingredients that are not naturally part of food raw materials, but are added to food to affect the properties or form of food [3]. According to Regulation of the Minister of Health of the Republic of Indonesia No. 722/Menkes/Per/IX/88 explained that BTP is an ingredient that is not usually used as food and is usually not a *ingredien* food characteristics, having or not having nutritional value,

which are deliberately added to food for technological purposes in the manufacture, processing, packing, packaging, storage or transportation of food to produce a component or affect the characteristic properties of the food.

One of the types of food additives that are allowed is preservatives. Preservatives (*Preservative*) is a food additive to prevent or inhibit fermentation, acidification, decomposition, and other damage to food caused by microorganisms [5]. Preservatives are generally used to preserve food that has perishable properties. These ingredients can inhibit or slow down fermentation, acidification, or decomposition processes caused by microbes. However, it is not uncommon for producers to use it in relatively durable foods with the aim of extending the shelf life or improving the texture. Preservatives that are widely sold on the market and used to preserve various foods are benzoates, which are generally found in the form of sodium benzoate or potassium benzoate which are more soluble [6].

Preservatives may be added in small quantities or within the limits of permissible provisions. If the amount of preservatives used exceeds the prescribed limit, it can trigger health problems. The increasing growth of the food industry in Indonesia, there is an increase in the production of food circulating in the market containing preservatives including sodium benzoate, borax, formalin and others (Faroch *et al.*, 2021). The general requirements of preservatives are, can inhibit the growth of spoilage microbes in food, both pathogenic and non-pathogenic, can extend the shelf life of food, does not reduce the nutritional quality, color, taste and smell of preserved food [8].

Sodium Benzoate (C₆H₅COONa) is a sodium salt from benzoic acid that is commonly used in foodstuffs. In foodstuffs, Sodium Ben zoate will decompose into its active form, namely benzoic acid. Due to its properties, sodium benzoate is used to preserve food products with an acidic pH, such as porridge and fruit pulp, jams, pickles, pickled herring

and mackerel, margarine, olives, beer, fruit yogurt, canned vegetables, and salads. Most often, sodium benzoate is added to carbonated drinks, sauces, mayonnaise, margarine, tomato paste, and fruit preserves.

While in its natural form, it is found in cinnamon, mushrooms, cranberries, blueberries, and cloves. Therefore, sodium benzoate is classified as a compound with a broad safety profile (Justyna and Herbet, 2022).

Sodium benzoate is a compound as an antimicrobial to inhibit the growth of yeast and bacteria in food. Sodium benzoate is generally chosen as a preservative because of its high solubility, which effectively inhibits enzyme activity and prevents oxidation of certain compounds. This property makes it a reliable preservative in maintaining the stability of the product over time [10]. The addition of preservatives to food aims to extend the shelf life of food without reducing quality and without disturbing health. The safety of chemical compounds in food ingredients needs to be considered, both chemical compounds added from outside the food and chemical compounds that are naturally found in the food itself [6].

Maximum limit of sodium benzoate that can be used according to the requirements SNI (Indonesian National Standard) 01-3546-2004-2002 and based on Regulation of the Head of the Food and Drug Supervisory Agency Number 11 of 2019 concerning the maximum limit for the use of sodium benzoate preservative food additives is 1 g/kg of material weight [12]. Restrictions on the use of benzoic acid aim to prevent poisoning. Excessive consumption of benzoic acid in a food ingredient is not recommended because the amount of preservatives that enter the body will increase with more and more frequent consumption [13]. Short-term exposure to sodium benzoate can irritate the eyes, skin, and respiratory tract, and long-term exposure or repeated exposure can cause Skin sensitization [14]

➤ *The Negative Impacts of Excessive Sodium Benzoate use on the Human Body are as follows [15]:*

- Long-term use of sodium benzoate preservatives can cause Lupus disease (*Systemic Lupus Erythematosus/SLE*).
- Other side effects that can arise are edema (swelling) due to retention (retention of fluid in the body) and can also be due to an increase in blood pressure as a result of increased plasma volume due to water binding by sodium.
- It can cause cancer because sodium benzoate acts as a carcinogenic agent. For example, in isotonic drinks where vitamin C (*ascorbic acid*) added to isotonic drinks will react with sodium benzoate to produce benzene. Such benzene is known as an air pollutant and can cause cancer.
- Benzoic acid and sodium benzoate can cause allergic reactions and neurological diseases.
- Causes death with symptoms of hyperactivity, canker sores, persistent urination and weight loss.
- Sodium benzoate is thought to damage DNA.

Based on the description above, the researcher is interested in conducting a study entitled "Analysis of Sodium Benzoate Levels in Tomato Sauce Sold in Gadingrejo Market Using the U-HPLC (*Ultra High Performance Liquid Chromatography*) Method".

II. METHODS

This type of research is qualitative and quantitative observational analysis. The method used to analyze the level of sodium benzoate in tomato sauce sold at Gadingrejo Market with U-HPLC.

A. Tools and Materials

The tools used for sample preparation are analytical scales (*Ohaus*, UK), separator funnels (*Pyrex*®, France), 250 ml glass beaker (*Pyrex*®, France), litmus paper (DR Gray, Spain), Erlenmeyer (*Pyrex*®, France), *whatman* filter paper no.1, porcelain cups (*Pyrex*®, France), *hot plate* (*Stirrer*, USA) parchment paper, stirring rod, measuring cup (5 ml, 25 ml). The tools used in qualitative analysis are test tubes (*Pyrex*®, France), and droppipettes (*Pyrex*®, France). The instruments used for the manufacture of the master raw solution are analyte scales (*Ohaus*, UK), and 100 ml measuring flasks (*Pyrex*®, France). The instruments used for the manufacture of standard series solutions are 10 ml volume pipettes (*Pyrex*®, France), 10 ml flasks (*Pyrex*®, France). The instrument used for the determination of sodium benzoate levels in tomato sauce samples is an *octadesyl silica* (ODS), U-HPLC (*Cromai*®, USA) column.

The ingredients used for sample preparation were 10 g of tomato sauce obtained from the gadingrejo market, aquadest (H_2O), NaOH (Sodium Hydroxide) 10%, HCl (Hydrochloric Acid) 1 M, diethyl ether ($C_4H_{10}O$), ethanol (C_6H_5O) 96%. The material used for the qualitative analysis was a sample of tomato sauce that had been prepared, Ammonia (NH_3), $FeCl_3$ (Iron (III) Chloride) 5%. The material used for the manufacture of master raw solutions and the manufacture of standard series solutions is sodium benzoate (100 ppm), *methanol grade* U-HPLC. The materials used for the determination of sodium benzoate levels in tomato sauce samples are 5 g tomato sauce samples and *methanol grade* U-HPLC, motion phase: *methanol grade* U-HPLC and aquabidest (50:50), stationary phase: *octadesyl silica* (ODS).

B. Sample Preparation

- The sample was weighed as much as 10 g and then put into a 250 ml glass beaker
- 100 ml of aquadest is added, then crushed with a mortar. Transfer the solution on the *beaker glass*.
- Add 10% NaOH until the solution becomes alkaline (red litmus paper turns blue).
- Then strain the solution with filter paper.
- 50 ml of filtrate is put into Erlenmeyer.
- HCl 1 M is added until it is acidic (blue lime paper turns red). Then put it in a separate funnel.

- Extracted 3 times with 15 ml of diethyl ether each (to prevent emulsification, shake continuously each time extraction by rotation).
- Diethyl ether is formed in 2 layers, namely the upper layer or the ether layer is separated into erlenmeyer glasses.
- The ether extract was washed 3 times each with 5 ml of aquadest.
- The washed ether extract is put into a porcelain cup, evaporated on a water bath.
- The residue obtained is dissolved with aquadest to taste.
- Then it is heated on a water bath.
- The dry extract is dissolved with 96% ethanol as much as 25 ml [16], [17].

➤ Qualitative Analysis

- A sample of tomato sauce that has been prepared is taken as much as 5 ml and put into a test tube.
- Add a few drops of NH₃ until the solution becomes alkaline.
- Added a few drops of FeCl₃ 5% b/v.
- The formation of brown deposits indicates the presence of sodium benzoate (Faroch *et al.*, 2021).

➤ Quantitative Analysis

• Preparation of Master Raw Solution

- ✓ Weighed as much as 10 mg of sodium benzoate.
- ✓ Dissolved with *methanol grade* U-HPLC In a flask, measure 100 ml to the limit mark, so that a concentration of 100 ppm is obtained (Hadriyati *et al.*, 2020).

• Standard Series Solution Manufacturing

- ✓ Pipetted a raw solution of sodium benzoate (100 ppm) 4 ml, 6 ml, 8 ml and 10 ml put in a 10 ml measuring flask.
- ✓ Plus *methanol grade* U-HPLC up to the limit mark.
- ✓ Dilution was obtained with concentrations of 40 ppm, 60 ppm, 80 ppm, and 100 ppm (Hadriyati *et al.*, 2020).

• Standard Solution Calibration Curve Generation

- ✓ Standard series solutions with concentrations of 40 ppm, 60 ppm, 80 and 100 ppm.
- ✓ Then the solution is injected as much as 20 µL through the injector and carried by the motion phase towards the column (Hadriyati *et al.*, 2020).

• Determination of Sodium Benzoate Levels in Tomato Sauce Samples

- ✓ Each sample of 5 g was dissolved with 10 ml of *methanol grade* U-HPLC.
- ✓ Then take 2 ml and then put it into a 10 ml measuring flask and add *methanol grade* U-HPLC to the limit mark.
- ✓ The sample solution is injected as much as 20 µL through the injector and carried by the moving phase towards the column.
- ✓ For each sample, 3 test repetitions were performed.

➤ The Condition of U-HPLC is as follows:

- Motion phase: *methanol grade* U-HPLC and aquabidest (50:50).
- Stationary phase : *Octadesil silica* (ODS).
- Flow rate : 1 ml/min.
- Detector : UV, λ 225 nm (Hadriyati *et al.*, 2020).

III. RESULTS

A. Results of Quantitative Analysis

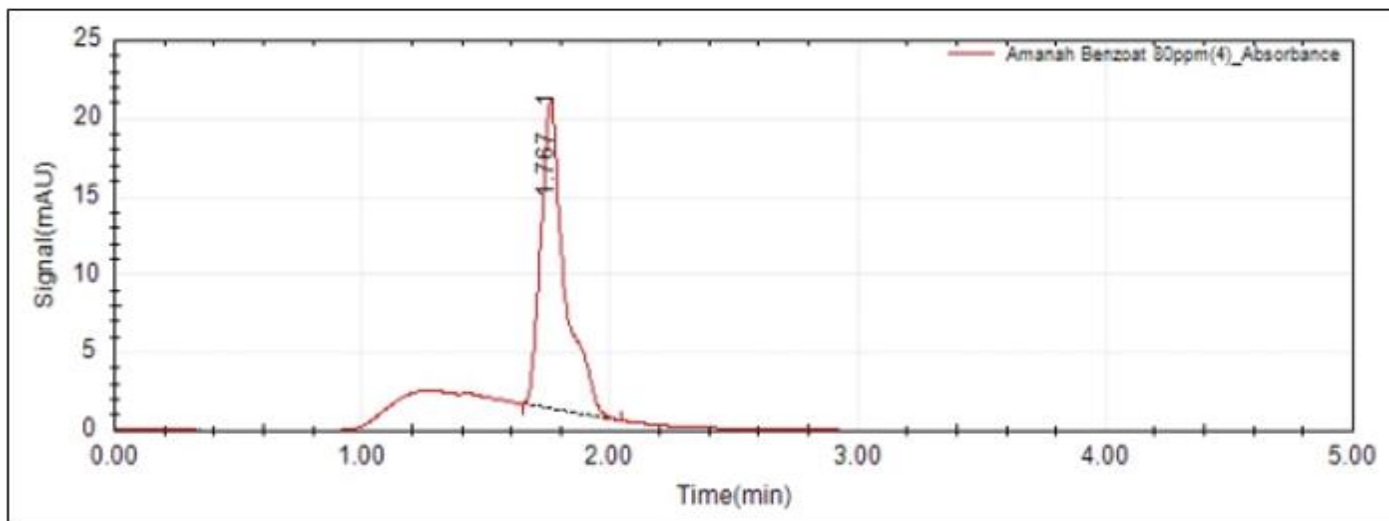
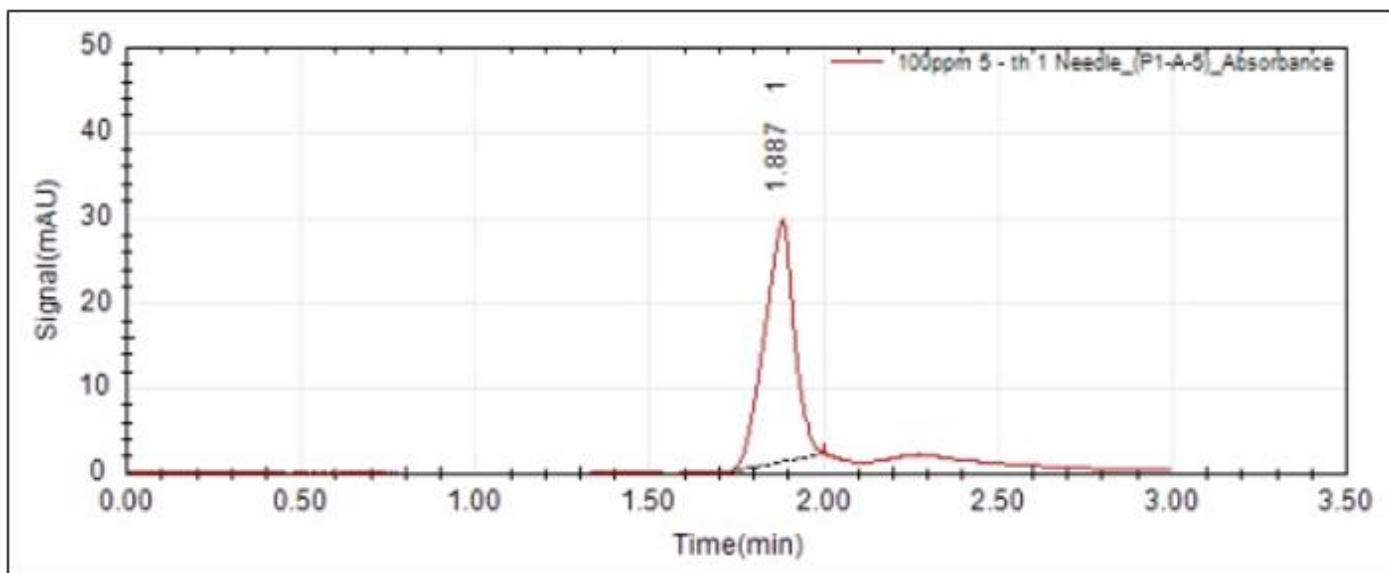
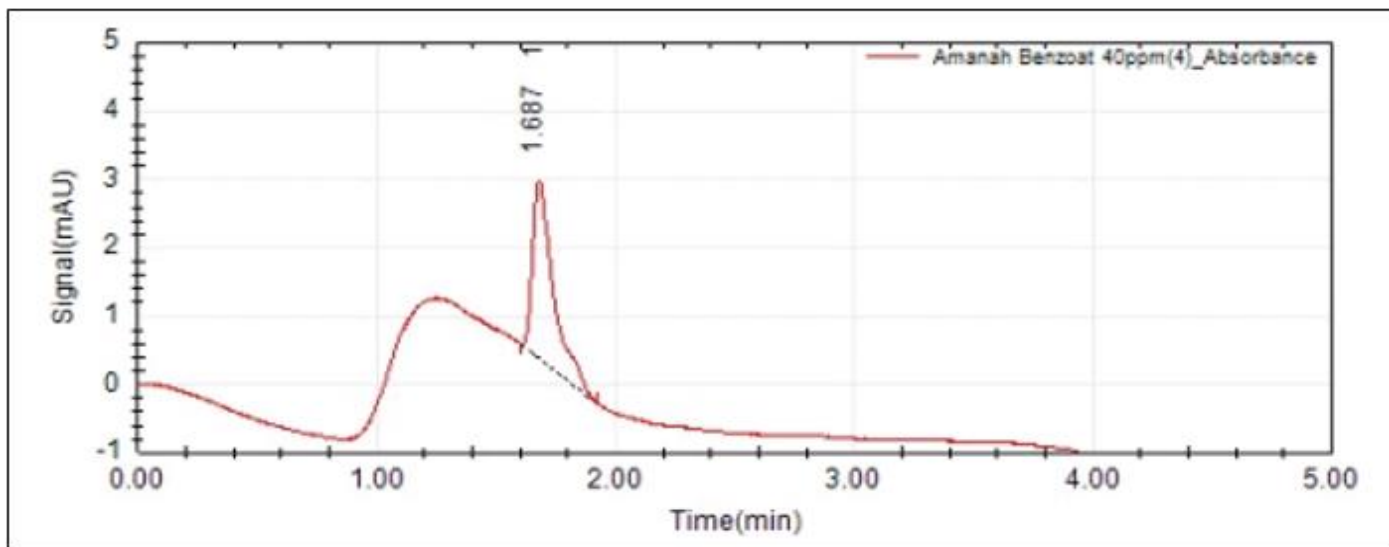
Table 1 Qualitative Test Results of Sodium Benzoate

Standard Sodium Benzoate	Sample Code	Result Observation	Information
1 g/kg weight of material (BPOM, 2019).	ST1	Deposits Brownish	(Positive) +
	ST2	Deposits Brownish	(Positive) +
	ST3	Deposits Brownish	(Positive) +
	ST4	Deposits Brownish	(Positive) +

B. Results of Quantitative Analysis

➤ Sodium Benzoate Curve Measurement Results

The chromatogram results of the sodium benzoate raw solution can be seen in the following figure:



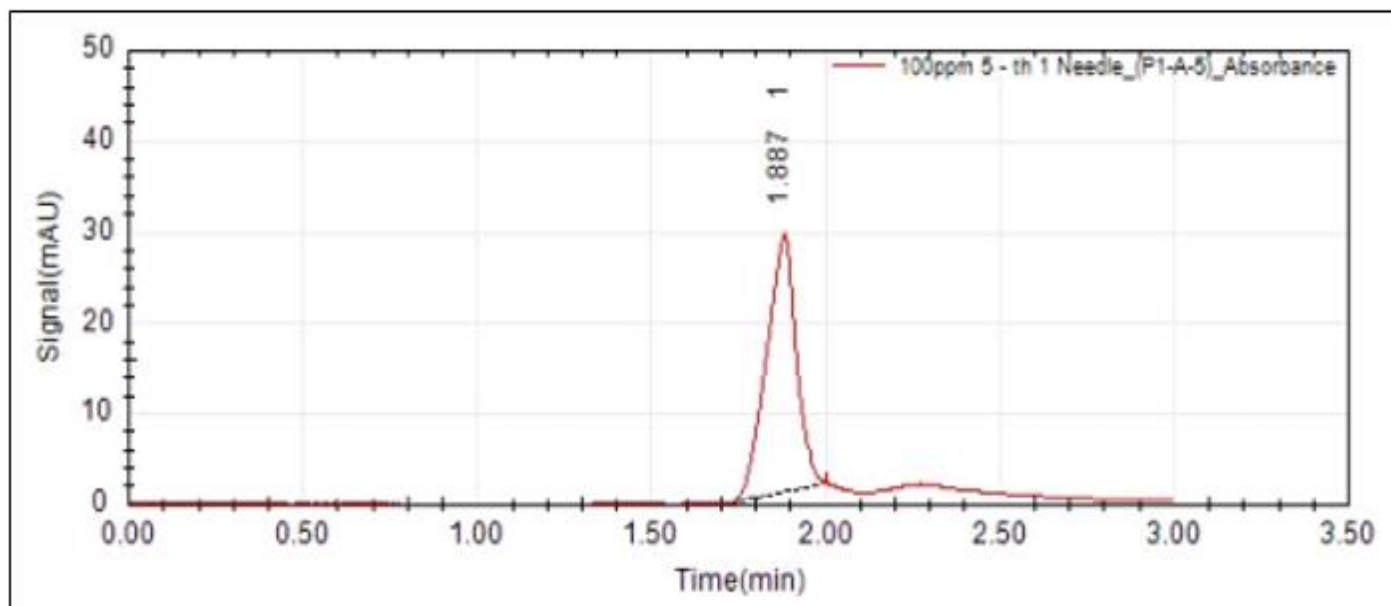


Fig 1 Motion Phase Composition Chromatogram Methanol: Aquabidest (50:50)

➤ *Results of Determination of Sodium Benzoate Levels in Samples*

Table 2 Sodium Benzoate Levels in Samples

Sample Code	AUC	Concentration (ppm)	Rate % b/b	Average Sodium Benzoate % b/b (mg/g)
ST1a	66,632	35,104	1,40	1,4
ST1b	43,322	35,096	1,40	
ST1c	2,626	35,081	1,40	
ST2a	15,336	35,085	1,40	1,4
ST2b	6,114	35,082	1,40	
ST2c	39,210	35,094	1,40	
ST3a	3,751	35,081	1,40	1,4
ST3b	3,850	35,081	1,40	
ST3c	4,128	35,081	1,40	
ST4a	5,009	35,081	1,40	1,4
ST4b	64,661	35,103	1,40	
ST4c	3,974	35,081	1,40	

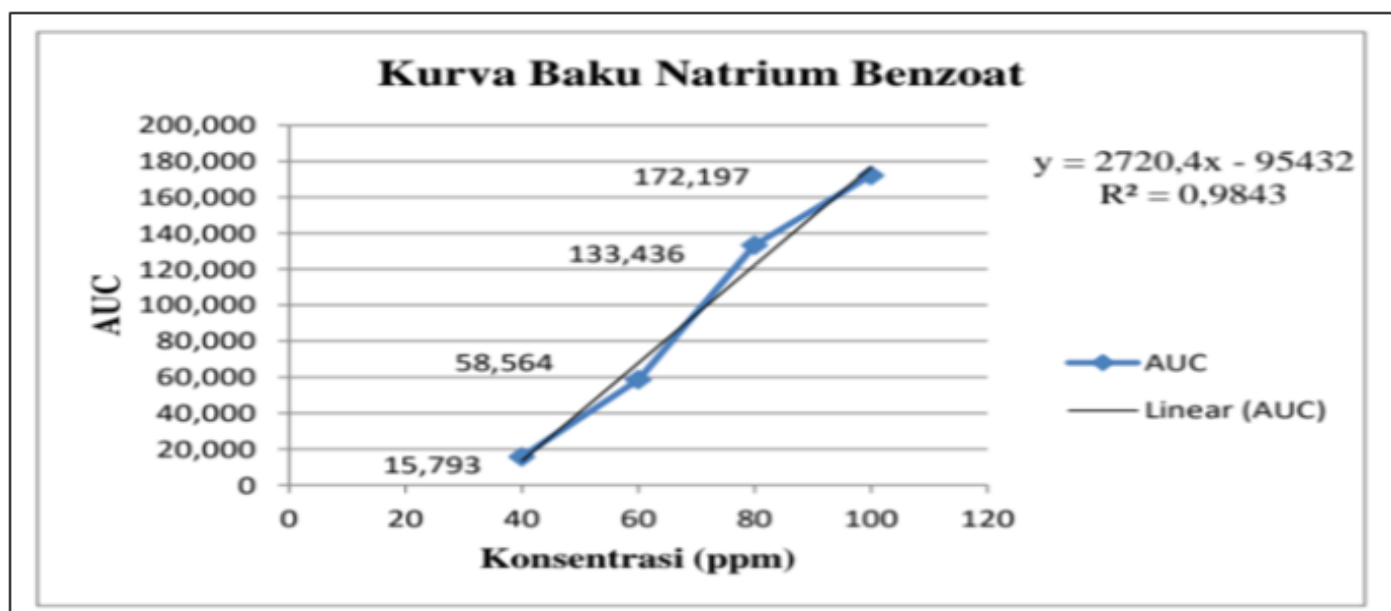


Fig 2 Sodium Benzoate Raw Curve

IV. DISCUSSION

➤ Results of Qualitative Analysis

The sample used in this study is tomato sauce sold at the Gadingrejo market using the *Simple random sampling*. A total of 4 tomato sauces were obtained and given codes ST1, ST2, ST3, and ST4 for both qualitative and quantitative analysis. Before qualitative and quantitative tests of sodium benzoate in tomato sauce samples, sample preparation was carried out first. The purpose of sample preparation is To minimize the presence of impurities that can interfere with the analysis process by eliminating components-Components other than analytes [19].

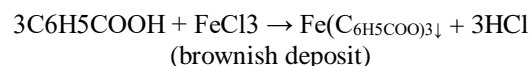
Sample Preparation is carried out by dissolving the sample into aquadest and then adding NaOH until it is alkaline which functions as a catalyst for the sample solution so that all benzoates contained in the sample change into the form of salt. The filtered samples are added with HCL until they are acidic, the addition of this solution aims to make benzoic compounds into benzoic acid that is insoluble in water, but can be dissolved in organic solvents such as diethyl ether [17].

The separation method carried out is liquid-liquid extraction. Liquid-liquid method or *liquid-liquid extraction (LLE)* namely the separation of a chemical component between two phases of solvent that do not mix with each other where some of the components are soluble in the first phase and partially soluble in the second phase, then the two phases containing the dispersed substance are shaken and left to rest until separation occurs and two layers of liquid phase are formed, where the upper layer or ether layer has a specific weight of 0.789 g/ml and the lower layer or water phase has a specific weight of 1.00 g/ml [20].

The extraction process of sodium benzoate is carried out in a fume hood with a sample solution added with a diethyl ether solvent, a diethyl ether solvent is a type of non-polar solvent. The reason for using diethyl ether is because sodium benzoate is insoluble in water but soluble in non-polar solvents so that diethyl ether solvents are felt to be able to dissolve and attract sodium benzoate (Johar and Mustikaningrum, 2023). The ether extract obtained is then washed with aquadest which aims to make the colored solution clear [22].

Heating is carried out on the samples, which aims to obtain a dry extract with a brownish tint. Then the residues obtained are dissolved with aquadest and then reheated, this process is carried out with the aim of clarifying the color change that will indicate a positive sample of sodium benzoate. The dry extract obtained is dissolved with 96% ethanol. Then the sample was added ammonia (NH₃) which aims to attract the dyes contained in tomato sauce [23]. Furthermore, the sample was added FeCl₃ with the aim of finding out which samples were positive for sodium benzoate with the presence of a brownish precipitate color.

The results of the qualitative test using the FeCl₃ reagent showed that the entire sample, which consisted of 4 samples that were positively analyzed to contain sodium benzoate, was seen from the color change that occurred in the sample after being added with FeCl₃. Discoloration that occurs in a sample after addition with FeCl₃ that is, cloudy and there are brownish deposits, This can occur due to the formation of a bond between 3 benzoate molecules of sodium benzoate and ferri-ion (Fe³⁺) from FeCl₃ which forms ferribenzoate chelate compounds with hydrochloric acid molecules [17]. The reaction that occurred was as follows [16].



The results of this study are in line with the research [24] who researched tomato sauce at the Beriman market in Tomohon City, where five samples were qualitatively tested using FeCl₃ reagent. It is proven that it contains all sodium benzoate compounds with the formation of brownish precipitates. Several subsequent studies obtained different results as in the Rahmania *et al.*, (2020) Of the eight samples, all contained sodium benzoate compounds.

➤ Results of Quantitative Analysis

The determination of sodium benzoate levels in tomato sauce samples in this study used U-HPLC. Tomato sauce samples that have been known to be positive for sodium benzoate from the results of qualitative analysis, namely all samples with codes ST1, ST2, ST3, ST4 are continued to be carried out for quantitative analysis which aims to determine the levels of sodium benzoate contained in tomato sauce samples. The determination of sodium benzoate content begins with the measurement of AUC in the raw solution of sodium benzoate, the measurement of AUC aims to obtain a curve of the raw solution that provides a linear regression equation to determine the level of sodium benzoate in the sample. Based on the data from the raw solution measurement results in Figure 4.1, a linear regression equation is obtained $y = 2720.4x - 95432$ with a correlation coefficient value $r = 0.9843$. The price of the correlation coefficient (r) is close to 1 expresses a linear relationship between concentration and the resulting absorption, in other words the increase in the AUC value of the analyte is directly proportional to the increase in its concentration in accordance with the acceptance criteria of a good correlation coefficient (r), because a good linear relationship if $r = 1$ or close to 1 [16].

This study uses a reverse phase, with the stationary phase used as *Octadesil silica* (C18), which is nonpolar. Use of the stationary phase *Octadesil silica* Because it is able to separate low, medium and high polarity [26] The phase of motion uses methanol:aquabidest (50:50) which is polar. The use of methanol and aquabidest motion phases is because sodium benzoate has good solubility in both phases of motion. The function of the motion phase is to dissolve the mixture of substances, lift and carry the components to be separated through the stationary phase. In U-HPLC, the reverse phase of polar compounds will be crystallized earlier. Wavelength 225 λ, analysis time 3 minutes, injection volume

20 µL With a flow rate of 1 ml/minute and a chromatogram peak is obtained at 1.7-1.9 minutes, because the larger the flow rate, the faster the retention time.

Validation of the U-HPLC method is carried out to prove that the method used meets the requirements to produce valid data. The parameters used are linearity, accuracy and

precision. The linearity parameter is used to determine the linear relationship between the analyte concentration and the detector response obtained from the standard curve measurement data (Figure 2). from this data, it can be said that it is linear because the correlation value (R^2) is close to the number 1.

Table 3 Accuracy Test Results

Sample	Average % Recovery
ST 1	104,88%
ST 2	104,24%
ST 3	103,64%
ST 4	104,40%

Table 4 Precision Test Results

Sample	% RSD	Average % RSD
ST 1	24,364 %	0.17 %
ST 2	14,768 %	
ST 3	0,196 %	
ST 4	28,957 %	

Accuracy test is expressed as a percentage of recapture (% *Recovery*) analytes added. The acceptance conditions of this method are vulnerable to 80%-120%, where the results obtained are ST1 104.88%, ST2 104.24%, ST3 103.64% and ST4 104.40%. These results are still vulnerable to accuracy test requirements. In the precision test, it has an RSD limit of <2% and a result of 0.17% is obtained. Based on the RSD obtained does not exceed the limit of %RDS and the method used can be said to be thorough because it does not exceed the number 2. On the LOD test (*Limit Of Detection*) and LOQ (*Limit Of Quantification*) obtained from the linear regression equation of the calibration curve, with a LOD value of 0.0097 mg/ml, a LOQ value of 0.0326 mg/ml. from these results, it was stated that the LOD and LOQ values were lower than the concentration of sodium benzoate in the determination of levels so that it could be used for quantitative analysis [26].

Based on the results of sample analysis using U-HPLC, it was found that all four samples had the same sodium benzoate level, which was 1.4 mg/g and this result was in accordance with the standard for the use of sodium benzoate which did not exceed the maximum limit of sodium benzoate or it can be said that all samples met the requirements set by the Regulation of the Head of the Food and Drug Supervisory Agency Number 11 of 2019 with a maximum limit of 1 g/kg of material weight [12].

Some previous studies have also shown mixed results. Research at the Medan City Traditional Market showed that out of 9 samples, none exceeded the threshold for sodium benzoate use [29]. Meanwhile, in the research (Wardani and Rahayu, 2021) showed that 3 of the 12 samples tested exceeded the threshold for sodium benzoate use.

Sodium benzoate has a function as an artificial preservative that aims to make food or beverages durable. The use of sodium benzoate is safe to consume, but in low levels [31]. The use of benzoate in the human body is

absorbed from the small intestine and activated through binding to CoA (*coenzyme A*) to produce *benzoyl coenzyme A*. Next *benzoyl coenzyme A* conjugated with glycine in the liver to form hyporic acid which is then excreted through the urine, if there is a disorder in the liver organ then sodium benzoate will accumulate (accumulate) cannot be excreted (Wahyuningsih and Nurhidayah, 2021). If the liver loses its function, sodium benzoate will accumulate in the body so that it can cause physical disorders, especially attacking the nervous system such as Alzheimer's, causing stomach cramps, fatigue, skin allergies, and cancer (Faroch *et al.*, 2021).

Consumption of sodium benzoate preservatives too often should be avoided because it will cause a buildup of preservatives in the body. Excessive intake of sodium benzoate can cause nausea, headaches and throat irritation. Sodium benzoate that enters the body will enter the bloodstream, be absorbed by the stomach and can irritate the stomach. The maximum limit on sodium benzoate is carried out because the use of sodium benzoate preservatives is not always safe, especially if used in excessive amounts can cause stomach cramps, numbness in the mouth, for those who are tired or have rashes (Suryani H, 2019).

There are several preservatives that are safer to use than sodium benzoate in terms of the risks that can be caused if added to food or drinks, one of which is vitamin C and vitamin E which are natural preservatives. In addition to playing the role of an essential vitamin that has the potential to prevent cancer and as a nutritional supplement. Vitamin c can also be used as a preservative. Vitamin c is rich in content that functions to prevent oxygen from being damaged. So that the food will also remain fresh. Because it plays a dual role as a vitamin and preservative, vitamin c (*ascorbic acid*) used in a variety of foods. Vitamin c is used as a food preservative because its antioxidant properties can prevent food from spoiling [33]. Vitamin e is one of the ingredients that can be

used as a preservative. Vitamin E is well-known for its health benefits and is recommended to be consumed regularly and is also used in the food industry as a natural preservative to protect food from oxidation due to its antioxidant properties [34].

V. CONCLUSIONS

➤ *Based on the Results of the Research that has been Carried out, Conclusions are Obtained:*

- There was sodium benzoate content in the four samples of tomato sauce sold at the Gadingrejo market as seen from the color change in the qualitative test.
- Sodium benzoate levels in tomato sauce with sample code ST1 (1.4 mg/g), sample code ST2 (1.4 mg/g), sample code ST3 (1.4 mg/g) and sample code ST4 (1.4 mg/g).

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