Analysis of Preservatives in Packaged Drinks with Chromatography Techniques

Merry Yunita
Department of Physics, Faculty of Mathematics and Natural Sciences, University of North Sumatra, Indonesia

ABSTRACT
A research has been conducted on qualitative and quantitative of soft drinks preservative analysis. The analysis used chromatography. The calibration curve of real standard was used to determine the concentration of preservative. The concentrations of the drinks preservative would be suitable for the standard of preservative of Minister of Publik Health R.l No. 722/Menkes/per/IX/1988. The data was analyzed by using correlation and signification method between area and concentration and the result of the research has fulfilled quality standard of preservative.

Keywords:
Chromatography
Packaged Drinks
Preservative

INTRODUCTION
The development of the industrial world, especially the food industry is very fast along with the increasing market demand. This is due to the increasing number of people who act as consumers. Food and drink are objects that cannot be separated in everyday life. In other words, food and drink are our basic needs as living beings.

For marketing continuity, the food industry must always strive so that the products produced have good quality. However, it does not rule out the possibility of fraud committed by the food industry but only thinking about the profits obtained.

The use of preservatives in the food industry is not something we encounter in our daily life. Especially in the beverage packaging industry. Preservatives are used as additives to drinks so that drinks can be consumed in a long time. Many types of packaged drinks that we encounter in the market that use preservatives. In general, packaged beverage manufacturers include the composition, nutritional value, expiration limit of the packaged drinks they produce, and the type of preservative used is usually listed on the packaging label. But sometimes we are often deceived and believe too much with what is written without us knowing the truth. Thus it is certain that we as consumers are the most disadvantaged in this case.
The use of preservatives as additional ingredients in packaged drinks has rules both in terms of the type used and in terms of concentration. This is in accordance with the Regulation of the Minister of Health stated in the Minister of Health No. 722/Menkes/Per/IX/1988.

The benefit of this research is that by knowing the type and concentration of food preservatives used in beverage packaging, we can determine or be more selective about the drinks to be consumed. Meanwhile, the objectives of this research are as follows: 1. Determine the type of preservatives from several beverage packages. 2. Determine the concentration of preservatives in some beverage packaging. 3. Observing whether the use of preservatives is in accordance with regulation no. 722/Menkes/Per/IX/88.

1.1 Theoretical basis

Chromatography was first developed by the Russian botanist Michael in 1903 to separate the colored pigments in plants by locating petroleum ether extracts in a glass column containing calcium carbonate (CaCO3). The history of the development of chromatography is summarized in table 2.1. Currently, chromatography is the most common and most frequently used separation technique in analytical and qualitative, quantitative, or preparative chemistry in the pharmaceutical, environmental, industrial and so on. Chromatography is a separation technique that uses a stationary phase and a mobile phase.

Chromatography techniques have developed and have been used to separate and quantify a wide variety of complex components, both organic and inorganic components.

1.2 Chromatographic Division

Based on the separation mechanism, chromatography is divided into:

a. Adsorption chromatography
b. Partition chromatography
c) Ion pair chromatography
d.Ion exchange chromatography
e.Size exclusion chromatography
f. Affinity chromatography

Based on the tools used, chromatography can be divided into:

a. Paper chromatography
b) Thin layer chromatography, also known as planar chromatography
c.High performance liquid chromatography (HPLC)
d. Gas chromatography (KG)

1.3 Polarity

Polarity is used as an indication of the nature of the solvent, adsorbent, and compounds that are separated (solutes). Water, which is the solvent, its electron configuration and molecular geometry can produce a very strong permanent dipole, therefore water is considered to have a very strong polarity, even the strongest when compared to other solvents.

1.4 Molecular Absorption of UV and Visible Rays

The absorption (absorption) of UV and visible light is generally produced by the excitation of bonding electrons, as a result of which the wavelength of the absorbing band can be related to the possible bonds in a molecule. There are three types of absorption of ultraviolet and visible energy, namely:

1. Absorption by transition of bonding electrons and antibonding electrons
2. Absorption by the d and f electron transitions of the complex molecule.
3. Absorption by charge transfer.

1.5 Qualitative and Quantitative Aspects of UV-Vis Spectrophotometry

UV-Vis spectra can be used for qualitative information as well as for quantitative analysis.

1. Qualitative Aspect

UV-Vis spectra data alone cannot be used for qualitative identification. However, it must be combined with other means such as infrared spectroscopy, nuclear magnetic resonance, and
mass spectroscopy, so that it can be used for the purpose of identification or qualitative analysis of a compound. The data obtained from UV-Vis are maximum wavelength, intensity, pH effect, and solvent; all of which are compared with published data (Published Data). For example, from the obtained spectra, it can be seen that the absorption (absorbance) changes or not because of its solubility.

2. Quantitative Aspect
In the quantitative aspect, a beam of radiation is applied to the sample (solution of the sample) and the intensity of the transmitted radiation beam is measured. The radiation absorbed by the sample is determined by comparing it with the intensity of the light absorbed in the absence of other absorbing species. The intensity or power of radiation is proportional to the number of photons that pass through one unit of cross-sectional area per second.

1.6 High Performance Liquid Chromatography (HPLC)
High Performance Liquid Chromatography or HPLC or also known as HPLC (High Performance Liquid Chromatography) was developed in the late 1960s and early 1970s. Currently, HPLC is a widely accepted separation technique for the analysis and purification of certain compounds in a sample in a number of fields, including: pharmaceutical, environmental, biotechnology, polymer, and food industries. Some of the latest HPLC developments include: miniaturization of the HPLC system, the use of HPLC for nucleic acid analysis, protein analysis, carbohydrate analysis, and analysis of chiral compounds.

RESEARCH METHOD

2.1 Ingredients
The materials used are:
1. Raw material for comparison (Na Benzoate)
2. Methanol as solvent
3. Acetonitrile (E. Merck)
4. Aquabides (Ikapharmindo)
5. Phosphate buffer (KH₂PO₄)

2.2 Sample
The beverage sampling process is carried out based on the brands circulating in the market (supermarket) in the Medan area and a code is used to maintain the industrial code of ethics.
1. MST1
2. MST2
3. MST3
4. KNA1
5. KNA2
6. MGK1
7. MGK2
8. M8P1
9. M8P2
10. MPP1
11. MPP2
12. GRS2
13. GRS3
14. GRS4

2.3 Equipment
1. HPLC consisting of a pump model LC -6A, latex C-18 column (15 cm x 4.0 mm ), UV detector, recorder and integrator model C-R4A Chromatopac (Shimadzu), UV-Vis Spectrophotometer 1601 (Shimadzu).
2. Analytical balance (Ohaus)
3. Filters
4. Chemical glassware.

2.4 Research Flowchart

![Research Flowchart]

2.5 Research procedure
2.5.1 Reagent Setup
1. Prepared a standard solution for the mother liquor of the preservative. Because all beverage packaging labels contain sodium benzoate as a preservative, 1.0 g of Na-Benzoate is dissolved in 200 ml of methanol and diluted to 1.01 with deionized water. Standard solutions were made with various concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, 120 ppm, 160 ppm.
2. The mobile phase for HPLC consisted of 0.05 KH2PO4 with pH = 2.65 and acetonitrile in a ratio of 60:40 (v/v). The mixture was filtered through a 0.45 m Duropore filter.

2.5.2 Sample Preparation
1. 6 grams of the sample was put into a 50 ml centrifuge tube and centrifuged 1500 x G for 5 minutes.
2. Sep-pack C-18 cartridges were prepared by diluting 2 ml of methanol followed by 4 ml of water.
3. One milliliter of the supernatant from the sample was pipetted in a syringe and injected through the prepared cartridge.
4. After the cartridge was washed with 4 ml of hexane, the preservative was eluted with 3 ml of methanol, and filtered through a 0.45 m duropore.

2.5.3 Chromatographic Condition
1. The Shodex column used measuring 150 mm x 6.0 mm is filled with polystyrene-divinylbenzene resin, assisted by RP-18 guard coulumn measuring 40 mm x 3.4 mm.
20 l aliquots of sample or standard were injected into the column. The preservative was eluted isocratically at a rate of 1.0 ml/min. Detection was carried out by UV spectrometry at a wavelength of 225 nm. Quantitatively the retention time and area are compared with the standard.

### Sampling Technique

Samples were taken from markets and supermarkets in the city of Medan and preservative standards were obtained from BPFI (Indonesian Pharmacology Research Agency with a purity level of 99.99%) then analyzed at the Inorganic Development Laboratory at PTKI Medan.

### Data analysis technique

#### Qualitative Analysis Techniques

For qualitative analysis or to determine the type of preservative, namely by comparison between unknown retention data and standard retention data under the same conditions.

#### Quantitative Analysis Techniques

For quantitative analysis, an external standard method is used, namely by using a calibration plot using an external standard. That is, the detector response (area) is plotted against the concentration. Then the concentration of the sample obtained was compared with the regulation of the minister of health stated in regulation No. 722/Menkes/Per/IX/1988.

#### Statistical Analysis Techniques

Statistical testing was carried out using the SPSS (Statistical Product and Service Solution) version 13 program and tested using the correlation and significance method between area and concentration.

### RESULTS AND DISCUSSIONS

#### Qualitative Analysis

The variable observed in this study was the retention time of the sample and compared it to the retention time of the standard for several types of beverages.

| No | Sample | Retention time |
|----|--------|----------------|
| 1  | MST1   | 7.089          |
| 2  | MST2   | 7.105          |
| 3  | MST3   | 7.066          |
| 4  | KNA1   | 7.104          |
| 5  | KNA2   | 7.119          |
| 6  | MGK1   | 7.028          |
| 7  | MGK2   | 7.026          |
| 8  | M8P1   | 7.101          |
| 9  | M8P2   | 7.127          |
| 10  | MPP1 | 7.087          |
| 11  | MPP2 | 7.099          |
| 12  | GRS2  | 6.996          |
| 13  | GRS3  | 7.008          |
By comparing the retention time of the standard to the retention time of the sample on the graph, it is determined that the preservative in the sample is Natrum Benzoate. (graphics of standard standard chromatogram results and each sample are attached)

2.1 Quantitative Analysis
Another variable that was observed was the area (absorbance) with a large concentration of preservatives that varied with the standard.

| No | Sample | Retention time |
|----|--------|----------------|
| 14 | GRS4  | 7.017          |

Table 4.2 Area data and concentration of standard standard

| No | Concentration (Mg/l) | area    |
|----|----------------------|---------|
| 1  | 20                   | 1023245 |
| 2  | 40                   | 2152156 |
| 3  | 60                   | 3231876 |
| 4  | 80                   | 4372369 |
| 5  | 120                  | 6284870 |
| 6  | 160                  | 8346124 |

Based on the table above, a calibration curve is made to obtain an equation that will be used to determine the concentration of preservatives in each sample using the Microsoft Excel program. The steps for making the curve are as follows:

1. Click the insert icon and then click the chart wizard.
2. To display data graphs, use the scatter graph type.
3. Fill in the Data Range by selecting cells A2 to B6.
4. Complete the steps in the Chart Wizard dialog box. And next we will get the data points in the chart. And look at the trend of the shape of the graph. For this graph tends to be linear (straight line).
5. We can display the equation of a straight line to represent the trend of the data in the figure. The steps we have to complete are as follows:
   1. Click one of the data contained in the excel chart.
   2. Do a right click on the data
   3. Select Add Trendline on the shortcut menu that appears.
   4. Fill out the Add Trendline dialog box.
      a. On the Type tab, in the Trend / Regression type section, select a linear regression type because the data points show a tendency to form a straight line.
      b. Click the Options tab in the Add Trendline dialog box
      c. Check the Display equation on chart option to display the equation of the line on the graph. And check the display R-squared value on the chart to show the level of accuracy of the equation.
From the graph, the equation of the regression line is $y = ax + b$ where $a = 52063$ and $b = 72337$ then the equation of the line obtained is $y = 52063x + 72337$ and this equation will be used to calculate the concentration of preservatives in each sample by entering the area value ($y$) into the equation, the amount of sample concentration can be calculated.

For example, in the MST1 sample, it is known that the area ($y$) = 4372834 and by plugging it into the equation $y = 52063x + 72337$ then $x = (y - 72337)/52063$.

| No | Sample | Area   | Concentration (mg) |
|----|--------|--------|--------------------|
| 1  | MST1   | 4372834| 82.60              |
| 2  | MST2   | 4416217| 83.44              |
| 3  | MST3   | 4151906| 78.34              |
| 4  | KNA1   | 127560 | 1.06               |
| 5  | KNA2   | 109555 | 0.71               |
| 6  | MGK1   | 2545155| 47.49              |
| 7  | MGK2   | 2517206| 46.96              |
| 8  | M8P1   | 3810745| 71.80              |
| 9  | M8P2   | 3816328| 71.91              |
| 10 | MPP1   | 4198206| 79.24              |
| 11 | MPP2   | 4154292| 78.40              |
| 12 | GRS2   | 1942701| 35.93              |
| 13 | GRS3   | 1974833| 36.54              |
| 14 | GRS4   | 1946037| 35.99              |

Graph 4.2. The relationship between concentration and area in each sample.
Correlation and Significance Analysis

To prove whether there is a relationship or influence between the area variable and the concentration variable, how is the direction of the relationship and how big is the relationship, statistical tests are carried out.

| The value of concentration | Interpretation                                      |
|---------------------------|----------------------------------------------------|
| Between 0.8 to 1          | The relationship between the two variables is very strong. |
| Between 0.6 to 0.8        | The relationship between the two variables is strong. |
| Between 0.4 to 0.6        | The relationship between the two variables is moderate. |
| Between 0.2 to 0.4        | The relationship between the two variables is weak.    |
| Between 0.0 to 0.2        | There is no relationship between the two              |

The correlation test with SPSS was carried out with the following steps:
1. From the SPSS main menu, enter the area variable value and the concentration variable value. Select the Analyze menu then the correlate submenu, and select bivariate...
2. Contents of Variables are area variables and concentration variables to be correlated.
3. Correlation Coefficients or correlation coefficient calculator. Choose Pearson
4. Test of significance, select Two-tailed
5. Enable Flag significance correlations
6. Click the Options button and enable Exclude cases pairwise
7. Continue with OK. And you will get the following display:

| area     | Concentration |
|----------|---------------|
| Pearson correlation | 1.000**       |
| Significance (two-sided) | .000       |
| N (number of samples) | 14          |

| area     | Concentration |
|----------|---------------|
| Pearson correlation | 1.000**       |
| Significance (two-sided) | .000       |
1. Meaning of correlation number
   Between area and concentration, the correlation number is +1, this means that the greater the concentration, the greater the area (absorbance), and vice versa.

2. Significance of Hypothesis correlation results:
   Ho = There is no significant relationship between area concentration and concentration.
   Ha = There is a relationship between area and concentration
   The test is carried out on both sides.

   Basis of decision making (based on probability):
   a. If the probability > 0.05 (or 0.01) then Ho is accepted.
   b. If probability < 0.05 (or 0.01) then Ho is rejected. Note: 0.05 or 0.01 depending on selection.

   Decision:
   Because the probability number is 0.000, the area variable and the concentration variable are significantly correlated. This can also be seen from the ** sign in the correlation number, which means the same, namely the correlation number is significant. The output states that SPSS considers the correlation significant at the 0.01 or 1% level. Of course if tested with a level of 5% will be significant too.

2.4 Comparison of Sample Concentration With Regulation No. 722/Menkes/Per/IX/1988.
   The permissible concentration of Sodium Benzoate in soft drinks under these regulations is 600 mg/l.

   Table 4.6 Comparison of concentrations with regulations of the Minister of Health.

   | No | Sample | Permitted concentration (mg/l) | Concentration of preservatives in sample (mg/l) | Specification |
   |----|--------|-------------------------------|-----------------------------------------------|--------------|
   | 1  | MST1   | 600                           | 82.60                                         | Well         |
   | 2  | MST2   | 600                           | 83.44                                         | Well         |
   | 3  | MST3   | 600                           | 78.34                                         | Well         |
   | 4  | KNA1   | 600                           | 1.06                                          | Well         |
   | 5  | KNA2   | 600                           | 0.71                                          | Well         |
   | 6  | MGK1   | 600                           | 47.49                                         | Well         |
   | 7  | MGK2   | 600                           | 46.96                                         | Well         |
   | 8  | M8P1   | 600                           | 71.80                                         | Well         |
   | 9  | M8P2   | 600                           | 71.91                                         | Well         |
   | 10 | MPP1   | 600                           | 79.24                                         | Well         |
   | 11 | MPP2   | 600                           | 78.40                                         | Well         |
   | 12 | GRS2   | 600                           | 35.93                                         | Well         |
   | 13 | GRS3   | 600                           | 36.54                                         | Well         |
CONCLUSION

Based on the analysis conducted on preservatives which includes qualitative analysis, namely determining the type of preservative and quantitative analysis, namely determining the concentration of preservatives in packaged drinks using chromatographic techniques, the conclusion is that:

1. By comparing the results of the standard standard chromatograms with the results of the sample chromatograms in both graphic form and retention times which are almost the same, the preservatives used in beverages are MST1, MST2, MST3, KNA1, KNA2, MGK1, MGK2, M8P1, M8P2, MPP1, MPP2, GRS2, GRS3, GRS4 is a Sodium Benzoate preservative.

2. Based on quantitative analysis using external standard methods, the concentration of preservatives in the packaging can be determined. The concentration of Sodium Benzoate in each package is as follows: MST1 = 82.60 mg/l, MST2 = 83.44 mg/l, MST3 = 78.34 mg/l, KNA1 = 1.06 mg/l, KNA2 = 0.71 mg/l, MGK1 = 47.49 mg/l, MGK2 = 46.96 mg/l, M8P1 = 71.80 mg/l, M8P2 = 71.91 mg/l, MPP1 = 79.24 mg/l, MPP2 = 78.40 mg/l, GRS2 = 35.93 mg/l, GRS3 = 36.54 mg/l, GRS4 = 35.99 mg/l.

3. By comparing the concentration obtained against regulation No.722/Menkes/per/IX/1988 regarding food additives where the concentration of the preservative sodium benzoate allowed in syrup drinks is a maximum of 600 mg/l, the concentration of sodium benzoate used in beverages is MST1, MST2, MST3, KNA1, KNA2, MGK1, MGK2, M8P1, M8P2, MPP1, MPP2, GRS2, GRS3, and GRS4 are still in accordance with the rules and are well used.

4. From the statistical tests carried out, it was found that the correlation between area (absorbance) and concentration was 1, meaning that there was a significant relationship between area and concentration where the greater the concentration of Sodium Benzoate in packaged drinks, the greater the absorption of UV waves.

ACKNOWLEDGEMENTS

We would like to thank all those who have contributed to this research, so that the research can be carried out properly.

REFERENCES

Adnan, M. 1997. Chromatographic Techniques for the Analysis of Foodstuffs. Yogyakarta, ANDI Publisher

Johnson, E and Stevenson, R. 1991. Fundamentals of liquid chromatography. Kosasih Padmawinata Translation. Bandung: ITB Publisher

Nana, Dana Priatna .2005. Introduction to Statistics. Yogyakarta: Graha Ilmu.

Park, GL and DB Nelson. 1981. HPLC Analysis of Sorbic acid in Citrus fruit. JFd.

Rohman, Abdul. 2007. Pharmaceutical Chemical Analysis. Yogyakarta: Student Library Publisher.

Santoso, Singgih. 2000. SPSS Parametric Statistics Exercise Book. Jakarta: Publisher PT Elex Media Komputindo.

Satiadarma, Kosasih. 2004. Principles of Development of Analytical Procedures. Surabaya: Airlangga University Press.

Widiyatmoko, Joko. 2006. MicrosoftExcel.Yogyakarta, Yescom Publisher
Zweigh, G. and Sherma, J. 2000. CRC Handbook of Chromatography: General Data and Principles. Florida: CRC Press, Inc.