Microfluidic Paper based Analytical Device (µPADs) for Analysis of Benzoic Acid in Packaged Beverages

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Abstract. Microfluidic Paper-based Analytical Devices (µPADs) are a simple analytical platform that satisfy combination of low cost, portability, and ease-to-use with selectivity, sensitivity, and accuracy. In this experiment, the µPADs devices are prepared using a chromatographic paper and designed at appropriate pattern prior to printing by mean of a solid ink (wax) printer to produce hydrophobic barriers and hydrophilic channels. Then, the µPADs are heated at 120°C for 3 min to allow a wax penetrates in the both side of a paper. The resulted µPADs are used for the detection of benzoic acid through alkalimetry principle. For this purpose, the devices are implanted with 5M NaOH in the detection zone and a 2% phenolphthalein indicator in the sample zone before dried in open air. When the devices have dried, the µPADs are ready to be used to detect benzoic acid. To avoid color interferences, the samples are distilled before introduced to the sample zone for quantitative detection. The result soft the color changes can be immediately seen by naked eye, and their intensities are quantified by the Image J software. Excellent linearity is achieved in the range of benzoic acid concentration of 20-120 ppm with the linear equation of $Y = 0.0102x + 0.0077$ and the correlation coefficient ($R^2$) of 0.9999. The optimized µPADs devices are successfully applied to the quantitative analysis of benzoic acid in the 5 kinds of commercially packaged beverages. There is no significantly different of the analytical results obtained by the µPADs in comparison to the spectrophotometric method. Overall, the results obtained in this study indicate that the µPADs devices are a reliable tool for high throughput and on-site determination of benzoic acid content in commercial drinks.

Keywords: µPADs, Benzoic Acid, Low cost detection, Microfluidic Paper-based devices

1. Introduction

Food and beverages are essential for the survival of human being. To be practically consumed anytime and anywhere, food and beverage are usually served in packaging. The packaging is aimed to make the food fresh and taste good for any time consuming. Therefore, packaged food and beverage are added by additional ingredient so that they can last long. One of the additional ingredients included in food and beverages is preservative. Preservative which are widely used to preserve various food ingredients is benzoate, which is commonly found in more soluble form of sodium benzoate [1]. Sodium benzoate is frequently used in a variety of food and beverage products, such as soft drinks, fruit juices, and yogurt, tomato sauce, jam, butter, cheese, fish products, herbs, soy sauce, mayonnaise, salads, etc [2].
Many methods are used to detect the concentration of sodium benzoate such as Titration methods, Spectrophotometry, High Performance Liquid Chromatography (HPLC), Mass Spectrometry Gas Chromatography (GC-MS), Mass Spectrometry Liquid Chromatography (LC-MS), and Electrophoresis, [9, 10]. HPLC, GC-MS and LC-MS methods are more promising because they provide high sensitivity and accuracy [7]. Although those methods are promising, there are some disadvantages attach to those methods. The disadvantages are non-portable using instrument, time consuming sample preparation and testing time [1, 9, 10], difficulty to identify the entire chromatogram peak correctly [9], and the expensive price of the instruments [11]. Therefore, it is necessary to develop a new method to overcome the disadvantages.

Microfluidic Paper-Based Analytical Devices (µPADs) is a renewable method in research technology. The concept of µPADs is to provide a simple test method on a sheet of paper [12] which has the same concept as litmus paper which is used to measure acidity in the sample [13]. µPADs uses chromatographic paper with hydrophilic and hydrophobic patterns which are designed using computer software and printed using certain methods [14]. The studies about the using of µPADs in determining the benzoate acid for packaged food and/or beverages in Indonesia are very less to be found, therefore this study was aimed to determine the benzoate acid level in typical packaged food/beverages that are used to take by the Indonesian kids.

2. Material and Methods

2.1. Material, Chemical, and Apparatus

The chemicals used in the study were Sodium Hydroxide (Merck, Indonesia), Phenolptalein (Merck, Indonesia), Benzoic Acid (Merck, Indonesia), Ethanol 96% (Merck, Indonesia). Whatman No.1 Chromatography Paper (GE Healthcare companies) was used for the preparation of µPADs. Analytical Balance, beaker glass, Erlenmeyer, micropipette, volume pipette, drop pipette, measuring flask, UV-1800 spectrophotometer (Shimadzu, Japan), hotplate, simple distillation device consisting of heating mantle, round bottom flask, condenser, and adapter, burette, clamp, stative were used to support the study. The µPADs pattern was designed using Corel Draw X3 and Xerox ColorQube 8580 DN2 printer wax was used to design the hydrophobic and the hydrophilic barriers on the chromatography paper. Canon MP230 printer scanner and computer with Image J applications were used to observed the RGB value of the µPADs.

2.2. µPADs Preparation and Optimization

The chip was made by using Whatman filter paper with the design presented below, in which this device was designed using Corel Draw X3 (Figure 1) and printed using the ColorQube Xerox 8580 DN2 Printer. After printing, µPADs was heated or penetrated at 120°C for 3 minutes which aimed to generate a pattern of hydrophobic resistance on the chromatography paper.
2.3. Preparation of Sample and Standard
The sample used in the study was the packaged beverage that varies in taste, packaging, and production. Samples of packaged beverage used were tea, orange juice, grapes jelly drink, strawberry juice, and apple cider flavored beverages. Hundred mL of the samples were distilled prior to the benzoic acid determination.

The standard production of sodium benzoate was made from 0.002%; 0.004%; 0.008%; 0.006%; 0.008%; 0.01%; 0.03% and 0.05%. This standard solution would be measured using spectrophotometry. Then it was dripped on the µPADs device called Standard.

2.4. Determination of Benzoic Acid Level
The distilled samples were dripped to the µPADs design using a micropipette. The detection reagent was dipped to the µPADs prior to the samples. The µPADs allowed the sample to flow through the channel, dispersed with the reagent and reacted in the detection zone. The color change that occurred was scanned and observed using the J Image application. The color changed obtained from the samples were then compared to the color changed obtained by the standard benzoic acid to determine the benzoic acid level of the samples.

3. Result and Discussion
This research was conducted to determine the benzoic acid levels in packaged beverages through titrimetric method. This method had the principle that there would be a color change that occurred due to the acid-base reaction. NaOH (as the alkaline reagent) was applied to reaction zone and the PP indicator was applied at the detection zone. When the sample was dripped on µPADs, it flowed and reacted with NaOH. The sample and NaOH would react and flowed towards the detection zone which has PP Indicator. The reaction between sample and NaOH was shown with the color changes to pink-purple that obtained by the PP indicator.

The color intensity corresponds to benzoic acid concentration. The higher benzoic acid concentration, the higher color intensity level produced which indicates that the RGB level would also be bigger

3.1. µPADs Optimization
The temperature of 120°C with penetration period of three minutes was discovered as the maximum optimization for µPADs. The µPADs designs (after printing and after the penetration) were presented in Figure 2. It showed that the penetration of the wax ink, enhance the hydrophobic barrier of µPADs. The penetration temperature generated a solid hydrophobic barrier to prevent any leakage during the test.

![Figure 2. µPADs design; after printing (a) and after a wax ink penetration (b)](image_url)

3.2. Standard of Benzoic Acid
The calibration of benzoic acid was made by measuring the variation of benzoic acid concentration from 20-120 ppm as shown in Figure 3. In making a standard solution, the results of standard benzoic acid’s wavelengths measurement obtained maximum absorption at 275 nm wavelength and obtained the
standard curve with the regression equation \( y = 0.0102x + 0.0077 \). Moreover, the standard RGB value of benzoic acid with different concentration were presented in Table 1.

![Standard Curve of Benzoic Acid](image)

**Figure 3.** The standard curve of Benzoic Acid at 275 nm wavelength.

### Table 1. Standard RGB Value of Benzoic Acid.

| Concentration | Red (μm) | Green (μm) | Blue (μm) |
|---------------|----------|------------|-----------|
| 0.002%        | 112.272 ± 1.84 | 34.373 ± 1.24 | 85.369 ± 1.70 |
| 0.004%        | 125.980 ± 1.8  | 37.520 ± 1.38 | 102.409 ± 1.76 |
| 0.006%        | 146.410 ± 2.0  | 41.698 ± 1.65 | 112.702 ± 1.10 |
| 0.008%        | 154.900 ± 1.76 | 57.832 ± 1.53 | 124.129 ± 1.55 |
| 0.010%        | 164.857 ± 1.60 | 65.234 ± 2.01 | 145.766 ± 1.33 |
| 0.030%        | 176.955 ± 1.28 | 73.574 ± 1.34 | 162.138 ± 1.92 |
| 0.050%        | 189.931 ± 1.48 | 84.559 ± 1.24 | 174.132 ± 1.77 |

#### 3.3. Application of µPAD to analyze Benzoic Acid level in packaged beverages with RGB method

The result of benzoic acid tests in µPADs were shown in Figure 4. The calculation result of benzoic acid levels in packaged beverage samples that have been distilled by UV-Vis Spectrophotometry were presented in Table 2. The samples that have been distilled were also analyzed using µPADs, and the RGB values were later analyzed by using Image J RGB values compared to standard values.

### Table 2. Level of sample after distillation.

| Sample | Absorbance (A) | Level (mg/100mL) |
|--------|----------------|-----------------|
| A      | 0.056          | 4.9             |
| B      | 0.513          | 50.6            |
| C      | 0.197          | 19.0            |
| D      | 0.031          | 2.4             |
| E      | 0.035          | 2.8             |
Table 2 showed that the benzoic acid levels obtained from each distilled packaging beverage was different. From the measurement of packaged beverage levels using the µPADs method, the µPADs method can be used as a substitute method in determining benzoic acid levels due to the use of fewer reagents and can accelerate the analysis process. However, to test the accuracy of the method, it is necessary to test the validation of the method between the µPADs method and the standard method of determining benzoic acid using the UV spectrophotometry method so that the accuracy of the analysis data can be obtained.

![Figure 4](image)

**Figure 4.** Test result of µPADs samples which have been distillated (a) Sample A, (b) Sample B, (c) Sample C, (d) Sample D, and (e) Sample E.

In Table 3, the obtained RGB values on packaged beverage which have been distilled was compared to the standard value of benzoic acid by looking at the value that was close to standard benzoic acid. The constituent color of pink which was a combination of red and purple was compared to the RGB value focusing on red and blue color. From the results of Table 4, it shows that from the two µPADs methods with the spectrophotometric method, were not significantly different in terms of measurement data comparison. It shows that the data obtained using the µPADs method has fairly good accuracy.

**Table 3.** RGB value in packaged beverage after distillation.

| Sample | Red          | Green        | Blue         |
|--------|--------------|--------------|--------------|
| A      | 124.914 ± 0.31 | 36.200 ± 0.72 | 103.367 ± 1.44 |
| B      | 188.836 ± 1.70 | 85.461 ± 1.40 | 173.972 ± 1.78 |
| C      | 168.455 ± 0.84 | 66.331 ± 0.83 | 147.161 ± 1.42 |
| D      | 116.353 ± 0.82 | 33.973 ± 1.60 | 84.160 ± 0.83  |
| E      | 122.370 ± 1.44 | 35.737 ± 0.316| 94.755 ± 1.46  |
Table 4. Comparison of benzoic acid levels using the µPADs method using the spectrophotometric method.

| Sample | µPADs Method (%) | Spectrophotometry Method (%) |
|--------|------------------|-----------------------------|
| A      | 0.004            | 0.0049                      |
| B      | 0.050            | 0.0506                      |
| C      | 0.010            | 0.0190                      |
| D      | 0.002            | 0.0024                      |
| E      | 0.002            | 0.0028                      |

4. Conclusion
From this study, it can be proven that the µPADs method and the results of the spectrophotometric method did not differ significantly. This µPADs method has good accuracy that can be applied to determine the level of benzoic acid in packaged beverages.

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