Derivative Uv-Spectroscopic determination of theophylline, salbutamol sulfate and glycerylguaiacolate in syrup mixture

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Abstract. This study aimed to determine theophylline, salbutamol sulfate and glycerylguaiacolate using ultraviolet spectrophotometry derivative. Research conducted in syrup was analyzed using zero crossing wavelength method. NaOH 0,1 N was selected as the solvent. Zero crossing wavelength of theophylline was 275 nm on the first derivative, salbutamol sulfate was 262 nm on the second derivative and glycerylguaiacolate was 243 nm on the third derivative. Results showed that the recovery of theophylline and glycerylguaiacolate were between 112,913% - 118,353% and 116,129% - 117,655%, respectively, while the relative standard deviation was between 1,090% - 1,903% for theophylline and 1,013% - 1,922% for glycerylguaiacolate. Limits of detection of theophylline and glycerylguaiacolate were 1,626 ppm and 6,375 ppm, respectively while limits of quantification were 4,927 ppm and 19,319 ppm. This results Thus, it was concluded that this method could be applied to determine theophylline and glycerylguaiacolate in syrup mixture.

1. Introduction

Theophylline (TH) has the IUPAC name 1,3-dimethyl-7H-purine-2,6-dione; it has maintained an important role as a potent and useful bronchodilator [1]. Salbutamol sulphate (SB) is, chemically known as bis [(1RS)-2- [(1, 1-Di-methyl-ethyl) amino] -1- [4-hydroxy- 3- (hydroxyl methyl) phenyl] ethanol] sulphate. It is beta-adrenoceptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease [2]. Glycerylguaiacolate(GG) is (RS)-3-(2-methoxyphenoxy) propane-1,2-diol reportedly increases the volume and reduces the viscosity of tenacious sputum [3]. Combinations of TH, SB, and GG are indicated for the prophylaxis and relief of reversible bronchospasm associated with acute and chronic asthma, bronchitis, and other chronic obstructive airway diseases in which reversible airway narrowing occurs [4].

Determination of the levels of these three substances can be done by various methods, including HPLC or High Performance Liquid Chromatography [5]. The HPLC method has a high analytical sensitivity but requires relatively expensive costs and relatively long analysis time [6], thus an alternative analytical method is developed which is simpler in its work, faster analysis time and cheaper operational costs. One of methods which meets the requirements is ultraviolet derivative spectrophotometry [7].

Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. Under computer-controlled instrumentation, derivative spectrophotometry is playing a very important role in the multicomponent analysis of mixtures by UV
molecular absorption spectrophotometry [8]. Binary mixtures can be easily resolved by means of a spectrophotometric method, which is based on the simultaneous use of “zero crossing” method [9,10]. The aim of this study was to determine theophylline, salbutamol sulfate and glycerylguaiacolate levels in multicomponent syrup preparations simultaneously using ultraviolet derivative spectrophotometry.

2. Material and method

2.1. Instrument and materials
The instruments used were Analytic Jenna Specord 200 UV-Vis Spectrophotometer, analytical scales, measuring cups, volumetric flasks, ultrasonicators, drip pipettes, micropipets, funnels, spades, beakers, cuvettes and other tools used in analytical laboratories.

Materials used were standard glycerylguaiacolate, standard salbutamol sulfate, theophylline, citric acid, sodium citrate, sodium benzoate, glycerin, aquadest, product A containing salbutamol sulfate 0.5 mg and 50 mg theophylline and product B containing salbutamol sulfate 2 mg and glycerylguaiacolate 75 mg, ethanol pro analysis and NaOH 0.1 N.

2.2. Preparing standard solutions of theophylline, salbutamol sulphate and glycerylguaiacolate
To prepare standard solution of theophylline, the first step was weighing carefully the 20 mg of theophylline and putting it in a 20 mL volumetric flask. Second step, the solution was dissolved with ethanol pro analysis using ultrasonicator. After dissolving, the researcher placed the volume up to the boundary mark (stock solution 1). Then, the stock solution was taken as much as 1 mL and was put in a 10 mL volumetric flask followed by adding 0.1 N NaOH to the boundary mark (stock solution 2).

Then, similar steps were carried out in preparing standard solution of salbutamol sulfate. Firstly, 25 mg of salbutamol sulfate was carefully weighed and put in a 25 mL volumetric flask. Secondly, the solution was dissolved with ethanol pro analysis using ultrasonicator. After dissolving, the researcher placed the volume to the boundary mark (stock solution 1). Then, the stock solution was taken as much as 1 mL and put in a 10 mL volumetric flask followed by adding 0.1 N NaOH to the boundary mark (stock solution 2).

Similarly, standard solution of glycerylguaiacolate was also prepared in the following steps. First step was 20 mg of glycerylguaiacolate was carefully weighed and put in a 20 mL volumetric flask. Then, the researcher dissolved it with ethanol pro analysis using ultrasonicator followed by placing the volume up to the boundary mark (stock solution 1). Finally, the stock solution was taken as much as 1 mL and put in a 10 mL volumetric flask followed by adding 0.1 N NaOH to the boundary mark (stock solution 2).

2.3. Determination of the maximum absorption wavelength (λ max) of theophylline, salbutamol sulphate and glycerylguaiacolate
Theophylline, salbutamol sulfate and glycerylguaiacolate solutions were prepared with a concentration of 10 ppm for theophylline, 10 ppm for salbutamol sulfate and 24 ppm for glycerylguaiacolate. Then each of the solutions was measured in the range of 200 - 300 nm with NaOH as blank.

2.4. Determination of measurement wavelength on the first derivative, second derivative, third derivative and fourth derivative
Salbutamol sulfate, glycerylguaiacolate and theophylline solutions were made with concentrations of 10 ppm for theophylline, 10 ppm for salbutamol sulfate and 24 ppm for glycerylguaiacolate. From the solution, the first, second, third and fourth derivative absorption spectra were made to obtain the measurement wavelength.

2.5. Making a standard curve
For the measurement of the theophylline standard curve at the measurement wavelengths of salbutamol sulfate and glycerylguaiacolate, a series of mixed solutions containing salbutamol sulfate,
glycerylguaiacolate, and theophylline with a ratio of the following concentrations of 10, 24, 2.5; 10, 24, 5; 10, 24, 7.5; 10, 24, 10; 10, 24, 12.5; 10, 24, 15 was created. Each solution was measured at wavelengths where salbutamol sulfate and glycerylguaiacolate did not provide absorption.

To measure the standard curve of salbutamol sulphate at the wavelengths of measurements of glycerylguaiakolate and theophylline, a series of mixed solutions containing salbutamol sulfate, glycerylguaiacolate, and theophylline was created with a concentration ratio as follows: 5, 24, 10; 10, 24, 10; 15, 24, 10; 20, 24, 10; 25, 24, 10; 30, 24, 10. Each solution was measured at wavelengths where glycerylguaiacolate and theophylline did not provide absorption.

For measurement of standard glycerylguaiacolate curves at salbutamol sulphate and theophylline wavelengths of measurements, a series of mixed solutions containing salbutamol sulfate, glycerylguaiacolate and theophylline were made with the following concentration ratio: 10, 12, 10; 10, 24, 10; 10, 36, 10; 10, 48, 10; 10, 60, 10; 10, 72, 10. Each solution was measured at wavelengths where salbutamol sulfate and theophylline did not provide absorption.

2.6. Method validation

2.6.1. Linearity test. The standard curve was made by measuring theophylline standard solution with a concentration series of 2.5, 5, 7.5, 10, 12.5 and 15 ppm, salbutamol sulfate with a concentration series of 5, 10, 15, 20, 25 and 30 ppm and glycerylguaiacolate with concentration series 12, 24, 36, 48, 60 and 72 ppm at predetermined derivative wavelengths. The mixed series were made with different concentrations as in making a standard curve; therefore, the linear regression equation was obtained y = bx + a.

2.6.2. Accuracy. Simulation samples were made with a ratio of 50 mg of active substance theophylline, 2 mg salbutamol sulfate, and 75 mg glycerylguaiacolate (50: 2: 75). Sample solution was made with 3 concentration levels in the range of 80%, 100%, and 120% of the actual concentration of each of the 3 replications. Each solution was measured in the order and wavelength selected for analysis. From the results of measurement, the percentage of acquisition value was calculated.

2.6.3. Precision. Sample solutions were made with concentration levels in the range of 80%, 100%, and 120% of the actual concentration of each of the 3 replications. Each solution was measured in the order and wavelength selected for analysis. From the results obtained, the relative standard deviation was calculated.

2.6.4. LOD and LOQ test. The limit of detection and limit of quantity were determined using the standard intersection and the slope of the calibration curve.

2.7. Determination of theophylline, salbutamol sulfate and glycerylguaiacolate levels in syrup available in the market

Determination of salbutamol sulfate levels in syrup was carried out in the following steps. First, pharmaceutical preparations taken as much as 5 mL were then put into a 25 mL volumetric flask, and diluted using ethanol pro analysis. Second, preparation of the salbutamol sulfate substance was prepared by taking 12.5 mL of the filtered mixture. Third, adding 0.1 N NaOH to the boundary mark on the 100 mL volumetric flask was the final step to carry out.

Meanwhile, for the measurement of glycerylguaiacolate, preparation was prepared by taking 12.5 mL of the above stock solution and putting it in a 100 mL volumetric flask. Then, the researcher added 0.1 N NaOH to the boundary mark. Finally, the mixture was diluted again by taking 0.5 mL of added 0.1 N NaOH in a 10 mL volumetric flask.

In addition, for the measurement of theophylline, the preparation was carried out by taking 12.5 mL of the filtered mixture, Next, the researcher added 0.1 N NaOH to the boundary mark on the 100 mL volumetric flask. Then, the mixture was diluted again by taking 0.5 mL of added 0.1 N NaOH in a 10 mL volumetric flask.
mL volumetric flask. Furthermore, absorbance measurements were carried out using a predetermined wavelength.

3. Result and discussion

3.1. Determination of maximum wavelength

Determination of maximum wavelength was carried out to find out maximum absorption of each compound. This was because maximum absorption could be used for qualitative analysis of a compound where the value of maximum $\lambda$ was specific for each compound.

3.1.1. Theophylline absorption spectrum. Figure 1 show the maximum absorption of theophylline with 0.1 N NaOH solvent was obtained at a wavelength of 277 nm, and the measured solution concentration was 10 ppm with an absorbance of 0.538 A.

3.1.2. Absorption spectrum of salbutamol sulphate. Figure 2 show the maximum absorption of salbutamol sulfate with 0.1 N NaOH solvent was obtained at 247 nm wavelength, the measured solution concentration was 10 ppm with absorbance of 0.379 A.

3.1.3. Glycerylguaiacolate absorption spectrum. Figure 3 show the maximum uptake of glycerylguaiacolate with 0.1 N NaOH was obtained at a wavelength of 275 nm, and the measured solution concentration was 24 ppm with an absorbance of 0.241 A.
3.2. The maximum absorption spectrum of theophylline, salbutamol sulfate and glycerylguaiacolate

Maximum absorbance of theophylline, salbutamol sulfate and glycerylguaiacolate are at adjacent wavelengths. This caused overlapping of the total absorption spectrum as seen in Figure 4. The overlapping absorption caused an analysis of a mixture of theophylline, salbutamol sulfate, and glycerylguaiacolate which could not be carried out in a conventional way because of the disturbance of absorption from other analytes so that derivatization methods were needed to obtain specific measurement wavelengths for quantification.

3.3. Determination of measurement wavelengths

Based on the maximum absorption spectrum of theophylline, salbutamol sulfate and glycerylguaiacolate that had been obtained, the derivative was made between $dA / d\lambda$ to $\lambda$, $d^2A / d\lambda^2$ to $\lambda$ to obtain the measurement wavelength using the zero crossing method. Zero crossing was a point where when measuring the wavelength of one substance, the other two substances did not provide absorption.

Figure 3. Glycerylguaiacolate Absorption Spectrum

Figure 4. The absorption spectrum of theophylline, salbutamol sulfate and glycerylguaiacolate.

Figure 5. The spectrum of the first derivative, theophylline, salbutamol sulfate and glycerylguaiacolate.
In the first derivative spectrum had shown a fairly clear separation. This was indicated by the measurement of salbutamol sulfate at 245 nm wavelength wherein theophylline and glycerylguaiacolate did not provide absorption. Figure 5 show the measurements of theophylline could be carried out at a wavelength of 275 nm where salbutamol sulfate and glycerylguaiacolate did not provide absorption. While the first derivative glycerylguaiacolate measurement was not possible because no wavelength was found where the theophylline and salbutamol sulfate did not provide absorption.

Figure 6. The spectrum of the second derivative, theophylline, salbutamol sulfate and glycerylguaiacolate.

In the second derivative spectrum showed that the measurement results of theophylline could be carried out at the wavelength of 295 nm, salbutamol sulfate was at wavelength 262 nm and glycerylguaiacolate was at wavelength 243 nm. It showed in figure 6

3.4. Determination of the standard curve of theophylline, salbutamol sulphate and glycerylguaiacolate

From the wavelength of the measurements obtained, a series of concentrations of theophylline, salbutamol sulfate and glycerylguaiacolate were made. Determination of measurement wavelengths was assessed from the best linearity gain. The absorption measurements for the theophylline standard curves were performed on the first derivative spectrum at 275 nm wavelength, giving a linear line with the regression line equation $y= 0.0008571x + 0.0003333$ with $r^2 = 0.9941$.

The absorption measurement for the standard salbutamol sulfate curve was carried out on the second derivative spectrum at 262 nm wavelength, giving a linear line with the regression equation $y = 0.0003486x + 0.0004$ with $r^2 = 0.9936$. The absorption measurement for the glycerylguaiacolate standard curve was carried out on the second derivative spectrum at 243 nm wavelength, giving a linear line with the regression equation $y = 0.0001786x - 0.0003333$ with a value of $r^2 = 0.9941$.

Making standard curves for theophylline, salbutamol sulfate and glycerylguaiacolate were 2.5–15 ppm, 5-30 ppm and 12-72 ppm, but the concentrations above the standard curve range were not linear and the resulting spectrum of derivatization showed many peaks. Therefore the range used became limited and narrow. The very large comparison of theophylline, salbutamol sulfate and glycerylguaiacolate (50: 2: 75) made dilution necessary so that the concentration obtained was theophylline at 12.5 ppm, salbutamol sulfate at 0.5 ppm and glycerylguaiacolate at 18.75 ppm which meant concentration of salbutamol sulfate was not in the range of standard curves that had been determined. Thus, the determination of salbutamol sulfate levels could not be done. Only the concentration determination of theophylline and glycerylguaiacolate was carried out.
3.5. Validation of analysis method

3.5.1. Linearity test. The linearity test of the analysis method was carried out using a series of solutions which differed in concentration as in making a standard curve. The linearity test was carried out to prove the linearity of the absorbance and the concentration indicated by the value of the correlation coefficient of the standard curve. The theophylline standard curve provided the correlation coefficient value of 0.9941 and the variance coefficient of 0.056. The standard curve of Glycerylguaiacolate provided a correlation coefficient value of 0.9941 and a variance coefficient of 0.060.

The requirement for a method was considered good as the coefficient of correlation was more than 0.99 and the variance coefficient was less than 2%. According to these conditions, the linearity test of this method met the linearity test requirements. The complete data can be seen in appendix 7.

3.5.2. Accuracy test. Accuracy test was obtained from the percentage of substances acquisition using direct comparison with the content of the simulated substance weighed. The measurement was also determined by adding excipients which were found in syrup preparations. 9 measurements were used with 3 different concentrations of 80%, 100%, and 120% concentration, and the results of percent theophylline recovery were 117.879%; 118.353%; 112.913% and glycerylguaiacolate 116.336%; 117.655%; 116.129%. The results of the acquisition met the standard validation testing accuracy that was 80% - 120%. This showed that the determination of theophylline and glycerilguaiakolate levels by derivative spectrophotometry method had good accuracy.

3.5.3. Precision test. Precision tests were conducted to determine the accuracy of the analysis method. The precision test was carried out 9 times with 3 different concentrations namely 80%, 100%, and 120%. The relative standard deviation values obtained were theophylline 1.090%; 1.385%; 1.903% and glycerylguaiacolate 1, 127%; 1.922%, 1.013%. The results obtained fulfilled a good precision test requirement of less than 2%. This showed that the determination of theophylline and glycerilguaiakolate levels by derivative spectrophotometry method had good precision.

3.5.4. Limit of Detection (LOD) and Limit of Quantity (LOQ). Detection limit calculation (LOD) and quantization limit (LOQ) were carried out with the linearity standard curve equation so that the theofin detection limit value was 1.626 ppm and the quantization limit value was 4.927 ppm. Meanwhile glycerylguaiakolate was detected by 6.375 ppm and the quantization limit value was 19.319 ppm. This showed that the lowest concentrations of theophylline and glycerylguaiacolate which could still be detected without interference from the analyte were 1.626 ppm and 6.375 ppm and the lowest concentrations of theophylline and glycerylguaiacolate which could still be measured precisely and accurately were 4,927 ppm and 19,319 ppm.

3.6. Determination of the level of syrup samples distributed in the market
The measured levels of each substance in the drug sample distributed in the market were obtained by putting the absorbance value into the standard linear regression curve equation. Determination of the levels for theophylline measurements gave an average yield of 54.136 mg with a percent recovery of 108.272% and relative standard deviation of 0.670%. Meanwhile, the level determination for salbutamol sulfate could not be carried out since the absorbance value was too small from the range of the standard curve of salbutamol sulfate.

4. Conclusion
From the results of research had been conducted, it was concluded that the ultraviolet derivative spectrophotometry method could be used to simultaneously determine the theophylline, salbutamol sulfate and glycerylguaiacolate levels where theophylline was performed on first-order derivatives. Meanwhile glycerylguaiacolate and salbutamol sulfate were carried out on second-order derivatives.
Determination of salbutamol sulfate levels could not be tested further since the values were not included in the standard curve range.

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