International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.6, pp 273-283, 2015

# Determination of Mixture of Theophylline and Ephedrine Hidrochloride in Tablets by Derivative Spectrophotometric Method

**Siti Morin Sinaga, Tika Oktaria Tarigan and Muchlisyam**

**Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Sumatera Utara Jalan Tri Dharma No.5 Pintu 4, Kampus USU, Medan Indonesia, 20155**

**Abstract:** The aim of this study was to test the validation of derivative spectrophotometric method in determination the content theophylline and ephedrine HCl in tablets. The method of this research was done by purposive sampling to theophylline and ephedrine HCl mixture of the sample Grafasma®and Ifasma® in tablets content using derivative spectrophotometric with zero-crossing technique and determination in HCl 0.1 N.
The research results were obtained the theophylline and ephedrine HCl content of the sample Grafasma® tablets were (99.89 ± 1.67) % and (99.35 ± 2.74) % and ephedrine HCl content of the sample Grafasma® in tablets (98.88± 3.21) % and Ifasma® (96.59 ± 2.32) %. Based on the results of analysis determine the sample content of theophylline and ephedrine HCl compound in tablet supply theophylline fulfilled the requirements in *United States Pharmacopoeia* (USP) 30th edition (2007) and ephedrine HCl fulfilled the requirement of tablet in Farmakope Indonesia V edition (2014). The results of validation test on the Grafasma tablets, the percent recovery for theophylline is 99.67 %, relative standard deviation (RSD)= 1.07% and for ephedrine HCl, the percent recovery = 100.07%, RSD= 1.07%.
**Keywords:** Theophylline, Ephedrine HCl, Derivative Spectrophotometric, Zero-Crossing, Second Derivatives, Validation.

## Introduction

Various preparations of drugs are available in the market with combination two or more active substances in one preparation. The combination aims to enhance the therapeutic effect. One of them is a combination of theophylline and ephedrine HCl, which is used to relieve the symptoms of respiratory disorders such as bronchial asthma, and allergic bronchial spasms[1]. Theophylline as a bronchodilator that serves as a direct relaxation of smooth muscle in the bronchi, while ephedrine HCl work against α and β-adrenoceptor used for bronchodilators, nasal decongestants and decongestants eye[2].

According to the *United States Pharmacopoeia (USP)* 30th (2007) for theophylline tablet is not less than 94.0% and not more than 106.0% of the amount listed on the label, while according to the Farmakope Indonesia Edition V (2014) the requirements for dosage contents of ephedrine HCl tablet is not less than 92.5% and not more than 107.5% of the amount listed on the label.

Determination of theophylline and ephedrine HCl, in a single form can be determined by ultraviolet spectrophotometric in acid solvent, theophylline at a wavelength of 270 nm and ephedrine HCl at a wavelength of 251-257 nm[3]. It also can be done by High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC)[4,5,6].

The research also be done determination mixture of theophylline and ephedrine HCl in the tablet by HPLC, TLC and spectrophotometric of derivative method showed the result that derivatives spectrophotometric with spectra ratio method is faster, simpler, cheaper and more accurate than HPLC[7].

Ultraviolet-Visible spectrophotometric (UV-Vis) had been evolving in line with the developing of science, so that it can be used to establish the contents of a mixture on several substances, namely through the application of derivative spectrofotometric method. Spectrofotometric method derivative or derivative curve method is one of the spectrofotometric method which can be used for mixtures analyze with directly without having to perform the separation in advance although the adjacent wavelengths. The most common procedure to determine the mix of overlapping spectrum is by zero-crossing method. This analysis method is determined the wavelength where the compound has an absorption wavelength becomes to zero and also the analysis of other substances in the mixture[8].

In the assay mixture of several substances with derivative spectrofotometric method must meet the validation requirements with several parameters: accuracy which is expressed in percent recovery determined by using the standard additions method, precision performed is used on the parameters and limits of detection and RSD limit of quantitation is determined by using the formula Limit of Detection (LOD) and Limit of Quantitation (LOQ)[9].

Accordingly, in this research will be conducted as the determination of theophylline and ephedrine HCl mixture in tablets by derivative spectrofotometric method with zero-crossing method.

## Experimental

### Tools

Tools used in this study is UV-Visible spectrophotometer, Personal Computer (PC) equipped with software Probe 2.42 UV (UV-1800 Shimadzu), analytical balance (Mettler Toledo), cuvette, filter paper, rubber ball, spatula, tools - glassware and equipment - other tools required in sample preparation.

### Materials

Materials used were 0.1 N HCl[11] , theophylline BPFI, ephedrine HCl BPFI, Grafasma® and Ifasma®.

### Sampling

Sampling was done by purposive, which is determined on the basis of the consideration that the samples drawn have characteristics similar to those studied. The samples used are Grafasma® tablets (PT. Graha Farma) and Ifasma® tablets (PT.Ifars), each of which contains 130 mg of theophylline and ephedrine HCl 10 mg.

### Maximum Absorption Spectrum Manufacture Theophylline

Taken as much as 0.8 mL of theophylline concentration 100 µg/mL was then inserted into a 10 mL flask and then diluted with HCl 0.1 N. Subsequently the solution is diluted with the same solvent until the line mark, then shaken until to obtain a homogeneous theophylline solution with a concentration of 8 µg/mL . Absorbance was measured at a wavelength of 200-400 nm.

### Maximum Absorption Spectrum Manufacture Ephedrine HCl

Taken as much as 0.5 mL of ephedrine HCl concentration 100 µg/mL ) was then inserted into the flask 10 ml to be diluted with 0.1 N HCl solvent until the line mark, then shaken until homogeneous to obtain a solution with a concentration of 5 µg/mL . Absorbance was measured at a wavelength of 200-400 nm.

### Absorption Spectra Manufacture Derivatives Theophylline

Standard solution made in 10 mL flask containing a concentration of each of the 4 µg/mL , 6 µg/mL , 8 µg/mL , 10 µg/mL , and 12 µg/mL , by diluting as much as 0.4 mL; 0.6 mL; 0.8 mL; 1.0 mL; sequentially and 1.2 mL of theophylline concentration 100 µg/mL using HCl 0.1 N. Then solvent absorption spectrum is made,

then the spectrum is transformed into the first derivative absorption spectrum and the second derivative at a wavelength of 200-400 nm with $\Delta\lambda = 2$ nm.

**Derivative Absorption Spectrum Manufacture Ephedrine HCl**

Taken by 0.2 mL; 0.3 mL; 0.4 mL; 0.5 mL; and 0.6 mL of ephedrine HCl concentration 100 µg/mL, then each put in a 10 mL flask 5, reconstituted with the solvent HCl 0.1 N. Then add with the same solvent to create a standard solution with a concentration of 2 µg/mL ; 3 µg/mL , 4 µg/mL ; 5 µg/mL ; and 6 µg/mL . Then made the absorption spectrum, then the absorption spectrum is transformed into a first derivative absorption spectrum and the second derivative at a wavelength of 200-400 nm with $\Delta\lambda$=2nm.

**Determination of Zero-Crossing**

Determination of the zero-crossing overlapping absorption spectrum obtained by each derived in different concentration of the solution. Zero-crossing each substance shown by the wavelength that has a zero uptake at various concentrations.

**Determination of Wavelength Analysis**

Created theophylline solution with a concentration of 8 µg/mL, ephedrine HCl solution with a concentration of 4 µg/mL , and a mixed solution of theophylline 8 µg/mL and ephedrine HCl 4 µg/mL . The third solution is then measured absorbance at a wavelength of 200-400 nm. Then absorption spectrum is transformed into the first and second derivatives of each single substance from a mixture of theophylline and ephedrine HCl. The second derivative absorption spectrum from a single substance solution and a mixture of both overlaid. Were chosen to be the wavelength analysis is that at a particular wavelength, the absorption single one of the compounds zero while single absorption partner compound and a mixture of both is almost the same or exactly the same. Because at these wavelength can selectively measure the uptake of one of the compounds without being bothered by the uptake of compounds partner.

**Preparation and Determination Linearity Calibration Curves Theophylline and Ephedrine HCl**

Created theophylline standard solution with a concentration of 4 µg/mL ; 6 µg/mL ; 8 µg/mL ; 10 µg/mL ; and 12 µg/mL , then the second derivative absorption measured ($\Delta\lambda$ = 2 nm) in wavelength analysis has been determined. Then do the analysis of the relationship between concentration and absorbance values thus obtained linear regression equation y=ax+b. And based on the absorption at a wavelength analysis, also conducted the calculation of the LOD and the LOQ .To determine the LOD and the LOQ can be used formula.

$$\mathbf{SD} = \sqrt{\frac{\Sigma(y - yi)^2}{n - 2}}$$

$$LOD = \frac{3 \times \mathbf{SD}}{Slope}$$

$$LOQ = \frac{10 \times SD}{Slope}$$

Description:
SD = standard deviation
LOD = Limit Of Detection
LOQ = Limit Of Quantitation

**Determination of Contents of Theophylline and Ephedrine HCl in Tablets**

Weighed 20 tablets commercial tablet containing 130 mg of theophylline and 10 mg ephedrine HCl and then crushed in a mortar until smooth and homogeneous. Furthermore, carefully weighed amount of powder equivalent to 50 mg of theophylline, ephedrine HCl calculated equality contained therein (weighing powders do as much as 6 repetitions). Subsequently incorporated into the flask 50 mL, and diluted with 0.1N HCl (the sonicator for 15 minutes), and then paid back with 0.1 N HCl until the line mark, shaken until homogeneous. The solution is then filtered, approximately 10 mL of the first filtrate discarded. The filtrate subsequently accommodated. Then the filtrate is pipetted much as 0.08 mL, was added 0.44mL of ephedrine HCl (for the addition), inserted into the flask 10 mL, add with 0.1N HCl to mark the line in order to obtain a

solution in which there is a concentration of theophylline 8 µg/mL and ephedrine HCl concentration of 4 µg/mL . Measured absorption at a wavelength of 200-400 nm, further absorption spectrum is transformed into a second derivative absorption spectrum Δλ 2 nm in wavelength analysis of theophylline and ephedrine HCl respectively 244.2 nm and 213.8 nm.

**Validation test**

**Accuracy Test**

Accuracy test was conducted by the addition of raw materials, namely by making the three concentrations of the analyte sample with a specific range of 80%, 100%, 120%. Where in each specific range is used 70% and 30% of raw samples to be added.

Then mix the sample and standard absorbance was measured at a wavelength of 200-400 nm, further absorption spectrum is transformed into a second derivative absorption spectrum Δλ 2 nm in wavelength analysis of theophylline and ephedrine HCl respectively 244.2 nm and 213.8 nm. Percent recovery can be calculated by the formula[9].

$$\% \text{ Recovery} = \frac{C_F - C_A}{C^*_A} \times 100\ \%$$

Description:
$C_F$ = concentration of the sample after the addition of raw materials
$C_A$ = concentration of the sample prior to the addition of raw materials
$C^*_A$ = Number of raw added

**Precision Test**

Precision is measured as relative standard deviation or coefficient of variation. Precision is measured indicates the degree of correspondence between individual test results when a method is repeated for a homogeneous sample. Relative standard deviation value which meets the requirements showed a precision method performed.

Based on the results of the recovery of theophylline and ephedrine HCl standard deviation determined theophylline and ephedrine HCl. To compute the standard deviation (SD) used the formula:

$$SD = \sqrt{\frac{\sum\left(X - \bar{X}\right)^2}{n - 1}}$$

Description:
SD = Standard deviation
X = The number of substances in samples
$\overline{X}$ = Amount of substance content of the sample
n = number of repetitions

Based on the standard deviation values obtained, the relative standard deviation calculated theophylline and ephedrine HCl. Relative standard deviation can be calculated with the following formula:

$$RSD = \frac{SD}{\overline{X}} \times 100\%$$

Description:
RSD = Relative Standard Deviation
$\overline{X}$ = The number of substances sample average
SD = Standard deviation

## Results and Discussion

### Results Determination of the Maximum Absorption Curves

Determination of maximum absorption spectra performed at a wavelength of 200-400 nm. Measurement of the concentration of theophylline in the 8 µg/mL, where as for ephedrine HCl at a concentration of 5 µg/mL . Based on the research results, obtained the maximum wavelength at 272.40 nm theophylline and ephedrine HCl at 206.80 nm.

### Results Determination of Absorption Curves

When done orientation with various concentrations of 130 µg/mL : 10 µg/mL , 40 µg/mL : 10 µg/mL , 10 µg/mL : 5 µg/mL , 8µg / mL: 4µg / mL concentration is best turns 8µg/mL : 4 µg / mL with a ratio of 2: 1. Results of the determination of the absorption spectrum was made against theophylline solution with a concentration of 8 µg/mL and ephedrine HCl solution with a concentration of 4 µg/mL , then made spectral absorption at a wavelength of 200-400 nm.

### Results Determination of Zero-Crossing at First Derivatives

Absorption spectrum theophylline and ephedrine HCl concentrations 8 µg/mL and 4 µg/mL respectively transformed into a first derivative absorption spectrum with $\Delta\lambda$ = 2 nm. Results of the determination of the zero-crossing first derivative absorption spectrum for each substance. Zero-crossing in the first derivative spectrum of each wavelength is shown by agents who have zero absorption. Overlapping absorption spectrum theophylline and ephedrine HCl in the first derivative can be seen in the Figure 1.

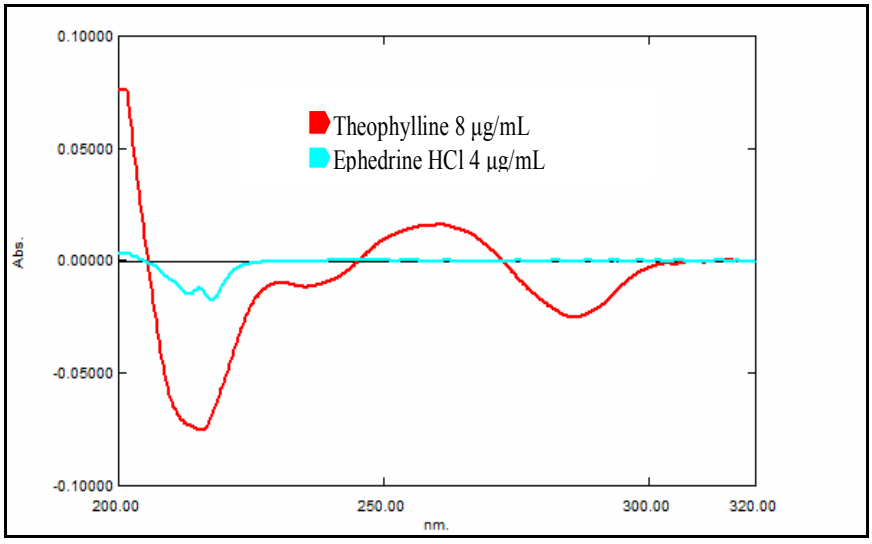


**Figure1. Overlapping absorption spectrum of theophylline and ephedrine HCl at first derivative**

### Determination of Absorption Zero-crossing on Second Derivatives

Results of the second derivative absorption spectrum determination is made by first making theophylline absorption spectrum of a solution with a concentration of 8 µg/mL and ephedrine HCl solution with a concentration of 4 µg/mL at a wavelength of 200-400 nm. Absorption spectra have been obtained is transformed into a second derivative absorption spectrum $\Delta\lambda$ 2 nm. The second derivative absorption spectrum of each of these substances overlaid. Overlapping absorption spectrum of theophylline and ephedrine HCl on the second derivative in Figure 2.

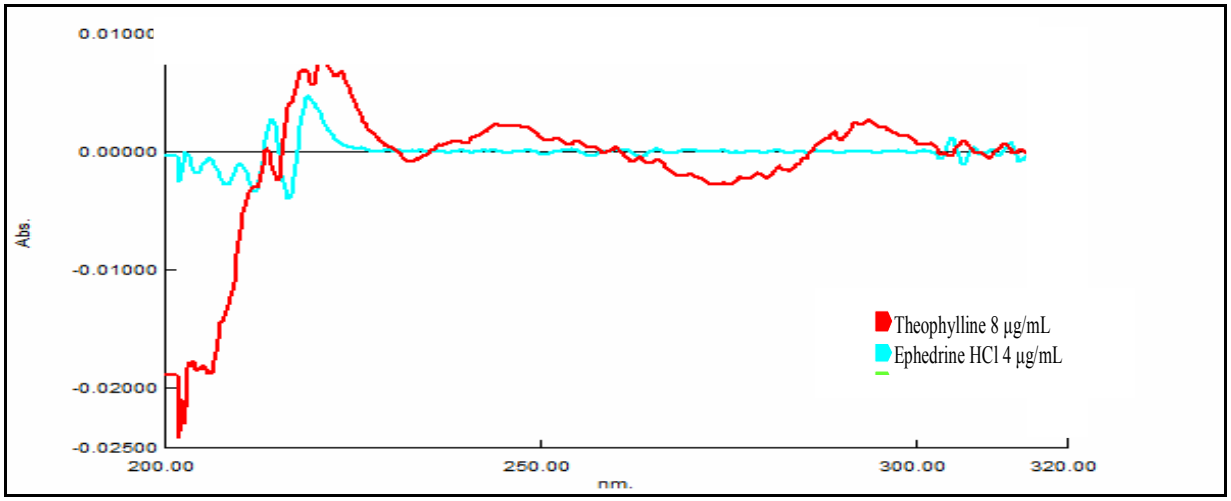


**Figure 2. Overlapping absorption spectrum of theophylline and ephedrine HCl on the second derivative**

**Results Determination of Wavelength Analysis**

Determination of the wavelength of the analysis done by making a solution of theophylline 8 µg/mL, ephedrine HCl solution of 4 µg/mL and mixed solution of theophylline 8 µg/mL and ephedrine HCl 4 µg/mL. Then made the first derivative of the absorption spectrum of each - each theophylline solution of 8 µg/mL, ephedrine HCl 4 µg/mL and mix theophylline 8 µg/mL and ephedrine HCl 4 µg/mL, subsequently superimposed, the same was done for the second derivative spectra. To determine the wavelength analysis of the absorption spectrum for each derivative is done by observing the absorption wavelength shows zero partner compounds and other compounds and mixtures thereof uptake has absorption value equal or nearly equal. Analysis wavelength of theophylline and ephedrine HCl can be seen in Figure 3.

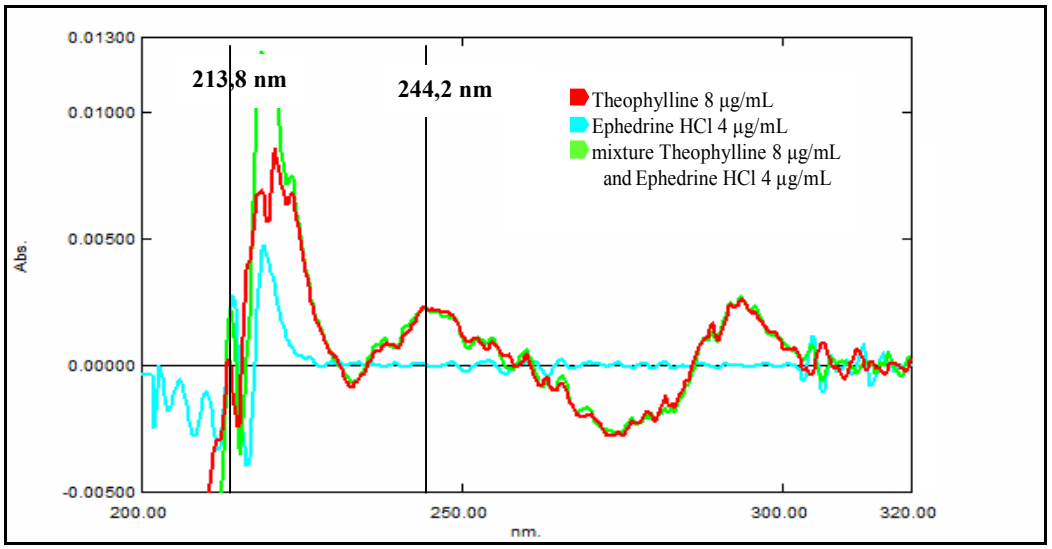


**Figure 3. Analysis wavelength theophylline and ephedrine HCl**

Based on the above figure, obtained by the wavelength that can be used to determine the content of a mixture of theophylline and ephedrine HCl is the second derivative absorption. It is known by wavelength selection for each derivative analysis. The wavelength of the analysis is obtained by determining the zero-crossing for theophylline and ephedrine HCl.

Analysis wavelength is determined by the way overlapping absorption spectrum of each derivative theophylline, ephedrine HCl and a mixture of theophylline and ephedrine HCl. Further specified wavelength where the absorbance of one of the substances currently on the value of zero, while other substances having almost the same absorption value. At first derivative absorption, wavelength analysis for ephedrine HCl can be found. However, the wavelength analysis for theophylline was not found, so the assay mixture of theophylline and ephedrine HCl in tablet dosage can not be done on the first derivatives. Therefore, it made the second

derivative absorption spectrum, then made the determination wavelength analysis in the same manner as in the first derivative.

Based on the results of the second derivative absorption spectrum, it is known that the zero-crossing for theophylline is at a wavelength of 213.80 nm; 305.20 nm and 319.60 nm and ephedrine HCl at a wavelength of 227.40 nm; 238.80 nm; 244.20 nm; 249.00 nm; 252.20 nm; 288.00 nm and 290.60 nm. After the second derivative absorption spectrum of both substances and mixtures overlaid, wavelength analysis obtained at 244.20 nm for theophylline and ephedrine HCl at 213.80 nm. Analysis wavelength and absorbance at the second derivative can be seen in Table 1.

**Tabel 1. Wavelength Analisys and Absorbance at Second Derivative**

| Wavelength (nm) | Absorbance | | |
|---|---|---|---|
| | Theophylline 8 µg/mL | Ephedrine HCl 4 µg/mL | Mixture Theophylline and Ephedrine HCl |
| **213.80** | **0.0000** | **0.0023** | **0.0022** |
| 227.40 | 0.0017 | 0.0000 | 0.0018 |
| 238.80 | 0.0008 | 0.0000 | 0.0008 |
| **244.20** | **0.0023** | **0.0000** | **0.0023** |
| 249.20 | 0.0017 | 0.0000 | 0.0018 |
| 290.60 | 0.0015 | 0.0000 | 0.0017 |
| 305.20 | 0.0000 | 0.0009 | 0.0005 |
| 319.60 | 0.0000 | 0.0001 | 0.0004 |

Based on Table 1 was obtained wavelength analysis of theophylline and ephedrine HCl were used respectively is 244.20 nm and 213.80 nm. Determination of the wavelength of the analysis is based on the third absorbance values of the solution at these wavelengths.

At a wavelength of 244.20 nm, ephedrine HCl absorbance value is zero, while the absorbance value for ephedrine HCl and mixed solution of theophylline and ephedrine HCl has the same absorption value is 0.0023, so to theophylline wavelength analysis is at 244.20 nm. Analysis of ephedrine HCl wavelength used was 213.80 nm because at these wavelengths, the absorbance values of theophylline is zero, while ephedrine HCl and mixed solution of theophylline and ephedrine HCl having almost the same absorption value for ephedrine HCl 0.0023 and 0.0022 for mixtures theophylline and ephedrine HCl.

**Determination Results Linearity Calibration Curves**

The linearity of the calibration curve showed a linear relationship between the absorbance with concentration. Theophylline regression equation, Y = 0,00029X + 0.00001 with a correlation of r = 0.9999 and ephedrine HCl, Y = 0.00057X + 0.00005 with a correlation of r = 0.9993. R values > 0.995 showed a linear correlation between X and Y [11]. The calibration curve theophylline and ephedrine HCl at each wavelength 244.20 nm and 213.80 nm can be seen in Figure 4 and 5.

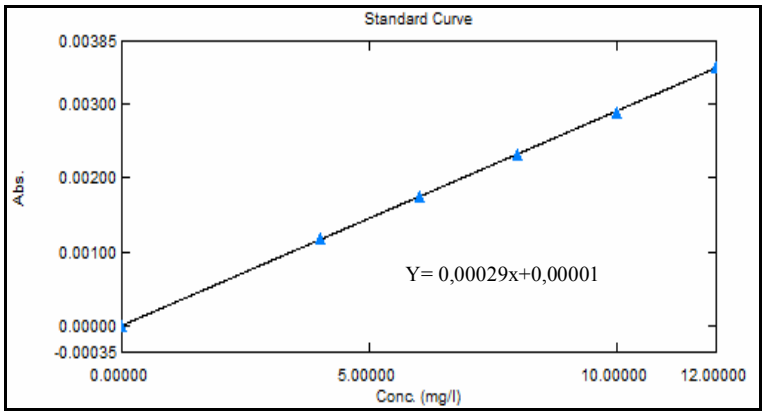


**Figure 4. Calibration curve of theophylline with wavelength 244,20 nm**

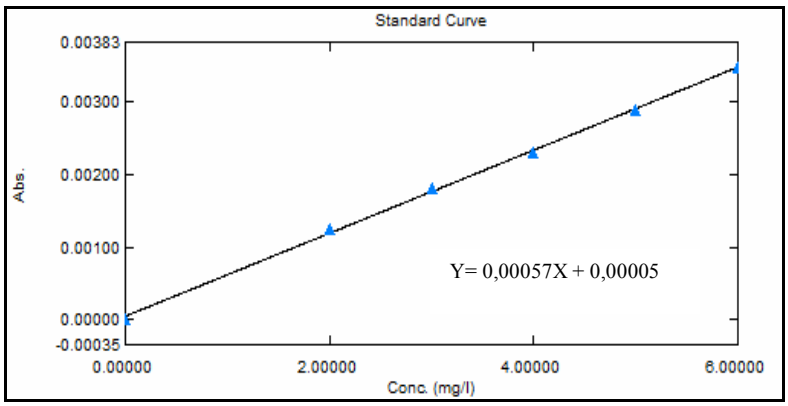


**Figure 5. Calibration curve of ephedrine HCl with wavelength 213,80 nm**

**Detection Limits and Quantitation Limits**

Limits of detection and quantitation limits calculated from the regression equation obtained from the calibration curve. Detection limits theophylline and ephedrine HCl is 0.1715 µg/mL and 0.2658 µg/mL, respectively and quantitation limits of theophylline and ephedrine HCl is 0.5718 µg/mL and 0.8859 µg/mL, respectively. It shows that the determination of the concentration of theophylline with 8 µg/mL and ephedrine HCl with a concentration of 4 µg/mL can be detected and measured using spectrofotometric methods derivatives.

**Application of the Method in Commercial Tablet**

Determination of assay theophylline and ephedrine HCl tablets circulating in commercial tablet each containing 130 mg of theophylline and 10 mg ephedrine HCl with trade name Grafasma® and Ifasma®, while the measurement of theophylline and ephedrine HCl with ratio 2:1 raw on both individual stocks theophylline 8 µg/mL and ephedrine HCl 4 µg/mL. In order to achieve the comparative test is carried addition with reference standards as much as 3.384 µg/mL after which it can be measured on derivative spectrofotometric method.

The prepared sample is then measured at a wavelength of 200-400 nm. Furthermore, the results of the absorption spectrum is transformed into a second derivative absorption spectrum with $\Delta\lambda = 2$ nm. Based on the absorbance spectrum can be determined theophylline and ephedrine HCl at a wavelength analysis has been obtained previously is wavelengths 244.20 nm and 213.80 nm.

Absorbance ephedrine HCl in the analyzed samples obtained negative value, this absorption is called absorption background. Absorbance ephedrine HCl in different samples by absorbance raw ephedrine HCl where measured have a positive value. This is probably due to:

1. There is no content of ephedrine HCl in the tablet being tested.
2. The presence of matrix interference with a greater concentration so as to cover the absorbance spectrum of ephedrine HCl in the sample.
3. Disruption of this matrix may also react with most of ephedrine HCl in the equilibrium form other compounds to form larger molecules. Larger molecules which direct the bathochromic effect on increasing the wavelength of the compound toward larger. Wavelength changes will alter the wavelength of the analysis so that the measurement of the amount of substance at the beginning of the wave length can not be done.

According Nurhidayati (2013), the contribution of background absorption disorders, which can be described as the second and third derivative polynomial lost on the fourth derivative. In practice, the matrix bullies can not always be eliminated by increasing order. In certain circumstances the standard addition method should be used to compensate the influence of the matrix, therefore, the assay of ephedrine HCl in the sample followed by the addition of the technique is the addition of a number of standard into the sample and measured the derivative ultraviolet spectrophotometric at a wavelength analysis.

According to Lambert-Beer law is the absorbance is proportional to the number of molecules that absorb radiation at specific wavelengths. This principle applies if there is more than one compounds that can absorb radiation. All multicomponent quantitative methods are based on the principle that the absorbance at each wavelength of the mixture is equal to the number of absorbance of each component in the mixture at a wavelength (Owen, 2000).

Theophylline and ephedrine HCl absorbance before and after the addition 3.384 µg/mL of raw ephedrine HCl can be seen in Table 2.

**Tabel 2 Absorbance of theophylline and ephedrine HCl before and after the addition of raw ephedrine HCl on the second derivative**

| Sample | Absorbance of theophylline at λ 244,20 nm | Absorbance ephedrine HCl at λ 213,80 nm | Absorbance ephedrine HCl at λ 213,80 nm |
|---|---|---|---|
| | | Before addition | After addition |
| Grafasma® | 0,00232 | -0,00013 | 0,00222 |
| Ifasma® | 0,00234 | -0,00017 | 0,00228 |

Based on the results of these measurements, the addition of raw ephedrine HCl in the sample was found to restore the ephedrine HCl absorption at a wavelength of 213.80 nm to the absorbance of the positive and the results of these measurements do not affect the absorbance of theophylline. Measurement results theophylline and ephedrine HCl with the standard addition method after the calculated levels tested further validation to show the truth and validity (valid).

Data calculation results theophylline and ephedrine HCl concentration in preparations the Grafasma® tablets and Ifasma® tablets after statistical analysis can be seen in Table 3.

**Table 3. Results of quantitation of theophylline and ephedrine HCl in commercial tablet by derivative spectrophotometric method**

| No | Drug | Grafasma® (%) | Ifasma® (%) | Label claim /tablet (mg) | Requirements (%) |
|---|---|---|---|---|---|
| 1. | Theophylline | 99.89 ± 1.67 | 99.35 ± 2.74 | 130 | 94.00-106.00 |
| 2. | Ephedrine HCl | 98.88± 3.21 | 96.59 ± 2.32 | 10 | 92.50-107.50 |

Can be seen in Table 3 contents of theophylline and ephedrine HCl in the tablet Grafasma® and Ifasma® meet the requirements.

**Test Results Validation**

Validation parameters tested were accuracy, precision, limit of detection and quantitation limits. Accuracy is expressed in % recovery were determined using the standard addition method. Test precision is done by using parameters RSD[9].

**Accuracy Test Results**

Test accuracy with parameter % recovery is done by using the tablet Grafasma®. Standard addition method by adding a certain amount of standard solution to the sample which has addition. Then the solution is measured absorbance wavelength corresponding analysis used.

**Table 4. Recovery of theophylline and ephedrine HCl with standard addition method in Grafasma® tablets.**

| Average (%) | % Recovery of | |
|---|---|---|
| | Theophylline | EphedrineHCl |
| 80 | 100.55 | 99.46 |
| | 99.26 | 99.96 |
| | 101.33 | 98.45 |
| 100 | 98.49 | 99.51 |
| | 99.93 | 99.11 |
| | 98.90 | 101.37 |
| 120 | 100.90 | 100.25 |
| | 99.86 | 100.82 |
| | 98.32 | 101.70 |
| *% recovery*<br>*Standard Deviation* (*SD*)<br>*Relative Standard Deviation* (*RSD*)(%) | 99.67<br>1.07<br>1.07 | 100.07<br>1.07<br>1.07 |

Table 4 shows that the average % recovery obtained had qualified for the accuracy of the validation of theophylline and ephedrine HCl is 99.67% to 100.07% respectively. Percent recovery for theophylline and ephedrine HCl meet the requirements the range of 98-102% [9].

**Precision Test Results**

Precision test is done by calculating the relative standard deviation. Based on the calculation of data on contents of theophylline and ephedrine HCl, relative standard deviation obtained for theophylline and ephedrine HCl is 1.07%. Results relative standard deviation for theophylline and ephedrine HCl meet the requirements that ≤ 2% [9].

**Table 5. Validation parameters for derivative spectrophotometric**

| Parameters | Theophylline | Ephedrine HCl |
|---|---|---|
| Corr. Coef. (r) | 0.9999 | 0.9993 |
| Slope | 0.00029 | 0.00057 |
| Intercept | 0.00001 | 0.00005 |
| Accuration (%) | 96.67 | 100.07 |
| LOD (µg/mL) | 0.1715 | 0.2658 |
| LOQ (µg/mL) | 0.5718 | 0.8859 |

## Conclusions and Suggestions

## Conclusion

Based on the research conducted, it can be concluded spectrophotometric method with zero-crossing derivatives can be used to establish the contents of theophylline and ephedrine HCl mixture, which required an additional method to establish the contents of ephedrine HCl addition technique. Theophylline contents meet the requirements of *USP* 30th (2007) and ephedrine HCl meets the requirements of Farmakope Indonesia V edition (2014). Validation test conducted on tablets Grafasma® and Ifasma® indicate that meet the requirements of derivative spectrophotometric method validation. The method is simple as there is no need for solvent, rapid and low cost.

## Suggestion

Suggested for subsequent research can perform the determination mixture of theophylline and ephedrine HCl using derivative spectrofotometric method with other solvents such as methanol.

## References


1. Charles. Bronchodilator Effect of Ephedrine and Theophylline after Two to Four Weeks of Repeated Dosing. Journal Publications. 1978, 73(6): 1019.
2. Siswandono, dan Soekardjo, B. Kimia Medisinal 2. Surabaya: Airlangga University Press. 2002; 400-402.
3. Moffat, A.C., Osselton, M.D., Widdop, B. Clarke's Analysis of Drug and Poisons. Fourth Edition. London: Pharmaceutical Press. 2005: 965, 1028, 1173, 1521, 1772, 1988, 2017, 2038 dan 2142.
4. Ditjen POM. Farmakope Indonesia edisi V. Departemen Kesehatan RI: Jakarta. 2014: 1250.
5. Kanakal, M.M., Aman S.A.M., Noor, S.A., dan Amin, M.S.A.M. Buffer-Free High Performance Liquid Chromatography Method for the Determination of Theophylline in Pharmaceutical Dosage Forms. Tropical Journal of Pharmaceutical Research. 2014, 13(1): 149-153.
6. United State Pharmacopoeia. The National Formulary. 30$^{th}$ edition : The United States Pharmacopoeia Convention. 2007: 3324-3325.
7. Senturk, Z., Nevin, E., Sibel, A.O. Determination of Theophylline and Ephedrine HCl in Tablets by Ratio-spectra Derivative Spectrophotometric and LC. Journal of Pharmaceutical and Biomedical Analysis. 2002 (29): 291-298.
8. Talsky, G. Derivative Spectrophotometric. Low and Higher Order. Weinheim Germany: VCH VerlagsgesellschaftmbH. 1994: 16 - 43.
9. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2005: 6-13.
10. Ditjen POM. Farmakope Indonesia edisi III. Departemen Kesehatan RI: Jakarta. 1979: 53.
11. Watson, D.G. Pharmaceutical Analysis: A Textbook for Pharmacy Students and Pharmaceutical Chemists. 2 edition. Jakarta: EGC. 2000: 126.


*****