Two Novel Ultraviolet Spectrophotometric Method For Determination Binary Mixture Of Isoniazide And Rifampicin In Tablet Dosage Form.

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Abstract : A combination of INH and RIF is one of the combination dosage in the treatment of tuberculosis. Each INH and RIF can be assayed by ultraviolet spectrophotometry. This study aims to establish the levels of both these drugs by comparing the derivative spectrophotometric method with zero crossing and spectrophotometry ultraviolet with multiple wavelength method in the matrix calculation.

The methodology used to determine the assay of INH and RIF in tablet dosage form is conducted by derivative spectrophotometric method with zero crossing, using 0.1N HCl solvent and spectrophotometric method with multiple wavelength in the matrix calculation with HCl 0.1 N as a solvent.The result showed that with the method of zero crossing, the two substances is measured at the second derivative in wavelength analysis of 300.80 nm for RIF and 240.20 nm to INH, while the multiple wavelength method measured at wavelength 215 nm, 225 nm, 232 nm , 250 nm, and 267 nm. Using the zero crossing method, the RIF levels obtained in Rimactazide® tablet was 100.51% ± 4:43% and INH levels was 99.7% ± 1:52% with recovery percentage for RIF was 100.39% with RSD=0.63%, and for INH, the recovery percentage was 101.05%, with RSD= 0.57%. Using the matrix calculation method by multiple wavelength, the levels of INH (97.83±0,12)%, with the value of CV= 0.0787% and RIF (97.40 ± 0.30)% with CV= 0.1951%. So it can be concluded that the derivative spectrophotometric method in zero crossing and matrix calculation in multiple wavelength can be used to determine of INH and RIF in tablet dosage form.

Keywords : RIF, INH, Derivative spectrophotometry, Zero Crossing, multiple wavelength, matrix calculation.

1. Introduction

Drugs used for tuberculosis is divided into two groups i.e. primary drufs and secondary drugs. The primary drug groups, namely isoniazide (INH), rifampicin (RIF), ethambutol and pyrazinamide. These medications are the most effective and lowest toxicity, but can lead to a quickly resistance when used as a single drug. Therefore the use of the drugs always by using combinations of the drugs [1].

Isoniazide (INH) is one of the effective anti-tuberculosis drμgs. The use of INH is usually combined with other antituberculosis drμgs such as Rifampicin. **Rifampicin (RIF)** is rather difficult to dissolve in water, easily soluble in chloroform, soluble in ethyl acetate and methanol[2]. Chemical structure of INH and RIF can be seen in Figure 1..

(a)   (b)
Figure 1. Chemical structure of (a) INH, (b) RIF [2].

Assay of each INH and RIF can be determined by ultraviolet spectrophotometry method. RIF has a maximum absorption in an acid solution at wavelength 231 nm, while the INH at wavelength 266 nm [3].

Several studies on the assay of INH and RIF had been done before, among others, the visible spectrophotometry and the spectrophotometric derivatives methods, the HPLC method, the electroanalysis method, and ultraviolet spectrophotometry method by multicomponent [4,5,6,7].

The derivative spectrophotometric method is one of the spectrophotometric method which can be used for analysis the mixtures of several substances directly without having to make prior separation, even though with the adjacent wavelength.The most common procedure used in the determination of a mixture of substances that the spectrum is overlapping is the method of zero crossing[8]. The modification of ultraviolet spectrophotometry method for multicomponent analysis without having prior separation is conducted with the principle of regression equation through matrix calculation of absorption observations of binary mixtures at some point wavelength [9,10]. INH and RIF has a wavelength that is adjacent. RIF has a maximum wavelength of 231 nm and INH has a maximum wavelength of 266 nm which causes the spectrum overlap. Therefore the derivative spectrophotometric method with zero crossing and the matrix calculation of multiple wavelength can be used to determine the levels of binary mixture of INH and RIF.

2.Experiment

2.1 Apparatus and Materials

UV-Visible spektrofotometer (Shimadzu), Personal Computer(PC) equipped with UV Probe 2.42 software (UV-1800 Shimadzu),HCl 37%, INH (Indonesian Pharmacopoeia Reference,BPFI), RIF (ASEAN Reference Substance, ARS), Rimactazide®(PT. Sandoz).

2.2 Preparation of INH and RIF solution

Carefully weighed 50 mg INH BPFI raw materials and dissolved with 0.1N HCl so a solution with a concentration of 50 ug/mL is obtainable. 50 mg of the reference standard RIF ARS weighed carefully and diluted with 0.1N HCl, so a solution with a concentration of 50 ug/mL is obtainable.

2.3. Determination of INH and RIF by Zero Crossing Derivative Spectrophotometric

2.3.1. Preparation the standard solution of INH and RIF

The solution of INH of 50 ug/ml is taken and 0.1N HCl is added in order to obtain a solution with a concentration of 5 ug/mL, 7.5 ug/ml, 10 ug/mL;12.5 ug/ml and 15 ug/mL. The RIF solution of 50 ug/ml is dissolved in HCl 0.1 N in order to obtain a solution with a concentration of 6 ug/mL;9 ug / ml, 12 pg / mL;15 ug / ml;and 18 ug/mL.

2.3.2. Determination of the Maximum Absorption Spectrum

The INH solution with a concentration of 50 mg / mL is taken and then inserted into the 10 mL flask and dilluted with 0.1 N HCl until the line marking in order to obtain a solution with a concentration of 11 ug/mL. Then, the RIF solution 50 mg / mL is taken and then put into a 10 mL flask and dissolved with HCl 0.1 N until the line marking in order to obtain a solution with a concentration of 13.5 ug/mL. The absorbance is measured at wavelength 200-400 nm.

2.3.3 Determination Derivative Absorption Spectrum of INH and RIF

Make absorption spectrum from INH solution with a concentration of 5;7.5;10;12.5; and 15 ug / mL and RIF solution with a concentration 6, 9, 12, 15, and 18 ug/mL at 200-400 nm wavelength.Then the spectrum is transformed into first and second derivatives absorption spectra with $\Delta\lambda = 2$ nm.

2.3.4. Determination of Zero Crossing

Determination the zero crossing is conducted by overlapping the absorption spectra each derivative in a range of the solution concentration. The zero crossing of each material is indicated by wavelength that has zero absorption at various concentrations.

2.3.5. Wavelength Determination Analysis

INH solution is made with a concentration of 10 ug /mL and RIF with a concentration of 15 ug / mL, and mixed solution of 15 ug/mL RIF and 10 ug /mL INH. Then all three of these solutions's absorbance is measured at wavelength 200-400 nm. Then it transformed into the second derivative absorption spectrum from a single substance solution of and a mixture of both is overlapped.

2.3.6. Preparation and Determination Calibration Curve Linearity

Make INH solution with a concentration of 5 ug/mL;7.5 ug/mL;10 ug/mL;12.5 ug/mL, 15 ug/ mL and RIF solution with a concentration of 6 ug/mL;9 ug/mL;12 ug/mL;15 ug/mL;and 18 ug/ mL, then measured the uptake of the second derivative ($\Delta\lambda = 2$ nm) at a predetermined wavelength analysis.Then analysis the relationship between concentration and absorbance values thus obtained by linear regression equation $y = ax +$ b and the calculation of LOD and LOQ [11,12,13].

2.3.7. Determination of Levels of INH and RIF in Tablet Dosage Forms

Twenty tablets are weighed and crushed homogeneous. The powder weighed equivalent to 50 mg of RIF and the equality of INH contained in there is calculated.It should be weighed up to six repetitions. Then the absorbance is measured at predetermined wavelength.

2.4. Determination of INH and RIF by multiple wavelength Spectrophotometric

2.4.1 Determination of Absorption Spectrum with multiple wavelength

The INH solution with a concentration of 5.0 ug/mL, 7.5 ug/mL, 10.0 ug /mL, 12.5 ug/ mL, and 15.0 ug/mL and RIF solution concentration of 6.0 ug/mL; 9.0 ug / mL, 12.0 ug / mL, 15.0 ug / mL;and 18.0 ug / mL each of which has made up to six repetitions, absorbance was measured at a wavelength of 200-400 nm.

2.4.2 Determination of Wavelength Analysis and the absorbance spectrum

Make INH solution with a concentration of 5.0 ug/mL and RIF solution with a concentration of 7.5 ug/mL.Then both of these solution'sabsorbance was measured at a wavelength of 200-400 nm.Furthermore absorption spectrum of each of the components in overlaid. Then searches 5 point as the wavelength to be used.Wavelength selection is taken from the absorption spectrum of components.the absorbance measured at predetermined multiple wavelengths. Both materials' absorbance are determined using linear regression method operated in the concentration and absorbance data of each compound at each wavelength measurements.

2.4.3 Determination binary mixture of INH and RIF.

Carefully weighed 10.0 mg of reference material of RIF ARS and then inserted into the 10.0 mL flask, diluted with 0.1 N HCl until dissolved to obtain a solution with a concentration of 7.5 ug/mL RIF and Carefully weighed 10.0 mg of reference material of INH BPFI and then inserted into the 10.0 mL flask, diluted with 0.1 N HCl until dissolved to obtain a solution with a concentration of 5.0 ug/ mL INH. Furthermore, the absorbance measured at 5 predetermined wavelength.

2.4.3 Determination binary mixture of INH and RIF in tablet dosage form

Twenty tablets are weighed and crushed homogeneous. The powder weighed equivalent to 50.0 mg of RIF and the equality of INH contained in there is calculated. It should be weighed up to six repetitions.

2.4.4 Calculation of RIF and INH levels in the binary mixture.

The calculation of the levels of each component in the mixture is conducted on the basis of the mixture absorbance (Ac) and absorbance of each component at rhe multi wavelength known from the measurement results using the matrix equation [10]:

[c] = [[a] x [a 1]]-1x [A] x Ac]
Descriptions :
[C]: the levels of components of the mixture
[A]: matrix of absorbance mixture of constituent compounds
[a 1]: transpose matrix of absorbance mixture of constituent compounds
[[a] X [a 1]]-1:inverse matrix times transpose matrix of absorbance mixture of constituent compounds
Ac: sample absorbance value

3. Results and Discussion

3.1 Determination of INH and RIF by Zero crossing Derivative Spectrophotometric

3.1.1 Maximum Absorbance Spectrum

Determination of maximum absorbance spectrum performed at a wavelength of 200-400 nm. INH at a concentration of 11 ug / mL,and RIF measurements performed at a concentration of 13.5 ug/mL, the results obtained maximum wavelength INH at 266.60 nm and 231 nm for RIF.The maximum absorbance spectrum of RIF concentration of 13.5 ug/mL, and the maximum absorbance spectrum of INH concentration of 11 ug each of which can be seen in Figures 2 and Figure 3
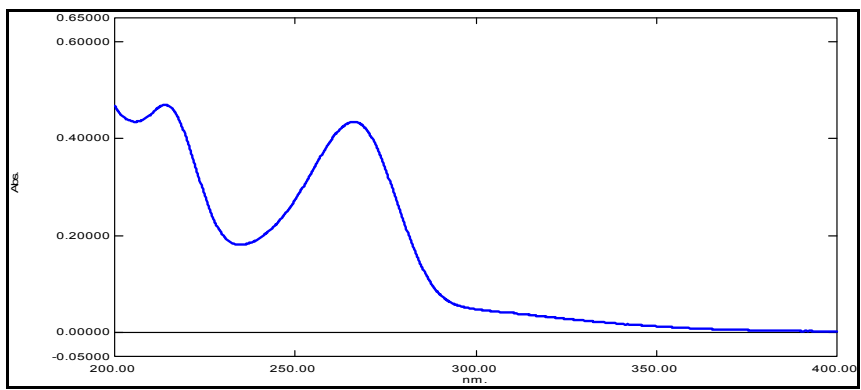
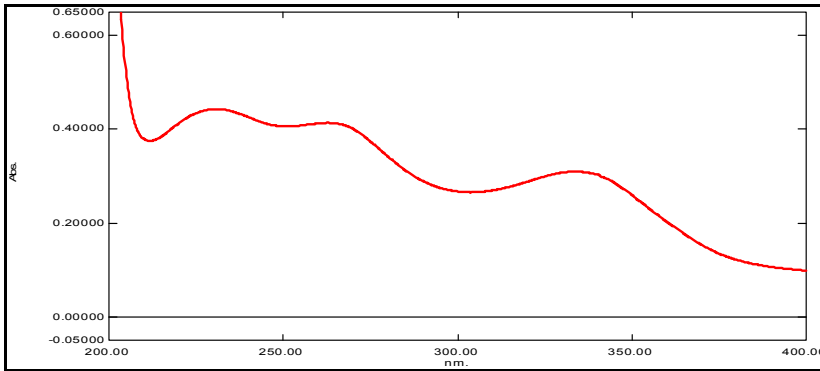**Figure 2. Maximum absorbance spectrum of INH concentration of 11 ug / mL**

Figure 3 Maximum absorbance spectrum for 13.5 ug/mL of RIF

3.1.2 The Determination of Absorbance Spectrum

The determination of the absorbance spectrum is obtained from INH solution with a concentration of 10 ug / mL and RIF solution with a concentration of 15 ug/mL. Then absorption spectrum at a wavelength of 200-400 nm is made.This is done because the comparison of content of RIF and INH in tablet dosage used was 450 mg: 300 mg (3: 2), and when the orientation of the various concentrations is conducted.. The absorbance spectrum of RIF concentration of 15 ug/mL, the absorption spectrum of INH concentration of 10 ug / mL and absorption spectrum of overlapping RIF and INH each of which can be seen in figures 4.
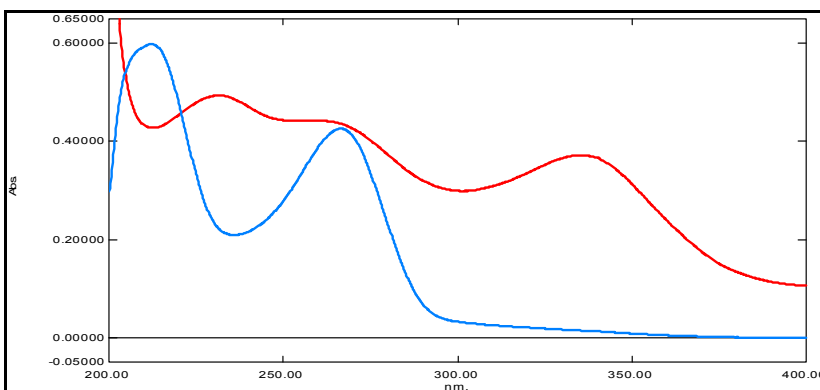
Figure 4. Absorbance spectrum of overlapping RIF and INH

Description :   ■Isoniazide 10 ug/mL
                 ■Rifampicin 15 ug/mL

Based from Figure 4 it can be seen of overlapping absorbance of RIF and INH, there is a two-way overlapping. A two-way overlapping means there is no wavelength of each compound can be measured without interference by other [9]. Based on the results of the overlapping absorbance spectrum of RIF and INH above, conduct a derivatization to obtain zero crossing of individual components.

3.1.3 Determination of zero crossing at second derivatives absorbance

Determintation of the second derivative absorbance spectrum is made by making the absorption spectrum of RIF solution with a concentration of 15 ug/ml and INH solution with a concentration of 10 ug/ml at a wavelength of 200-400 nm. Absorbance spectrum, that have been obtained, transformed into a second derivative absorbance spectrum with $\Delta\lambda = 2$ nm. Second derivative absorbance spectrum of each substances was overlapped The second derivative absorbance spectrum of 15 ug/mL RIF concentration, the second derivative absorbance spectrum 10 ug/mL INH concentration. The overlapping spectrum of second derivative absorbance of RIF and INH can be seen in Figures 5 below.
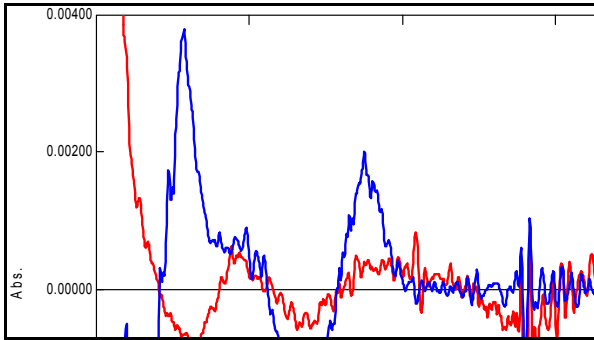
Figure 5 The overlapping spectrum of second derivative absorbance of RIF and INH

Description : ▪ Isoniazide 10 ug/mL
▪ Rifampicin 15 ug/mL

Based of Figure 5, it can be seen that overlapping absorption RIF and INH result zero crossing at the second derivative absorbanceat the wavelength 256.00 nm, 278.80 nm, 300.80 nm and 312.00 nm for RIF.As for INH obtained at a wavelength of 220.40 nm, 240.20 nm, 260.20 nm and 284.00 nm.The result of the zero crossingof RIF and INH obtained, used for the assays of these two substances.Wavelength and absorbance analysis on the second derivative can be seen in Table 1.

Table 1. Wavelength and Absorbance Analysis on the Second Derivatives

Wavelength (nm)	Absorbance		
	RIF 15 μg/mL	INH 10 μg/mL	Mixture of RIF and INH
220.40	-0.0000	-0.0004	-0.0010
240.20	**0.0000**	**0.0008**	**0.0008**
256.00	-0.0000	0.0000	-0.0007
260.20	-0.0000	-0.0008	-0.0015
278.80	-0.0000	0.0000	0.0006
284.00	0.0000	0.0010	0.0019
300.80	**0.0003**	**0.0000**	**0.0003**
312.00	0.0023	-0.0000	0.0003

3.1.4 Determination of Wavelength Analysis of RIF and INH

Determination of the wavelength analysis is conducted by making 15 ug/mL RIF solution, 10 ug /mL INH solution and mixed solution of 15 ug/mL RIF and 10 ug/mL INH. Then made the second derivative of the absorbance spectrum of each solution 15 ug/mL RIF, 10 ug/mL INH and mixture of 15 ug/mL of RIF and 10 ug/mL of INH, subsequently overlapped. Determination of the wavelength analysis of the absorbance spectrum of each derivative is conducted observing wavelengths that showed zero absorbance partner and the other substance and its mixtures has an equal or nearly equal absorption value.

At first derivative absorbance, the wavelength analysis for INH can be found. However, the wavelength analysis for RIF was not found, so the assay of the mixture of RIF and INH in tablet dosage can not be performed on the first derivative.Therefore, the second derivative absorbance spectrum is made, and wavelength analysis of RIF and INH are both obtained on the second derivative.Wavelengths for RIF obtained at 300.80 nm, and INH at 240.20 nm.

At a wavelength of 300.80 nm, the absorbance value of INH is zero, while the absorbance value for RIF and the mixed solution of RIF and INH have the same absorption value which is 0.0003. Therefore, selected wavelength analysis for RIF is at 300.80 nm. For INH wavelength analysis used was 240.20 nm because at these wavelengths, the absorbance value of the RIF is zero, while the absorbance value to INH and the mixture of RIF and INH have the same absorption value which is 0.0008. The second derivative absorbance overlapping

spectrum of RIF and INH, RIF's wavelength analysis spectrum λ = 300.80 nm, and the wavelength analysis spectrum for INH λ = 240.20 nm.Each can be seen in Figure 6 and Figure 7.
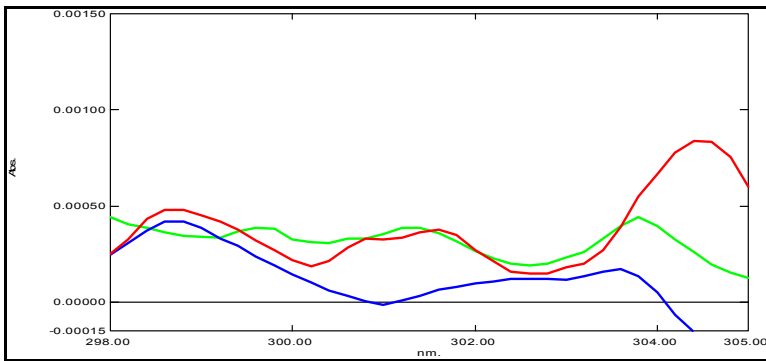

Figure 6. RIF's wavelength analysis spectrum with λ = 300.80 nm
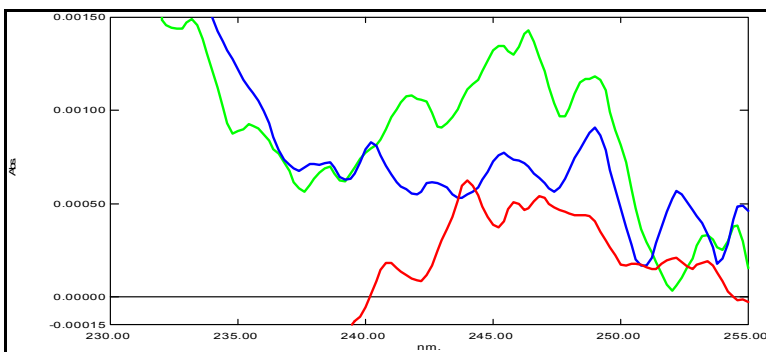

Figure 7. The wavelength analysis spectrum for INH with λ = 240.20 nm

Description :   ■Isoniazide 10 ug/mL
                ■Rifampicin 15 ug/mL
                ■Rifampicin 15 ug/mL and Isoniazide 10 ug/mL Mixture

3.1.5 Determination of Linearity of Calibration Curve

The linearity of the calibration curve shows the linear relationship between the absorbance with concentration.RIF regression equation is $Y = (0.2185X + 0015) .10^{-4}$ with correlation r = 0.9996 and INH, $Y = (0.8379X + 0.087) .10^{-4}$ with a correlation of r = 0.9991.Value r> 0995 showed a linear correlation between X and Y [14]. The obtained value of r for RIF and INH show that the calibration curve RIF and INH has a linear relationship and meet the requirements, i.e. r> 0.995.65-66. RIF calibration curve at a wavelength of 300.80 nm and INH calibration curve at a wavelength of 240.20 nm can be seen in Figure 8 and Figure 9
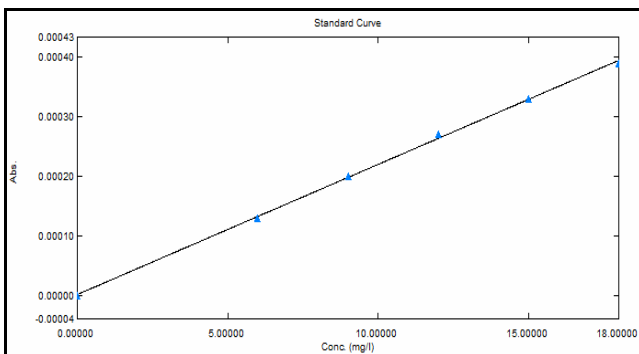

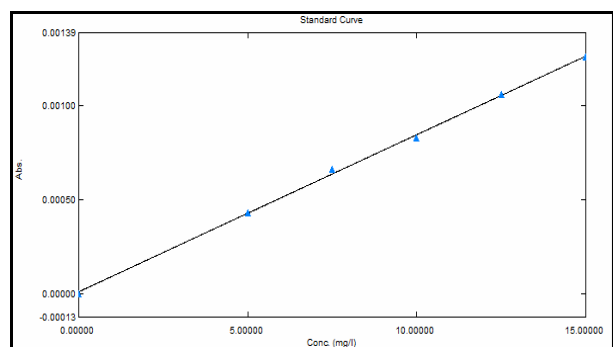**Figure 8. RIF calibration curve at a wavelength of 300.80 nm**

Figure 9. INH calibration curve at a wavelength of 240.20 nm

3.1.6 The Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the regression equation obtained from the calibration curve.The LOD of RIF and INH is 0.5931 ug/mL and 0.5445 ug/mL, respectively, and the LOQ of RIF and INH is 1.9771 ug/mL and 1.8152 ug/mL, The LOD is the lowest concentration of the analyte in the sample that can still be detected. The LOQ is defined as the lowest analyte concentration in a sample that can still meet the criteria for careful and thorough respectively [11,12,13]. It shows that the assay of RIF with a concentration of 15 ug/mL and INH with a concentration of 10 ug/mL can be detected and measured using spectrophotometric derivatives methods.

3.1.7 Determination of RIF and INH levels in Rimactazide[®] Tablets

Assay of RIF and INH is done using Rimactazide[®] tablet containing 450 mg of RIF and 300 mg of INH. Raw RIF and raw INH measurements is 15 ug/mL RIF and 10 ug/mL INH, which is tailored to the content ratio of the two substances in the preparation, which is 450: 300 or 3: 2.

The prepared samples were measured at a wavelength of 200-400 nm.Furthermore, the results of the absorbance spectrum is transformed into a second derivative absorbance spectrum with $\Delta\lambda$ = 2 nm.The spectrum can be determined based on the absorbance of RIF and INH at a wavelength analysis has been obtained previously, ie, wavelengths 300.80 nm and 240.20 nm. Levels RIF and INH in Rimactazide tablet can be seen at Table 2

Table 2. Content of RIF and INH in tablet Rimactazide ®

No.	Substances	Rimactazide[®]	Requirements
1.	RIF	$(100.51 \pm 4.43)\%$	90 – 110 %
2.	INH	$(99.7 \pm 1.52)\%$	90 – 110 %

It can be seen from Table 2 that the levels of RIF and INH in tablet Rimactazide[®] meets the requirements stated in the levels of WHO[15], which contains a RIF and INH is not less than 90% and not more than 110% of the amount listed on the label[16]. The results indicate that the tablet dosage Rimactazide[®] on the market meet the requirements.

3.1.9 Accuracy Test Results

Accuracy Test with parameter of recovery percentage is done by raw material addition method by adding a certain amount of raw material into the sample solution[11,12,13]..Then the solution is measured its absorbance at the wavelength of the analysis, which is 300.80 nm to 240.20 nm for the RIF and INH.

Recovery percentage of RIF and INH, it is obtained an average percent recovery for the RIF is 100.39% with SD = 0.64.and to INH is 101.05% with SD = 0.57. These results meet the accuracy requirements for the validation of analytical procedures, because in between theranges that have been established, ie 98-102% [11,12,13]. Results of RIF and INH recovery by raw material addition addition method Rimactazide[®] standard in the tablet can be seen in Table 3.

Table 3. RIF And INH Recovery By Raw Material Addition Metho In Rimactazide®- Tablet

Specific Range Spesifik (%)	RIF Recovery (%)	INH Recovery (%)
80	101.61 100.47 100.85	101.59 100.47 100.85
100	99.97 99.97 99.36	100.77 100.77 100.17
120	100.45 100.20 100.71	101.61 101.37 101.86
Average (% *recovery*) *Standard Deviation* (*SD*) *Relative Standard Deviation* (*RSD*) (%)	100.39 0.64 0.63	101.05 0.57 0.57

Based on the validation test at Table 3 that the derivative spectrophotometric method meets the validation requirements and can be used to assay mixture of RIF and INH in Rimactazide® tablets.

Precision test is done by calculating the relative standard deviation.Based on calculation data on levels of RIF and INH, obtained relative standard deviation = 0.63% for RIF and INH = 0:57%. The results meet the requirements, ie ≤ 2% [11,12,13].

3.2. Determination of INH and RIF by multiple wavelength spectrophotometric

3.2.1 Determination of Multiple Wavelength Analysis

The results of measurements on some chosen absorption point for the determination of the measurement matrix by multiple wavelength can be seen in the Figure 10 below

Based on the Figure 10 that the five wavelength used are 215 nm, at this wavelength RIF and INH started giving absorption, at a wavelength of 225 nm is the point of intersection of RIF and INH, at a wavelength of 232 nm INH still provide sizeable absorbance and RIF's absorbance began to decline, 250 nm is the point of intersection of RIF and INH and RIF 267 nm gives a sizeable absorbance and absorbance of INH began to decline.
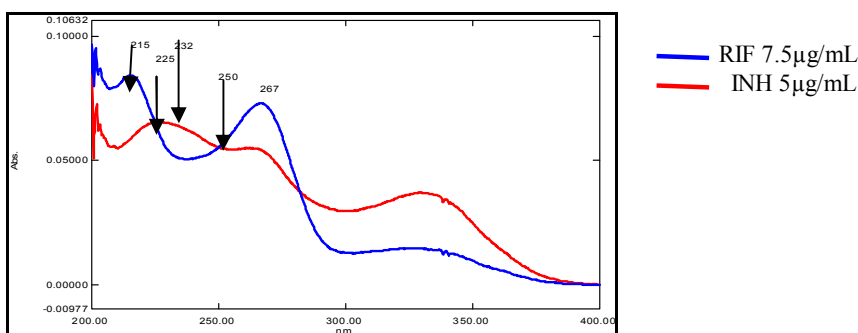

Figure 10 Five points of wavelength to determination by multiple wavelength

3.2.2 Determination absorbance spectrum of multiple wavelength method.

The absorbance value is a value that indicates how big the contributions of a substance's absorbance to the mixture of compounds' absorbance at a particular wavelength[10]. The absorbance value is conducted by measuring absorbance of each solution of RIF and INH at wavelengths of 215, 225, 232, 250, and 267 nm.In this study, the determintation of absorbance value is conducted by operating the absorption data at each wavelength to the concentration of the solution in linear regression equation which analogous to the equation in

Beer's law [9]. The linear regression equation is: $Y = aX + b$. In the linear regression equation, Y shows the absorbance (A), a show absorbance, The absorbance Spectrum of RIF's and INH's can be seen at Table 4 and Table 5

The used absorbance value (a) is the absorption value of RIF and INH at Table 4 and Table 5. The selection of this absorbance value (a) is determined based on the price of (r) count. The (r) count value compared with the value of (r) table with a level of 95% is 0.8114. Based on these data, it appears that the value of (r) count of RIF and INH on repetition VI is greater than the value of (r) table. This means that the equation has good linearity, because the value of (r) count ranges with values $-1 \leq r \leq 1$ [10]. The obtained absorbance data is then used to establish the levels of RIF and INH in the mix with matrix calculation [10].

Table 4. Absorbance Spectrum of RIF's

Concentration µg/mL	λ1 215 nm	λ2 225 nm	λ3 232 nm	λ4 250 nm	λ5 267 nm
6	0.14207	0.16347	0.16655	0.14554	0.14562
9	0.21659	0.23586	0.23371	0.20213	0.20129
12	0.30078	0.33188	0.33019	0.28771	0.29032
15	0.39561	0.44412	0.4449	0.38926	0.39637
18	0.44356	0.49872	0.49858	0.43784	0.44996
	a =0.02544 b=-0.0047 r=0.9980	a=0.0285 b=-0.006 r=0.9975	a=0.02845 b=-0.0055 r=0.9972	a=0.02495 b=-0.0058 r=0.9969	a=0.02561 b=-0.0089 r=0.9964

Table 5. Absorbance Spectrum of INH's

Concentration µg/mL	λ1 215 nm	λ2 225 nm	λ3 232 nm	λ4 250 nm	λ5 267 nm
5	0.24441	0.15952	0.11219	0.14694	0.22101
7.5	0.36850	0.23969	0.16707	0.21873	0.33251
10	0.50411	0.32890	0.23027	0.30012	0.45386
12.5	0.60127	0.38758	0.27069	0.35790	0.54596
15	0.73853	0.47607	0.33221	0.43916	0.67052
	a =0.04901 b =0.0011 r =0.9995	a=0.03158 b=0.0021 r =0.9993	a=0.02203 b=0.0018 r =0.9992	a=0.02912 b=0.0011 r =0.9995	a=0.04449 b=-0.0001 r =0.9997

3.2.3 Content, Coefficient of Variation, Acuracy and precesion of RIF and INH in Rimactazide tablet

The calculation of the levels of measurements of RIF and INH in preparation tablet Rimactazide circulating in pharmacies, containing respectively 450 mg RIF and 300 mg INH. The RIF and INH of measurements each 7.5 ug/mL and 5.0 ug/mL can be seen at Figure 11.
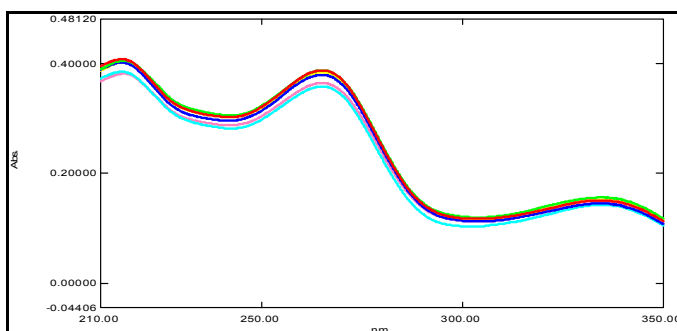

Figure 11. Absorbance spectrum of the sample preparation of Rimactazide® tablet

Based of Figure 11 that the prepared samples are then measured at a wavelength of 200-400 nm.The spectrum can be determined based on the absorbance of RIF and INH at a wavelength analysis has been obtained previously [10], for this research the wavelengths are 215. 225. 232. 250. and 267 nm.

The data of absorbance of the sample solution that has been obtained is used to measure the levels of each substances by entering the data available on the matrix calculation formula. Then from the calculation, it will be obtained the concentration of each component with the accuracy from the results of the matrix and the coefficient of variation (CV)[10].

Matrix calculation of INH and Rif in Rimactazide, for example sample preparation number one :

$$\begin{pmatrix} C1 \\ C2 \end{pmatrix} = \left(\begin{pmatrix} 0.02508 & 0.02789 & 0.02783 & 0.02426 & 0.02466 \\ 0.04221 & 0.02809 & 0.02029 & 0.02624 & 0.03956 \end{pmatrix} \begin{pmatrix} 0.02508 & 0.04221 \\ 0.02789 & 0.02809 \\ 0.02783 & 0.02029 \\ 0.02426 & 0.02624 \\ 0.02466 & 0.03956 \end{pmatrix}^{1} \right)^{-1} \times \begin{pmatrix} 0.02508 & 0.02789 & 0.02783 & 0.02426 & 0.02466 \\ 0.04221 & 0.02809 & 0.02029 & 0.02624 & 0.03956 \end{pmatrix} \begin{pmatrix} 0.40197 \\ 0.34361 \\ 0.3073 \\ 0.31409 \\ 0.37708 \end{pmatrix}$$

$$\begin{pmatrix} C1 \\ C2 \end{pmatrix} = \begin{pmatrix} 7.303201 \\ 5.092248 \end{pmatrix}$$

The result of the calculation of the levels and the Coefficient of Variation of RIF and INH levels in the Rimactazide® Tablet can be seen in Table 6.

Table 6. Accuray, Precision and Coefficient of Variation of INH and RIF in Rimactazide®- Tablet

Sample	RIF			INH		
	Measurable levels (µg/mL)	Theoretical levels (ug / mL)	The accuracy of the matrix (%)	Measurable levels (µg/mL)	Theoretical levels (ug / mL)	The accuracy of the matrix (%)
1	7.30	7.50	97.58	5.09	5.30	97.85
2	7.78	7.97	97.78	5.09	5.26	98.54
3	7.78	7.99	97.54	4.84	5.05	97.72
4	7.68	7.92	97.18	4.84	5.04	97.92
5	8.30	8.56	97.07	4.96	5.15	98.17
6	7.50	7.72	97.31	4.87	5.06	97.85
Mean			97.41	Mean		98.00
% CV			0.1951	% CV		0.0787
Accuracy			good	Accuracy		good
Precision			good	Precision		good

Based from Table 6 can be seen that RIF and INH levels in preparation Tablet Rimactazide® meets the requirements according to World Health Organization (WHO)[15] , namely for the preparation of RIF tablets and INH tablets that is not less than 90.0% and not more than 110.0% of the amount listed on the label [16].

Accuracy of matrix calculation are used to determine the accuracy of an analytical method while the coefficient of variation (% CV) is used to determine the precision of an analytical method. The accuracy of an analytical method for the drug substance with low concentrations considered good when the value range between 90-107% accuracy, while an analytical method is said to have good precision if the coefficient of variation (% CV) <2% [11,12,13]. The coefficient of variation (% CV) obtained in RIF and INH is 0.4898% and 0.0787%, respectively. It means having a good precision.

Conclusion

1. Derivative spectrophotometric method with zero crossing and multiple wavelength spectrophotometric method with matrix calculation can be used to set the mixture levels of INH and RIF.
2. Levels of INH and RIF in tablet dosage Rimactazide® can determinate with the derivative

spectrophotometric methods and multiple wavelength with matrix calculation spectrophotometric method meets the requirements of the combination tablet levels of INH and RIF are listed in the WHO.
3. Validation tests performed on the tablets Rimactazide® indicates that the derivative spectrophotometric methods and multiple wavelength with matrix calculation spectrophotometric method meet the validation requirements, which include the parameters of accuracy and precision.

Acknowledgement

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