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The $-NO_2$ vibrational spectra of metronidazole for analytical method development using Fourier Transform Infrared compared to the UV-VIS spectrophotometry

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ABSTRACT

This study aimed to develop a green method in analyzing metronidazole compound using Fourier Transform Infrared (FTIR) and to ensure that this method is suitable to be done for tablet content determination, and then was compared to the established spectrophotometry ultraviolet-Visible (UV-Vis). Both methods were compared in terms of analysis parameters, effectiveness, and cost required. The experiment was started by screening to find the best spectra was performed, supported by derivation mode to increase the specificity. After that, the method was developed by tests its validation parameters, and then it was applied for tablet content determination. All observation results were compared to an established spectrophotometry UV-VISVis, which also has been verified concurrently. As a result, metronidazole showed a specific spectrum of $-NO_2$ at 1388.5-1338.36 cm^{-1}, which is not interfered by matrices. The calibration curve yielded revealed the correlation regression (r) = 0.99985. All validation parameter requirements can be fulfilled by this method, which had the limit of detection and limit of quantification of 0.36 ppm and 1.19 ppm, respectively. As the comparison to UVVis spectrophotometry, in terms of effectiveness, the FTIR method is relatively more practical and more straightforward, it was evidenced by the shorter processing time than the counterpart. In terms of cost, the FTIR method also required a relatively less cost. Besides, it is more environmentally friendly due to the absence of solvent usage.

Keywords: *Metronidazole, FTIR, UV-Vis spectrophotometry, Analysis, effectiveness, cost.*

1. INTRODUCTION

Medicine is a commodity that is very important and needed by society. The availability of drugs in the community is the responsibility of the government and also cannot be separated from the role of the pharmaceutical industry as the party that produces and supplies drugs to the community. In fact, it is not only the problem of drug availability that needs attention, but the main elements of the drug, namely quality, effectiveness, and safety must also be an essential factor to consider. The pharmaceutical industry must produce products that are good quality, effective and safe for consumption by the community by regulating and managing all things related to the process of making the drug as well as possible, from the selection of raw materials to the product marketing process. Medicines that are safe for consumption can be known as the results through examination with specific analytical methods, and the results are compared with the conditions set.

Metronidazole is useful in the treatment of anaerobic brain abscesses [1]. This active compound is still considered as a standard gold antibiotic [2]. Some antibiotics that have anaerobic activity must be compared with metronidazole both in terms of antimicrobial activity, pharmacokinetics and pharmacodynamics, side effect profiles, and in terms of financing [3]. To guarantee the quality, the benefits of the composition and safety of the use of these medicinal products, quality control is needed to avoid the possibility of circulating drugs having the quality that does not meet the requirements. Fourier transform infrared [FTIR] is still rare to be utilized for the routine analysis, whereas it is relatively cheap, efficient, and clean due to no solvent needed. However, some studies have been reported the suitability of this instrument to be used in the quantitative analysis [4], but not yet for metronidazole.

This study aims to find an effective and efficient method for analyzing metronidazole compounds and ensure that this method is suitable to be an alternative method in the analysis of metronidazole compounds. Next, this method was compared to the established UV-Vis spectrophotometry [5]. The principal disadvantages of the vibrational method are the lack of specificity due to almost bindings in the molecule will produce spectrums. Hence, the thorough selection of the specific wavenumber of the target-compound, which is not influenced by other constituents, must be performed in advance. Fortunately, nowadays, the instrument was commonly completed by the automatic program to derivate the spectra, which can separate to overlaid spectrums and increase their resolution. It was hoped that the outcome of this study is to produce a method that is truly specific, selective, precise, and accurate in analyzing metronidazole compounds. We have reported some works about FTIR method drug analysis in a variate matrices, mainly tablets. In 2019, Nugrahani was reported Quantitative Vibrational Methods Development And Its Performance Comparison To Colorimetry On The Assay Of Kanamycin Sulfate [6], The rapid and green hair analysis method development using FTIR for papaverine HCL determination was reported by Nugrahani [7]. Rapid assay development of diclofenac sodium coated tablet assay using FTIR compared to HPLC method was reported too [8].

The utilization of FTIR method is very broad, one of which is Development of the NSAID-L-Proline Amino Acid Zwitterionic Cocrystals was reported by Nugrahani, Utami, Permana, and

Ibrahim [9]. The comparison has done and the results are decent enough for alternative testing.

2. MATERIALS AND METHODS

2.1. Reagent, Material, and Standards

The materials used include metronidazole pure reference standard (Indonesian Pharmacopoeia's Reference Standard), potassium bromide crystal (KBr) (Sigma Aldrich, Indonesia), acetone analytical grade (Sigma Aldrich, Indonesia), 0.1N HCl solution, aqua-demineralisata (Bandung Institute of Technology, Indonesia), metronidazole tablet dose 500 mg preparation (Kimia Farma, Indonesia).

2.2. FTIR Method Development

The parameters of validation checked were linearity, accuracy, precision, and specificity, following the criterion which is required in the references. From the regression equation, LOD and LOQ were generated, respectively [10, 11].

2.2.1. Linearity

A series of BPFI metronidazole concentrations were made in KBr between 1.00-6.00 %w/w, then measured using FTIR. The resulting spectrum in the form of a function of % transmission with wavenumbers in the range 4000 cm^{-1} to 400 cm^{-1} was then converted into an absorbance function. Then plotted between the BPFI metronidazole concentration in KBr %w/w and the area under the curve (AUC) then determined its linearity. The linearity acceptance criterion is r (correlation coefficient) ≥ 0.999, and the regression coefficient of variance (Vxo) $\leq 2.0\%$ [12].

2.2.2. Accuracy

A series of metronidazole sample concentrations were made in KBr 3.2; 4; and 4.8% w/w. Then measured using FTIR, replicated three times (triplo). Then calculate the% recovery, the acceptance criteria accuracy with the acquisition value of 98-102% [13].

2.2.3. Precision

The concentration of metronidazole in KBr 4% was made; the precision validation parameter was done by repeating the measurement six times with the same concentration. Measurements were also made on three days when the analysis was different. The precision acceptance criterion was to look at the RSD (Relative Standard Deviation) value [14].

2.2.4. Specificity

An overlay between the metronidazole spectrum of BPFI and the spectrum of the tablet matrix was carried out; the selected wave range was based on the best linearity value.

2.2.5. Limit of Detection (LOD) and Limit of Quantity (LOQ).

LOD and LOQ can be estimated by the standard deviation approach of the response and the slope of the calibration curve. LOD can be stated as:

$$LOD=\frac{3\sigma}{S}$$

Where:
LOD = Limit of Detection
σ= Standard Deviation
S= Slope of the Calibration Curve

$$LOQ=\frac{10\sigma}{S}$$

LOQ can be stated as:
Where:
LOQ= Limit of quantitation
σ= Standard deviation
S= Slope of calibration curve [15]

2.3. Determination of Metronidazole Active Substance in Metronidazole Tablet Preparations

Determination of the concentration of samples of metronidazole tablet preparations. A total of 20 tablets were weighed and then crushed until smooth and homogeneous. Weighed equivalent to 4 mg and mixed with KBr so that the mass is 100 mg. Then put in the KBr plate and pressed. The sample then was measured by FTIR and determined the area under the curve in the appropriate wavenumber range. Then the levels are calculated using the standard curve equation [16,17,18]

2.4. UV-Vis Spectrophotometry Method Verification [5]

2.4.1. Determination of Linearity Calibration Curves for Metronidazole in 0.1 N HCl Solution.

From LBI II piped 3 mL each; 4.5 mL; 6 mL; 7.5 mL; and 9 mL, put in a flask measuring 50 mL, then the volume is filled with 0.1 N HCl to the marked line, so that the concentration of metronidazole is 6 ppm each; 9 ppm; 12 ppm; 15 ppm; and 18 ppm. Measured absorption at the maximum wavelength using 0.1 N HCl as blank [19].

2.4.2. Accuracy.

A series of 9.6 ppm, 12 ppm and 14.4 ppm metronidazole sample concentrations were made each of three replications, then measured for absorption at the maximum wavelength determined. Then the% recovery is calculated. Criteria for acceptance of accuracy with the acquisition value of 98-102% [20].

2.4.3. Precision

Metronidazole sample solution was made with a concentration of 12 ppm, then measured its absorption at the maximum wavelength. The precision verification parameter was done by repeating the measurement six times with the same concentration. Measurements were also made on three days when the analysis was different. The criteria for receiving precision are to look at the RSD (Relative Standard Deviation) value [14].

2.4.4. Specificity

Specificity was carried out by overlaying the standard metronidazole absorption curve with the metronidazole tablet sample.

2.5. Determination of Metronidazole Content of Tablets

Carefully weighed 20 metronidazole tablets, recorded each weight, then calculated the average weight of tablets. After that, the whole tablet was crushed until homogeneous. Weighed a number of powders equivalent to 100 mg of metronidazole powder, then put into an Erlenmeyer and added 10 mL of acetone and stirred, then the clear liquid was transferred in another Erlenmeyer (carried out as much as four times). The dried extract obtained was then weighed carefully as much as 50 mg and put into a 100 mL measuring flask and then dissolved with 0.1 N HCl and supplied to the marked line, shaken homogeneously, then a solution with a concentration of 500 ppm was obtained [21]. From the solution pipetted as much as 10 mL, put in a flask measuring 50 mL and filled with 0.1 N HCl to the marked line, then obtained a concentration of 100 ppm solution. Then from the solution pipetted as much as 6 mL put into a flask measuring 50 mL and filled with 0.1 N HCl until the signed line, then obtained a solution with a concentration of 12 ppm. Then the absorption is measured

at the maximum wavelength using 0.1 N HCl as blank [5]. Metronidazole tablets contain metronidazole, $C_6H_9N_3O_3$, not less than 90.0% and no more than 110.0% of the amount indicated on the label [22].

3. RESULTS

3.1. FTIR Spectrum Measurement of Metronidazole Standard

In the spectrum of derivatives formed by the standard metronidazole (Figure 1), several ranges of wave numbers will be selected which provide a significant area compared to the others. The results of the calculation of the area of the derivative curve presented in Table 1. The conversion into absorbance must be done because followed Lambert Beer's rule the absorbance value will be directly proportional to the concentration [23], so it can correlate with the area of the curve. Subsequently, derivatization of the spectrum has been converted into its absorbance form.

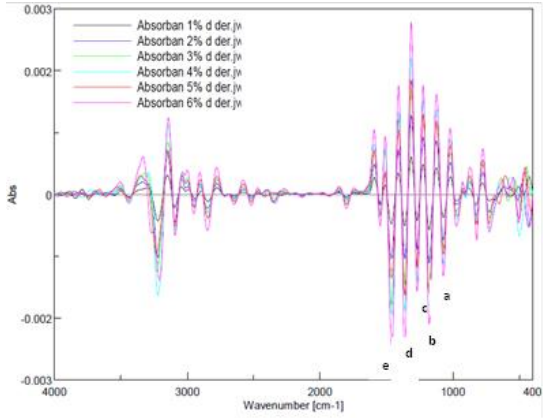

Figure 1. Combined metronidazole raw derivative spectrum at wave number 4000-400 cm^{-1}. (a) 1095.37-1045.23 cm^{-1}; (b) 1203.36-1149.37 cm^{-1}; (c) 1288.22-1249.65 cm^{-1}; (d) 1388.5-1338.36 cm^{-1}; (e) 1496.49-1434.78 cm^{-1}

Table 1. area of derivate curve of metronidazole tablet

Concentration	Area of the curve				
	Peak a	Peak b	Peak c	Peak d	Peak e
1%	0.0132	0.0231	0.0131	**0.0239**	0.0216
2%	0.0227	0.0384	0.0227	**0.0333**	0.0425
3%	0.0259	0.0524	0.0298	**0.0421**	0.0571
4%	0.0307	0.0580	0.0336	**0.0512**	0.0665
5%	0.0351	0.0685	0.0395	**0.0593**	0.0849
6%	0.0383	0.0722	0.0438	**0.0688**	0.0988
r	0.9831	0.9792	0.9876	**0.9999**	0.9957

Derivation was performed by correcting the spectrum baseline and then converting it into its derivative form. Then, the area of the curve was calculated from the derivative spectrum with the axis = 0 in the specific wavenumber range. Specific spectrum selection based on several research results, such as, specific spectrum for acetosal is 41407.86 cm^{-1}, caffeine is 39062.5 cm^{-1}, and paracetamol is 38684.72 cm^{-1} [24, 25]

From the measurement results showed in Table 1. The range of wave numbers between 1388.5-1338.36 cm^{-1} which represented the $–NO_2$ group [26] has the best linearity, indicated by the highest (r) which is 0.99985. The spectra from a series of concentrations here are revealed in Figure 2. Also, after correction of the excipient matrix adjusted, this spectra only owned by metronidazole and as not possessed by the matrice's spectral derivatives. So this range of wavenumbers is used as the basis for the quantitative calculation of metronidazole levels in tablets.

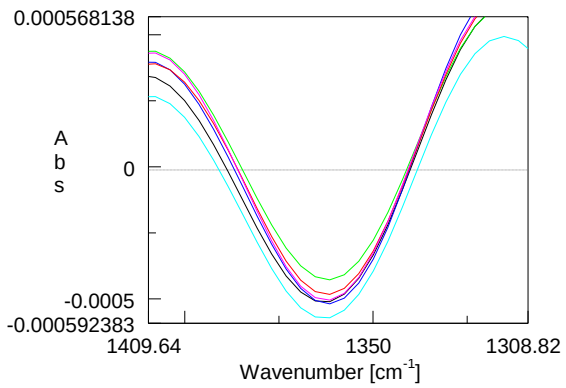

Figure 2. Overlay of derivate curve range at 1388.5-1338.36 cm^{-1}, represented the – NO_2 group.

3.2. Comparison of Analysis Parameters between FTIR Method and The Established UV-Vis Spectrophotometry Method

3.2.1. Linearity

Determining linearity used a range between points of concentration that were large enough to facilitate the differences in responses produced but still produce results that were following the requirements.

In the FTIR method, the standard curve of metronidazole was used in the concentration of 1.00; 2.00; 3.00; 4.00; 5.00; and 6.00% w/w. The calibration curve between the concentration and the response of the instrument was made by plotting the value under the spectrum of each predetermined peak to the concentration. The linearity of the standard curve is expressed by the correlation coefficient (r) in the linear regression analysis of the standard curve line equation y = bx + a. The results are shown in Table 2 and Figure 3.

Table 2. Calibration data of metronidazole standard concentration FTIR method

Concentration (% w/w)	Area of the curve
1	0.0239±0.0014
2	0.0333±0.0012
3	0.0421±0.0013
4	0.0512±0.0006
5	0.0593±0.0008
6	0.0688±0.0009

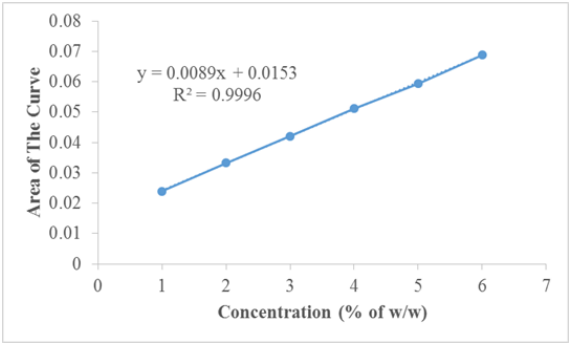

Figure 3. Metronidazole standard calibration curve using FTIR.

From the determination of the linearity of the FTIR, the line equation was: y = 0.0089x+ 0.0152 with a correlation coefficient (r) of 0.99985 (or r^2=0.9996) and V_{x0} value of 1.1001%.

3.2.2. Measurement of Maximum Absorption Wavelength of Metronidazole with UV-Vis Spectrophotometer

The standard metronidazole solution was made in 0.1 N HCl with a concentration of 12 ppm. then its absorption is measured in the wave range 200-400 nm and as a blank was used 0.1 N HCl. The maximum absorbance was shown at 277 nm. The calibration curve yielded can be seen in Figure 4.

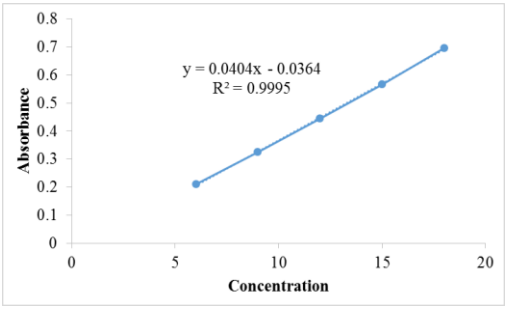

Figure 4. Calibration curve of metronidazole using UV-Vis spectrophotometry method

The linearity equation of the UV-Vis spectrophotometry method is: y = 0.0404x + 0.0364 with a correlation coefficient (r) of 0.99975 or r^2 of 0.9995 and V_{x0} value of 0.9924%. From these results, it can be concluded that this method was similar in terms of linearity with the FTIR method and both accordance with the requirements of the r-value greater than or equal to 0.98 and the value of V_{x0} was smaller or equal to 2.0% [27].

3.2.3. Accuracy and Precision Test

In determining the accuracy of this FTIR method, a sample concentration series was prepared in KBr 3.2; 4; and 4.8% w/w. While the accuracy-test for the UV-Vis spectrophotometry method used a sample concentration series of 9.6; 12; and 14.4 ppm. The results met the percent recovery acceptance criteria of 98-102% [28].

Furthermore, the RSD value obtained in the precision test with the FTIR method was 0.4264%, whereas the UV-Vis spectrophotometry method obtained an RSD value of 1.2741%. The results met the acceptance criteria; namely, the RSD value was less than 2% [29].

3.2.4. Specificity

In the FTIR method, specificity test was done by comparing the derivatives of the matrix spectrum with the matrix spectrum derivatives which have been added by analytes. The combined spectrum of matrix derivatives and spectrum of matrix derivatives mixed with analytes is shown in Figure 5.

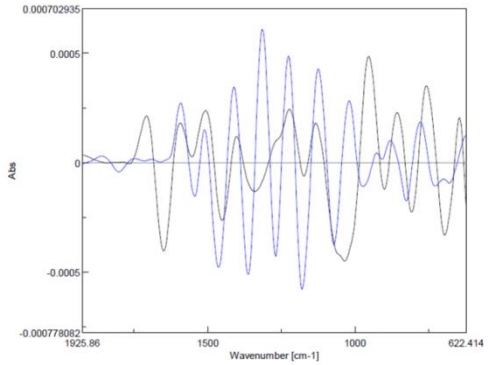

Figure 5. Combined spectrum of matrix and matrix derivatives mixed with analytes.

3.2.5. LOD and LOQ

From this study, LOD values of 0.116% w/w or 0.116 mg of active ingredients in a 100 mg KBr mixture were obtained. Whereas for the UV-Vis spectrophotometry method, the LOD value is 0.3573 ppm. From this study obtained LOQ value of 0.385% w/w or 0.385 mg of the active substance in a mixture of 100 mg KBr. Whereas for the UV-Vis spectrophotometry method, LOQ values were obtained at 1.1909 ppm.

3.3. Determination of Active Substance Concentration in Metronidazole Tablets

Once validated assay method and proved that this method is valid, then performed an assay of active substance metronidazole in the tablet dosage form. The assay was conducted on three product metronidazole tablets on the market, obtained the results shown in Table 3.

Table 3. Data content in preparation metronidazole tablets with FTIR method

Product	Average of Area of Spectra NO_2	Concentration (in mg)	Concentration (%w/w)
I	0.0502±0.0027	490.88	98.18
II	0.0509±0.007	501.20	100.24
III	0.0514±0.0009	507.91	101.58

From the measurement results, it was concluded that the three products on the market that meets the criteria required by the Indonesian Pharmacopoeia V, not less than 90% and not more than 110% w/w [5].

The assay was conducted on three product metronidazole tablets on the market. Data obtained measurement results are shown in Table 4.

Table 4. Data Content in preparation Metronidazole Tablets by UV-Vis Spectrophotometry Method

Product	Average of Absorbance	Concentration (in mg)	Concentration (%w/w)
I	0.468±0.0023	445.03	104.02
II	0.466±0.0097	442.83	103.58
III	0.467±0.006	444.44	103.91

From the measurement results, it was concluded that the three products on the market that meet the criteria required by the Indonesian Pharmacopoeia V, not less than 90% and not more than 110%.

3.4. Comparison of FTIR Methods and UV-Vis Spectrophotometry Methods.

3.4.1. Based on Analysis Parameters.

Comparison of FTIR method with UV-Vis spectrophotometry based on aspects of analysis parameters is shown in Table 5.

Table 5. FTIR method comparison data and UV-Vis spectrophotometry method based parameter analysis

NO	Parameters	Method	
		FTIR	UV-Vis
1	Linearity	r= 0.999 V_{x0}=1.100	r= 0.999 V_{x0}=0.992
2	Accuracy	% Recovery = 99.89± 0.624	% Recovery = 99.75± 1.688
3	Intra Day Precision	% RSD = 0.1884	% RSD = 0.7973
4	Inter Day Precision	% RSD = 0.4264	% RSD = 1.2741
5	LOD & LOQ	LOD = 0.116 % w/w LOQ = 0.385 % w/w	LOD = 0.3573 ppm LOQ = 1.1909 ppm

From the above data it can be concluded that in terms of linearity, both methods above provide a linear curve, which means that the increase in concentration is directly proportional to the response generated by the two instruments. When viewed from the coefficient of variance of regression methods, UV-Vis spectrophotometer has better grades than FTIR method, which means that the distribution of data from UV-Vis spectrophotometry method is smaller in comparison with the FTIR method.

Based on the validation parameters of accuracy and precision, both methods can be regarded as an accurate and precise method. However, among them, the FTIR method is more accurate and precise than the method of UV-Vis spectrophotometry. It can be confirmed from the value of % recovery of the FTIR method instead of the UV-Vis because of the greater, the more accurate method. For the validation parameters of precision method of FTIR is also better than the method of UV-Vis. It is seen from the % RSD, where the value of the FTIR method smaller than the methods of UV-Vis, which means that the method was more precise than the counterpart. The UV-Vis spectrophotometry method has the LOD and LOQ values smaller than the FTIR method, which means that the accuracy of the UV-Vis Spectrophotometry method is better than the developed FTIR method. However, totally, for the standard dose of tablet content determination, the FTIR method still fulfilled the validation requirements.

The other method that can be used in the analysis of metronidazole is to use the RP-HPLC method, such as the research conducted by Minal et al. Analysis of metronidazole using this method gives a LOD value of 0.6 ppm and a LOQ value of 1.9 ppm. This shows that in terms of sensitivity, the FTIR method is still better than the UV-Vis Spectrophotometry methods and RP-HPLC. While in terms of accuracy, the RP-HPLC method gives an average value of recovery of 99.3% not much different from the accuracy of the FTIR method and UV-Vis Spectrophotometry [30].

3.4.2. Based on Effectiveness

Methods FTIR and UV-Vis spectrophotometry compared based on their effectiveness, it can be concluded that the FTIR method was more effective than UV-Vis spectrophotometry method. This effectiveness in terms of processing time and ease of implementation procedures. The processing time of the FTIR method will be shorter because it does not take time for making reagent solutions as was done in the UV-Vis spectrophotometry method. From the research that has been carried out the time required in the FTIR method working procedure starting from the preparation of materials to measurements in the instrument more or less requires 8-11 minutes, while the working procedure UV-Vis spectrophotometry method takes 13-15 minutes calculated starting from the prepared sample to measurement in the instrument.

3.4.3. Based on Costs. Cost calculations of the FTIR method analysis versus the UV-Vis spectrophotometry method are shown in Tables 6 and 7.

Table 6. Cost analysis of FTIR methods data

Materials	Function	Amount	Price	Total Price
Aseton	Solvent	200 mL	Rp. 1,900/mL	Rp. 380,000.00
Kbr	Mixed with sample in FTIR plate	20 gram	Rp. 2,600/g	Rp. 52,000.00
Total				Rp. 432,000.00

Table 7. Cost analysis of spectrophotometry UV-Vis method

Materials	Function	Amount	Price	Total Price
Aseton	Solvent	200 mL	Rp. 1,900/mL	Rp. 380,000.00
Concentrate Hydrochloride Acid	Blank	15 mL	Rp. 2,300/mL	Rp. 34,500.00
Aquabidestilata	Solvent	2 L	Rp. 38,000/ L	Rp. 76,000.00
Total				Rp. 490,500.00

From the data above the cost analysis results, it can be concluded that the costs involved in the process of metronidazole assay with FTIR method require less cost compared to the method of UV-Vis spectrophotometry.

When compared with the RP-HPLC method which has been used routinely based on the compendia requirement [31], the FTIR method is also much more efficient in terms of costs because in the RP-HPLC method it uses a large and varied amount of organic solvents, of course, this affects the costs incurred to obtain these solvents. In addition, the use of many solvents also affects the environment because the solvent used will eventually produce much waste. So, if it viewed from the impact on the environment, the FTIR method is an environmentally friendly method.

4. CONCLUSIONS

The specific infrared spectra peak for metronidazole quantification using FTIR is owned by -NO_2 at 1388.5-1338.36 cm^{-1}. Assay of metronidazole in tablets using FTIR method meets the criteria validation parameters: linearity, accuracy, interday precision, intraday precision, detection limit, quantitation limits, and ranges. This method was then used to measure the levels of metronidazole tablets on the market and showed results that qualify. Compared with UV-Vis spectrophotometry method, this method is more accurate, precise, effective, efficient, and relatively cheap and easy.

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6. ACKNOWLEDGEMENTS

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