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Original Article

DEVELOPMENT AND VALIDATION ANALYSIS OF ACYCLOVIR TABLET CONTENT DETERMINATION METHOD USING FTIR

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ABSTRACT

Objective: The aim of this research is to develop the fourier transforms infrared spectroscopy (FTIR) as an alternative method for determination acyclovir content in the tablet dosage form.

Methods: Infra-red spectrum of acyclovir were transformed to be its first derivative, then measured the AUC (area under the curve). Calibration curve was made in a series of the concentration of 0.2-1.2% w/w, and some wavenumbers spectra's area were choiced. Some validation parameters: specificity, linearity, limit of detection, limit of quantification, accuracy and precision were evaluated, then this validated method was used to determine the content of acyclovir in three tablet dosage forms found from the market.

Results: The optimal wavenumber obtained was shown at 3700-3440 cm^{-1}, next it was used during validation test, which produced the excellent yields of all validation parameters. Furthermore, this method was used for tablets content determination.

Conclusion: All the results from this research showed that FTIR can be used as a validated alternative method, more rapid and simple for determine the content of acyclovir in a tablet.

Keywords: FTIR, Quantitative, Acyclovir, Tablet, Validation

INTRODUCTION

Acyclovir is a synthetic purine nucleoside analogue, which commonly used in the treatment and prophylaxis of infectious herpes simplex virus types 1, 2, and varicella-zoster virus. This action requires intracellular conversion of acyclovir by the thymidine-kinase to the monophosphate with subsequent conversion by cellular enzymes to the diphosphate and activated triphosphate. This activation inhibits DNA synthesis and its replication by inhibit the herpes virus's DNA polymerase enzyme; as well as being incorporated into the DNA. This process is highly selective for the infected cell.

This drug structure is shown in fig. 1 below. Some quantitative analytical methods of acyclovir have been reported and standardized in compendia using high-performance liquid chromatography and uv-vis spectrophotometry. However, these methods need many toxic solvents, more consuming time, and relatively costly [1-6].

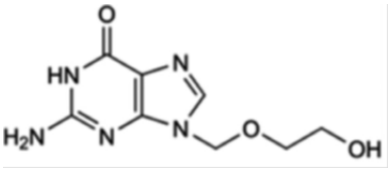


Fig. 1: Acyclovir molecule structure

On the other side, FTIR has several significant advantages compares to the dispersive types infra-red spectrophotometry instruments. Three of these are the Fellgett (multiplex) and Jacquinot (throughput) advantages. The Fellget's is due to an improvement in the signal-to-noise ratio (SNR) per unit time, proportional to the square root of the number of resolution elements being monitored. This results from a large number of resolution elements being monitored simultaneously. In addition, FTIR does not require a slit or other restricting device, so the total source output can pass the sample continuously. This results gain in energy to the detector, hence translating to higher signals and improved SNR. This is known as Jacquinot's advantage. Besides, FTIR has the higher speed advantage, because the mirror in FTIR instrument can move short distances quite rapidly. Three main advantages of FTIR have explained, make this spectrophotometry can obtain infra-red spectra on a millisecond timescale [7-9].

In the analytical field, almost all compendias stated that infra-red spectrophotometry used as an identification (qualitative) method for some substances [5, 6]. The aim of this research was to develop FTIR as a more rapid, simple, and cheaper method for determination acyclovir in a tablet. Infra-red spectrum of acyclovir will be changed to be the first derivative spectra then measuring its AUC (area under the curve). This method was carried out, in purpose to sharpening the peak which will improve the data that easier to calculate. In the other term, the derivative method was used to increase the method selectivity [9, 10].

In this research, there was no sample pretreatment, such as extraction or purification. The derivative method was used to remove spectral interferences from the other components. Besides the other infra-red instruments such as Raman spectroscopy; recently, FTIR also has been reported can be used as a quantitative method, includes for ibuprofen and azithromycin content determination in tablets [11, 12], cimetidine in its suspension [13], and acyclovir in a nano-particle product [14]. This method was expected to provide some advantages, such as: more economic, fast, simple, free of solvent, and showed the adequate of its sensitivity, precision, and accuration.

MATERIALS AND METHODS

Reagents and samples

Standard acyclovir was USP 32 grade and obtained from PT Indofarma (Cikarang, Indonesia), potassium bromide crystal (KBr) in its infra-red spectra grade, acyclovir tablet samples made by different pharmaceutical manufacturing companies, which were obtained from some drug stores in Bandung (Indonesia).

Apparatus

FTIR (fourier transforms infrared) spectrophotometer Jasco 4200 type used to obtain the infra-red spectrum. FTIR spectrums were

recorded in the wavenumber range 4000-400 cm^{-1}, averaging scanning rate performed was 2 cm/s with a nominal resolution of 16 cm^{-1}, triglycine sulphate (TGS) used as a detector of FTIR. Spectra manager II software was used to collect and analyze the data.

Calibration curve

A calibration curve was prepared from six different acyclovir standard concentrations within the range of 0.2-1.2% w/w. An appropriate quantity of acyclovir was diluted with potassium bromide to get each concentration and triturated to ensure sample homogeneity. Infra-red spectra of each measurement were converted to be the first derivative spectra. Area under the curve (AUC) of each calibration standard was measured at five different wavenumbers, namely 3700-40, 3012-2870, 1955-1680, 1176-1072, and 721-609 cm^{-1}. Each calibration standard was analyzed in the replicates of three, and an average from three measurements was used to obtain a calibration curve.

Validation method

The developed FTIR method was validated by specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, and accuracy test.

Specificity

The specificity of the method was assessed by comparing standard acyclovir spectra with sample spectra.

Linearity

Linearity was evaluated by preparing standard acyclovir concentrations within the range of 0.2-1.2% w/w through the same procedure to make calibration curve at five different wavenumbers. Each measurement in FTIR was analyzed three times. Linearity was evaluated by calculation the correlation coefficient (r) and a variation coefficient of linear regression (Vxo) from the regression curve. Additionally, the wavenumber which showed the highest r value was used for subsequent measurement.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) was assessed by calculation from the regression curve. LOD was calculated by formula LOD = 3.3 (Sy/x)/b. Meanwhile; LOQ was calculated by formula LOD = 10 (Sy/x)/b. *Sy/x* itself is the residual standard deviation of linear regression and *b* is the slope of the regression curve.

Precision

The precision of the method was assessed by repeatability and intermediate precision. Repeatability was performed by analyzing acyclovir standard at a concentration of 1% w/w six times on the same day thoroughly. Intermediate precision of assay method was evaluated by repeating studies inter-day (three different days).

Accuracy

The accuracy of the assay method was evaluated by the standard addition method at three different concentrations (80, 100, and 120% w/w). For the pre-analyzed tablet sample powder preparation, a known amount of acyclovir powder corresponding to 80, 100, and 120% of label claim were added. Recovery was calculated according to the following equation:

$$\text{Recovery}\,(\%) = \{(C1 - C2)/C3\} \times 100\%$$

where C1 is the concentration of the spiked sample, C2 is the measured concentration of sample without spiking, and C3 is the actual standard concentration added to the sample.

Acyclovir tablet content determination

Three different brands of acyclovir tablets were used to determine their drug content. For each brand of the tablet, ten tablets were weighed accurately, their average determined, and finely powdered. An appropriate quantity of each tablet powder was diluted with potassium bromide to get 1% w/w of acyclovir. The solid samples were mixed thoroughly by triturating. The analysis was performed three times.

RESULTS AND DISCUSSION

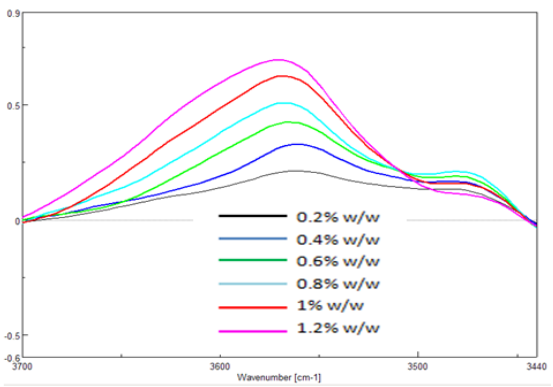
Nowadays, FTIR commonly is not used to quantitative analysis, because of its unspecific/unclear spectra showed. In this development method, the derivatization will sharpen the spectra, then increasing its specificity and sensitivity. The spectra which shows the best linear data between a series of concentration to their area under the curve (AUC) was a choice and used to be a test of its validity parameters, such as LOD, LOQ, accuration, and precision.

The calibration curve was made in a range of 0.2-1.2% w/w standard acyclovir at five different wavenumber, namely 3700-3440, 3012-2870, 1955-1680, 1176-1072, and 721-609 cm-1. Based on the result of linearity test listed in table 1, it was decided to use the peak at 3700-3440 cm-1 for validation and quantitative analysis of acyclovir, since it showed the best linearity than other wavenumbers. The peak at 3700-3440 cm-1 showed N-H scissoring of amide in acyclovir structure.

Table 1: Linearity test data at five different wavenumbers (n=3 for each measurement point)

Wavenumber (cm^{-1})	Regression equation	Correlation coefficient (r)
3700–3440	$y = 54.922x + 17.479$	0.9999
3012–2870	$y = 7.9131x + 6.5191$	0.7945
1955–1680	$y = 59.97x + 23.606$	0.9859
1176–1072	$y = 10.694x + 14.649$	0.7927
721–609	$y = 13.604x + 5.9508$	0.9342

The area under the curve (AUC) of the peak at 3700-3440 cm^{-1} was used to arrange the calibration curve as shown in fig. 2.



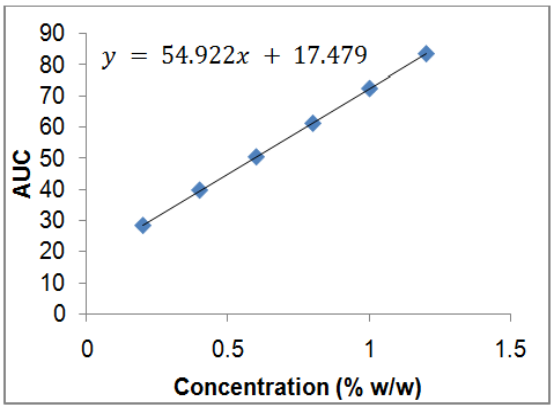
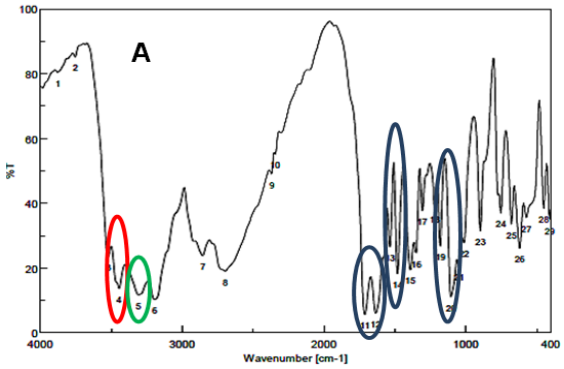


Fig. 2: Derivative spectra (A) and calibration curve at 3700-3440 cm^{-1}(B)-(n=3 for each measurement)

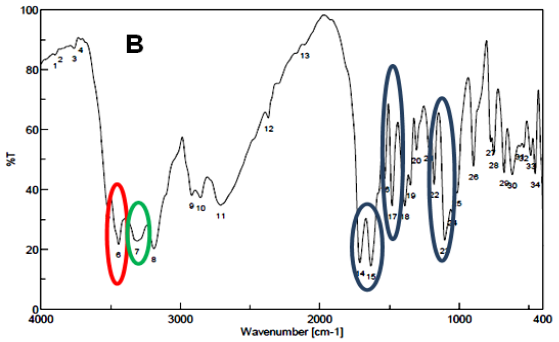
The calibration curve described by the equation y = bx+a, where y represents AUC and x represents the concentration of acyclovir (fig. 2B). The corresponding linear regression equation was y = 54.922x+17.479 and the correlation coefficient for calibration were 0.9999. The limit of detection and limit of quantification was obtained at 0.01 and 0.03 % w/w. These markedly demonstrate that FTIR had good sensitivity.

Fig. 3 shows FTIR spectras of standard acyclovir and tablet sample containing acyclovir as an active constituent, as follows:



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity
1	3876.22	80.4287	2	3752.8	85.1687
3	3521.38	23.5042	4	3444.24	13.6584
5	3309.25	11.6557	6	3193.54	10.1679
7	2854.13	23.7624	8	2696	18.9569
9	2368.16	49.0997	10	2345.02	55.0572
11	1712.48	5.53975	12	1635.34	5.96822
13	1585.66	26.4044	14	1481.06	18.1715
15	1392.35	19.4195	16	1349.93	24.6618
17	1307.5	37.6548	18	1218.79	38.2799
19	1180.22	26.6237	20	1103.08	11.0611
21	1049.09	20.5031	22	1014.27	27.7137
23	898.666	31.394	24	752.102	36.8964
25	678.82	33.5466	26	620.966	25.9913
27	574.683	35.5728	28	451.261	38.3883
29	408.835	34.8819			



Result of Peak Picking

No.	Position	Intensity	No.	Position	Intensity
1	3899.36	84.851	2	3860.79	86.7555
3	3760.51	87.0192	4	3714.23	89.9899
5	3521.38	34.213	6	3444.24	21.5574
7	3309.25	22.6557	8	3189.68	20.1393
9	2919.7	37.886	10	2854.13	37.1254
11	2711.42	34.6819	12	2368.16	63.7086
13	2109.74	88.241	14	1712.48	15.4814
15	1635.34	14.2609	16	1535.06	43.5513
17	1481.06	34.4012	18	1392.35	34.411
19	1349.93	41.3173	20	1307.5	52.9727
21	1218.79	53.9109	22	1180.22	41.536
23	1103.08	22.9287	24	1049.09	32.2164
25	1018.23	38.6775	26	898.666	47.6806
27	775.244	55.7402	28	752.102	51.5281
29	678.82	45.5117	30	620.966	44.8217
31	566.969	54.2449	32	539.971	53.9959
33	485.974	51.2717	34	455.118	45.0469

Fig. 3: Standard acyclovir (A) and sample infra-red spectra (B)

Fig. 3 shows FTIR spectra of standard acyclovir and tablet sample containing acyclovir as an active constituent. Acyclovir has some specifics wavenumbers, namely 1717, 1632, 1485, and 1104 cm^{-1} (blue circle). Besides, FTIR acyclovir spectra also showed OH scissoring of acyclovir structure (green circle). A prominent band in the range of 3700-3440 cm^{-1} was selected for the quantification (red circle). The purpose of the newly developed method was completely accomplished as there seems no major interference by the excipients present in tablet formulation as the spectra of tablet samples stretch over the surface acyclovir standard spectra indicating the negligible interference of the other sample matrix. These result showed that the method is specific.

As a comparison, in spectrophotometry, the drug is treated with a fixed amount of perchloric acid-crystal violet mixture and absorbance of the resultant violet colour is measured at 570 nm and is related to drug concentration shows the sensitivity of 1.78 × 10^4 L mol^{-1} cm^{-1} and 12.68 x 10^{-6} g. cm^{-2}, respectively. The limits of detection and quantification are calculated to be 1.696 and 5.654/g ml^{-1}, respectively. This method also was successfully applied to the determination of acyclovir in tablets. The validity of the methods was further ascertained by parallel determination by a reference method and by recovery studies via standard-addition technique [3].

Table 2 shows the validation analysis result of the parameter, repeatability and intermediate precision of acyclovir standard. Meanwhile, table 3 shows the accuracy, measured by FTIR.

Table 2: Repeatability and intermediate precision data (n=6)

Day	AUC	SD	RSD (%)	Horrat
1	72.04	0.0033	0.33	0.08
	72.09			
	72.07			
	72.18			
	72.46			
	72.41			
2	72.59	0.0027	0.27	0.07
	72.54			
	72.25			
	72.32			
	72.32			
	72.25			
3	72.65	0.0038	0.38	0.09
	72.66			
	72.44			
	72.28			
	72.67			
	72.19			

The precision was expressed by the coefficient of variation or relative standard deviation (RSD) and Horrat value. It was assessed by repeatability and intermediate precision at a concentration of 1% w/w. Repeatability assay gave RSD value 0.33% while the precision was evaluated from three different days of measurement and gave RSD value 0.38%. These results were within the acceptable variable limits. Based on the result of precision studies (table 2), the FTIR method had a good precision.

The accuracy of the FTIR method was evaluated by standard addition method at three different levels (80, 100, and 120% of the content at the label claimed) and the recovery data summarized in table 3. Good recoveries of acyclovir were obtained in the range of 99.98-101.21%.

Table 3: The recovery data of accuracy analysis (n=10)

Label claim (mg)	Amount of standard added (mg)	Total (mg)	AUC	Amount recovered (mg)	Recovery (%)*	Average of recovery (%)
400	320	720	72.51	725.08	101.59	101.21±0.0017
			72.75	722.68	100.84	
			72.76	723.89	101.22	
400	400	800	72.79	801.56	100.39	100.98±0.0026
			72.61	805.06	101.26	
			72.69	805.17	101.29	
400	480	880	72.21	876.99	99.37	99.98±0.0029
			72.48	881.28	100.27	
			72.49	881.52	100.32	

Based on all the results of validation test, this method can be applied to determine the content of the active compound in the solid dosage form such as capsule, tablet, powder, etc.

In this research, the proposed validated method was applied to quantify acyclovir content in tablet dosage form. As experimental samples, three different brands of acyclovir tablets were analyzed using the developed method, and the results summarized in table 4.

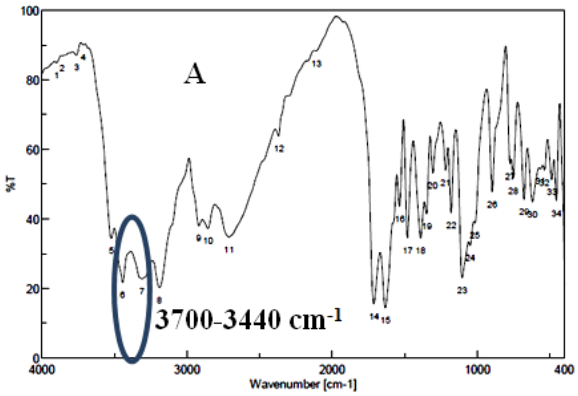
Table 4 shows the results of AUC measurement of tablet A, B, and C with each was 400 mg dosage claimed.

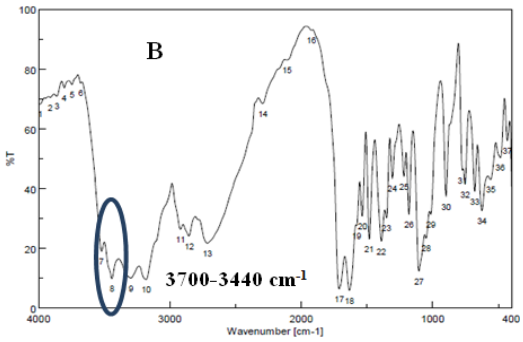
Table 4: Acyclovir tablets content determination results (n=3)

Tablet	Label claim (mg)	AUC	Amount recovered (mg)	Recovery (%)	RSD (%)
A	400	63.18	414.61	103.65	0.14
		63.23	415.09	103.77	
		63.10	413.97	103.49	
B	400	63.54	409.34	102.33	0.57
		63.48	408.77	102.19	
		63.06	405.06	101.27	
C	400	63.37	395.67	98.92	0.64
		63.88	400.09	100.02	
		63.37	395.66	98.92	

Furthermore, fig. 4 below, shows the infrared spectra of tablet A, B, and C, measured by FTIR.

The FTIR spectrum of tablet samples in fig. 4, indicates that there was no interference from excipients used in the formulation of tablet dosage form. Acyclovir tablet A, B, and C contained acyclovir 103.49-103.77; 101.27-102.33; and 98.92-100.02% respectively, with the RSD values, were within the range of 0.14-0.64. The recovery of label claim was in a good agreement and within the acceptable limits of the Indonesian Pharmacopoeia V, restricted not less than 90.0% and not more than 110.0% of the stated amount of active acyclovir compound.





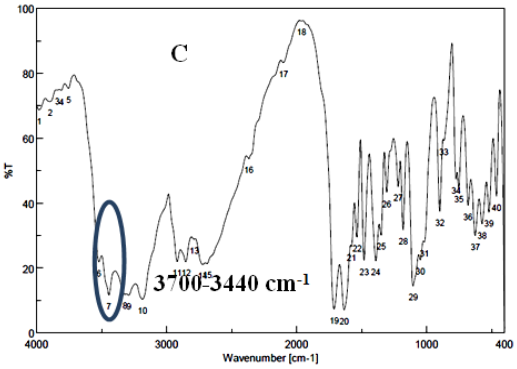


Fig. 4: FTIR spectra of sample A, B, and C

All of data shows that this method can be proposed as an alternative m ethod for compendia after an advance test, the further testing to assess the validity and feasibility with a robustness testing completely in several comparison laboratories. This method shows an easy sample treatment and shorter time compared to other procedures such as visible/ultraviolet spectroscopy, potentiometry, and HPLC. The other advantage is no organic solvent should be used, so it will be less costly besides this is in line with the green pharmacy issue.

CONCLUSION

FTIR spectrophotometry has been validated as a developed method for quantitative analysis acyclovir in a tablet. The method can be used to determine the acyclovir content explained in the Indonesian Pharmacopoeia 5-th edition limit to its tablet dosage form. All the results from this research shows that FTIR can be proposed as an alternative method for the content determination of acyclovir tablets.

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CONFLICTS OF INTERESTS

Declare none

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