Original Article

SIMULTANEOUS ASSAYS OF METFORMIN HCL AND GLIBENCLAMIDE MIXTURE USING TWO ANALYTICAL METHODS OF SPECTROPHOTOMETRY

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ABSTRACT

Objective: Area Under Curve method (AUC) and the Multiple Wavelength Spectrophotometric (MWS) method are practice and simple methods for simultaneous assays of Metformin HCl and Glibenclamide on the tablet dosage form.

Methods: The AUC method is measured for the absorption spectrum with a concentration 4 mg/l Metformin HCl by calculating the area spectrum at wavelength 230-240 nm and the absorption spectrum with a concentration 8.7 mg/l Glibenclamide by calculating the area at wavelength 225-235 nm. The MWS by determining the absorption spectrum and the five wavelength points for the absorption value at 225 nm, 229.4 nm, 236.6 nm, 233 nm, and 243 nm and calculated using matrix operations.

Results: The validation test of the AUC method for Metformin HCl obtained accuracy = 99.35%, linearity = 0.9881, precision = 0.39%, LOD = 0.4459 mg/l. LOQ = 1.4864 mg/l and for Glibenclamide obtained accuracy = 100.79%, linearity = 0.9993, precision = 0.65%, LOD=0.4372 mg/l, LOQ = 1.4072 mg/l and the MWS method for Metformin HCl obtained accuracy = 100.76%, linearity 0.9949, precision = 0.65%, LOD = 0.9103 mg/l, LOQ = 3.0431 mg/l, and for Glibenclamide with accuracy = 100.07%, linearity = 0.9993. precision = 0.36%, LOD = 0.9205 mg/l. LOQ = 3.0431 mg/l , and appropriate the requirements of ICH guidelines.

Conclusion: These Methods successively applied to determine of Metformin HCl and Glibenclamide mixture in tablet dosage form and fulfill the validation requirements.

Keywords: AUC, Glibenclamide, Metformin HCl, MWS, Spectrophotometry methods, Validation

INTRODUCTION

The combination of Metformin HCl (MFM) and Glibenclamide (GBC) is widely used as an antidiabetic drug. MFM is a hypoglycemic group for reduces glucose production in the liver and increases muscle tissue sensitivity and adipose to insulin. The GBC is a hypoglycemic drug and a group of Sulfonylureas and works to stimulate insulin secretion from pancreatic Langerhans β cell granules. The combination of MFM and GBC will make absorption ability of GBC is increased because the stimulation of insulin secretion by GBC in response to a meal is essential [1, 2].

Determination of MFM and GBC levels in a single form can be carried out by the ultraviolet spectrophotometry method, MFM has maximum absorption at 236 nm and 229 nm for GBC and also can be determined by HPLC methods [3, 4]. The method of simultaneous determination of MFM and GBC with the HPLC method was published in the article using the mobile phase consisting of a mixture of 20 mmol ammonium buffer format at pH 3.5 and acetonitrile, with a ratio of 45:55 v/v. [5].

Various studies on the development of Spectrophotometry methods have been published as examples: UV spectrophotometry methods for simultaneous estimation, Double Divisor Spectra derivative spectrophotometry ratio, and MCR method [6-9].

Some researchers have made a simultaneous determination of MFM and GBC mixture of development of spectrophotometry methods such as a derivative spectrophotometry method, simultaneous equation. The discovery of new course spectrophotometry methods has been validated and appropriate requirements of ICH guidelines and can leave the classic spectrophotometry method for multi-component analysis of drug [10-13].

The AUC method is a simple, economical, and relatively faster way that has also been developed. Research conducted by Ilango and Kumar (2012) for the determination of Medoxomil and Hydrochlorothiazide levels, and Karajgi's research, *et al.* (2016) for simultaneous determination of Artemether and Lumefantrine levels

in drug preparations provided accurate, precise and selective results. The Area Under Curve method in spectrophotometry was carried out and offered exact, and particular [14-17].

The MWS methods are a spectrophotometry method for the simultaneous determination of the drug mixture by determining the absorption point at various wavelengths whose absorption curves have overlapped and the level calculations performed by a matrix operation [18].

The AUC method is a spectrophotometry method which simultaneously determines MFM and GBC overlapping absorption curves between MFM and GBC, whose absorption measurements are not at maximum absorption, but used in areas that have no maximum spectrum curve but which are carried out in a parabolic spectrum area. This method has a value at two selected wavelengths, λ1 and λ2. The obtained AUC value is determined by the levels of each drug in the regression equation [14-17]. The MWS spectrophotometry method has a procedure to determine the five points on the overlapped absorption curves of the MFM and GBC are used together, then the absorption is determined and calculated by the matrix equation [18].

The aims of this research are to validation and simultaneous assays of AUC and MWS methods for simultaneous determination of MFM and GBC.

MATERIALS AND METHODS

Instrumentation and methods

The U. V. Spectrophotometer (Shimadzu 1800) which combined with a set of Personal Computers (P. C. s) equipped with 2.42 UV-Probe software and software with the Minitab®17 application. 99.8 % Metformin HCl, 99.4% Glibenclamide from Kimia Farma Plant Medan, Sumatera Utara, Indonesia, 100.02% Metformin HCl and 99.98% Glibenclamide (Indonesian Standard) Methanol absolute and 25% NH4OH, E. Merck, German from Rudang Medan Indonesia. Local Product of Metformin and Glibenclamide by Sanbe, Bandung, Indonesia.

Preparation of MFM and GBC working solutions

The working solutions are prepared by dissolving 50.3 mg MFM and 50.4 mg GBC powder, transferred to 50 ml volumetric flasks respectively and dissolved with methanol absolute, then taken 5 ml of MFM and 10 ml of GBC accurately and transferred to 50 ml volumetric flasks respectively, then disappeared with methanol pure to get 50.3 mg/l MFM and 100.8 mg/l GBC, and both of them used as working solutions.

Preparation absorption spectrum

The working solution is taken 0.8 ml of MFM and 0.9 ml of GBC respectively, and then it's measured at the wavelength of 200-400 nm.

The validation test

The validation of these methods was performed based on the U. S. Food and Drug Administration (FDA) guidelines for precision, accuracy, linearity, the limit of detection (LOD), Limit of Quantitation (LOQ), linearity and coefficient of variation are validation parameter for spectrophotometry methods [19-21].

Accuracy

The accuracy method was determined using the standard addition method by preparing a sample with three specific ranges of 80%, 100%, and 120%, with six repetitions. The formula for accuracy:

$$\% \text{ recovery} = \frac{CF - CA}{AC*} \times 100\%$$

Note:

CF = Concentration after addition

CA = Theoretical concentration before addition

AC = Concentration of the added standard substances

Precision

The calculation of the relative standard deviation (RSD) value is used to determine the precision in the validation method. RSD is a formula as follows:

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

Note:

RSD = Relative standard deviation

SD = Standard deviation

X = Average data.

Linearity

The linearity of the analytical method in the validation method was determined by calculating the correlation coefficient [19-21].

The regression equation is a formula as follows:

$Y = ax+b$

Notes:

Y = Absorption

a = Slope

x = Concentration (mg/l^{-1})

b = Constant.

Limits of detection (LOD) and limits of quantitative (LOQ)

According to the absorbance at the analytical wavelength, the LOD and LOQ calculations are performed [19-21].

$$SD = \sqrt{\frac{\sum y - y_1}{n - 2}}$$

$$LOD = \frac{3,3 \times SD}{\text{slope}}$$

$$LOQ = \frac{10 \times SD}{\text{slope}}$$

Note:

SD = Standard deviation

Slope = a ($y = ax+b$)

Area under curve method (AUC)

Construction of absorption spectrum of MFM and GBC

The working solution is carefully taken at 4.0 mg/l MFM and 8.7 mg/l GBC concentrations and measured their absorption spectrum respectively and used a U. V. probe 2.42 software to calculate the area values in the area range 230-240 nm for MFM and 225-235 nm areas for GBC. The Concentration of Metformin and Glibenclamide can be determined as follows [14-17]:

$$C^{MFM} = \frac{X_{\lambda_1-\lambda_2}^{GBC} AUC_{\lambda_3-\lambda_4} - X_{\lambda_3-\lambda_4}^{GBC} AUC_{\lambda_1-\lambda_2}}{X_{\lambda_3-\lambda_4}^{GBC} X_{\lambda_1-\lambda_2}^{MFM} - X_{\lambda_3-\lambda_4}^{GBC} X_{\lambda_1-\lambda_2}^{MFM}}$$

$$C^{GBC} = \frac{X_{\lambda_1-\lambda_2}^{MFM} AUC_{\lambda_3-\lambda_4} - X_{\lambda_3-\lambda_4}^{GBC} AUC_{\lambda_1-\lambda_2}}{X_{\lambda_1-\lambda_2}^{MFM} X_{\lambda_3-\lambda_4}^{GBC} - X_{\lambda_3-\lambda_4}^{MFM} X_{\lambda_1-\lambda_2}^{GBC}}$$

Note:

C = Concentration

$X_{\lambda_1-\lambda_2}$ = AUC $_{\lambda_1-\lambda_2}$/Concentration in mg/l^{-1}

$X_{\lambda_3-\lambda_4}$ = AUC $_{\lambda_3-\lambda_4}$/Concentration in mg/l^{-1}

Construction of calibration curve for the AUC method

The calibration curve can determine by the value of the area in the curve between two selected wavelengths such as λ1 and λ2 (AUC value), The AUC value of each spectrum in various concentrations is calculated to get the analytical wavelength, then calculated and plotted with varying concentrations to obtain the regression line equation using U. V. Probe 2.42 software [14, 16]:

$$Y = aX + b$$

Note:

Y = AUC value

a = intercept

b = regression coefficient

Multiple wavelength spectrophotometry methods

Construction of spectrum selection point

The working solution is construction to 4.0 mg/l^{-1} MFM and 8.7 mg/l^{-1} GBC concentrations, and these solution's absorption measured at 200-400 nm wavelength. Furthermore, the absorption spectrum overlapped Then searches five points as the wavelength to use. Wavelength selection is taken from the absorption spectrum of components, the absorption measured at predetermined multiple wavelengths. Both compound's absorption is determined using the matrix equation method, which operated in the concentration and absorption data of each compound at each wavelength measurement

Determination of MFM and GBC absorption curves

Carefully weighed 4.0 mg MFM (Indonesian Standard) and 8.7 mg of GBC (Indonesian Standard) and then transfer into the 10.0 ml flask, diluted with methanol to obtain a solution with a concentration of 4.0 mg/l^{-1} MFM and of 8.7 mg/l^{-1} GBC, then the two solutions are measured for each at a wavelength of 200-400 nm. Furthermore, the two curves are overlapped and determined the five points.

Assay procedures for a tablet by AUC and MWS

Twenty tablets were weighed and finely powdered. The powder obtained was sufficiently considered in such a way to contain 50 mg of MFM and transferred to a 50 ml volumetric flask, dissolved with methanol. Then, pipette 0.1 ml sample solution and added 4.3 ml (433.44 µg) of GBC working solution for standard addition.

The measured absorption spectrum is created in the area of 230-240 nm for MFM and 235–245 nm for GBC at the AUC method. Level calculations are done using the regression equation [14-17].

The MWS methods are the methods for measurement of MFM and GBC mixture based on five-point absorption from the overlapping curve of raw material. The content of each component in the mix is calculated based on the absorption capacity of drug samples (Ac) and the absorption value and obtained from measurements of predetermined wavelengths, and component levels obtained using a matrix equation [18].

$$[c] = [[a] \, x \, [a1]]^{-1} \, x \, [a] \, x \, Ac$$

Note:

[c]	= components concentration of mixture
[a1]	= transpose absorption matrix of mixed compounds
{a}	= matrix of the compound mixture absorption
$[[a] \, x \, [a1]]^{-1}$	= matrix inverses multiplied by the transpose absorption matrix of compound mixture
[Ac]	= matrix of sample absorption value

RESULTS AND DISCUSSION

The principle of development method on U. V. spectro-photometry

The Principle of the method of molecular absorption in U. V. spectrophotometry has been widely used for the determination of drug components in pharmaceutical preparations. This method can be used for pharmaceutical analysis, which has a spectrum matrix constraint of the active drug, overlapped and making it difficult as the simultaneous determination of the drug mixture [3-4].

Absorption spectrum of MFM and GBC

Determination of maximum absorption spectrum of Metformin and Glibenclamide was making in the wavelength range of 200-400 nm. The results obtained that the qualitative absorption spectrum of 4.1 mg/l[-1] Metformin at the 235.6 nm (fig. 1) and 8.7 mg/l[-1] Glibenclamide at 228.2 nm (fig. 2).

The maximum absorption of MFM and GBC at 235.6 nm and 228.2 nm is qualified because that Moffat (2011) wrote that the maximum wavelength of MFM is located at 236 nm, GBC was located at 225 nm as methanol is a solvent. Based on Indonesian Pharmacopeia that the difference in the maximum wavelength of each MFM and GBC was no less and no more than 5 nm [3, 4]. It means that the maximum wavelength of each component is qualified.

Validation methods

The result from the production of validation test must be achieved for chemical analysis activities. The validation test result is described as linearity, LOD, LOQ, R[2.] accuracy, and precision (table 1).


No.	Wavelength nm.	Absorbance
1	235,60	0,436
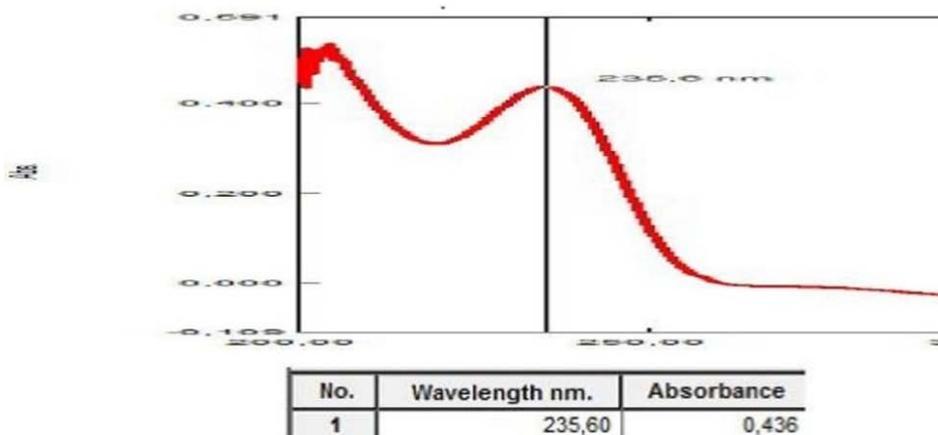
Fig. 1: MFM absorption spectra

No.	Wavelength nm.	Absorbance
1	228,20	0,449
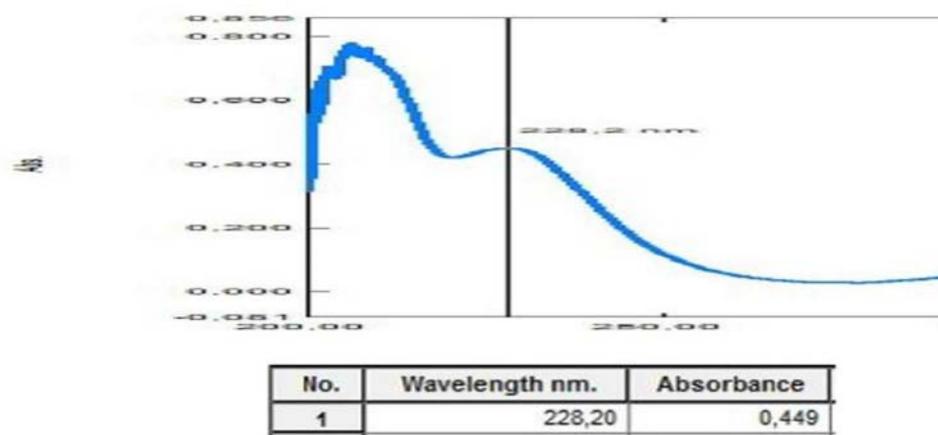
Fig. 2: GBC absorption spectra

Table 1: Validation of the AUC method and MWS method

Parameter	MFM		GBC	
	AUC	MWS	AUC	MWS
Solvent	Methanol		Methanol	
Linearity (%)	0.9881	0.9993	0.9949	0.9987
LOD (mg/l)	0.4459	0.4372	0.9103	0.9205
LOQ (mg/l)	1.4864	1.4072	3.0344	3.0431
R²	0.9881	0.9993	0.9946	0.9993
Accuracy(%)	99.35	103.16	100.76.	100.07
Precision (%)	0.39	0.26%	0.65	0.

It can be stated that the AUC and MWS method have excellent accuracy and precision, besides that all carried out measurements are in the 0.2–0.6 absorption area which means that the sample measurements are also above the detection limit and the quantitation limit because all parameters of validation test have according to the validation requirements of ICH 2015. It means that both of these methods have appropriated the validation requirements [15-17].

Result of AUC methods and MWS methods for sample

The spectrum absorption curve of sample for the market product

The curve of the spectrum absorption from a mixed sample of 250 mg Metformin HCl and 1.25 mg Glibenclamide on market products can be seen in the fig. 3 below.


Wavelength
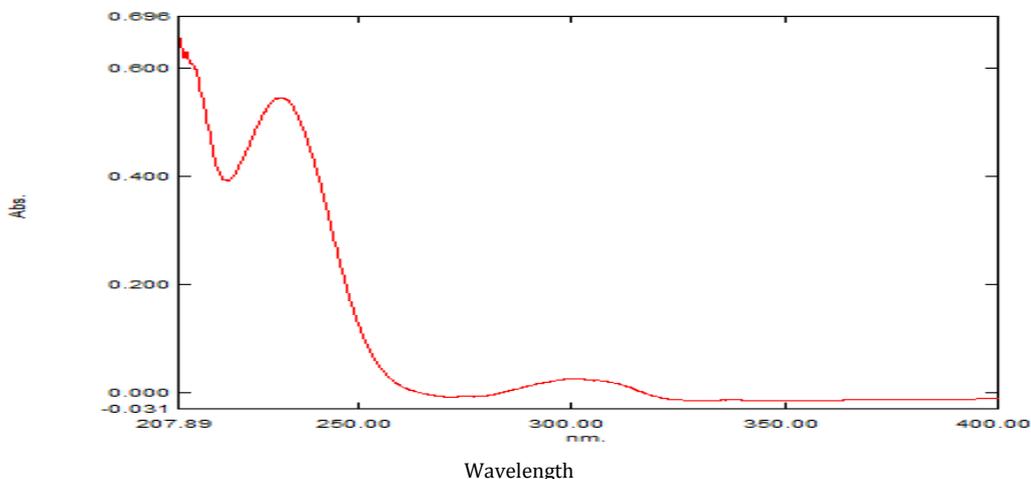
Fig. 3: The curve absorption spectrum of sample from market products

Based on the fig. 3 above, it can be seen that the concentration of GBC in the mixture is minimal. Based on the validation method for LOQ results of the AUC method has obtained LOQ = 3.0344 mg/l While the MWS method is LOQ = 3.0431 mg/l and it is true that content of GBC in tablets sample containing 50 mg of MFM, It's small below LOQ which is 0.2499 mg. According to the law of lambert beer, this naturally produces in a low GBC concentration, namely 0.0199 mg/l while the GBC concentration is according to the level of MFM. So the GBC working solution was added 8.6801 mg/l which total was added 433.44 µg in 50

ml sample solution. So, using the AUC and MWS methods, the content of glowing levels in the sample can be known.

Overlapped absorption spectrum for AUC method

The AUC method begins by calculating the AUC value in the spectrum in the wavelength range of 227 nm-248 nm with concentrations of (2. 3. 4. 5. 6) mg/l for MFM and wavelength range 225 nm-236 nm with a level of (4.7. 6.7. 8.7. 10.7. 12.7) mg/l for GBC the range of λ (wavelength) selected to give the best linearity relationship between the AUC and Concentration (fig. 4) as indicated by the value of the correlation coefficient (r ≤ 1)and can be seen at table 2 [15, 16].

Table 2: Wavelength of analysis for metformin and glibenclamide

Component	Wavelength (nm)	Δλ	Linierity
Metformin	G227-231	5	0.9688
	234-238	5	0.9797
	230-240	10	0.9881
	231-241	10	0.9880
Glibenclamide	227-231	5	0.9757
	228-232	5	0.9934
	225-235	10	0.9949
	226-236	10	0.9934

Based on table 2 shows that the excellent wavelength range is (230-240) nm for GBC have a good the value of the correlation coefficient, so the selected wavelengths to determine MFM and GBC by

spectrophotometry UV with AUC method were 230-240 nm and 225-235 nm, respectively. It concludes that there is no overlapping AUC of each wavelength ranges both for MFM (230-240) nm and

GBC (225-235) nm and affecting one to another. The absorption value that appropriates the requirements is obtained with the wavelength range. That's mean AUC method can be used to determine simultaneously the drug content, which consisting of more than one component [15, 17].

The determination of the AUC in the tablet market production is carried wavelength (230-240) nm for MFM and (225-235) nm for GBC with calculations based on the following formula:

$$C^{MFM} = \frac{X_{\lambda1-\lambda2}^{GBC}AUC_{\lambda3-\lambda4} - X_{\lambda3-\lambda4}^{GBC}AUC_{\lambda1-\lambda2}}{X_{\lambda3-\lambda4}^{GBC}X_{\lambda1-\lambda2}^{MFM} - X_{\lambda3-\lambda4}^{GBC}X_{\lambda1-\lambda2}^{MFM}}$$

$$C^{GBC} = \frac{X_{\lambda1-\lambda2}^{MFM}AUC_{\lambda3-\lambda4} - X_{\lambda3-\lambda4}^{MFM}AUC_{\lambda1-\lambda2}}{X_{\lambda1-\lambda2}^{MFM}X_{\lambda3-\lambda4}^{GBC} - X_{\lambda3-\lambda4}^{MFM}X_{\lambda1-\lambda2}^{GBC}}$$

Note:

C = Concentration

$X_{\lambda1-\lambda2}$ = AUC $_{\lambda1-\lambda2}$/Concentration in mg/l^{-1}

$X_{\lambda3-\lambda4}$ = AUC $_{\lambda3-\lambda4}$/Concentration in mg/l^{-1}

Based on the calculation above, the AUC values are obtained, which result in table 3 for MFM and table 4 for GBC below.

Table 3: AUC value of MFM

No.	Concentration (µg/ml)	AUC (Y)
1	0.0000	0.0000
2	2.0	0.1840
3	3.0	0.2880
4	4.0	0.3130
5	5.0	0.3760
6	6.0	0.4680

Table 4: AUC value of GBC

No.	Concentration (µg/ml)	AUC (Y)
1	0.0000	0.0000
2	4.7	0.1170
3	6.7	0.1850
4	8.7	0.2430
5	10.7	0.2930
6	12.7	0.3860

Based on the AUC value in table 3 and table 4. then each regression equation can be calculated whose results can be shown in table 5 for MFM and table 6 for GBC.

Table 5: Calculation of regression equations on MFM

No.	X	Y	XY	X²`	Y²
1.	0.0000	0.00000	0.00000	0.00000	0.0000000000
2.	2.0	0.1840	0.368	4	0.033856
3.	3.0	0.2880	0.864	9	0.082944
4.	4.0	0.3130	1.252	16	0.097969
5.	5.0	0.3760	1.88	25	0.141376
6.	6.0	0.4680	2.808	36	0.219024
	ΣX = 20	ΣY = 1.629	ΣXY = 1.1953	ΣX² = 90	Σ Y² = 0.575169
	$\overline{X}$ = 3.3333	$\overline{Y}$ = 0.2715			

Table 6: Calculation of regression equations on GBG

No.	X	Y	XY	X²`	Y²
1.	0.0000	0.00000	0.00000	0.00000	0.0000000000
2.	4.7	0.1170	0.5499	22.0900	0.013689
3.	6.7	0.1850	1.2395	44.8900	0.034225
4.	8.7	0.2430	2.1141	75.6900	0.059049
5.	10.7	0.2930	3.1351	114.4900	0.085849
6.	12.7	0.3860	4.9022	161.2900	0.148996
	ΣX = 43.5	ΣY = 1.224	ΣXY = 11.9407	ΣX² = 418.45	Σ Y² = 0.341808
	$\overline{X}$ = 7.25	$\overline{Y}$ = 0.204			

Baesd on table 5 and table 6 can be calculated the regression equation by formula (19)

$$Y = aX + b$$

Note:

Y = AUC value

a = intercept

b= regression coefficient

Based on calculations from the above formula, the regression equation for MFM and GBC is obtained y = 0.074 x+0.022 and y = 0.029 x-0.011. The calculation of the sample is carried out with replications six times followed by statistical calculations so that the average level is obtained in table 7, table 8 and table 9.

Table 7: The metformin HCl levels on the market product samples

No	Weight of MFM in the sample (mg)	Weight of the study results (mg)	Percentage of measure result (%)
1	50.06	51.12	101.37%
2	50.09	52.00	103.05%
3	50.11	52.12	103.24%
4	50.12	52.25	103.47%
5	50.14	52.75	104.40%
6	50.15	52.62	104.10%

Table 8: The glibenclamide levels on the market product samples

No	Weight of GBC in the sample (mg)	Addition weights of raw GBC to the sample (mg)	Weight of the study results (mg)	Percentage of measure result (%)
1	0.2498	108.50	108.75	99.04%
2	0.2505	108.62	108.87	97.06%
3	0.2507	108.50	109.00	96.11%
4	0.2511	108.50	108.75	98.06%
5	0.2515	108.62	108.87	99.39%
6	0.2526	108.62	108.87	98.46%

Table 9: Statistical calculation of MFM and GBC on the market product

No	Component of drug	Statistical calculation	Content in etiquette	Level requirements
1	MFM	(103.27±1.7521)%	250 mg	(95–105)%
2	GBC	(98.02±2.0404)%	1.25 mg	(95-105)%

Based on table 9 above, after statistical calculation of MFM and GBC in the market production mixture, the levels were (103.27±1.7521)% for MFM and (98.02±2.0404)% for GBC to meet the requirements for tablet dosage according to Indonesian Pharmacopoeia Edition V is (95-105)%. It means that the AUC method can be used to simultaneous determine the mixture of MFM and GBC in a tablet.

Result of five absorption points for MWS methods

The simultaneous determination of MFM and GBC is combined of two spectra, to make a five of wavelength points (for analysis of MFM and GBC mixture). The five used to measure the absorption of each standard solution MFM and GBC was obtained (fig. 4).
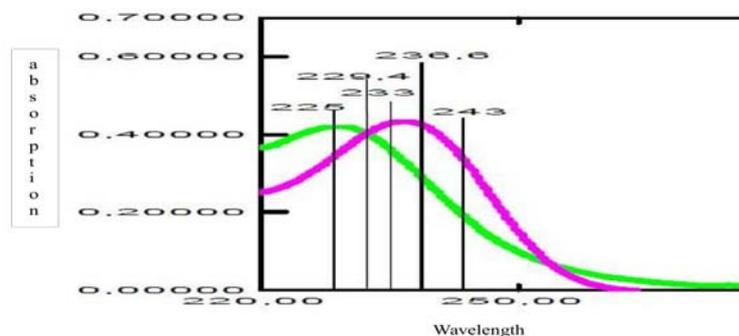

Fig. 4: Five-wavelength analysis used in the multiple wavelengths method

After measuring the absorption of each solution by various concentrations at the five wavelengths are obtained that five wavelengths used is 225 nm, at a wavelength of MFM and GBC have begun to provide absorption. The wavelength of 229.4 is the maximum absorption GBC, and wavelength of 233 nm is the point of intersection of MFM and GBC, the wavelength of 236.6 nm is the maximum wavelength of MFM, and a wavelength of 243 nm, either MFM or GBC absorption begins to decrease. The obtained absorption data is used to establish the levels of MFM and GBC in the mixture with matrix calculation.

Results of determination of absorption at five wavelengths

Absorption value is the ability of the absorption of a compound to absorb from a mixture of compounds at the selected wavelength. The determination of the absorption value is done by measuring the absorption of each solution of MFM and GBC at a wavelength of 225 nm; 229.4 nm; 233 nm; 236.6 nm; and 243 nm. The measurements result of MFM and GBC uptake can be seen in table 10.

The selection of absorption values (a) can be determined based on the calculated r-value. The estimated r-value is compared with the r-value of the table with a confidence level of 95% with df four, which is 0.8114. Based on table 10 and table 11 above, the value of r count metformin and glibenclamide are higher than the value of r table. It means that the equation has excellent linearity because the value of r count is ≤1.

Simultaneous determination results of MFM and GBC with the MWS method

The market preparations contain MFM 250 mg and GBC 1.25 mg. With a ratio of 1: 200 so that the GBC level is challenging to analyze. To overcome this, add the GBC working solution so that measurements can be made with six repetitions, with the aim that the data obtained is more accurate. Then the absorption is measured at the five wavelengths of 225 nm; 229.4 nm; 233 nm; 236.6 nm and 243 nm. The absorption data of the mixed samples of MFM and GBC samples were obtained, then carried out by calculating the matrix so that the level and percentage of the coefficient of variation can be seen in table 12 below.

Table 10: Result of absorption value of MFM

Concentration (µg/ml)	Wavelength 225 nm	Wavelength 229.4 nm	Wavelength 233 nm	Wavelength 236,6 nm	Wavelength 243 nm
0.0	0.0000	0.0000	0.0000	0.00000	0.0000
2	0.1268	0.1834	0.2278	0.24738	0.1936
3	0.1904	0.2600	0.3140	0.34029	0.2742
4	0.2118	0.3167	0.4017	0.44580	0.3586\
5	0.2788	0.4054	0.5092	0.56339	0.4579
6	0.3338	0.4877	0.6142	0.67867	0.5494
	a= 0.062	a= 0.084	a= 0.101	a= 0.110	a=0.075
	r= 0.9995	r= 0.9981	r= 0.9990	r= 0.9993	r= 0.9996

Table 11: Result of absorption value of GBC

C (µg/ml)	Wavelength 225 nm	Wavelength 229.4 nm	Wavelength 233 nm	Wavelength 236,6 nm	Wavelength 243 nm
0.0	0.0000	0.0000	0.0000	0.0000	0.0000
4.7	0.2385	0.2448	0.2290	0.1927	0.1199
6,7	0.3290	0.3420	0.3220	0.2713	0.1661
8.7	0.4416	0.4483	0.4180	0.3521	0.2207
10.7	0.5146	0.5351	0.5036	0.4259	0.2664
12,7	0.6384	0.6669	0.6281	0.5291	0.3219
	a= 0.052	a= 0.051	a= 0.041	a= 0.048	a= 0.020
	r= 0.9991	r= 0.9992	r= 0.9992	r= 0.9993	r= 0.9997

Table 12: Result of matrix calculation of MFM and GBC by MWS methods

No.	MFM The concentra-tion of matrix calculations (µg/ml)	The concentra-tion of weighing results (µg/ml)	Percentage of Level (%)	GBC The concentra-tion of matrix calculations (µg/ml)	The concentra-tion of weighing results (µg/ml)	Percentage of level (%)
1	4.07517	4.0054	100.99	8.70025	0.02	100.24
2	4.22861	4.0183	104.46	8.70033	0.02	100.63
3	4.23732	4.0247	104.51	8.70097	0.02	103.84
4	4.24082	4.0248	104.59	8.70018	0.02	99.90
5	4.04323	4.0377	99.40	8.69972	0.02	97.61
6	4.22542	3.9924	105.06	8.66894	0.02	98.24
	Average		103.16	Average		100.07
	Coefficient of variation (%)		0.2628	Coefficient of variation (%)		0.3641

Based on table 12 above, that MFM and GBC levels in market products meet the requirements according to Indonesian Pharmacopoeia Edition V (2014), namely for tablet preparations containing MFM and GBC not less than 90% and no more than 110% of the amount indicated on etiquette. It means that the MWS method can be used for simultaneous determination of MFM and GBC.

The result of the application of AUC and MWS methods for MFM and GBC

The result of simultaneous determination from the MWS method and the AUC method for MFM and GBC levels has been calculated (table 2).

Table 2: Statistic calculation of MFM and GBC in mixture formula

Component	AUC		MWS	
	MFM	GBC	MFM	GBC
Self-made Product (%)	100.38±0.97	100.23±1.52	100.02±0.29	98.28±1.78
Local Product (%)	103.27	98.02	103.16	100.07
Coefficient Variation (%)	1.75	2.04	0.26	0.36
Range of Level (%)	90-110	90-110	90-110	90-110

The result of the statistical calculation of this method was not significant, although with a different work procedure. Both ways have given MFM and GBC levels in laboratory self-made and local products, and it's appropriate the requirements according to Indonesian Pharmacopoeia edition V (2014). It means that AUC methods and MWS methods have good accuracy because they are in the range of 90%-110% and also have excellent precision because the percentage of coefficient variation of MFM and GBC are<2% [15-17].

recovery and accuracy makes them applicable in quality control laboratories and were validated according to ICH guidelines for simultaneous assays of Metformin and Glibenclamide in laboratory self-made and local Product.

Therefore the Area Under Curve method and Multiple Wavelength methods have successfully applied the simultaneous determination of Metformin and Glibenclamide on the pharmaceutical formulation.

CONCLUSION

The Area Under Curve method and Multiple Wavelength method can simultaneous assays of Metformin and Glibenclamide mixture in methanol solvent with overlapping spectra, and have a functional

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CONFLICT OF INTERESTS

I declare that there is no conflict of interest

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

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