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SIMULTANEOUS ASSAY OF METFORMIN AND GLIBENCLAMIDE IN COMBINED DOSAGE FORM BY MEAN CENTERED OF SPECTRA RATIO METHODS

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ABSTRACT

The Mean Centered of Spectra Ratio (MCSR) method is a practical and straight forward method to the determination of Metformin and Glibenclamide in tablet preparations using mathematical software models. The MCSR method has manipulated an absorption to amplitude and change the wavelength of Metformin from 235.6 nm to 244.4 nm and Glibenclamide from 228.2 nm to 210.3 nm. The method had met the requirements of method validation following ICH 2005. The statistical results showed that the levels were in accordance with the requirements of tablet preparations according to Indonesian Pharmacopoeia Edition V. The MCSR application had met the validation requirements for Metformin and Glibenclamide levels in tablet preparations.

Keywords: Metformin, Glibenclamide, MCSR, Spectrophotometry, Validation

INTRODUCTION

A retained glucose tolerance and an obstruction of fasting glucose tolerance is a clinical manifestation, that causes a person at risk of developing type 2 diabetes mellitus[1]. Metformin and Glibenclamide are the right combinations for patients with type 2 diabetes mellitus as Metformin increases glucose uptake and decreases insulin resistance while working Glibenclamide stimulates insulin secretion granules β Langerhans cells of the pancreas.[2-3] Assays of Metformin and Glibenclamide in a single form was determined with maximum absorption at wavelengths of 236 nm for Metformin and 229 nm for Glibenclamide by the ultraviolet spectrophotometry method and an HPLC methods.[4-5] Various studies have been published for quantitative determination methods for simultaneous assays of drug mixtures have been developed by researchers using the HPLC method[6], RP-HPLC[7], matric methods[8], intersection absorption spectrum method[9], UV-visible spectrophotometry.[10-11]

The combinations of Metformin and Glibenclamide preparations have adjacent absorption and interfere with each other simultaneously assay with the classic spectrophotometry method. Therefore, it is necessary for one simultaneously assay without separation by spectrophotometry methods.

Nowadays, methods of spectrophotometry which simultaneously assays a mixture of compounds without a separation have been developed using a combination of mathematics and computers, and one of the methods is Mean Centering Ratio Spectra (MCR).[12-13] Afkhami (2005) principally in the assay mixture of one of the compounds are used as a dividing factor or a divisor in determining the ratio of spectra.[12]

Several studies have been published for determination of the mixture of acetaminophen and ibuprofen[14], chlorphenoxamine hydrochloride and caffeine[15], hydrochlorothiazide and benazepril hydrochloride[16], hydrochlorothiazide mixture with candesartan in tablet preparation with the Mean Centering of Ratio Spectra (MCR) method.[17]

The goal of the study was to validate and simultaneous assays of Metformin and Glibenclamide mixture in a pharmaceutical form with the MCSR method.

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EXPERIMENTAL

Material and Methods

The spectrophotometry analysis for Metformin and Glibenclamide by Shimadzu model 1800 double beam UV-Visible spectrophotometer. Software used in the study was Spectra spontaneously accessed by UV-Probe system software and MATLAB version 9.0. Spectrophotometry Conditions include spectrum mode, high speed, area measurements Wavelength 200-400 nm with a scale of absorbance 0.002 A -2.00 A and determination of correction at the baseline using ethanol.

Material

Raw materials were Metformin (Dexa Medica), Glibenclamide (Indofarma), local tablet products, and methanol pro analysis.

General Procedure

MCSR method or mean centering ratio spectra[12-13] are the methods for simultaneous assay the combination of Metformin and Glibenclamide with methanol solution, first performed the absorption of every single substance in the maximum wavelength of 235.6 nm to 4 µg. ml^{-1} Metformin and 228.2 nm to 8.7 µg. ml^{-1} Glibenclamide. Then conducted by construction spectrum ratio use of software Probe 2:42 and construction of MCSR with MATLAB software to obtain the value of MC (amplitude) and the wavelength of 4 µg.ml^{-1} Metformin are obtained at 244 nm while 8.7 µg. ml^{-1} Glibenclamide at 211 nm. Tablet sample preparation was carried out by adding Glibenclamide as a standard addition. This was done to match the ratio with Metformin levels in measurement. So when construction Glibenclamide as a dividing factor, the absorption concentration used was in accordance with the Lambert-Beer law that measured good samples, namely absorption in area 0.2-0.6.[12-13]

The Preparation of Working Solutions

Accurately weighed raw materials of 50.3 mg Metformin and moved to separate 50 ml volumetric flasks and dissolved in methanol solvent. The Metformin solution was pipetted 5 ml, and moved with it to 100 ml volumetric flask and added methanol solvent to the mark in order to make 50.3 µg. ml^{-1} of the Metformin working solution. Accurately weighed raw materials of 50.4 mg Glibenclamide. And moved to separate 50 mL volumetric flasks and dissolved in methanol solvent. The Glibenclamide solution was pipetted 2.5 ml, and moved to 25 mL volumetric flask, and added methanol solvent to the mark line in order to make 100.8 µg.ml^{-1} Glibenclamide working solution[5.18].

The Construction Absorption Spectrum Of Metformin And Glibenclamide

Pipetted a working solution of 0.75 ml Metformin and 0.87 ml Glibenclamide. Each working solution is moved to the 10 mL volumetric flask, and added with methanol solvent to the markline, and obtained a working solution of 4 µg.ml^{-1} Metformin and 8.7 µg.ml^{-1} Glibenclamide. Then each solution is measured the absorbance in a wavelength range of 200-400 nm.[4-5,18]

The Construction of Spectrum Absorption of Raw Material Combination of Metformin And Glibenclamide.

Carefully weighed each 10 mg of Metformin and 10 mg of Glibenclamide, respectively. Transferred into the 10 mL volumetric flask, diluted with methanol and stirred until dissolved, then pipetted 0.75 ml of Metformin solution and 0.87 ml of Glibenclamide. Mixed into 10 mL volumetric flask and added with methanol. Pipetted 1 ml of the mixed solution and moved to into 10 mL volumetric flask. Then the solution is measured a absorption in the wavelength range of 200-400 nm[12-13].

The Preparation Absorption Spectrum Ratio of Metformin and Glibenclamide and It's Combination.

The Metformin absorption spectrum at various concentrations was measured at a wavelength of 235.8 nm with 8.7 µg.ml^{-1} Glibenclamide as a divisor. The Glibenclamide absorption spectrum at various concentrations was measured at a wavelength of 228.2 nm with 4 µg.ml^{-1} Metformin as a divisor. The

spectral absorption was manipulated with the type of data sets, then been operating appropriations a spectrum of combinations with the spectral of Glibenclamide and Metformin[13]. They are followed by the measurement of an absorption spectrum of Metformin and Glibenclamide combination by dividing each with an absorption spectrum of 8.7 µg.ml^{-1} Glibenclamide for Metformin and 4 µg.ml^{-1} of Metformin for Glibenclamide[12].

The MCSR Absorption Spectrum Preparation

The Metformin absorption spectrum was divided by the absorption spectrum of 8.7 µg.ml^{-1} the Glibenclamide and the absorption spectrum of Glibenclamide were divided by the absorption spectrum of 4 µg.ml^{-1} Metformin with the help of UV 2.42 probe. While to get the first spectrum ratio, the treatment was carried out with the help of MATLAB software version 9.0.[12-13]

The Calibration Curve Construction By MCSR

The mean centering (MC) value or the amplitude of the first spectrum of Metformin and Glibenclamide ratios, were calculated and plotted with the concentration to get the regression line. Calibration graphs of each Metformin and Glibenclamide were obtained by construction the mean center (MC) values versus corresponding concentrations, and the regression equations of Metformin and Glibenclamide were obtained[12.19].

Validation Test

Accuracy

The accuracy was calculated by the measured recovery percentage in three specific points which were: 80%, 100%, and 120%. At each test point, it is used in the ratio of 70% of the sample and 30% of the pure active substance (standard addition method). The results obtained are compared with the level in the sample etiquette. In this method, the levels of materials obtained are compared with the levels in etiquette, so the accuracy of the process can be known. The levels of substances were obtained as the ratio of the obtained results to the theoretical results with general formula below: [20-22]

$$Percentage\ recovery = \frac{Ca - Cb}{Cc}\ X\ 100\%$$

Where: C_a=Concentration after addition; C_b=Sample concentration before addition; Cc=Concentration of the standard substances added.

Linearity

Standard solution of Metformin and Glibenclamide for spectral absorption was constructed made and measured at the selected wavelength points (235.8 nm for Metformin and 228.2 nm for Glibenclamide). The amplitude values of both active substances were determined using the regression equation for each component at its selected wavelengths. The obtained regression equation general formula is as below:[20-22]

$$Y = ax + b$$

Where: Y=Amplitude; a=Slope; x=Concentration (µg/mL); b=Constant.

Precision

Determination of precision or the relative standard deviation (RSD) is obtained from the regression equation that was calculated in the calibration curve with the following formula:[20-22]

$$RSD = [SD/X] \times 100\%$$

Where: RSD = Relative standard deviation; SD = Standard deviation; X = Average Data

Limit of Detection (LOD) and Limit of Quantification (LOQ) Test

Based on the absorbance at the wavelength analysis, LOD and LOQ calculations were also performed.[20-22]

$$LOD = [3xSD/Slope]$$

$$LOQ = [10xSD/ Slope]$$

Where: SD = Standard Deviation; Slope= a (y = ax+b)

Determination of Metformin and Glibenclamide in Pharmaceutical Tablet

Twenty tablets were weighed and powdered in a mortar. Furthermore, weighed powder equivalent to 50 mg Metformin and moved to a 50 mL volumetric flask, then dissolved by methanol solvent to the mark line and shaken until homogeneous and filtered[4-5]. Pipped 0.1 mL solution and moved to 50 mL volumetric flask and added methanol solvent, then added 4.3 mL Glibenclamide working solution and dissolved with methanol till mark-line. The solution was contain of 3.9984 µg.ml^{-1} Metformin and 8.6801 µg.ml^{-1} Glibenclamide.[4-5] The absorption of the solution was recorded in the wavelength range of 200-400 nm.

Pippeted 0.1 mL, and moved to 25 mL volumetric flask , and added . Standard solution of Glibenclamide (50.3 µg.ml^{-1}) was pipetted 4.3 mL, and moved to a 50 mL volumetric flask, and dissolved by methanol solvent. The mixture was made up to mark line with methanol solvent to obtain of 3.9984 µg.ml^{-1} Metformin and 8.6801 µg.ml^{-1} Glibenclamide[4-5].

The MC values of each Metformin and Glibenclamide in tablets can be obtained by the same procedure above, then calculated based on the regression equation at the wavelength that has been acquired by MCSR method.[12-13,18-19]

RESULTS AND DISCUSSION

This study was conducted as Afkhami (2005) and Darwish (2011) for the MCSR method, which began with the measurement of the maximum absorption spectrum of the component of drug[4-5]. The overlapping combination is shown in Fig.-1, Fig.-2 and Fig.-3.

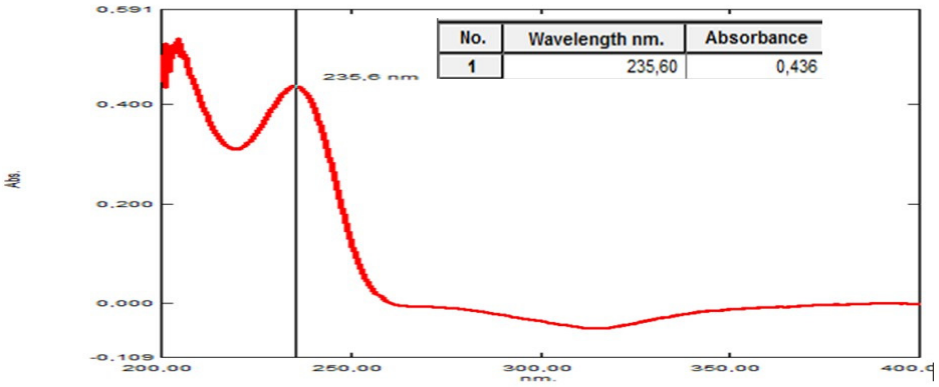


Fig.-1: Absorption Spectrum of 4 µg.ml^{-1} Metformin

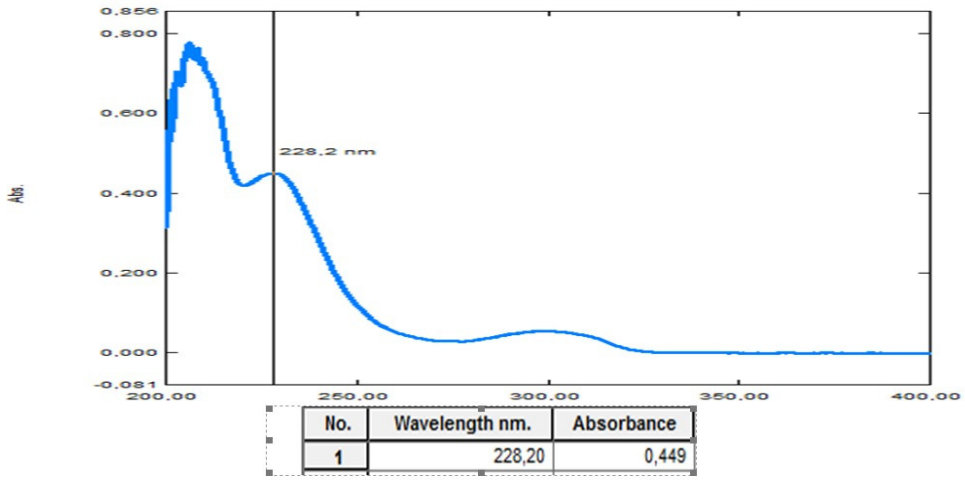


Fig.-2: Absorption Spectrum of 8.7 µg.ml^{-1} Glibenclamide

Based on Fig.-1 and Fig.-2 are shown, that the absorption spectrum of Metformin was at 235.6 nm and Glibenclamide at was at 228.2 nm. That means the maximum absorption of both components of the drug was slightly different from in the literature, which is 236 nm to 229 nm for Metformin and Glibenclamide. The difference did not affect the study, because according to the Indonesian

Pharmacopoeia 4th Edition 2014 that the difference spectrum absorption was allowed if not exceeding 5 nm[4].

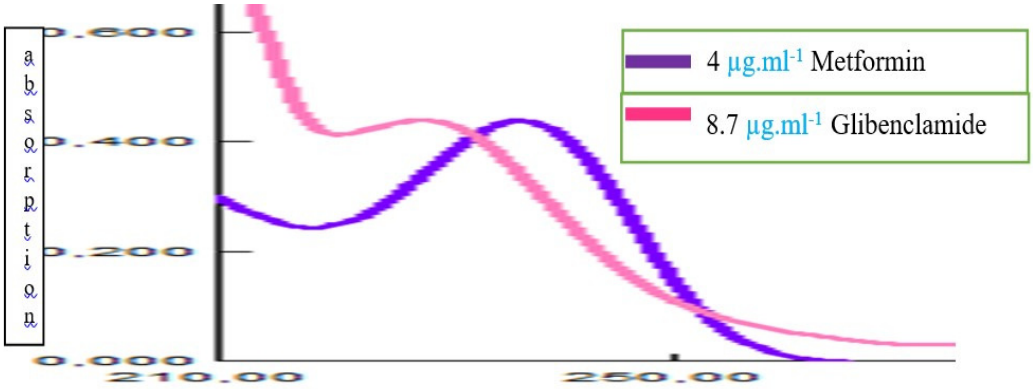


Fig.-3: Overlapping Absorption Spectrum of 4 µg.ml^{-1} Metformin and 8.7 µg.ml^{-1} Glibenclamide

Based on Fig.-3 above, it can show that the absorption curve of Metformin has overlapped with the absorption curve of Glibenclamide. So the MCSR methods were used in order to obtained Metformin and Glibenclamide levels without separation. By using this method, the wavelengths of these two drugs will be separated[12-13].

Spectrum Metformin and Glibenclamide with Divisor Factor

The spectrum of Metformin and Glibenclamide after distribution by the divisor factor was shown in Fig.-4 and Fig.-5. Based on Fig.-4 and Fig.-5 above, it can be seen that after having been given another component drug as a divisor. After manipulation spectrum absorption with Matlab software equipment, then the wavelength shifts to of Metformin being to 244 nm and Glibenclamide at 210.3 nm. The absorption spectrum changed to amplitude and the regression equation would be based on the wavelength contra amplitude[12-13].

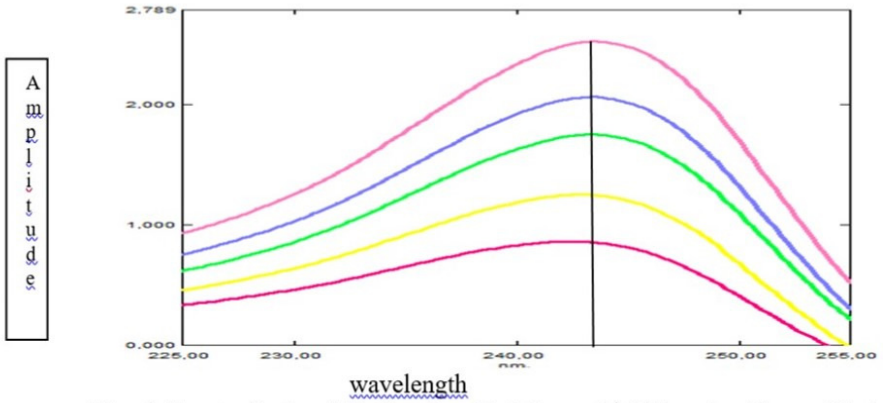
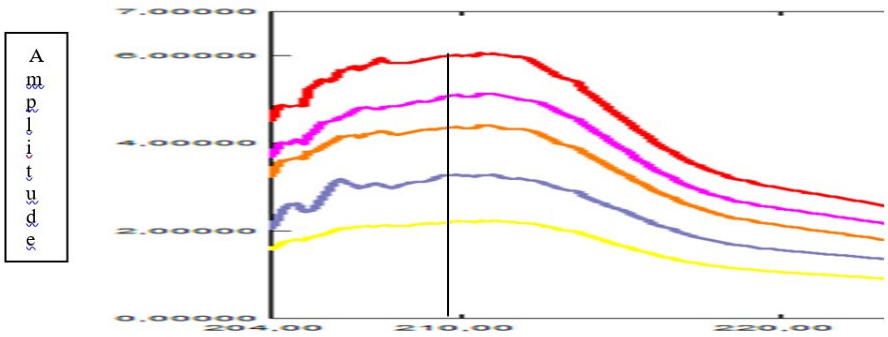


Fig.-4: Spectra Ratio of Metformin with 8.7 µg.ml^{-1} Glibenclamide as a Divisor

Construction the Mean Centered of Spectra Ratio for Metformin and Glibenclamide

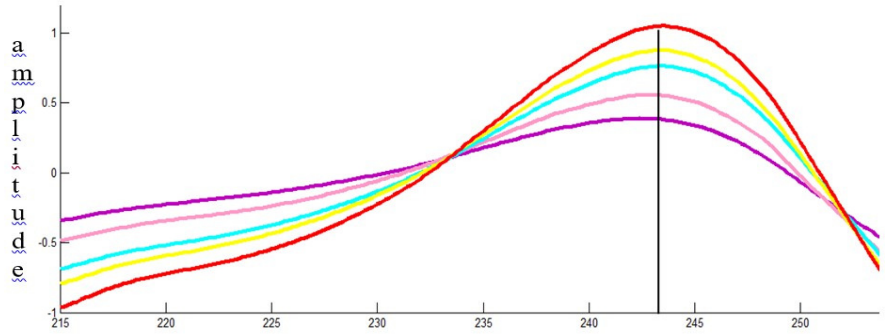
The results of the MCSR preparation from working solutions of Metformin, Glibenclamide, and overlap are shown in Fig.-6, Fig.-7 and Fig.-8.

Based on Fig.-6 and Fig.7, the absorption spectrum data of Metformin has divided with Glibenclamide as a divisor and Glibenclamide has divided with Metformin as a divisor, and has been determined using UV Probe software 2:42. The result of amplitude are obtained with the software MATLAB to get the MC (amplitude) of each of the spectrum, the plot between wavelength contra amplitude, and then entered into the regression equation[12-13].



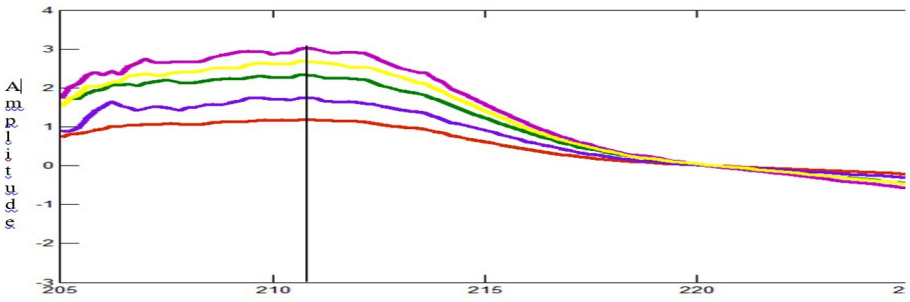
Wavelength

Fig.-5: Spectra Ratio of Glibenclamide with 4 µg.ml^{-1} Metformin as a Divisor



Wavelength

Fig.6-: Mean Centered of Spectra Ratio of Metformin



Wavelength

Fig.-7: Mean Centered of Spectra Ratio of Glibenclamide

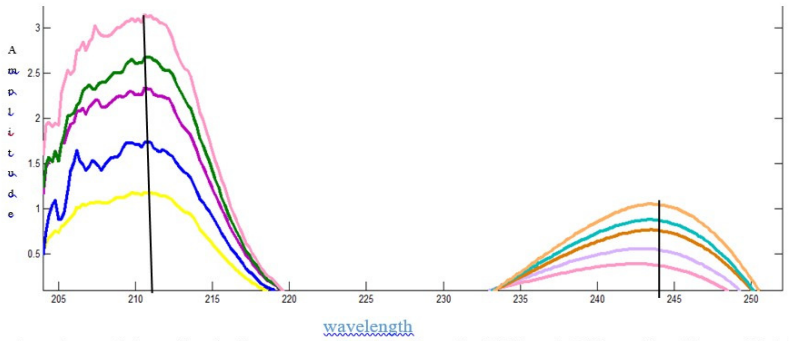


Fig.-8: Overlapping of Amplitude Spectrum for Metformin HCl and Glibenclamide with MCSR

Based on Fig.-8, that the spectrum of Metformin and Glibenclamide had been separated without interference from each substance. The wavelength of Metformin was changed from 235.6 nm to 244.4

nm, while for Glibenclamide from 228.2 nm to 210.3 nm, It could be stated that Metformin and Glibenclamide had have separated into single substances, so that they ~~it~~ can determine the level of each active substance without separation[12-13].

Method of Validation

The validation parameters measured are recovery test, RSD test, the limit of detection (LOD) and limit the quantitation (LOQ). The recovery test is determined by the addition method. Accuracy is carried out by three sample concentrations with a specific range of 80%, 100%, and 120%, calculated containing 70% analyte and 30% standard and are shown at Table-1 and Table-2 below[20-22].

Based on Table-1, Table-2, and Table-3 were shown that MCSR method had good accuracy and precision had met the accuracy, precision, LOD and LOQ requirements for method validation to obtain the amplitude value.[12,20-22] This means that MCSR methods had met the requirements of validation methods.

Table-1: Accuracy of Metformin with Standard Addition Method

No.	Range (%)	Weight			Percentage Recovery
		Before Raw Addition (mg)	After Raw Addition (mg)	Addition of Raw Material (mg)	
1.	80%	11.6809	11.7401	0.0591	100.16 %
2.		11.6788	11.7384	0.0591	100.84 %
3.		11.6812	11.7411	0.0591	101.35 %
4	100%	12.5856	12.6600	0.0734	101.36 %
5.		12.6501	12.7238	0.0734	100.40 %
6.		12.5860	12.6597	0.0734	100.40 %
7.	120%	16.5266	16.6112	0.0886	100.00 %
8.		16.1810	16.2697	0.0886	100.11 %
9.		16.5229	16.6125	0.0886	101.12 %
Average					100.63 %

Table-2: Accuracy of Glibenclamide With Standard Addition Method

No.	Range (%)	Weight			Percentage Recovery
		Before Raw Addition (mg)	After Raw Addition(mg)	Addition of Raw Material (mg)	
1.	80%	11.6809	11.7401	0.0591	100.16 %
2.		11.6788	11.7384	0.0591	100.84 %
3.		11.6812	11.7411	0.0591	101.35 %
4	100%	12.5856	12.6600	0.0734	101.36 %
5.		12.6501	12.7238	0.0734	100.40 %
6.		12.5860	12.6597	0.0734	100.40 %
7.	120%	16.5266	16.6112	0.0886	100.00 %
8.		16.1810	16.2697	0.0886	100.11 %
9.		16.5229	16.6125	0.0886	101.12 %
Average					100.63 %

Table-3: Result of Validation Test

No	Active Substance	Accuracy (%)	Precision (%)	LOD µg/mL	LOQ µg/mL
1	Metformin	100.23	0.43	0.5214	1.73
2	Glibenclamide	100.63	0.36	0.9001	3.01

LOD: Limit of detection, LOQ: Limit of quantification

Calibration Curve with Mean Centered of Spectra Ratio

Calibration curve in MCSR method is constructed by concentrations versus amplitude (MC results) graph.[12,19] The regression equation that obtained using MCSR method for Metformin and Glibenclamide have shown in Fig.-9 and Fig.-10[19].

Based on Fig.-9 and Fig.-10, it shows, that the Metformin's regression equation was Y = 0.1743 X + 0.0246 with the correlation coefficients of 0.9976. The glibenclamide regression equation was Y = 0.2499 X +0.0314 with a correlation coefficient of 0.999. The value of $r \leq 1$, it can be stated that the relationship between amplitude and concentration is linear, meaning the MCSR method can be used in this study[18-19]. Preparation of sample solution on a local product was made with standard addition method to achieve maximum concentration. The samples were measured containing Metformin and Glibenclamide in the ratio of 1: 200. wherein the measured absorption Glibenclamide did not fulfill the law of Lambert-beer, so it needed the addition of Glibenclamide**.** According to Harmita (2004), in the method of addition, a number of samples analyzed is added with analyte with the necessary levels of analyte concentration expected, mixed, and reanalyzed. The difference between the two results is compared with the actual level[21].

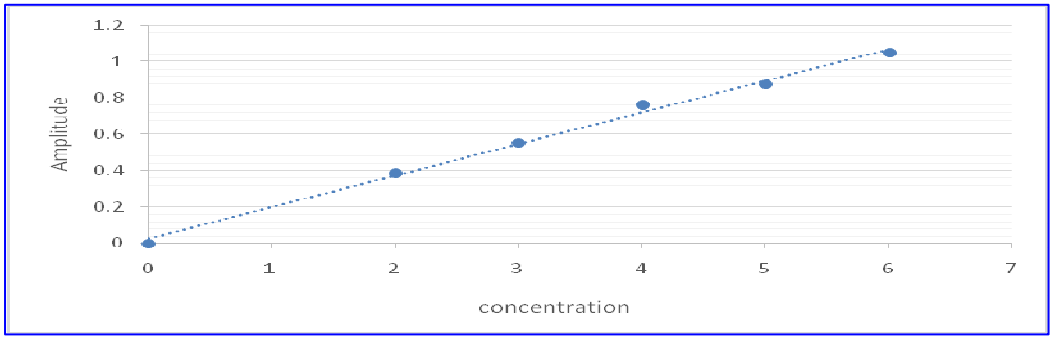


Fig.-9: MCSR Calibration Curve of Metformin

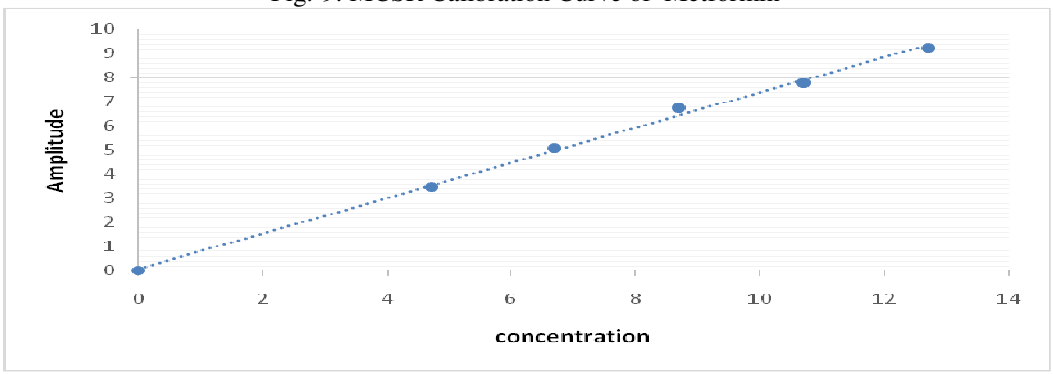


Fig.-10: MCSR Calibration Curve of Glibenclamide

The prepared sample was measured at a wavelength of 200-400 nm. The absorption spectrum of the samples obtained was divided by the spectrum with the 8.7 $\mu g.ml^{-1}$ Glibenclamide spectrum to produce the Metformin ratio absorption spectrum and 4 $\mu g.ml^{-1}$ Metformin for Glibenclamide. The MC value contra concentration was calculated and the result was shown in Table-4.

Table-4: MC values of Metformin and Glibenclamide in Local Product

Repetition	Metformin	Glibenclamide
1	0.7458	2.2057
2	0.7491	2.2058
3	0.7491	2.2058
4	0.7493	2.2057
5	0.7489	2.2057
6	0.7513	2.2058

The range of levels in the local product for Metformin and Glibenclamide was 95 - 105% and 95-105% respectively. The relative standard deviation (RSD) for Metformin and Glibenclamide is 0.43% and 0.36%. Metformin and Glibenclamide have good precision because both RSD values are less than 2%[20-22].

Table-5: The Statistical Levels of Metformin and Glibenclamide in Local Product

No.	Component	Level	Content in Etiquette	Level Requirements
1	Metformin	(102.76 ± 0.349)%	250 mg	(95-105)%
2	Glibenclamid	(103.58 ± 0.572)%	1.25 mg	(95-105)%

CONCLUSION

The assay with the mean-centered of spectra ratio methods is conducted with the separation of the drug component, by a factor of a divider and qualified validation, while and can be used for simultaneous assays of the Metformin and Glibenclamide combination in tablet dosage.

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