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Research Article

Development of Metformin and Glimepiride Analysis Methods Using TLC-Spectrofluorometry

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Abstract

Diabetes mellitus (DM) is a chronic metabolic disorder disease. DM presents in two main types 1 and 2. Type 2 DM was caused by genetic and lifestyle which was the biggest contributor to DM cases. Many patients with type 2 DM treated with combination therapy using Fix Dose Combination (FDC) preparation to increase patients compliance and therapeutic effect. FDC preparations contained metformin and glimepiride. Assay of these combination was not found in compendial. A rapid, simple, specific method analysis of metformin and glimepiride has been developed in this riset using TLC-spectrofluorometry. Metformin and glimepiride content in tablet were determined by the TLC-spectrofluorometry using dansyl chloride as derivatization agent under alkali conditions. In the TLC method, metformin and glimepiride were dissolved with methanol and gives Rf values of 0.52 and 0.70, respectively in system containing silika gel GF_{254} as stationary base and methanol:water: glacial asetic acid (6:4:0.25) as developing solvent. TLC results were scraped off and reacted using dansyl chloride 0.1% then fluorescence intensity measurements were carried out at emission wavelength 483 nm for metformin and 489 nm for glimepiride. Method validation is done by determining linearity, accuracy, precision, Limit Of Detection (LOD) and Limit Of Quantization (LOQ).

Keywords: Diabetes, Glimepiride, Metformin, TLC-Spectrofotometry.

Pengembangan Metode Analisis Metformin dan Glimepirid menggunakan KLT-Spektrofluorometri

Abstrak

Diabetes melitus (DM) merupakan penyakit gangguan metabolisme kronik. DM terdiri dari dua tipe yaitu 1 dan 2. DM tipe 2 disebabkan oleh genetik dan gaya hidup yang merupakan penyumbang terbesar kasus DM. Banyak pasien DM tipe 2 diobati dengan terapi kombinasi menggunakan sediaan *Fix Dose Combination* (FDC) untuk meningkatkan kepatuhan pasien dan efek terapeutik. Sediaan FDC salah satunya mengandung metformin dan *glimepiride*. Pengujian kombinasi ini tidak ditemukan pada kompendial. Penelitian ini telah mengembangkan metode analisis metformin dan *glimepiride* yang cepat, sederhana, dan spesifik dengan menggunakan KLT-spektrofluorometri. Kandungan metformin dan glimepiride dalam tablet ditentukan dengan KLT-spektrofluorometri menggunakan dansil klorida sebagai zat derivatisasi dalam kondisi alkali. Pada metode KLT, metformin dan *glimepiride* dilarutkan dengan metanol dan menghasilkan nilai Rf masing-masing sebesar 0,52 dan 0,70 dalam sistem yang mengandung silika gel GF_{254} sebagai fase diam dan metanol:air: asam asetat glasial (6:4:0.25) sebagai pelarut pengembang. Hasil KLT dikikis dan direaksikan menggunakan dansil klorida 0,1% kemudian dilakukan pengukuran intensitas fluoresensi pada panjang gelombang emisi 483 nm untuk metformin dan 489 nm untuk *glimepiride*. Validasi metode dilakukan dengan menentukan linearitas, akurasi, presisi, batas deteksi (LOD) dan batas kuantisasi (LOQ).

Kata Kunci: Diabetes, Glimepirid, Metformin, KLT-Spektrofluorometri

1. Introduction

Diabetes mellitus (DM) still a serious and complex disease faced by the world community, especially Indonesia. There are two types of DM, type 1 DM and type 2 DM. Type 2 DM is the biggest contributor in the case of DM caused by disruption of insulin production by the pancreas, or the ineffectiveness of the insulin produced. The deficiency of insulin leads to increased glucose concentration in the blood and causes complications of damage to the body system, especially blood vessels and nerves.[6,13] Type 2 DM patient is treated with combination therapy using Fix-Dose Combination (FDC) to improve patient adherence in consuming the drug. The content of FDC preparations is Metformin and Glimepiride which results in lower hyperglycemic levels when compared with the combination of Metformin and other Sulfonylurea group.[9]

Some literature reveals that the determination of separate or simultaneous levels of Metformin and Glimepiride has been carried out by the TLC method[1]; RP-HPLC[11]; LC-MS/MS[10]; and Ultraviolet spectrophotometry[7]. The combination of drugs in one preparation with two active substances can complicate quality control in determining levels. So it is necessary to develop an analytical method to determine the levels of metformin and glimepirid using thin layer chromatography (TLC) to support the two active substances which are then further analyzed using spectrofluorometry with dansyl chloride derivatization. So far no one has analyzed the levels of metformin and glimepiride using thin layer chromatography-spectrofluorometry complexed with dansyl chloride as an alternative to the analysis.

2. Method

2.1. Tools

The tools used include Shimadzu Spectrophotofluorometry RF-6000; Plate TLC GF_{254}; Chamber; Micropipettes Vial Analytical balance; Measuring cup; Measuring flask; Chemical cups; and glass tools commonly used in quantitative analysis.

2.2. Materials

The materials used include Metformin; Glimepirid; Dansyl Chloride (DNS); acetone; sodium carbonate; methanol; glacial acetic acid; all other chemicals from the class of analytical reagents.

2.3. Procedure

2.3.1. Solution Making

Each standard solution of Metformin and Glimepiride was 10 mg dissolved in a 100 mL measuring flask using methanol. The standard solution of Metformin and Glimepiride was diluted until the concentration of Metformin was 0.5-1.4 µg/mL and Glimepirid was 0.1-1 µg/mL. The derivation reagent such as chloride was dissolved using acetone and 0.1% concentration.

2.3.2. Development of Metformin and Glimepiride using Thin-Layer Chromatography

Metformin and Glimepiride that have been made series of concentrations was developed using TLC with a fluid developer in the form of methanol: water: glacial acetic acid (6: 4: 0.25) and left for 20 minutes. The silent phase used the 254 silica Type KLT plate that was previously activated in the oven with a temperature of 105°C for 10 minutes..

2.3.3. Derivation and Determination of Wavelength

For each series of concentrations of Metformin and Glimepiride that have been developed using TLC, the result of the TLC added a pH buffer 10 then filtered. The filter result was added 1 mL and 0.1% chloride and incubated for 10 minutes at room temperature. Then, conduct an analysis with a spectrofluorometer and measure fluorescence intensity at 483 nm emission wavelength for metformin and 489 nm for Glimepiride.

2.3.4. Interference Linearity

Measurement of linearity was evaluated by analyzing the various concentrations of the raw standard solution of Metformin and Glimepiride in which each concentration was performed three times of repetition. The

coefficient of determination (R2) and the coefficient of regression variation (Vx0) was evaluated for linearity.

2.3.5. Detection Limit and Quantization Limit Test

Limit of quantization (LOQ) method was determined through multiplication between the linear line of residual standard deviation (Sy/x) and the factor of 10/b with B was the slope of a linear regression line. Limit of detection (LOD) method was determined through multiplication between the standard deviation of the linear regression line (Sy/x) and the factor of 3.3/b. Through the equation obtained from the calibration curve, the slope of a linear regression line (Sy/x) obtained the root result of the number of the residual squared divided by n-2 where n is the amount of concentration analyzed.

2.3.6. Precision and Accuracy

Precision and accuracy were evaluated every three iterations of each of its concentrations. The precision used a concentration of 100% of the sample rate. Then done on Day 1, 2 and 3. The concentration of Metformin and Glimepiride was calculated using the calibration curve. Precision is represented by the relative standard deviation percent (% RSD), while the accuracy was expressed by the percent reacquisition. The precision and accuracy acceptable at the DL Point were 80%, 100%, and 120% of the sample rate, respectively.

3. Result and Discussion

The development of analytical methods for determination of metformin and glimepiride levels by spectrofluorometry must be carried out by derivatization using dansyl chloride reagents. This is due to dansyl chloride and the structure of metformin and glimepiride which produce dansyl derivatives if they are not reacted with dansyl chloride (DNS-Cl) reagent. DNS-Cl (1-dimethylaminoaphtalene-5-sulphonyl chloride) reacts quantitatively with primary amines, secondary, imidazoles and phenols under suitable conditions to produce fluorocated sulfonamides in a stable manner. Optimized the addition of 0.1% w / v dansyl chloride; 0.1 mL to 1 mL, derivate reagen can increase fluorescence intensity when adding 0.1 mL to 0.8 mL and a constant fluorescence intensity when adding 0.9 mL to 1 mL. The concentration of dansyl chloride is higher than the concentration analysis because in alkaline conditions the analyte will compete with the base component to react with dansyl chloride. The reaction between the basic components and dansyl chloride causes the hydrolysis of dansyl chloride to become dansyl hydroxyl, SO_2Cl group in dansyl chloride turns into a sulfonate group (SO_3H), where the dansyl hydroxyl is unable to react with the analyte (Blanco et al., 2000). A concentration of 0.1% with the addition of 1 mL DNS-Cl was determined as the optimal concentration and volume for derivatization.

3.1. Development of Metformin and Glimepiride using Thin-Layer Chromatography

The separation was using Thin-Layer Chromatography (TLC) to determine the retention factor (Rf) value as well as the

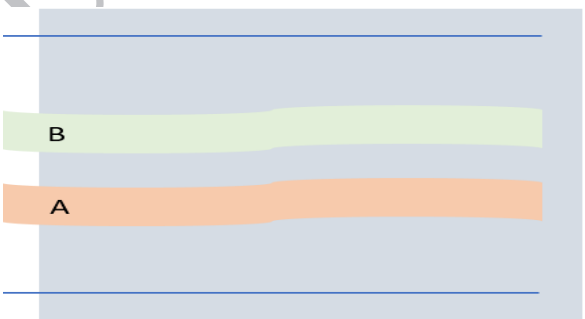

Figure 1. The illustration of the development of metformin and Glimepirid using TLC. (a) Metformin, (b) Glimepiride

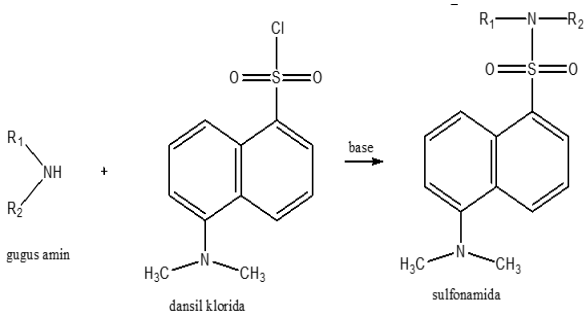

Figure 2. The Amin Group reaction with the chloride dansyl[8]

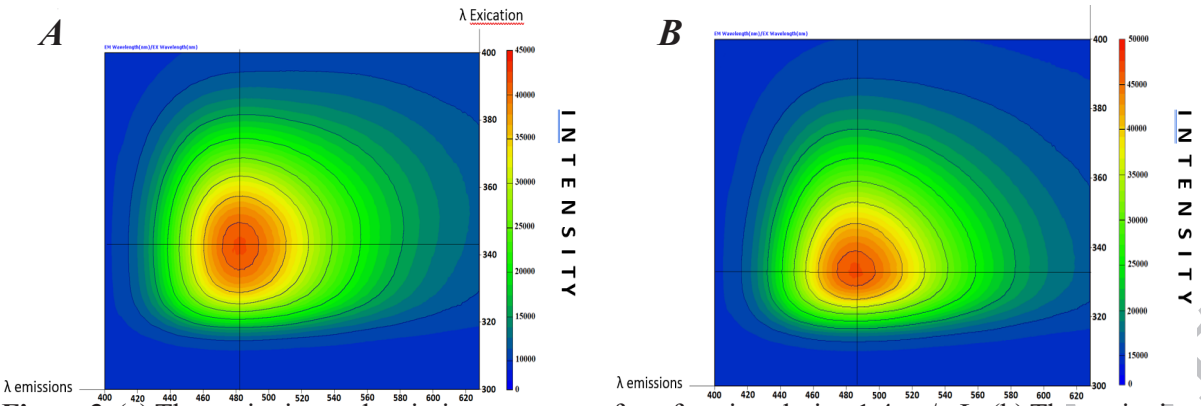

Figure 3. (a) The excitation and emission spectrum of metformin solution 1.4 µg/mL. (b) The excitation and emission spectrum of Glimepirid solution 1.0 µg/mL.

resolution value (Rs). The TLC process aims to process the separation of Metformin and Glimepiride in the combination of tablets. The silent phase used type of silica gel GF_{254} which was activated in advance with an oven at 105°C for 10 minutes to remove moisture in a well-assorted atmosphere in the silica gel GF_{254}. The phase of the motion used was methanol: water: glacial acetic acid (6: 4: 0.25) which was left for 20 minutes. The saturation of the motion phase in the chamber can affect the value of RF, where the saturated condition of the RF was lower than the RF with an unsaturated motion phase because concurrently the solvent migrated through the plate sorbent through the capillarity style and also interacted with the vapor of Eluen.[12]

Several interactions occur such as interactions between the Eluen vapor phase, Eluen phase, and adsorbed humidity in the plate with the plate sorbent itself. In the chamber, the unsaturated chamber, the Eluen was hight with 3-5 mm, the Eluen vapor will fill the room with a saturation of 75% at the center of the chamber and it will be even lower. When the plates were inserted in the chamber with low saturation, the conditions of saturation in the chamber change slightly and it took time to condition the new saturation. Metformin and Glimepiride resulted in a consecutive Rf value of 0.54 and 0.70. While the separation using TLC, this result in a resolution value (RS) was 1.9. This Rs value was an analysis condition ability to separate the two compounds in the sample. Rs value should be more than 1.5. The greater the value of the resolution the better the separation occurred.[12] Vizualization of TLC shown in Figure 1.

3.2. Derivation and Determination of Wavelength

The derivation was then carried out on the TLC result by reacting to the analytic with the attenuation of 0.1% chloride in the condition of pH 10 which was the optimum condition for the reaction and incubated for 10 minutes at room temperature. The derivation of a compound with a dansyl chloride was a base pH to support the acidic atmosphere caused by the results of the side reactions of the derivatization. In the acidic atmosphere, the dimethylamino group on the derivative body would be protonated and lead to decreased fluorescence efficiency from the derivative and derivatives.[5] Derivatization reaction shown in Figure 2.

The determination of excitation wavelength and raw solution emissions of Metformin and Glimepiride was carried out using the 3D spectrum spectrofluorimetry. It aimed to determine the wavelength of excitation and emissions simultaneously so that it can be seen the highest intensity resulting in the value of quantum efficiency approaching 1. The higher the value of quantum efficiency, the higher the fluorescence intensity observed from a molecule.[4]

The wavelength of emissions and excitation was measured against the raw solution of Metformin and Glimepiride. The raw solution of Metformin shown in Figure 3, from a wavelength range of excitation 300-400 nm obtained the highest fluorescence intensity at a wavelength of 483 nm. While, the raw solution of Glimepiride shown in Figure

Table 1. A nalytical performance data for fluorometric determination of Metformin and Glimepiride

Parameter	Result	
	Metformin	Glimepiride
Excitation wavelength (nm)	344	334
Emission wavelength (NM)	483	489
Average concentration (Ẋ)	0.92	0.52
Intercept (a)	35480.22	45462.79
Slope (b)	2665.92	1967.91
Sy/X	60.99	87.67
Correlation coefficient (R2)	0.997	0.996
Linierity range (µg/mL)	0.5 – 1.4	0.1 – 1.0
Vx0 (%)	4.5	2.3
Detection limit (µg/mL)	0.07	0.13
Quantization limit (µg/mL)	0.23	0.45

3, from a wavelength range of excitation 300-400 nm obtained the highest fluorescence intensity at a wavelength of 489 nm.

3.3. Linearity, LOD, and LOQ

The calibration curve is made from a series of metphromine standard solutions in a concentration of 0.5-1.4 µg / mL, while a series of glimepirid standard solutions in a concentration of 0.1-1.0µg / mL. Correlation coefficient (r) 0.997 from the linear regression equation for metformin and the line equation y = 2665.92x + 35480.22 while for glimepirid it produces a correlation coefficient (r) 0.996 with the line equation obtained y = 1967.92x + 45462.79 ; y is the fluorescence intensity and x is the concentration. In the requirements of the AOAC International Guideline for the validation of analytical methods, it is stated linear if the coefficient of correlation is more than 0.99. Thus the method developed is stated to meet the requirements of good linearity.[14,15]

The coefficient regression of variation (Vx0) was also proposed to meet the linearity requirements of the validation method. Vx0 is the percentage of the ratio Sy/x with multiplication b and the average concentration analyzed. The Vx0 values obtained for metformin and glimepiride were 4.5% and 2.3%, respectively. The value of Vx0 obtained also meets the requirements based on the AOAC International Guideline for validation of analytical methods where the requirements are met if Vx0≤5.0%. Data shown in Tabel 1.

The limit of quantitation (LOQ) is the lowest concentration of analyte that can still be determined by the quantitative method. The LOQ of the method is determined by multiplying the linear line resudual standard deviation (Sy / x) by a factor of 10 / b where b is the slope of the linear regression line. The limit of detection (LOD) is the lowest concentration of analyte that can still be detected by the method without needing to be determined quantitatively. The LOD of the method is determined by multiplying the residual standard deviation of the linear regression line (Sy / x) by a factor of 3.3 / b where b is the slope of the linear regression line. Through the equation obtained from the calibration curve, the slope of the linear regression line (Sy / x) is the root result of the sum of squares of the residual divided by n-2 where n is the number of concentrations analyzed. The BD and BK values for

Table 2. Metformin and Glimepiride data accuracy test

	Metformin			Glimepiride		
	Theoretical* (µg/mL)	Obtained* (µg/mL)	% Partition*	Theoretical* (µg/mL)	Obtained* (µg/mL)	% Partition*
80%	0.8	0.78	98.63	0.4	0.38	98.02
100%	1.0	0.98	98.94	0.5	0.47	98.15
120%	1.2	1.19	99.43	0.6	0.58	98.43

Table 3. Data Precision Test Metformin and Glimepiride

	Metformin			Glimepiride		
	Average concentration ± SD	SBR (%)	HORRAT	Average concentration ± SD	SBR (%)	HORRAT
Intraday*	1.00 ± 0.02	1.27	0.15	0.49 ± 0.01	1.49	0.17
Interday**	0.99 ± 0.01	1.14	0.14	0.48 ± 0.01	1.38	0.15

***Doing three times the replication in one day**

****Doing three days, one day three times the replication**

metformin from the method were 0.07 µg / mL and 0.13 µg / mL, respectively; whereas for glimepirid, BD and BK obtained from the method were 0.23 µg / mL and 0.45 µg / mL respectively which can be seen in Table 1.

3.4. Accuracy

Accuracy used to express the accuracy of the method based on the closeness of the results obtained from the tested analysis method with the real value. Proximity to yield is calculated as the percent rate of recovery (% recovery). The percentage of recovery is the percentage of the ratio of the concentration of the analysis results obtained to the theoretical concentration. The requirements based on the Indonesian Pharmacope V mentioned that Metformin Hydrochloride contains no less than 98.5% and not more than 101,0% $C_4H_{11}N_5$. HCl is calculated against dried substances. While Glimepirid contains no less than 98.0% and no more than 102.0% $C_{24}H_{34}N_4O_5S$, counts against anhydrous substances.[16,17] in this study, metformin and glimepirid met the accuracy requirements contained in the pharmacopoe. Data shown in Tabel 2.

3.5. Precision

Precision is used to state the suitability of analysis when analytical procedures are used repeatedly on the same analyte. Interday precision is determined based on the accuracy of the results obtained from testing on different days. The similarity of the results is expressed as the HORRAT value obtained from the comparison between the relative standard deviation (RSD) of experimental and theoretical results. Based on the requirements of the AOAC International Guideline, the method is declared precise if the relative standard deviation (RSD) is less than 2% and the HORRAT value is less than 2. On accuracy, metformin and glimepirid yield good RSD values.[18,19,20] Data shown in Tabel 3.

4. Conclusion

Validation methods performed by determining linearity, accuracy, precision, limit of detection (LOD) and the limit of quantitation (LOQ) As a result, the analytes have met the validation requirements, so metformin and glimepiride were able to do the analysis using KLT-spektrofluorosen method as an alternative analysis.

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