Introduction

Diabetes is a chronic metabolic disease characterized by elevated blood glucose levels which leads to serious damage to the heart, blood vessels, eyes, kidneys and nerves. Type 2 diabetes is the most common diabetes in adults, which occurs when the body becomes insulin resistant or no longer produces enough insulin. In the last three decades, the prevalence of type 2 diabetes has dramatically increased in all countries in the world [1,2]. As a results, oral hypoglycemic drugs with various mechanisms have been used and developed to restore and improve the function of pancreas in order to secrete insulin, reducing insulin resistance in body tissues or increasing glucagon-like peptide-1 (GLP-1). However, the glucose-lowering agents in monotherapy have over time shown increasing failure rate to control blood

Penetapan Kadar Metformin dan Glimepiride secara Simultan pada Tablet Kombinasi Dosis Tetap yang dijual di Pasar Pramuka menggunakan Metode Spektrofotometri UV Tervalidasi

(Simultaneous determination of metformin and glimepiride in fixed-dose combination tablets sold in pramuka market using validated uv spectrophotometric method)

Zilhadia*, Supandi & Salman AlFarisi

ABSTRACT: Combination of metformin and glimepiride as a diabetes mellitus drug has recently shown significant increase due to its benefits in improving patient compliance and offering cost effectiveness. This study aimed at analyzing metformin-glimepiride in fixed dose combinations purchased at Pramuka Market (the largest drug market in Jakarta) using validated simultaneous UV-spectrophotometry. The maximum wavelength of metformin and glimepiride is 237 nm and 228 nm respectively. The validation result of metformin and glimepiride at concentration range of 2-8 µg/mL and 5-17 µg/mL showed Linearity with 0.9999 and 0.9996, LOD values with 0.0962 µg/mL and 0.2954 µg/mL, LOQ values with 0.3208 µg/mL and 0.9846 µg/mL, and recovery (accuracy test) with 99.72% ± 1.88% to 103.86 ± 0.87%, respectively. The Relative Standard Deviation (RSD) as the precision test indicator for both drugs was less than 2%. The level of Metformin-glimepiride fixed-dose combination purchased at Pramuka Market was about 96.15% ± 3.56% to 102.71% ± 1.50%. The conclusion of this study showed that the level of metformin-glimepiride sold in the Pramuka market met the requirements.

Keywords: fix-dose combination; glimepiride; metformin; simultaneous; UV-Spectrophotometric.

ABSTRAK: Kombinasi metformin dan glimepiride sebagai obat diabetes mellitus memberikan hasil peningkatan yang signifikan dalam kepatuhan pasien dan menawarkan efektivitas biaya. Penelitian ini bertujuan menetapkan kadar tablet metformin-glimepiride dalam kombinasi dosis tetap yang dibeli di Pasar Pramuka. Metode yang digunakan adalah simultan spektrofotometri UV tervalidasi sebagai metode alternatif yang lebih cepat dan berbiaya murah. Panjang gelombang maksimum metformin dan glimepiride masing-masing adalah 237 nm dan 228 nm. Hasil validasi metformin dan glimepiride pada rentang konsentrasi 2-8 g/mL dan 5-17 g/mL menunjukkan Linearitas dengan 0.9999 dan 0.9996, nilai LOD 0.0962 g/mL dan 0.2954 g/mL, nilai LOQ 0.3208 g/mL dan 0.9846 g/mL. Hasil uji akurasi diperoleh masing-masing masing-masing 99,72% ± 1,88% hingga 103,86 ± 0,87%, dan uji presisi diperoleh Relative Standard Deviation (RSD) kurang dari 2%. Kadar metformin-glimepiride kombinasi dosis tetap yang dibeli di Pasar Pramuka berkisar antara 96,15% ± 3,56% sampai 102,71% ± 1,50%. Disimpulkan bahwa kadar metformin-glimepiride yang dijual di pasar pramuka memenuhi persyaratan menggunakan metode simultan spektrofotometer UV tervalidasi.

Kata kunci: kombinasi dosis tetap; glimepiride; metformin; simultan; spektrofotometri UV.
glucose, which ultimately requires a number of antidiabetic drugs in combination. The commonly used combination is metformin and glimepiride [3]. Metformin is classified as a guanidine widely known as IUPAC 3-(diaminomethylidene)-1,1-dimethylguanidine. It is an antidiabetic agent with antihyperglycemic activity [4]. Meanwhile, glimepiride, commonly called IUPAC 4-ethyl-3-methyl-N-[2-[4-[(4-methylcyclohexyl) carbamoyl]sulfamoyl]phenyl][ethyl]-5-oxo-2H-pyrrrole-1-carboxamide, is a third generation sulfonylurea with hypoglycemic activity [5]. Compared to other generation sulfonylurea, glimepiride is very strong and has a longer duration of action. This combination is an effective and complementary method to reverse the two main causes of type 2 diabetes [6]. To increase patient adherence, reduce treatment costs, minimize drug side effects, and reduce the risk of resistance, metformin and glimepiride in fixed dose combination tablets have been developed [7].

In Indonesia, this drug combination is widely sold in several drugstores including Pramuka market. The market is the largest drug market in the country which sells counterfeit, illegal and expired drugs [8]. For this reason, in this research we conduct the examine of contents of metformin-glimepiride sold at Pramuka market. The 6th edition of the Indonesian Pharmacopoeia mentions the metformin content in tablets is not less than 95.0% and not more than 105.0%, while the content of glimepiride is not less than 90.0% and not more than 110.0% based on the amount stated on the label [9].

The common methods used to analyze metformin and glimepiride are HPLC [10,11], LC-MS/MS [12], and UPLC-QToF-MS [13]. The principle of the three methods is separation based on polarity differences in 2 phases with high technology and higher cost [14]. A faster and more simple, inexpensive, precise and reproducible method is required for any drug analysis. HPLC, for example, has met the criteria in terms of accuracy and reproducibility, but it is more expensive, complicated and time consuming compared to UV-Vis Spectrophotometry. Therefore, in this research, the UV spectrophotometry was used in determining the contents of metformin and glimepiride in fixed dose combination tablets. Because the maximum wavelengths of the two compounds are close to each other or nearly overlapping, it was decided to use simultaneous method [15]. This method has been known to be simpler, cheaper, and more precise and required a shorter analysis time.

**Method**

**Material**

Metformin HCl and glimepiride were purchased from Merck, Germany. Methanol was purchased from Emsure, Germany. The samples were bought at Pramuka Market, Indonesia in January 2020. The dosage of metformin HCl and glimepiride is 250 mg and 1 mg, respectively. The criteria of the samples are as follow: 1) they were purchased from a pharmacy/drugstore at Pramuka market which practices or operates without licenses including the pharmacist license and sells a combination of glimepirid 1 mg and metformin HCl 250 mg, 2) they were not expired, and 3) they were in different batches.

**Instrumentation**

Spectrophotometer UV-Vis (HITACHI U-2910, Japan) with spectral bandwidth for 3nm and wavelength accuracy for ±1nm and 1cm quartz cells was used in this research.

**Preparation of Standard and Working Solution**

The standard stock solution was prepared by accurately measuring 10 mg of the standard metformin and glimepiride into a 100 ml volumetric flask and dissolving them in methanol. For working solution, the standard solution is diluted to obtain the desired concentration.

**Determination of Maximum Wavelength**

Metformin and glimepiride solutions were prepared at a concentration of 20 µl by diluting the standard solution of metformin HCl and glimepiride. In turn, the solutions were measured at a wavelength of 200–400 nm [15].

**Table 1. Absorptivity of metformin and glimepiride**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wavelengths (nm)</th>
<th>Absorptivity (L/g cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>237 nm (metformin)</td>
<td>0,0982 (ax1)</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>228 nm (glimepiride)</td>
<td>0,0746 (ax2)</td>
</tr>
<tr>
<td>Metformin</td>
<td>237 nm (metformin)</td>
<td>0,0415 (ay1)</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>228 nm (glimepiride)</td>
<td>0,0521 (ay2)</td>
</tr>
</tbody>
</table>
Determination of Metformin and Glimepiride Absorptivity

The standard solution of metformin was diluted to obtain a concentration of 2, 5, and 8 µg/mL. The absorption was then measured at the maximum wavelength of the obtained metformin. Glimepiride solution at concentrations of 5, 11 and 17 µg/mL was prepared in the same way. In the end, the metformin and glimepiride absorbance values were calculated using the Lambert-Beer formula [16].

Determination of Calibration Curve and Linearity of Metformin and Glimepiride

The absorbance of metformin and glimepiride solutions at concentration of 2; 3.5; 5; 6.5; 8 µg/mL and 5; 8; 11; 14; 17 µg/mL respectively were measured at the respective maximum wavelengths to obtain the equation of the calibration curve [16].

Determination of Limit of Detection (LOD) and Limit of Quantification (LOQ) as Sensitivity Criteria

The equation of the calibration curve was used to calculate the LOD and LOQ with the following formulas; LOD = 3SD/B and LOQ = 10SD/B [17,18]. The SD stands for Standard Deviation of Intercept, while the B means the slope of calibration curve.

Accuracy Test

Metformin and glimepiride solutions were prepared at a concentration of 80%, 100% and 120% according to the compound content stated on the label. Each concentration was repeated for 3 times and accuracy was expressed by measuring the percent recovery [17,18].

Precision Test

The precision test was measured using intra-day and inter-day variations (n = 6) with concentrations referring to the content stated on the label (metformin 250 mg and glimepiride 1 mg). The intraday variation was carried out at 2 different times within 24 hours intervals on the first day. Meanwhile, the inter-day variation was conducted on different days for 3 consecutive days. The precision test was obtained using the % RSD (Relative Standard Deviation) parameter as follows:

\[
\text{% RSD} = \frac{SD}{X} \times 100\%
\]

Note:

\[
\text{% RSD} = \text{Relative Standard Deviation}
\]

\[
SD = \text{Standard Deviation}
\]

\[
X = \text{average test}
\]

Sample Assay

20 tablets of fixed dose-combination (metformin and glimepiride) from the same batch were ground into a powder, and an accurately weighed sample of powdered tablets equivalent to 1 mg of glimepiride was dissolved in 100 ml of methanol, shaken until the solution was homogeneous, filtered and diluted using methanol. The absorbance of each drug was measured by reducing the absorbance effect of other drugs.

Table 2. The result of correlation coefficient (r), LOD, and LOQ

<table>
<thead>
<tr>
<th>Sample</th>
<th>Linier Regression</th>
<th>R</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>(y = 0.101x - 0.010)</td>
<td>0.9999</td>
<td>0.0962</td>
<td>0.3208</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>(y = 0.054x - 0.015)</td>
<td>0.9997</td>
<td>0.2954</td>
<td>0.9846</td>
</tr>
</tbody>
</table>

Table 3. Result of metformin and glimepride recovery

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured Concentration (%)</th>
<th>Measured Concentration (mg)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80%</td>
<td>200</td>
<td></td>
<td>103.86% ± 0.87%</td>
</tr>
<tr>
<td>100%</td>
<td>250</td>
<td></td>
<td>99.80% ± 0.30%</td>
</tr>
<tr>
<td>120%</td>
<td>300</td>
<td></td>
<td>103.37% ± 0.17%</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>25,8</td>
<td></td>
<td>101.68% ± 2.07%</td>
</tr>
<tr>
<td>100%</td>
<td>26</td>
<td></td>
<td>99.72% ± 1.88%</td>
</tr>
<tr>
<td>120%</td>
<td>26,2</td>
<td></td>
<td>100.13% ± 3.59%</td>
</tr>
</tbody>
</table>
**Result and Discussion**

The maximum wavelengths of metformin and glimepiride obtained were 237 nm and 228 nm, respectively. The maximum wavelength for the two compounds is according to the Certificate of Analysis of the standard used in this research. The spectrum of metformin, glimepiride and the combination of the two was illustrated in Figure 1. The maximum wavelengths of metformin and glimepiride were so close to each other or nearly overlapping (only 9 nm apart) that the obtained absorbance affected each other\[13\]. At the maximum wavelength of glimepiride, absorbance of metformin was found. In the same way, absorbance of glimepiride also appeared at the maximum wavelength of metformin. Hence, the determination of the contents of the two compounds used simultaneous method\[10,16,19\].

The determination of the two drugs absorptivity was carried out to measure the absorbance of metformin at the maximum wavelength of glimepiride and vice versa. The measurement was conducted using three series of concentration; low, medium and high concentrations at two wavelengths, 237 nm (metformin) and 228 nm (glimepiride). The absorptivity data was described in Table 1.

The calibration curve and linearity were plotted between the maximum wavelength and the absorbance. According to the ICH recommendation, 2005, 5 series of concentration are considered sufficient to obtain the absorbance data. With an optimization process, the optimal series of concentration which meets the Lambert-Beer law was 2; 3.5; 5; 6.5; 8 µg/mL for metformin and 5; 8; 11; 14; 17 µg/mL for glimepiride, respectively. In the meantime, the equation of the calibration curve obtained for metformin and glimepiride was $y = 0.101x-0.01$ and $y = 0.054x-0.0152$, respectively (illustrated in Table 2). Further, the R2 value for the two compounds was 0.999 indicating that the increase in concentration had a direct relationship with the increase in absorbance. This showed the linearity met the requirements\[17,18\].

The sensitivity of the analytical method was evaluated by measuring the Limit of Detection (LOD) and Limit of Quantification (LOQ) applying the formula mentioned in the research method. The Limit of Detection (LOD) is the smallest detectable amount of a substance in a sample that can still give a significant response, and the Limit of Quantitation (LOQ) is the smallest quantity of analyte in a sample that can meet the precise criteria for accuracy and precision\[20\]. The LOD and LOQ values were illustrated in Table 2. The LOD value in metformin and glimepiride was 0.0962 µg/mL and 0.2953 µg/mL respectively. This means the UV-Vis spectrophotometry was able to respond to 0.0962 µg/mL for metformin and 0.2953 µg/mL for glimepirid. The LOQ value was 0.3208 µg/mL and 0.9845 µg/mL for metformin and glimepiride respectively indicating the method used to analyze the metformin and glimepiride simultaneously was able to respond to the data accurately and precisely at a minimum concentration of 0.3208 µg/mL and 0.9845 µg/mL.

Accuracy is a parameter showing the degree of closeness of the analysis result to the actual analyte content. In the accuracy test, three concentrations were analyzed, 80% (metformin 200 mg and glimepiride 25.8 mg), 100% (metformin 250 mg and glimepiride 26 mg), and 120% (metformin 300 mg and glimepiride 26.2 mg).

**Table 4. The result of precision test**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nominal Concentration (mg)</th>
<th>Measured Concentration (mg)</th>
<th>Precision (% CV)</th>
<th>Measured Concentration (mg)</th>
<th>Precision (% CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>250</td>
<td>249.02 ± 0.99</td>
<td>1.24</td>
<td>251.66 ± 1.01</td>
<td>1.13</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>1</td>
<td>0.93 ± 0.60</td>
<td>1.79</td>
<td>0.88 ± 0.99</td>
<td>1.11</td>
</tr>
</tbody>
</table>

**Table 5. Determination of metformin and glimepiride purchased at Pramuka Market**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tablet X1</th>
<th>% Recovery (%)</th>
<th>Tablet X2</th>
<th>% Recovery (%)</th>
<th>Tablet X3</th>
<th>% Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>96.64% ± 1.29</td>
<td></td>
<td>102.71% ± 1.50</td>
<td></td>
<td>99.34% ± 1.10</td>
<td></td>
</tr>
<tr>
<td>Glimepiride</td>
<td>100.90% ± 4.11</td>
<td></td>
<td>96.15% ± 3.56</td>
<td></td>
<td>98.53% ± 2.06</td>
<td></td>
</tr>
</tbody>
</table>
This test was taken to ensure the accuracy of the method and the interference of the excipients [19,20]. In testing the accuracy, an additional method for glimepiride was made, adding standard glimepiride to the sample being tested in a certain concentration. The result of accuracy test (percent recovery) was illustrated in Table 3 which was in the 80-110% range indicating the accuracy test met the requirements.

The precision of an analytical method is the degree of agreement among each test result when the method is repeated to multiple samplings of a homogeneous sample. The precision is expressed as the Relative Standard Deviation (% RSD) of repeated intraday and interday measurements. Conducting intraday and interday testing is intended to see the effect of time differences on sample repeatability [19,20]. The intraday testing was completed by measuring the levels of the active compounds at 2 different times in a day. In the meantime, the interday testing was performed by measuring the levels of active compounds on different days for 3 consecutive days. The result of the precision test (Table 4) showed that the precision test met the requirements with a %RSD value <2%. The method used to determine the levels of metformin and glimepiride have got good repeatability.

The validation method on the levels of metformin and glimepiride with simultaneous UV spectrophotometry generated a result that matched the requirements. Accordingly, the research continued with an assay test on samples at the market.

The samples purchased at Pramuka market were fixed-dose combination tablets containing 250 mg of metformin and 1 mg of glimepiride. The tablets that met the inclusion criteria consisted of 3 brands, X1, X2 and X3. The result which was illustrated in Table 5 showed the determination of the levels ranged from 96.64% ± 1.29% to 102.71% ± 1.50% of the amount stated on the label. The fixed-dose combination tablets met the requirements, generating the level of 90-110% [12].

**Conclusion**

The validation method of fixed-dose combination tablets of metformin and glimepiride using simultaneous Ultraviolet (UV) spectrophotometry met the requirements. The fixed-dose combination purchased at Pramuka Market had the levels of 96.15% ± 3.56% to 102.71% ± 1.50%. These levels indicated the fixed dose combination tablets met the requirements.

**Acknowledgment**

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Referensi

[1]. World Health Organization, Diabetes, Available from: https://www.who.int/health-topics/diabetes#tab=tab_1. [Last accessed on January 17, 2021]


