DEVELOPMENT AND VALIDATION OF A TLC DENSITOMETRIC METHOD FOR DETERMINATION OF GLIMEPIRIDE IN TABLETS

PENGEMBANGAN DAN VALIDASI METODE TLC DENSITOMETRI UNTUK DETERMINASI GLIMEPIRIDE DALAM SEDIAAN TABLET

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ABSTRACT

A simple and valid TLC method has been developed for the determination of glimepiride in tablet formulation. After extraction of the analyte with a mixture of methanol and ammonia 0.2M (1:1, v/v), the extracts were spotted on precoated TLC silica gel F254 plates, which were developed with a mixture of toluene: methanol:ethyl acetate (75:20:5, v/v/v). Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 238 nm. The method was validated for specificity, linearity, accuracy and precision. Good linearity was achieved in the concentration range 100-800 ng/spot. The RSD of repeatability and intermediate precision were found to be less than 2%, whereas the mean of the recovery data was 100-101%. The detection limit and quantification limit were 22 and 74 ng/spot, respectively. The method is specific, linear, precise, and accurate; it can be used for the routine quality control testing of marketed formulations.

Keywords: glimepiride, TLC densitometric, validation of pharmaceutical methods, pharmaceutical analysis, antidiabetic drug

ABSTRAK


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INTRODUCTION

Glimepiride (Fig. 1) 1-[4-2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl sulphonyl]-3-(4-methylcyclohexyl)-urea is used as an antidiabetic drug in the treatment of type 2 diabetes. It is a second-generation sulfonlurea primarily activating specific receptors on pancreatic β-cell membrane. When glimepirides are bound to receptors, ATP-sensitive potassium channels close, thus resulting in an augmented trans-membrane calcium flux and insulin release from β-cells. Various liquid chromatographic methods have been reported for the determination of glimepiride in human plasma and biological fluids. In bulk drug and in pharmaceutical preparations, multiple analytical procedures have been reported for the analysis of glimepiride when used as a single active principle or in combined dosage forms. Some reported analytical methods involve time and money consuming. There is a need for a simple, rapid, cost effective and reproducible method for assay of glimepiride in its dosage forms. Therefore, it was thought of interest to develop simple, rapid, accurate, specific and precise TLC method for the analysis of glimepiride in its tablet formulation. The objective of the
current work is, to develop a simple, rapid, and validated TLC method for determination of glimepiride (GMP) in tablet formulations.

Figure 1. Chemical structure of glimepiride

EXPERIMENTAL

Materials

GMP reference substance was supplied by PT Dexa Medica (Palembang, Indonesia). Silica gel 60 F_{254} TLC plates (20 cm x 10 cm, Merck) as stationery phase. Methanol was from Sigma Aldrich, toluene was from Merck, ethyl acetate and ammonia were from Riedel-de Haën.

Two commercial tablets, the generic drug (GMP-G) and the Patent ones (GMP-P) containing 4 mg GMP were purchased from a local Pharmacy in Jember. GMP-G, GMP-P were produced in Indonesia.

Preparation of standard solutions

Stock standard solutions were prepared by dissolving accurately weighed GMP (25 mg) in 50 mL and (25 mg) in 25 mL a mixture of methanol and ammonia 0.2M (1:1, v/v). To ensure complete dissolution of the drug it was sonicated for 5 minutes. Various standards solutions were prepared from the two stock solution by dilution with the solvent. For basic linearity studies, solution were prepared containing 50, 100, 150, 200, 250, 300, 350, 400 μg mL⁻¹ ang 2 μL of each of these solutions was spotted on the TLC plate.

Sample Preparation

Two brands of tablets GMP-P and GMP-G were selected. Twenty tablets were weighed and the average weight was calculated. The tablets were then powdered and an amount equivalent to 5.0 mg of GMP was dissolved in 25 mL solvent mixture. To ensure complete extraction of the drug it was sonicated for 30 minutes. This solution was filtered through a filter paper.

TLC method and chromatographic condition

In this TLC method, the samples (2 μL) were applied on the precoated TLC plates 10 mm from the bottom. A Camag double-throght light-weight 20 x 10 cm chamber with glass lid was saturated for 60 min with the mobile phase containing a mixture of toluene:methanol:ethyl acetate in ratio of 75:20:5 (v/v/v). After chamber saturation, the plates were developed to a distance of 80 mm. Densitometric analysis was carried out using a Camag TLC Scanner 3 (Camag). The purity and identity of the analyte spots were determined by scanning in the absorbance reflectance mode from 200 to 700 nm. Quantitative evaluation was performed by measuring absorbance reflectance of the analyte spots at 238 nm. The slit dimension was kept at 6.00 mm x 0.30 mm and a scanning speed of 20 mm/s was employed. Quantitation of the analyte spots was performed by the winCATS software Version 1.4.1.8154 (2005) from CAMAG. Quantitative evaluations were performed via peak areas with linear regression, using at least four-point calibration on each plate.

Method validation

The method was validated for linearity, detection limit (DL), quantification limit (QL), accuracy, precision, and robustness according to the published methods with appropriate modification. For accuracy studies, a three-point standard addition method on commercial tablets (addition 30%, 45%, and 70% of the label claim) was performed on three different days. The precision was also evaluated by using commercial tablets.

RESULTS AND DISCUSSION

Validation of the method

Various mobile phases were evaluated to arrive at abest resolution, peakshape, and RF. The mobile phase consisting of toluene:methanol:ethyl acetate in ratio of 75:20:5 (v/v/v) gave peak shape, RF, and well separated spots of the GMP from the mixture. The Ri values was found to be 0.47±0.01 for GMP. Densitometric GMP was performed at 238 nm. Adequate separation of the drug enabled the
development of a selective and specific method of analysis.

The proposed TLC system demonstrated that all analyte spots in samples furnished in situ UV spectra that were identical to those of standards ($r \geq 0.9997$). Purity check of the analyte spots using the CATS software also showed that all analyte spots of the extracts were pure. The correlation of the spectra taken at the peak start slope and at the peak maximum ($r_{s0}$) was 0.99999 and the correlation of the spectra taken at the peak maximum and at the peak endslope ($r_{e0}$) was 0.9997, demonstrating that the proposed TLC method is highly selective.

**Linearit**

The linear regression data for the calibration plots ($n = 8$) showed a good linear relationship over a concentration range of $100 - 800$ng/2µL spot (concentration of analyte in the spotted solution) for GMP. The slope, intercept, correlation coefficient ($r$), and relative process standard deviation value ($V_{rel}$) are shown in Table 1.

**Limit of detection and quantification**

LOD and LOQ values were found to be 22 and 74 ng/2µl spot, respectively, and pointed towards adequate sensitivity of the method.

**Precision**

The repeatability of sample application and measurement of peak area were expressed in the terms of % CV and found to be 1.91. The results shown in Table 2 revealed intra- and inter-day variation of GMP in six replicates at a concentration of 200 µg/mL. The % CV for within and day-to-day analysis were found to be less than 5.3%.

**Accuracy**

The accuracy of this method was confirmed by determining the average percentage recoveries from the sample by applying the standard addition method. Table 3 demonstrates the high accuracy of the proposed method as revealed by percentage of mean recovery data. The mean percentage recoveries of GMP sample was in accordance with fixed limits of 100 up to 101%, indicating the suitability of the developed method in quantifying the concentration of GMP in pharmaceutical tablets.

**Analysis of commercial products**

A single spot of GMP was observed in the densitogram of the drug samples extracted from tablets. There was no interference from the matrix commonly present in the tablets. The drug content was found to be $99.6\% \pm 2.66$ and $98.1\% \pm 0.45$ for GMP-P and GMP-G respectively. It may therefore be inferred that degradation of GMP had not occurred in the marketed formulations that was analyzed by this method. The low % CV value, given in Table 4, indicated the suitability of this method for routine analysis of GMP in pharmaceutical dosage form.

**CONCLUSION**

A TLC method has been developed for the analysis and quantification of GMP in tablet formulation. This method was found to be specific, sensitive, precise, and accurate for determination and can be employed for the routine quality control analysis of GMP from tablets.

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\text{Figure 2: TLC - Chromatograms obtained from standard solution of GMP in toluene:methanol:ethyl acetate (75:20:5, v/v/v)}
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<table>
<thead>
<tr>
<th>Table 1: Linear regression data for the calibration curve</th>
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<tbody>
<tr>
<td>Parameters</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Linearity range (ng/spot)</td>
</tr>
<tr>
<td>Slope</td>
</tr>
<tr>
<td>Intercept</td>
</tr>
<tr>
<td>$V_{rel}$</td>
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</table>
Table 2: Results of precision evaluation

<table>
<thead>
<tr>
<th>Measurement&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RSD value [%] (n = 6)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.91</td>
</tr>
<tr>
<td>2</td>
<td>1.75</td>
</tr>
</tbody>
</table>

<sup>a</sup>Each measurement was performed by same analyst and on a different plate and different days (intermediate precision).

<sup>b</sup>Evaluated by one analyst on one plate (repeatability).

Table 3: Method accuracy results for GMP tablets

<table>
<thead>
<tr>
<th>Sample</th>
<th>GMP (μg/spot)</th>
<th>% Recovery&lt;sup&gt;a&lt;/sup&gt; (mean ± (RSD))</th>
</tr>
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<tbody>
<tr>
<td>Xc</td>
<td>Xf</td>
<td></td>
</tr>
<tr>
<td>GMP-P</td>
<td>770&lt;sup&gt;a&lt;/sup&gt;</td>
<td>a = 774 100.5% ± 1.55</td>
</tr>
<tr>
<td></td>
<td>782&lt;sup&gt;a&lt;/sup&gt;</td>
<td>b = 782 100% ± 2.01</td>
</tr>
<tr>
<td></td>
<td>794&lt;sup&gt;a&lt;/sup&gt;</td>
<td>c = 799 101% ± 0.94</td>
</tr>
</tbody>
</table>

Xc is nominal concentration of analyte in the spotted solution
Xf is measured concentration of analyte in the spotted solution
<sup>a</sup>Mean ± SD (n = 3; n = 9)
<sup>b</sup>addition 80%
<sup>c</sup>addition 100%
<sup>d</sup>addition 120%

Table 4. Results of analysis of Glimepiride in Commercial Product

<table>
<thead>
<tr>
<th>Sample</th>
<th>GMP (μg/spot)</th>
<th>% Recovery&lt;sup&gt;a&lt;/sup&gt; (mean ± (RSD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label claim</td>
<td>Result of measurement</td>
<td></td>
</tr>
<tr>
<td>GMP-P</td>
<td>403</td>
<td>401.5 99.6 ± 2.67</td>
</tr>
<tr>
<td>GMP-G</td>
<td>400</td>
<td>392 98.1 ± 0.45</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean ± SD (n = 3)

ACKNOWLEDGEMENTS

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