Majalah Farmaseutik
ISSN-e: 2614-0064 | ISSN-p: 1410-590x
Volume 19(3), 468-473

Development of Glibenclamide Validation Method Using UV-Spectrophotometry for Solubility Test in Mesoporous Mannitol

Nindya Kusumorini[1*], Adhyatmika[1], Sheria Itqan Biruni[2], Sukmarini Anugrahanti[2]
[1] Department of Pharmaceutic, Faculty of Pharmacy Universitas Gadjah Mada
[2] Undergraduate student, Faculty of Pharmacy Universitas Gadjah Mada
Corresponding author: Nindya Kusumorini; Email: nindya.kusumorini@ugm.ac.id
Submitted: 06-11-2023 *Revised: 01-12-2023* *Accepted: 01-12-2023*

ABSTRACT

This study was aimed to develop a validation method for glibenclamide in mesoporous mannitol using UV spectrophotometric method. This method was chosen because it is simple, sensitive, accurate, precise, reproducible and economical. The wavelength of glibenclamide (λ max) was obtained at 229 nm using ethanol and water in a ratio of 1:1. The linearity of glibenclamide was selected at a concentration of 5 - 17.5 µg/mL. Correlation coefficient (r) = 0.9998; intraday RSD < 1%; interday RSD < 2%, recovery 100 - 105%, LOD = 0.32 µg/mL and LOQ = 1.08 µg/mL, and has high sensitivity because the presence of mesoporous mannitol in the solution does not interfere with the reading process of glibenclamide. So it can be concluded that this method has good reproducibility based on the results of linearity, precision, and accuracy of glibenclamide and in accordance with ICH requirements, so this method can be used for routine analysis of glibenclamide compounds in mesoporous mannitol.
Keywords: glibenclamide; validation; spectrophotometric; mannitol; mesoporous

INTRODUCTION

Glibenclamide with the IUPAC name 5-chloro-n-[2-[4[[(cyclohexylamino)carbonyl] amino]-sulphonyl]phenyl]-ethyl]-2-methoxy benzamide (Figure 1) or often known as Glyburid is a second-generation sulfonylurea class hypoglycemic drug that is widely used to treat type 2 diabetes mellitus (DM). Gilbenclamide has a mechanism of action by increasing insulin secretion from pancreatic β-cells. In addition to reducing insulin secretion, glibenclamide can also reduce insulin clearance in liver and can increase plasma insulin levels (Aa et al., 2016; Rambiritch et al., 2014; Sola et al., 2015).

Based on Biopharmaceutics Classification System (BCS), glibenclamide is classified as BCS class II, which has low solubility in biological channels and high permeability in cell membranes. Based on literatures, glibenclamide has a water solubility of 0.018 mg/mL at 37 ^{o}C (Liu et al., 2014). This causes the low ability of glibenclamide to reach systemic circulation and results in low bioavailability of the drug, so it has low bioavailability which ranges from 40-45% (Ahmed et al., 2023; Elbahwy et al., 2017; Srivastava et al., 2022).

There are various ways to improve the solubility of glibenclamide, one of which is by engineering glibenclamide in cocrystal form using mesoporous mannitol (Al-Khattawi et al., 2015; Saffari et al., 2015, 2016a). Water-insoluble glibenclamide is loaded into mesoporous mannitol using organic solvents such as ethanol, chloroform or methanol, and then tested for solubility of glibenclamide that has been loaded in mesoporous mannitol. Mesoporous mannitol was chosen because it has a high-water solubility, non-hygroscopic, and has a high pore volume, making it possible to incorporate drugs into mannitol in large quantities (Saffari et al., 2016b).

This study was aimed to determine the validation of a simple, sensitive, accurate, precise and reproducible method in determining glibenclamide compounds in samples. The development of glibenclamide validation method using Spectrophotometry was carried out in accordance with ICH guidelines (Nash & Wachter, 2003).

METHODS

Instrument

Analytical balance (Ohauss PA 214), UV-spectrophotometer (Genesys 10S, USA), vortex (Heidolph Reax Top), stirrer (Stuart Hotplate Stirrer CB162), micropipette (Biologix, USA), and glassware (Pyrex) were used for sample preparation.

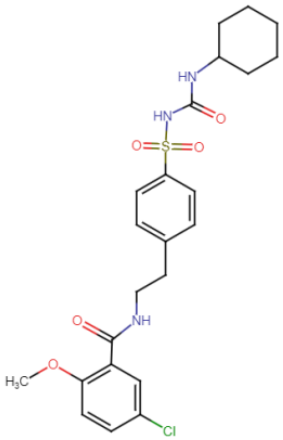

Figure 1. Chemical structure of glibenclamide

Material

Glibenclamide standard ($C_{23}H_{28}ClN_3O_5S$ ≥99%) (Sigma Aldrich), glibenclamide active pharmaceutical ingredient (PT Phapros), D-mannitol (Merck), ethanol 98% (Brataco, Indonesia), ammonium bicarbonate (Sigma Aldrich), aquadest.

Validation of glibenclamide:

Preparation of glibenclamide standard stock solution

Standard glibenclamide was carefully weighed approximately 25.0 mg using an analytical balance (Ohauss PA 214), dissolved in 25 mL of ethanol in a 25 mL volumetric flask and then sonicated for 5 minutes to obtain a glibenclamide standard stock solution of 1000 µg/mL (stock solution 1). Furthermore, 10 mL of stock solution 1 was taken with ethanol and water solvents in a ratio of 1:1 so as to obtain glibenclamide 100 µg/mL standard stock solution (stock solution 2).

Determination of maximum wavelength (λmax)

The 100 µg/mL stock solution was diluted 10x to obtain a concentration of 10 µg/mL and then scanned at a wavelength of 200 to 400 nm against the blank. The wavelength of maximum absorbance was then used for the preparation of the calibration curve.

Analytical method validation procedure

Linearity

A standard concentration series of glibenclamide at 5; 7.5; 10; 12.5; 15; 17.5 µg/mL was made, then the absorbance of each concentration was measured on a UV spectrophotometer.

Specificity

Specificity was evaluated by obtaining UV-Vis spectra of glibenclamide and excipients (D-mannitol, ammonium bicarbonate, mesoporous mannitol) to ensure no interference occurred in the absorbance region. All solutions were then scanned at a wavelength of 200-400 nm.

Accuracy

To determine the accuracy of the proposed method, the concentration of glibenclamide in mesoporous mannitol was made in several levels, namely at low concentration (6 µg/mL), medium concentration (7.5 µg/mL), and high concentration (9 µg/mL) which were then mixed with mesoporous mannitol. Accuracy was assessed as percentage recovery.

Precision

The precision test was determined using different sample concentrations (7.5; 10; 15 µg/mL) and analyzed on intra-day and inter-day. The same procedure was followed for three different days to study the inter-day variation (n=27). The percentage relative standard deviation (% RSD) of the concentration predicted from the regression equation was taken as precision.

***Limit of detection* (LOD) and *limit of quantification* (LOQ)**

The determination of LOD and LOQ values is based on standard deviation values of y intercept values and slope values in linear regression equations from linearity curves. The LOD value is calculated by the formula (ICH, 2005):

$$\text{LOD} = \frac{3{,}3\ x\ {Sy}/_{x}}{b}$$

The LOQ value is calculated using the formula:

$$\text{LOQ} = \frac{10\ x\ {Sy}/_{x}}{b}$$

with Sy/x values calculated using the formula:

$$\frac{Sy}{x} = \sqrt{\frac{\sum(yi - yc)^2}{n - 2}}$$

with: b= slope; yi= y measurement result; yc= y calculated from the regression equation

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Computerized data were statistically described using Microsoft Excel v.10.0 (Microsoft, USA).

RESULT AND DISCUSSION

Maximum wavelength

Based on the results of the maximum wavelength scan of glibenclamide from 200 - 400 nm, the maximum wavelength of 229 nm was obtained (figure 2). The maximum wavelength that has been determined is then used for the reading of glibenclamide compounds.

Specification

The specificity test of glibenclamide in mesoporous mannitol was determined by measuring the absorbance of glibenclamide, glibenclamide in mannitol, and glibenclamide in mesoporous mannitol at a wavelength of 200-400 nm. Based on the chromatogram results, the reading of glibenclamide compounds at the maximum wavelength is not affected by mannitol or mesoporous mannitol added (figure 3). So it can be concluded that mannitol and mesoporous mannitol do not interfere with the reading of glibenclamide at the maximum wavelength of 229 nm.

Linearity and range

Linearity is used to determine the ability of a method to obtain test results that are proportional to the concentration of the analyte in the sample used. Linearity was performed on the standard concentration series of glibenclamide 5 - 17.5 µg/mL. The linear regression equation obtained was y = 0.0514x + 0.0234 with a correlation coefficient of 0.9998. The correlation coefficient (r) obtained has met the specified requirements of >0.999 (Snyder et al., 1997). The standard curve of glibenclamide is shown in Figure 4.

Precision

The precision test is carried out to determine the closeness of the values produced in a measurement under the same analysis conditions. Based on the analysis results, it has been obtained % RSD repeatability (n = 6) (table II), % RSD intraday and % RSD interday (table I) less than 2%. The RSD results obtained have met the requirements of Horwitz and AOAC RSD values (Gustavo González & Ángeles Herrador, 2007).

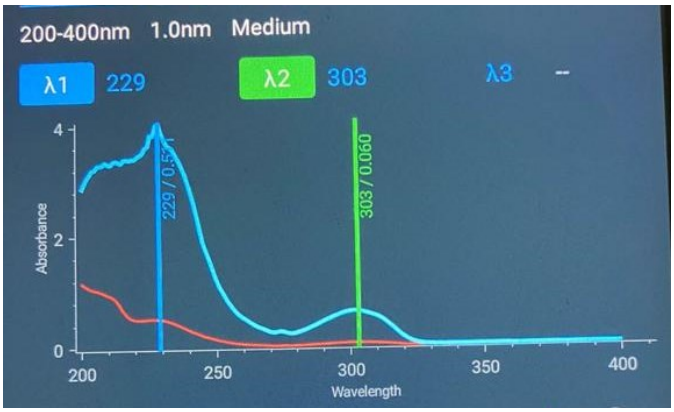

Figure 2. Maximum wavelength results of glibenclamide

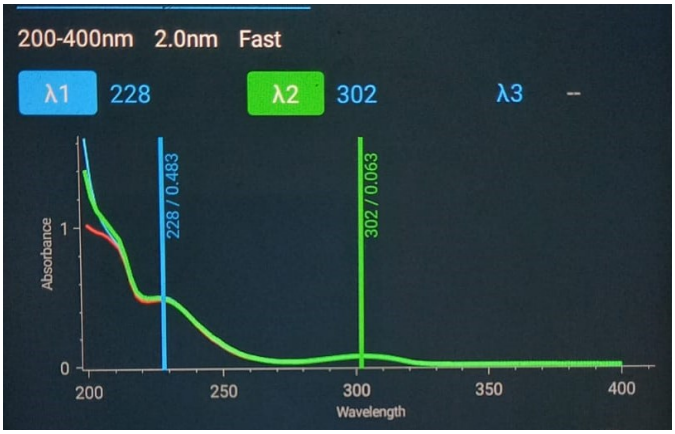

Figure 3. Specificity results of glibenclamide with mesoporous mannitol

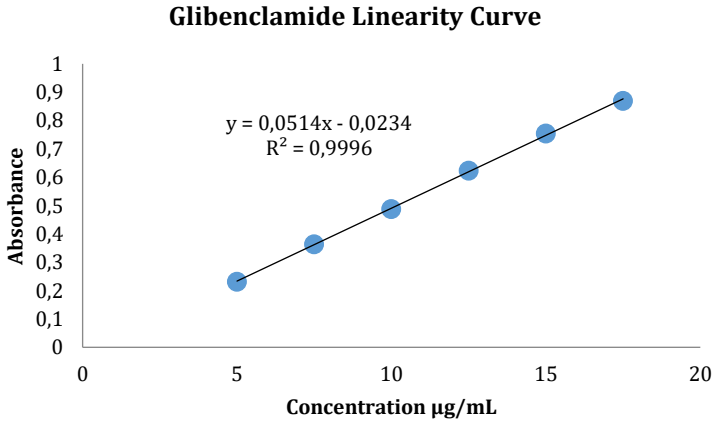

Figure 4. Linearity results of glibenclamide

Accuration

The accuracy test is carried out to determine the closeness of the value results obtained with the actual value. Accuracy is expressed by the percent recovery of the measured content to the actual content. Based on table 2. the resulting % recovery results have met the acceptance requirements of AOAC (Horwitz, 2002).

Table I. Repeatability of Absorbance's

Concentration	% RSD Intraday	% RSD Interday
7.5 µg/mL	0.17	1.69
10 µg/mL	0.11	1.3
15 µg/mL	0.15	0.006

Table II. Validation Parameter for Glibenclamide

No.	Parameters	Results
1.	Absorption maximum (nm)	229 nm
2.	Linearity range (µg/mL)	5 – 17.5 µg/mL
3.	Standard regression equation	y = 0.0514x - 0.0234
4.	Correlation coefficient (r)	R = 0.9998
5.	Specificity	Glibenclamide solution in mesoporous mannitol produces the same absorbance value as pure glibenclamide
6.	Accuracy (% recovery)	94.59 – 96.68 %
7.	Precision RSD Repeatability (n-6)	0.56 %
	Intra-day	0.11 – 0.17%
	Interday	0.005 – 1.69 %
8.	LOD	0.32 µg/mL
9.	LOQ	1.08 µg/mL

LOD and LOQ

LOD and LOQ were determined from the results of the standard regression equation for glibenclamide at a concentration of 5 - 17.5 µg/mL with the regression equation, y = 0.0514x - 0.0234 (figure 4). Based on the calculation results, the LOD value for glibenclamide was 0.32 µg/mL and the LOQ value for glibenclamide was 1.08 µg/mL. The LOD value represents the concentration of analyte that can still be detected and the LOQ value represents the smallest concentration of analyte that can still be quantified accurately and precisely.

CONCLUSION

The development of a validated analytical method of glibenclamide using a UV-spectrophotometer with ethanol and water solvents in a 1:1 ratio has resulted in good reproducibility. Based on the results of linearity, specificity, precision and accuracy, the results obtained have met the requirements of the ICH, so it can be concluded that this analytical method can be used for routine analysis of glibenclamide in mesoporous mannitol samples.

ACKNOWLEDGMENT

The authors would like to thank the Faculty of Pharmacy, Universitas Gadjah Mada for providing financial support during this research through the Young Lecturer Research Grant Program scheme in 2023.

REFERENCES

Aa, T., Ah, B., Cj, B., 2016. Pharmacolog1y and therapeutic implications of current drugs for type 2 diabetes mellitus. *Nature Reviews. Endocrinology*, 12(10).

Ahmed, N., Abo-zeid, Y., Sakran, W., 2023. Strategies Adopted to Improve Bioavailability of Glibenclamide: *Insights on Novel Delivery Systems. Journal of Advanced Pharmacy Research,* 7(1), 35–49.

Al-Khattawi, A., Koner, J., Rue, P., Kirby, D., Perrie, Y., Rajabi-Siahboomi, A., Mohammed, A. R., 2015. A pragmatic approach for engineering porous mannitol and mechanistic evaluation of particle performance. *European Journal of Pharmaceutics and Biopharmaceutics*, 94, 1–10.

Elbahwy, I. A., Ibrahim, H. M., Ismael, H. R., Kasem, A. A., 2017. Enhancing bioavailability and controlling the release of glibenclamide from optimized solid lipid nanoparticles. *Journal of Drug Delivery Science and Technology*, 38, 78–89.

Gustavo González, A., Ángeles Herrador, M., 2007. A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. *TrAC Trends in Analytical Chemistry*, 26(3), 227–238.

Horwitz, W., 2002. *AOAC Guidelines for Single Laboratory*.

ICH., 2005. *Q2 (R1),"Validation of analytical procedures: Text and methodology" ICH Harmonised Tripartite Guideline*. International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use.

Liu, H., Shang, K., Liu, W., Leng, D., Li, R., Kong, Y., Zhang, T., 2014. Improved oral bioavailability of glyburide by a self-nanoemulsifying drug delivery system. *Journal of Microencapsulation*, 31(3), 277–283.

Nash, R. A., Wachter, A. H., 2003. *Pharmaceutical Process Validation: An International*. Routledge & CRC Press. https://www.routledge.com/Pharmaceutical-Process-Validation-An-International/Nash-Wachter/p/book/9780824708382

Rambiritch, V., Maharaj, B., Naidoo, P., 2014. Glibenclamide in patients with poorly controlled type 2 diabetes: A 12-week, prospective, single-center, open-label, dose-escalation study. *Clinical Pharmacology : Advances and Applications*, 6.

Saffari, M., Ebrahimi, A., Langrish, T., 2015. Highly-porous mannitol particle production using a new templating approach. *Food Research International*, 67, 44–51.

Saffari, M., Ebrahimi, A., Langrish, T., 2016a. A novel formulation for solubility and content uniformity enhancement of poorly water-soluble drugs using highly-porous mannitol. *European Journal of Pharmaceutical Sciences: Official Journal of the European Federation for Pharmaceutical Sciences*, 83, 52–61.

Saffari, M., Ebrahimi, A., Langrish, T., 2016b. Nano-confinement of acetaminophen into porous mannitol through adsorption method. *Microporous and Mesoporous Materials*, 227, 95–103. https://doi.org/10.1016/j.micromeso.2016.02.047

Sola, D., Rossi, L., Schianca, G. P. C., Maffioli, P., Bigliocca, M., Mella, R., Corlianò, F., Fra, G. P., Bartoli, E., Derosa, G., 2015. State of the art paper Sulfonylureas and their use in clinical practice. *Archives of Medical Science*, 11(4), 840–848.

Srivastava, D., Fatima, Z., Kaur, C. D., Mishra, A., Nashik, S. S., Rizvi, D. A., Prasad, R., 2022. Glibenclamide–malonic acid cocrystal with an enhanced solubility and bioavailability. *Drug Development and Industrial Pharmacy*.