Maximum Wavelength And Overplay Of Glibenclamid And Its Metabolits 4-Trans-Hydroxyglibenclamid By Uv-Vis Spectrophotometry

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Abstract
Glibenclamide, another name for gliburid, is an oral antidiabetic drug (ADO) that works by blocking K+-ATP channels in pancreatic beta cells, thereby increasing insulin release. 4-trans-hydroxyglibenclamide is the main metabolite of glibenclamide, which also has a hypoglycemic effect. The aim of this study was to develop and validate the mean centering of ratio spectra (MCR) spectrophotometric method for the determination of glibenclamide and 4-trans-hydroxyglibenclamide levels in rabbit plasma. Validation of analytical methods includes linearity tests, determination of LOD and LOQ. This method is carried out in stages, namely making an absorption spectrum for each mixture, making a ratio absorption spectrum, and making an MCR absorption spectrum. Then they tested its validity with validation parameters. The results showed that the maximum wavelength of glibenclamide was at 300.60 nm, while 4-trans-hydroxyglibenclamide was at 299.50 nm. The two spectra overlap in a two way kind of overlap.
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Keyword : *Method Validation, Glibenclamide, 4-Trans-Hydroxyglibenclamide.*

I. INTRODUCTION

Glibenclamide is one of the oral antidiabetic drugs (ADO) that works by closing the K+-ATP channel in pancreatic beta cells, thereby increasing insulin release[1]. Inventive+ paraphrase 4-trans-hydroxyglibenclamide is the main metabolite of glibenclamide and pharmacologically active. It has low hypoglycemic activity with an insulin secretory mechanism. Drugs and their metabolites both have a hypoglycemic effect. Therefore, they have a high possibility of causing hypoglycemia, so it is important to monitor drug levels in the blood or Therapeutic Drug Monitoring (TDM)[2]–[4]. Because of the importance of measuring glibenclamide levels, it is necessary to have a reliable method to determine the levels of glibenclamide and its metabolites.Several studies have used LC-MS [3], [5] both in determining the levels of glibenclamide and its metabolites in plasma and urine.

However, the costs involved are very large. In addition, given the importance and frequent use of these measurements, a method that can be performed repeatedly and simply, that saves time and money, is very much needed. Spectrophotometric methods can be an alternative for estimating time, cost, and measurement sensitivity.The spectrophotometric method begins with knowing the maximum wavelength of each mixture, then an analysis of the overlap between the two mixtures is carried out to determine the type of overlap. This method has been successful and has been used in determining drug concentrations in several mixtures[6]–[9]. Determined drug levels in a binary mixture, namely theophylline and ephedrine HCl [10] , determined drug levels in a ternary mixture, namely salicylic acid, ascorbic acid and paracetamol [6], even Issa et al. , (2013) conducted assays of five drug mixtures at once using MCR and chemometric methods. All the results obtained indicate that the method can be used in determining the drug mixture [6], [9].

II. METHOD

1.1 Method

This research is an experimental research with Ultraviolet Spectrophotometry method). This research was conducted at the Laboratory of Quantitative Pharmaceutical Chemistry.

1.2 Tools and Material

The tools used in this study were complete ultraviolet-visible spectrophotometry (Shimadzu 1800) with a Personal Computer (PC) equipped with UV Probe 2.42 software, analytical balance (Boeco Germany), vocutainer, centrifuge, vortex, glassware, as well as other tools needed in preparation.The materials used in this study were glibenclamide (PT. Indofarma), 4 trans-hydroxyglibenclamide (Sigma) methanol pa, aquabidestylates.

1.3 Preparation of Standard Parent Solution (LIB) of Glibenclamide

Standard glibenclamide mother liquor was prepared with a concentration of 10.02 g/ml in 100 ml of methanol pa

1.4 Preparation of the maximum absorption spectrum of 4-trans-hydroxyglibenclamide

Standard stock solution of 4 trans-hydroxyglibenclamide was prepared with a concentration of 10.02 g/ml in 100 ml of methanol pa.

1.5 Preparation of the maximum absorption spectrum of glibenclamide and 4-trans-hydroxyglibenclamide

The absorption of glibenclamide was measured in a wavelength range of 200-400 nm, respectively.

1.6 Preparation of the maximum absorption spectrum of 4-trans-hydroxyglibenclamide

The absorption of 4-trans-hydroxyglibenclamide was measured in a wavelength range of 200-400 nm,respectively

III. RESULT

3.1 Maximum absorption spectrum of glibenclamide and 4-trans-hydroxyglibenclamide

The wavelength used for quantitative analysis is the wavelength that has the maximum absorbance. This is because (1) at the maximum wavelength, the sensitivity is also maximum; (2) around the maximum wavelength, the absorbance curve forms flat and under these conditions the Lambert-Beer law will be fulfilled; (3) if repeated measurements are made, the error will be very small [9]. The maximum absorption spectra of glibenclamide and 4-trans-hydroxyglibenclamide can be seen in Figure 1 and Figure 2.

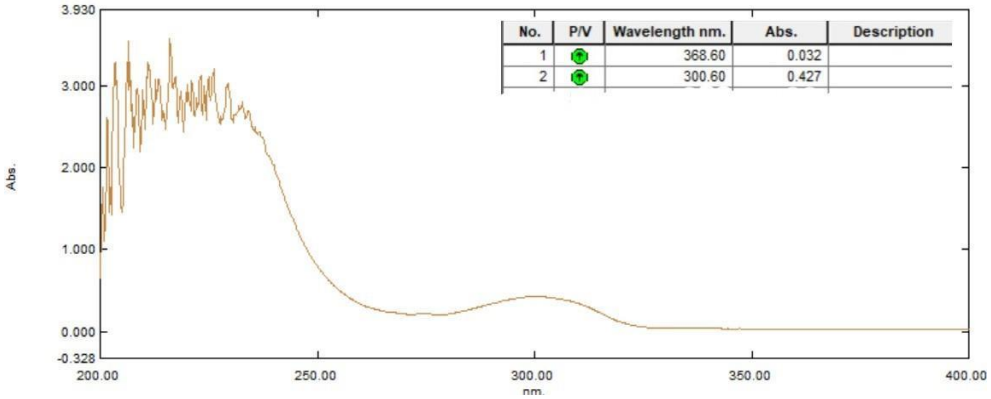

Fig 1. The maximum absorption spectrum of glibenclamide concentration 50µg/ml

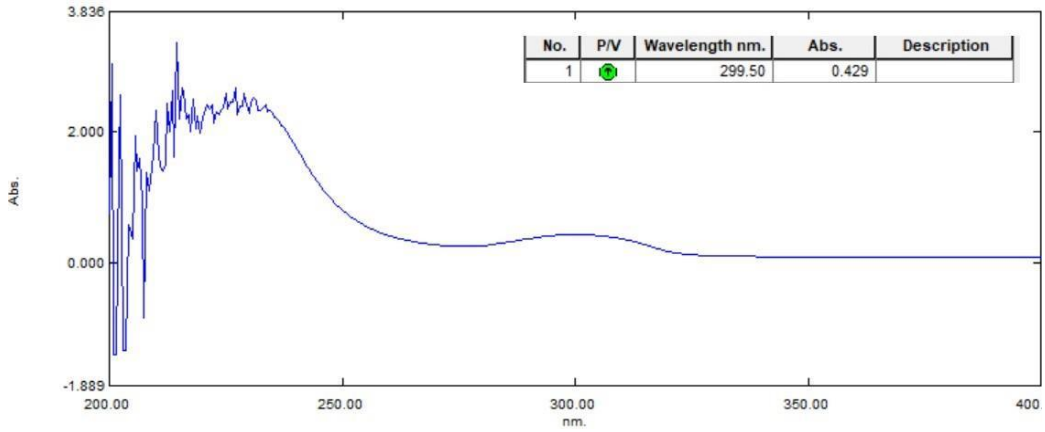

Fig 2. Maximum absorption spectrum of 4-trans-hydroxyglibenclamide at a concentration of 45µg/ml

Based on the results, the maximum absorption of glibenclamide (concentration 50 g/ml) at a wavelength of 300.60 nm and 4-trans-hydroxyglibenclamide (concentration 45 g/ml) at a wavelength of 299.50 nm was obtained. The results obtained are in accordance with Clark's provisions, where according to Clark the maximum wavelength of glibenclamide in methanol solvent is at 272 and 300 nm with valuesA_1^1=63a10Glibenclamide and 4-trans-hydroxyglibenclamide have very close wavelengths, this is because glibenclamide and its metabolites have very similar chemical structures and very close relative masses.

1.7 Overlap of the maximum absorption spectra of glibenclamide and 4-trans-hydroxyglibenclamide

The overlapping maximum absorption spectra of glibenclamide and 4-trans-hydroxyglibenclamide can be seen in Figure 3

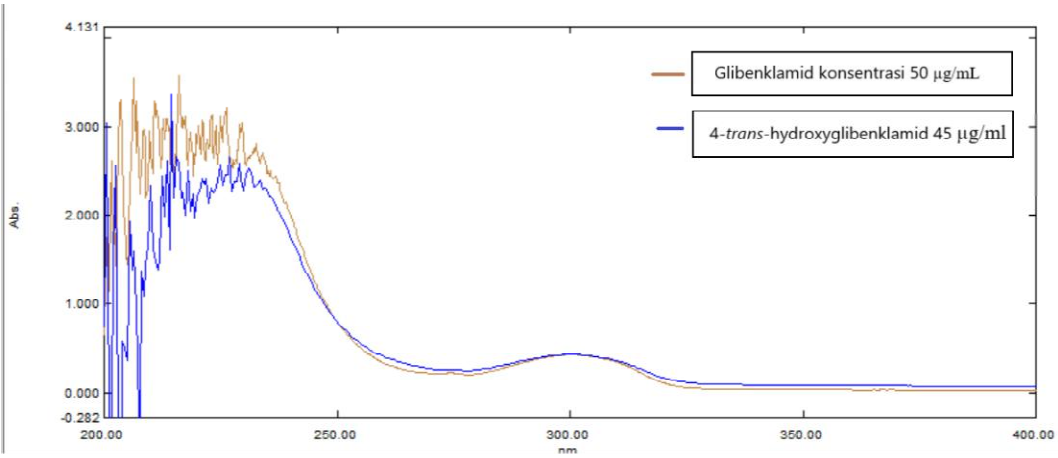

Fig 3. Overlapping the absorption spectrum of the maximum concentration of glibenclamide 50µg/ml and its metabolite 4-trans-hydroxyglibenclamide 45µg/ml.

Based on Figure 2 above, it is clear that what is happening is the third possibility (overlapping in two ways) i.e. there is no wavelength at which one component can be measured without interference by the other component, because the two spectra overlap as a whole. That is, at the maximum absorbance of glibenclamide at a wavelength of 300.60 nm, 4-trans-hydroxyglibenclamide also has its own absorbance. At the maximum absorbance of glibenclamide at a wavelength of 300.60 nm, 4-trans-hydroxyglibenclamide also has its own absorbance. Likewise, at the maximum absorbance of 4-trans-hydroxyglibenclamide at a wavelength of 299.50 nm, glibenclamide also has its own absorbance (Kamal et al., 2016;Afkhami and Bahram, 2005; Muchlisyam et al., 2018)8,10,11

IV. CONCLUSION

The Mean Centering of Ratio Spectra spectrophotometric method meets the method validation requirements

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