Spectrofluorimetric method for the determination of glibenclamide in pharmaceutical formulations

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Abstract:
A sensitive spectrofluorimetric method for the determination of glibenclamide in its tablet formulations has been proposed. The method is based on the dissolving of glibenclamide in absolute ethanol and measuring the native fluorescence at 354 nm after excitation at 302 nm. Beers law is obeyed in the concentration of 1.4 to 10 µg.ml⁻¹ of glibenclamide with a limit of detection (LD) of 0.067 µg.ml⁻¹ and a standard deviation of 0.614. The range percent recoveries (N=3) is 94 - 103.

Key words: Glibenclamide, spectrofluorimetric.

Introduction:
Glibenclamide or glyburide is chemically known as 5-chloro-N-[2-[4-[[[(cyclohexylamino) carbonyl] amino] sulfonyl] phenyl] ethyl]-2-methoxy benzamide as indicated in scheme-1 which shows the structural formula:

![Scheme -1 structural formula of glibenclamide](image)

It has been prepared by Aumuller et. al. in 1966, as crystals from methanol, melting point 169-170 °C, sparingly soluble in water and soluble in usual organic solvents [1], it has been considered as the second generation of sulfonyleureas. It has been widely used in treatment of type 2 diabetic patients after well establishing that this compound acts by increasing insulin release from the beta cells in the pancreas. The Literature survey shows that spectrophotometric methods have been employed for the determination of glibenclamide based on derivatization technique or coupling with another reagent [2-8]. High pressure liquid chromatography methods are the most commonly used for the determination of glibenclamide and different methods coupled with UV detection [9, 10, 11], fluorescence detection [12] or mass spectrometry [13]. Thin layer chromatography (TLC) has been employed for the detecting of glibenclamide [14]. Volta metric method was used [15]. Most of these methods are time consuming, expensive and some of the reagents used may be harmful to human and to the environment.

The aim of this work was to develop a simple and rapid spectrofluorimetric method for the determination of glibenclamide in pharmaceutical formulations with a simple physical treatment and utilizing the native fluorescence of

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glibenclamide at 354 nm after excitation at 302 nm.

Materials and methods:

Apparatus:
A Perkin-Elmer LS50 luminescence spectrophotometer equipped with a 1cm quartz cell has been used to carry out the fluorescence measurements using different values of the excitation and emission slit widths. A Hewlett Packard 8452A diode array spectrophotometer with a response time of 0.1 sec equipped with a 1cm quartz cell, has been used to carry out the absorbance measurements.

Reagents and standard solutions:
Glibenclamide of 99 % purity was purchased from Sigma-Aldrich (USA); absolute ethanol of 99.9 % was supplied by Sharlau (Barcelona, Spain). Ultrapure water with a resistivity of 18.2 MΩ.cm was obtained from a milipore Milli-Q (Bedford, MA).
A stock standard solution of glibenclamide was prepared by dissolving of 25 mg glibenclamide in to 100 ml of absolute ethanol in order to obtain 250µg.ml\(^{-1}\) and was mechanically shaked for 15 minutes to ensure a complete dissolving of glibenclamide. Ultrasonic bath was not used to avoid any type of degradation or cleavage which may occur. A working standard solution was prepared from the stock standard solution by diluting with 50% (v/v) ethanol. The stock standard solution and working standard solution were kept in refrigerator to avoid the effect of temperature and light and they being stable for several days. Glibenclamide tablets, labelled to contain 5 mg glibenclamide were obtained from local pharmacy as indicated in the table-1.

<table>
<thead>
<tr>
<th>No</th>
<th>Commercial names</th>
<th>Manufacturers</th>
<th>Average tablet weight ( g)</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glibil(^{®})</td>
<td>Hikma</td>
<td>0.1365</td>
<td>Jordan</td>
</tr>
<tr>
<td>2</td>
<td>Samaclamide (^{®})</td>
<td>S.D.I</td>
<td>0.19193</td>
<td>Iraq</td>
</tr>
<tr>
<td>3</td>
<td>Norglicem(^{®})</td>
<td>Rottapharm S.L</td>
<td>0.14935</td>
<td>Spain</td>
</tr>
<tr>
<td>4</td>
<td>Glibesyn</td>
<td>Medichemn LTD</td>
<td>0.1831</td>
<td>Cyprus</td>
</tr>
</tbody>
</table>

General procedures:
Construction of the external calibration curve:
Accurate volumes of working standard solution (56 µg.ml\(^{-1}\)) of 0.25, 0.5, 0.75, 1.00 and 1.50 ml were added into five volumetric flasks of 10 ml and the volumes were diluted to the mark with 50% (v/v) ethanol. The fluorescence intensity measurements were carried out at 354 nm after excitation at 302 nm.

Construction of the standard addition method:
This work deals with test pharmaceutical formulations which are complex in character, because the samples under testing were brought from different manufacturers and may have different excipients. The procedure for constructing of the standard addition method is based on taking 10 tablets from each formulation and were powdered using mar mol mortar and an accurate weights of 0.3413 (sample 1), 0.4799 (sample 2 ), 0.3734 (sample3), 0.4577 (sample 4) gm were dissolved in 30 ml of 50% ethanol and mechanically shacked for 15 minutes and filtered into 50 ml volumetric flask. The residue on filter paper was washed four times with 50% ethanol. The filtrate was diluted to 50 ml with ethanol solution. Working solutions were prepared by diluting with ethanol solution in order to obtain 56 µl\(^{-1}\) according to labels. Aliquots of 0.25, 0.5, 0.75 and 1 ml of glibenclamide working standard solution were
transferred into a series of 10 ml volumetric flasks and constant quantity of 0.8930 ml of sample extract working solution was added to each volumetric flask and diluted to 10 ml by adding 50% ethanol solution. This sequence has been done for each sample and the fluorescence intensity measurements were carried out at 354 nm after excitation at 302 nm.

Results and Discussion:

UV-Absorption spectrum of glibenclamide:

Fig.1, shows an absorption spectrum of 5.6 µg.ml⁻¹ glibenclamide dissolved into 50 % (v/v) ethanol which exhibits three absorption bands at 210, 229 and 302 nm and to avoid the absorbance of other substance or substances (excipients) being present might be added to the absorbance of glibenclamide under investigation, so the later absorption band at 302 nm is considered as the more selective with specific absorbance of 0.0195 and molar absorptivity of 16.250 L.mol⁻¹.cm⁻¹. As a consequence, poor sensitivity was achieved by direct spectrophotometric measurements.

Effect of alcohol percentage

Mixed aqueous-organic solvents are widely used in chemistry to enhance the reactivity, solubilities of a wide variety of chemical substances [16]. So the effect of alcohol percentage on the absorbance of glibenclamide was studied at constant concentration of 5.6 µg.ml⁻¹ glibenclamide. The obtained results show that on decreasing the percentage of alcohol, a hypschromic shift was observed, this may be due to the increasing of solvent polarity. Fig.2, shows that 15 - 50% (v/v) alcohol can be used to achieve an adequate absorbance, higher percentage of alcohol were neglected to avoid the background problems.

Fluorescence of glibenclamide

Scheme-1, shows that the structural formula of glibenclamide involves of two aromatic ring system and two carbonyl groups and it is well known that molecular photo excitation leads to a dramatic change in both $\pi \rightarrow \pi^*$ and non-bonding electrons of the ground state and the underlying cause of this is a result of redistribution of electrons following excitation.

The effect of alcohol percentage on the fluorescence intensity of 5.6 µg.mL⁻¹ was studied and the obtained results show that there was no significant difference in the fluorescence intensity measured at 354 nm and 50% alcohol was selected for the next work in order to ensure a complete dissolution of glibenclamide.

Also the effects of instrumental parameters through the changing of the excitation and emission slit width were studied. The obtained results show that on recording emission spectra, one should use a broad excitation band and minimise the emission slit width.
Significant increase in fluorescent intensity was achieved on using an emission slit width of 2.5 nm which was selected as the more appropriate to carry out the quantification of glibenclamide.

**Analytical figures of merits**

The proposed spectrofluorometric method was evaluated under the selected conditions by carrying out both the external and standard addition method. Table-2, shows that Beer’s law was obeyed in both external and standard addition calibration curve methods in the range of 0 to 10 µgml⁻¹ glibenclamide.

**Table - 2 Optical characteristics for the determination of glibenclamide.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>External calibration curve</th>
<th>Standard addition method</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda _{em} ) (nm)</td>
<td>354</td>
<td>354</td>
</tr>
<tr>
<td>Linearity range (µg mL⁻¹)</td>
<td>0-10</td>
<td>0-10</td>
</tr>
<tr>
<td>Limit of detection (µg mL⁻¹)</td>
<td>0.068</td>
<td>0.204</td>
</tr>
<tr>
<td>Standard deviation (S ) (n=3)</td>
<td>0.614</td>
<td>1.977</td>
</tr>
<tr>
<td>Regression equation ( Y= a+ bx )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>1.142</td>
<td>29.697</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>27.075</td>
<td>29.078</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9999</td>
<td>0.9998</td>
</tr>
</tbody>
</table>

* LOD = n*σ/slope;

**Analysis of pharmaceutical preparations:**

On using the external calibration curve method, the obtained recoveries did not resemble the real quantification of glibenclamide in the dosages due to the presence of excipients. A standard addition method approach proved useful. Table-2 shows good recoveries obtained which indicate that on using the standard addition method, the effects of the excipients was completely avoided.

**Table-3 Recoveries of glibenclamide using standard addition method**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labelled amount (mg)</th>
<th>Obtained amount (mg)</th>
<th>Recovery % ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample-1</td>
<td>5</td>
<td>5.11</td>
<td>102.20 ± 1.67</td>
</tr>
<tr>
<td>Sample-2</td>
<td>5</td>
<td>5.19</td>
<td>103.80 ± 2.49</td>
</tr>
<tr>
<td>Sample-3</td>
<td>5</td>
<td>4.75</td>
<td>95.00 ± 1099</td>
</tr>
<tr>
<td>Sample-4</td>
<td>5</td>
<td>4.90</td>
<td>98.00 ± 1.75</td>
</tr>
</tbody>
</table>

**Conclusion:**

The proposed method represent a simple, rapid, sensitive and inexpensive method which can be applied to the analysis of pharmaceutical preparations as no interference from common excipient in commercial preparations was observed.

In comparison with the other existing methods for glibenclamide analysis such as UV-Visible and HPLC, this method is very rapid and no consumes harmful reagents

**References:**


الطريقة تقدير الكليبنكلامايد بطريقة الفلوره في الخلطات الصيدلانية

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 مديرية تربية الكرخ – وزارة التربية.

الخلاصة:
يعرض البحث طريقة لتقدير مركب الكليبنكلامايد في الخلطات الصيدلانية باستخدام ظاهرة الفلوره. تعتمد هذه الطريقة على إذابة المركب في الكحول المطلق وقياس شدة الفلوره الطبيعية عند طول موجي مقداره 0.53 نانوميتر بعد إثارته عند طول موجي مقداره 0.36 نانوميتر. يشير الرسم البياني لشدة الفلوره مقابل التركيز بأن قانون بير ينطبق ضمن مدى التركيز من 3.3 إلى 33 مايكرو غرام/مليلتر وانحراف قياسي قدره 3.233.