SPECTROPHOTOMETRIC ESTIMATION OF METFORMIN IN HUMAN PLASMA

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ABSTRACT:
To develop a simple, selective and sensitive spectrophotometric method and validated for the determination of metformin in human plasma. The method was applied for the determination of metformin from pharmaceutical preparations and plasma samples and after spiking with metformin. The results were checked by standard addition method. A number of pharmaceutical additives and plasma matrix did not affect the determination of metformin. The proposed method was found to be accurate and precise for routine estimation of metformin hydrochloride in human plasma.

Keywords: Metformin, spectrophotometric, standard addition, plasma, pharmaceutical preparation

INTRODUCTION
Metformin HCl (1,1-dimethylbiguanide HCl), first developed in 1957, is one of the most commonly used oral anti-hyperglycemic agents for the treatment of Type II diabetes mellitus. It is currently recommended as first-line therapy in overweight or obese patients with this condition (1). However high concentration of metformin in plasma has been associated with an increase in the incidences of lactic acidosis, particularly in patient in acute renal failures. The methods for the quantitation of metformin from pharmaceutical preparations and biological fluids include spectrophotometry [5, 6], gas chromatography [7-9], high performance liquid chromatography-tandem mass-spectrometry (HPLCMS-MS) [10], reversed phase HPLC [11, 12], ion pair HPLC [13, 14]. The spectrophotometric methods are simple and required sensitivity and selectivity could be achieved. Regarding sample preparation from biological fluids, extraction and clean-up of the sample is a critical first step in bioanalysis and requires high selectivity. Organic liquid-liquid extraction is a simple and effective method of affording sample cleanup for most analytes.

Unfortunately, in the case of metformin, this approach is challenging because of the drug’s polar characteristics. Herein, a method that successfully employs the liquid-liquid extraction of metformin and capable of measuring metformin in small volumes with high sensitivity.

Figure 1: Chemical structure of metformin HCL

MATERIAL AND METHODS:

Chemicals and reagents:
metformin HCl were obtained as gift sample from pharmaceutical Laboratories Ltd. Perchloric acid, Acetonitrile and water used were of analytical grade (FINAR limited, hyderabad, India). All other chemicals and reagents used were analytical grade unless otherwise indicated.
**Instrumentation**

The proposed work was carried out on a ELICO UV-visible spectrophotometer (model SL 210), which possesses a silicon photodiode detector with a 1 cm quartz matched cell. All weighing was done on electronic balance (Wensar HPB 220).

**Selection of Solvents:**

The water solubility of metformin HCL was found to be 1.38 mg/ml, log p value -1.8 and pKa (strongly basic) 12.33. On the basis of solubility study perchloric acid and Acetonitrile was selected for extraction.

**Preparation of Standard Stock Solution of Metformin:**

**Metformin Stock Solution**

An accurately weighed quantity of Metformin (100 mg) was taken in 100 mL volumetric flask and dissolved in water (50 -mL) with the help of ultrasonication for about 5 min. Then the volume was made up to the mark using methanol to get metformin standard stock solution (1 mg / mL).

**Metformin Working Standard Solution**

Metformin standard stock solution 10mL was diluted to 100 mL using to get working standard solution 100 µg / mL.

**Preparation of biosample:**

500µL volume of plasma or erythrocyte from individuals not receiving metformin was transferred to 1.5mLpolypropylene microextraction tube. Extraction was performed by adding 50µL of metformin working standard to the tube and shaking for 60 seconds, followed by centrifugation at 10000g for 10 minutes, the whole organic layer was separated into another tube, then 200µL of perchloric acid 10% was added, the mixture was vortexed and centrifuged at 10000g for 5 minutes. From the organic layer 0.1mL of extract was transferred to 10 mL volumetric flask and volume was made up to 10mL with distilled water. The absorbance of sample is determined using water as blank solution at 233nm.

**Determination of λ Max of Metformin HCL:**

An appropriate aliquot portion of metformin (1 mL) was transferred to 10 mL volumetric flask, the volume was made up to the mark using distilled water to obtain 10ppm solution of Metformin. Drug solution were scanned between 200 nm to 400 nm. Metformin shows λ max at 233nm.

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**Figure 2: spectra of metformin HCL**

**Figure 1: calibration plot of metformin HCL**

**Table 1: Regression and Optical characteristics of metformin**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s law limit (µg/mL)</td>
<td>20-100µg/mL</td>
</tr>
<tr>
<td>Correlation Coefficient (r)</td>
<td>0.997</td>
</tr>
</tbody>
</table>

**Regression equation**

Slope 0.203  
Intercept 0.230
Accuracy [Recovery Study]

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the sample to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula. % Recovery = \( \frac{[A - B]}{C} \times 100 \)

Where,
A = Total amount of drug estimated
B = Amount of drug found on preanalysed basis
C = Amount of Pure drug added
The results are reported in (Table 4).

Table 2: Recovery Study.

<table>
<thead>
<tr>
<th>Drug in solution µg/ml</th>
<th>%recovery±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.10</td>
<td>100.04±0.021</td>
</tr>
<tr>
<td>60.02</td>
<td>99.56±0.056</td>
</tr>
<tr>
<td>80.05</td>
<td>99.59±0.032</td>
</tr>
</tbody>
</table>

S.D. = Standard Deviation

Robustness

The robustness was carried out to evaluate the influence of a small but deliberate variation in the spectrometric condition for determination of Metformin HCl in biosample. The Robustness data for variations in wavelength of detections (±5nm) and the absorbance and its analytical performance parameters of Metformin HCl were shown in Table

Table 3: Robustness Study.

<table>
<thead>
<tr>
<th>Wavelength in nm</th>
<th>metformin 20 µg/mL</th>
<th>Absorbance found in µg/mL Mean S.D. (n=3)</th>
<th>% R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>238</td>
<td>0.4202±0.001</td>
<td>0.0010</td>
<td>21</td>
</tr>
<tr>
<td>228</td>
<td>0.4109±0.019</td>
<td>0.0069</td>
<td>21</td>
</tr>
</tbody>
</table>

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REFERENCES:


