A simple and sensitive method was developed for estimation of Glimepiride (GLIM) in rat Plasma by reverse phase high performance liquid chromatography (RP-HPLC). The drug samples were extracted by liquid-liquid extraction with 300 µL of acetonitrile and 5 mL of diethyl ether. Chromatographic separation was achieved on C₁₈ column using methanol: water (85:15 v/v) as mobile phase at a flow rate of 1 ml/min and UV detection at 230 nm. The retention time of GLIM was found to be 2.5 min. The developed method was validated for accuracy, precision, linearity and recovery. Linearity studies were found to be acceptable over the range of 100 – 6000 ng/mL. The method was successfully applied for the analysis of rat plasma sample for application in pharmacokinetic study, bioavailability.

Keywords: glimepiride, RP-HPLC, rat plasma

INTRODUCTION

Glimepiride, 3-ethyl-4-methyl-N-(4-[N-((1r,4r)-4-methylcyclohexyl-carbamoyl]sulfamoyl]-2-oxo-2,5- dihydro-1H-pyrrole-1-carboxamide, is an
antidiabetic drug. The drug provokes a brisk release of insulin from pancreas by acting on the so called sulfonylurea receptor (SURI) present on the pancreatic β cell membrane. GLIM causes depolarization by reducing the conductance of ATP sensitive K+ channel which enhances Ca²⁺ influx causing degranulation which results in increased rate of insulin secretion at any glucose concentration [1]. The chemical structure of GLIM shown in Figure 1.

![Chemical structure of GLIM](image)

**Figure 1. Chemical structure of GLIM**

Literatures have been reported for the estimation of GLIM in the human plasma and biological samples such as the estimation of GLIM separately by micellar electrokinetic capillary chromatography with diode-array detection (DAD) or ultraviolet detection [2], high performance liquid chromatography (HPLC) with DAD [3], ultraviolet detection detection [4], UV detection by derivative spectrophotometry [5], liquid chromatography-electrospray ionization mass spectrometry (LC-ESI/MS) [6-10], an HPLC method for the quantification of GLIM in tablets [11], the determination of related substances in GLIM [12], an method for quantification of cis-isomer of GLIM by normal phase chromatography [13], the quantification of cis-isomer of GLIM in a bulk drug substance by reverse-phase chromatography [14] and determination of some other drug simultaneously estimated with Glimepiride[15-18] have been reported.

The present work describes a simple HPLC method involved with UV detection making use of methanol and water as components of mobile phase providing advantage over the reported HPLC methods making use of buffer as a component of mobile phase. Beside this the method is short and shows good degree of LOD and LOQ. The method has been successfully applied for pharmacokinetic studies in rats.

**Experimental**

**Instrumentation**

A double beam UV-Visible spectrophotometer, model UV-2401 PC (Japan) with 10mm matched quartz cell was used.

The HPLC instrument consisted of Thermo separation product quaternary gradient equipped with pump spectra system P-4000 having inline membrane degasser. Detector was a UV visible detector belonging to spectra system UV 1000, rhodyne 9725 manual injector with 20 μL loop. All the data was processed using data ace software. Separation was achieved using a prontosil C18 stationary phase (250 x 4.6 mm i.d. 5 μm particle size) and the analytical column was protected by a Phenomenex C18 guard column (4mm × 2.0 mm, i.d.).

**Materials and Reagents**

Glimepiride was gifted genorously by Zim laboratories pvt. ltd. All the reagent and chemical used were of AR analytical & HPLC grade. Methanol (Spectrochem) and water (Lobachem) used were of HPLC grade.
Chromatographic conditions

All determinations were carried out at room temperature. The isocratic separation of compounds was carried out by using mobile phase consisting of methanol: water (85:15 v/v). The flow rate was maintained at 1 mL/min. The volume of injection was 20 µL. The mobile phase was filtered through 0.45 µm membrane filter and degassed by ultrasonification.

Preparation of Standard Solutions

Glimepiride stock and working solution

The stock solution of GLIM was prepared by dissolving 10 mg in 100 mL of methanol and further dilutions were prepared in methanol to obtain working solution of GLIM in the range of 100-6000 ng/mL.

Preparation of Sample

Plasma samples were stored at −20°C and allowed to thaw at room temperature before processing. In brief, 100 µL plasma, 100 µL aliquot of working standard solution of GLIM was added in a polypropylene centrifuge tubes and was added with 300 µL of acetonitrile and 5 mL of diethyl ether. Then tubes were centrifuged for 10 min at 3000 rpm. The clear supernatent layer was transferred into another conical glass tube and organic layer completely evaporated at room temperature. After evaporation the residue was dissolved in mobile phase. Resultant samples were injected in developed chromatographic conditions. The three different concentrations of quality control samples for further validation of develop method was selected 100 ng/mL (LQC), 800 ng/mL (MQC) and 4000 ng/mL (HQC) of GLIM.

Application of the Assay

The above method was successfully applied for the Pharmacokinetic studies of GLIM. Sprague-Dawley rats (200 - 250 g) were housed with free access to food and water [CPCSEA registration no.- (650/02/C/CPCSEA/08)]. The rats were fasted overnight with free access to water before administration of drugs. After a single oral administration of 0.5 mg/kg of GLIM, rats were anesthetized and blood samples (0.5 mL) were collected from retro orbital plexus sinus at 0.5, 1, 2, 4, 6, 12 and 24 h time-points. Plasma was separated by centrifugation and stored at −20°C until analysis. Aliquots of 0.1 mL serum samples were processed and analyzed for GLIM.

The pharmacokinetic parameters were calculated with a Non-Compartmental model using Kinetica TM Soft-ware (version 4.4.1 Thermo Electron Corporation, U.S.A). Each value is expressed as Mean ± SD.

Results and Discussion

Method Validation

Selectivity and Specificity

Blank plasma was studied for endogenous interference. A representative chromatogram of the plasma blank is shown in Figure 2(a). No additional peaks of endogenous substances were observed. Figure 2(b) shows the chromatograms of calibration standard containing 500 ng/mL of GLIM in plasma.
Figure 2. HPLC trace of GLIM using Ultraviolet detection at 230 nm. (a) Blank plasma sample; (b) Quality control standard (500 ng/mL).

**Linearity and Limit of Quantitation:**

Linear calibration curves with correlation coefficients greater than 0.9999 were obtained over the concentration range 100 – 6000 ng/mL for GLIM in plasma. The coefficient of regression i.e. \( r^2 = 0.9924 \) for GLIM. The results shown that within the concentration range indicated there was an excellent correlation between peak area ratio and each concentration of GLIM.

The limit of quantification, defined as the lowest concentration analyzed with an accuracy of ±15% and a co-efficient of variation <15%, was 100 ng/mL and Limit of determination was 30 ng/mL for the determination of GLIM in plasma.

**Accuracy**

Accuracy study was performed for GLIM in terms of recovery studies. % recovery and % RSD were calculated (Table 1).

**Table 1. Results of Accuracy studies**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>QC Sample (ng/mL) GLIM</th>
<th>Recovered amount (ng/mL) GLIM</th>
<th>Accuracy (%) GLIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>100</td>
<td>95</td>
<td>97.5%</td>
</tr>
<tr>
<td>02</td>
<td>800</td>
<td>780</td>
<td>97.5%</td>
</tr>
<tr>
<td>03</td>
<td>4000</td>
<td>3984</td>
<td>99.6%</td>
</tr>
</tbody>
</table>
Precision

Inter-day and intra-day precision studies were done by injecting 3 serial dilutions in developed chromatographic conditions (n=6). For precision studies QC samples were injected (n=6). Peak areas were calculated for % RSD values, results for inter-day and intra-day precision are shown in table no II respectively.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>QC sample of drug solution (ng/mL)</th>
<th>Inter-day Precision</th>
<th>Intra-day Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Peak Area GLIM</td>
<td>%RSD</td>
</tr>
<tr>
<td>1</td>
<td>100ng</td>
<td>50.7±0.9</td>
<td>1.77</td>
</tr>
<tr>
<td>2</td>
<td>800ng</td>
<td>410.3±8.39</td>
<td>2.04</td>
</tr>
<tr>
<td>3</td>
<td>4000ng</td>
<td>1798.46±18.7</td>
<td>1.03</td>
</tr>
</tbody>
</table>

Averaged for six measurements at each concentration level (n = 6); % recovery = (response of extracted spike) / (response of post-extracted spike) × 100.

Extraction Recovery

Extraction recovery of GLIM was determined by comparing peak areas obtained from extracted plasma samples with those found by extracting blank matrices through the extraction procedure and spiking with a known amount of GLIM. The results showed that the mean extraction recovery of GLIM was >85% (Table 3). Different organic extraction solvents were evaluated in the experiment, including methanol, acetonitrile, chloroform and diethylether. Diethylether and acetonitrile combination proved to be the most efficient in extracting GLIM from plasma and had a small variation in extraction recoveries over the concentration range.

<table>
<thead>
<tr>
<th>QC sample of GLIM (ng/mL)</th>
<th>Mean % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>91.2</td>
</tr>
<tr>
<td>800</td>
<td>86.4</td>
</tr>
<tr>
<td>4000</td>
<td>89.7</td>
</tr>
</tbody>
</table>

Application of the Analytical Method in Pharmacokinetic Studies

The described method was applied to a pharmacokinetic study in rats. After a single oral administration of GLIM (0.5 mg/kg) to rats, plasma concentrations were determined over a period of 24 h after administration. The mean plasma concentration-time curve after an oral dose of GLIM (0.5 mg/kg) is shown in Figure 3 and the main pharmacokinetic parameters are summarized in Table 4. The Cmax of GLIM detected in the rats was 8.6 µg/mL, and the Tmax was 4 hrs.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>AUC0-t (µg.h/mL)</th>
<th>AUC0-∞ (µg.h/mL)</th>
<th>C max (µg)</th>
<th>Tmax (hr)</th>
<th>Kel</th>
<th>T1/2 (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLIM</td>
<td>35.57±0.329</td>
<td>36.05±0.04</td>
<td>8.6±0.1</td>
<td>4 hr</td>
<td>0.19±0.05</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Conclusion

In the present studies a simple, accurate, precise method was developed for the estimation of GLIM by HPLC. The developed method was simple employing water and not a buffer as component of mobile phase. The developed method was short with elution of both GLIM less than 5 min and specific with no interferences of blank matrix interfering the quantification of GLIM. The developed method was applied successfully for pharmacokinetic studies of GLIM. The applicability of method suggests its further application for bioequivalence, and bioavailability studies.

Acknowledgement

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References


Figure 3. Mean serum concentration-time profile of GLIM after oral administration of 2.5 mg/kg of GLIM in rats.


