Simultaneous Estimation of Antihypertensive and Antidiabetic Drugs by HPLC
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ABSTRACT
A simple, fast and economic reversed phase high performance liquid chromatographic (HPLC) method has been successfully developed and validated for simultaneous determination of an antihypertensive and an antidiabetic drug namely, valsartan and glimepiride in pure form (as API). Before the method development with HPLC, a complexation study was carried out with these drugs by DSC which demonstrated no complexation among these drugs. Therefore, a simple HPLC method was developed and validated according to the guidelines of ICH, FDA and USP with respect to accuracy, precision and linearity. For method development a C-18 bonded silica column (250 x 4.6 mm, 5 μ, Phenomenex, Inc.) and the mobile phase comprising 3% aqueous acetic acid in acetonitrile (60:40, v/v) at a flow rate of 0.7 ml/min were used. For accuracy, the recovery was found to be more than 99% for each of valsartan and glimepiride. Intra-day and inter-day precisions of the new method were less than the maximum allowable limit (RSD% ≤2.0) according to FDA guidelines. The method showed linear response with correlation coefficient of 0.9993 for valsartan and 0.9981 for glimepiride.

Therefore, the developed method was found to be simpler, accurate, reproducible, efficient and less time consuming and can be successfully applied for the simultaneous estimation of valsartan and glimepiride.

INTRODUCTION
Valsartan is an angiotensin II-receptor blockers (ARBs) used to treat heart failure patients who are intolerant to angiotensin-converting enzyme (ACE) inhibitors or have an unsatisfactory response to ACE inhibitors [1,2]. Glimepiride is a second generation hypoglycemic drug. It is a more potent oral antihyperglycemic drug belonging to sulfonylurea group and effective at low doses in patients with non-insulin-dependent diabetes mellitus [3]. The structures of the drugs are shown in Figure 1.

DSC technique is most commonly used to determine the drug-drug interactions [4,5]. Therefore, the complexation study was evaluated in vitro by DSC which is a thermoanalytical technique whereby the difference in heat flow between a sample and reference are measured as a function of temperature[6]. In HPLC, separation of drugs of closely related chemical structures can be efficiently achieved and characterized on the basis of polarity, solubility profile, pKa value etc. It is a widely used technique to estimation drugs.

To ensure the effectiveness of the drug, quality and efficacy assessments and maintenance of proper dosage schedule are of great importance. Manufacturers have to evaluate their products during and after manufacturing processes and at various time intervals during the shelf life of every product. Therefore, it is needed to study and determine the potency and efficacy of valsartan...
Several methods have been previously reported in the literature for determination of valsartan and glimepiride in the pharmaceutical formulations but there is no report on the simultaneous determination of these drugs in pharmaceutical dosage forms. To the best of our knowledge, this is the first report for the simultaneous determination of valsartan and glimepiride using non-buffer mobile phase containing 3% aqueous solution of acetic acid with acetonitrile.

Therefore, a rapid and sensitive reversed phase high performance liquid chromatographic method was developed and validated according to the guidelines of FDA, ICH, and USP with respect to accuracy, precision, specificity and linearity [7-11]. The developed method was found to be simpler, accurate, reproducible, efficient and less time consuming, and was applied successfully for the study of valsartan and glimepiride formulations.

**MATERIALS AND METHOD**

**Drugs and reagents**

Working standards of glimepiride (99.25%) and valsartan (99.9%) were kind gifts from Drug International Ltd., Dhaka, Bangladesh. HPLC grade acetonitrile and acetic acid were purchased from Active Fine Chemicals Ltd., Dhaka, Bangladesh.

**Instrumentation**

Differential Scanning Calorimeter (DSC) (Model: DSC-60 WS, Shimadzu Corporation, Japan) was used for recording DSC thermograms. High Performance Liquid Chromatography (HPLC) (UFLC Prominence, Shimadzu Corporation, Japan) equipped with an auto sampler (Model-SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for the analyses. The data was recorded using LC-solutions software. Phenomenex C-18 (4.6 × 250 mm, 5 μm) column was used for the analyses.

**Mixing for DSC study**

To study whether the drugs form complexation with each other, a mixture of these drugs was prepared by admixing a 1:1 (w/w/w) ratio of valsartan and glimepiride. Standard solutions of the pure drugs were prepared by dissolving equivalent to 10 mg each of valsartan and glimepiride in a 10 ml volumetric flask using mixture of water-acetonitrile (50:50). The resulting solution was evaporated to dryness by a water bath at below 60 °C and the mixture was indicated as C-1 and evaluated in vitro by DSC. Experiment was performed at a temperature ranging from 30 ºC to 600 ºC with the rate of 10 ºC/min increase of temperature under nitrogen gas at a flow rate of 20 ml/min. Then 4 mg of the drug mixture (C-1) was taken in an aluminum pan and sealed properly. The DSC thermograms were recorded for samples sealed in aluminum pan and the peak temperature of melting of the sample and the heat of fusion were determined from the DSC traces by thermal analysis program. The thermograms of individual drugs were also taken in the same manner. Indium (melting point 156.0 °C) was used as a standard for calibrating the instrument.

**Study by high-performance liquid chromatography (HPLC)**

HPLC is a chromatographic technique that can separate a mixture of compounds and is used to identify, quantify and purify the individual components from the mixture. Retention time and absorbance of peak of one species in solution may be changed due to the interaction with other species. In the present study, HPLC analyses were carried out according to following methods.

**Preparation of mobile phase:** To prepare a 3% aqueous acetic solution (pH 2.5), 30 ml glacial acetic acid was added in about 500 ml of double distilled water in a 1000 ml volumetric flask and finally water was added up to the mark. Then the solution was sonicated for 10 minutes followed by filtration through a 0.22 µm Millipore filter. HPLC grade acetonitrile was also filtered and degassed before use into the HPLC system.

**Chromatographic conditions:** All analyses were done at ambient temperature under isocratic condition. The mobile phase contained 3% aqueous acetic acid solution and acetonitrile in the ratio of 60:40 (v/v) at a flow rate of 0.7 ml/min. The injection volume was kept at 20 μl for all analyses. Before analysis, every standard and sample was filtered through 0.45 μm filter tip. The column eluate was monitored at 240 nm.
Preparation of standard solutions: Equivalent to 30 mg of each drug was weighed and taken in a 100 ml volumetric flask. At first the drugs were dissolved separately in 50 ml acetonitrile and distilled water (50:50) and then the solvent was added up to the mark to make the volume 100 ml. The concentration of the each solution was 240 µg/ml.

Then 50 mL was taken in a flask from each of the solutions of glimepiride and valsartan, and the solution mixed properly and termed as C-2 solution of which concentration was 100 µg/mL for each drug. Then by calculation and dilution procedure, solutions of various concentrations were prepared.

**METHOD VALIDATION**

**Specificity:** The specificity of the method was evaluated to ensure that there was no interference from the excipients present in the pharmaceutical product. The specificity was studied by injecting the standard solution and pharmaceutical product of valsartan and glimepiride.

**Linearity:** For linearity 40, 45, 50, 55 and 60 µg/ml of standard drug solutions were prepared. For this purpose 4 ml, 4.5 ml, 5 ml, 5.5 ml and 6 ml stock solution was transferred from C-2 solution in a 10 ml volumetric flask and the volume 10 ml was made by adding the solvent up to the mark of the volumetric flask. Then 20 µL from each solution was injected into the HPLC by auto-sampler. The analyses were monitored at 240 nm and repeated three times. The average peak areas were plotted against concentrations and calibration curves were used to calculate slope and intercept values as well as to evaluate the linearity of the proposed method by calculating the coefficient of correlation.

**Accuracy:** The accuracy of an analytical method expresses the closeness between the expected value and the value found. It is expressed by calculating the percent recovery (%R) of the drug recovered. In this case, three successive analyses for three different concentrations of standard solutions of valsartan and glimepiride (10 µg/mL, 20 µg/mL and 30 µg/mL) were carried out by using the developed method.

**Reproducibility:** Reproducibility expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample. It was checked by intra- and inter-day repeatability of responses after replicate injections and expressed as %RSD amongst responses using the formula [%RSD=(Standard deviation/mean) x 100%].

In the current method development and validation protocol, precision was determined by three replicate analyses of each of the concentrations of 20 µg/mL and 30 µg/mL of standard valsartan and glimepiride solutions using the proposed method.

**RESULTS AND DISCUSSION**

Before the method development with HPLC, a complexation study was performed with valsartan and glimepiride by DSC and found that no complexation occurred among these three drugs (Figure 2). After then, a reversed phase HPLC method was developed and validated as per ICH, USP and FDA guidelines for simultaneous determination of valsartan and glimepiride. The DSC thermogram of mixture of valsartan and glimepiride showed the absence of any interaction between the drugs.

![Figure 2. HPLC chromatogram of valsartan and glimepiride.](image)

In HPLC analyses all the drugs were detected at 240 nm using mobile phase comprising of 3% aqueous solution of acetic acid and acetonitrile in the ratio of 60:40 (v/v) at ambient temperature with a flow rate of 0.7 ml/min. The injection volume was kept at 20 µL for standards and samples. The retention time of valsartan and glimepiride were found at 8.6 ± 0.1 and 13.7 ± 0.1 min, respectively (Figure 2).

A mixture of market preparations containing valsartan and glimepiride were analyzed to observe the specificity of the method. No peak was detected close to the retention time of valsartan and glimepiride which proved the high degree of specificity of the method.

To get the calibration curve, five different concentrations (40 µg/mL, 50 µg/mL, 60 µg/mL, 70 µg/mL and 80 µg/mL) were used. When peak areas were plotted against these concentrations, good correlation coefficients (r²), 0.9993 for valsartan and 0.9981 for glimepiride were obtained, which were within the acceptable range of guidelines. These revealed good linear relationship of the newly developed method (Figure 3).
The accuracy was evaluated at three different concentrations which were conducted in successive analysis (n=3) using the proposed method and the values were expressed as percentage of recovery between the mean concentrations found and added concentrations for both of these drugs. The average percentage of recovery was found to be within 98-103% for valsartan and glimepiride at 10 μg/mL, 20 μg/mL, 30 μg/mL (Table 1) which were within the acceptable range. The acceptable range for% recovery for API drug is 97-103% (Ref). The reproducibility of the proposed method was checked by intra- and inter-day repeatability of responses after replicate injections of standard solutions (20 and 30 μg/mL). The reproducibility is expressed as%Relative Standard Deviation (%RSD). Levels were analyzed three times within the same day (intra-day variation) and three other days (inter-day variation) (Table 2).

Table 1. Accuracy of valsartan and glimepiride.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Injected conc. (μg/mL)</th>
<th>Recovered conc. (μg/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valsartan</td>
<td>10</td>
<td>10.30</td>
<td>103.00</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.73</td>
<td>98.65</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.68</td>
<td>98.33</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>10</td>
<td>10.12</td>
<td>101.20</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.10</td>
<td>100.50</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>29.88</td>
<td>99.60</td>
</tr>
</tbody>
</table>

Table 2. Intra-day precision of valsartan and glimepiride.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Day</th>
<th>Injected conc. (μg/mL)</th>
<th>Mean recovered (μg/mL)</th>
<th>SD</th>
<th>Intra-day %RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valsartan</td>
<td>Day 1</td>
<td>20</td>
<td>19.81</td>
<td>0.23</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>20</td>
<td>19.34</td>
<td>0.24</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>20</td>
<td>19.95</td>
<td>0.19</td>
<td>0.93</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>Day 1</td>
<td>20</td>
<td>19.14</td>
<td>0.22</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>20</td>
<td>19.34</td>
<td>0.21</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Day 3</td>
<td>20</td>
<td>19.95</td>
<td>0.17</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Since, the method was developed and validated according to the guidelines of FDA, ICH and USP with respect to accuracy, precision and linearity, it may be suitable for routine analysis of samples and APIs.

CONCLUSION

The efficacy of a drug depends upon some absolute requirements such as quality, potency, etc. It is obvious that a little change in the formulation or variations in the manufacturing process or use of low quality materials including APIs can affect the efficacy of the drugs leading to harmful effects to the patients. Therefore, quality and efficacy assessment and maintenance of proper dosage schedule are strongly needed to ensure the effectiveness of the drug. Hence, we planned to study the potency of most commonly used antihypertensive and antidiabetic drugs namely, valsartan and glimepiride which are frequently prescribed in Bangladesh.

To attain this objective, a rapid and sensitive reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated according to the guidelines of FDA, ICH and USP with respect to accuracy, precision, specificity and linearity. The newly developed method has been found to be simpler, accurate, reproducible, efficient and less time consuming, and has been applied successfully for the simultaneous determination of an antihypertensive and an antidiabetic drugs namely, valsartan and glimepiride.

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