Simultaneous Determination of Ibuprofen and Sitagliptin by HPLC

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ABSTRACT

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID), widely used over the counter drug worldwide. Co-administration of antidiabetic drugs with non-steroidal anti-inflammatory drug (NSAID) such as Ibuprofen is quite common. Rapid liquid chromatographic procedure proposed for analysis of Ibuprofen and Sitagliptin in pharmaceutical preparations using methanol: water (80:20 v/v) as a mobile phase, adjusting pH to 3.2 with UV detection at 275 nm. The advantages of this method include good and rapid separation, well resolved peaks, and only a small amount of sample is required for assay that display adequate precision. The method showed good linearity in the range of 231.25-1000 μg mL⁻¹ for Ibuprofen and Sitagliptin with a correlation coefficient of 0.9999. The recovery of Ibuprofen and Sitagliptin was > 99.99% and > 99.96%, respectively. The proposed method may be used for the quantitative analysis of Ibuprofen and sitagliptin alone or in combination in bulk drugs and combined dosage formulations.

Keywords: Ibuprofen, Sitagliptin, Formulation, HPLC, Analysis

INTRODUCTION

Ibuprofen belongs to a non-steroidal anti-inflammatory drug (NSAID) that comprises of arylpropionic acid derivatives. Chemically ibuprofen is [(RS)-2-[[4-(2-methylpropyl)phenyl]] propanoic acid (figure 1). [1]. Molecular weight is 206 and structural formula is C13H18O2.[2][ It shows analgesic, anti-inflammatory, and antipyretic activity [3,4].

Sitagliptin is an oral antihyperglycemic drug that belongs of the dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs[5]. This drug is used either alone or in combination with other oral antihyperglycemic agents for treatment of type 2 diabetes mellitus[6].

There are a number of liquid chromatographic methods reported in the literature for the determination of Ibuprofen by HPLC [7-9] Several methods also reported for determination of Sitagliptin [10-14]. Although there are a number of methods reported for the determination of both Ibuprofen and Sitagliptin separately or in combination with other drugs [15], no method is reported for the simultaneous determination of these drugs using RP-HPLC in pharmaceutical formulations.

METHODOLOGY

Standard bulk drug sample of Ibuprofen were supplied by Glaxo Smith Kline, tablet formulation of Ibuprofen was used including Brufen 400mg Abbott Laboratories Pakistan Ltd, Standard bulk drug sample of Sitagliptin were supplied by Highnoon Laboratories Ltd. HPLC grade acetonitrile and methanol
were obtained from RCI Labscan Limited, Darmstadt, Germany.

**Instrumentation**

High pressure liquid chromatography system model Shimadzu LC-20 AT, equipped with UV–visible detector model SPD-10A(V) vp, connected by CBM-102 communication Bus Module Shimadzu to Intel Pentium 4 machine with Shimadzu_ Class LC-20 Version 1:62 is used for integration and processing of chromatograms. HPLC Column Mediterranea Sea-18 of dimension 150 mm 4.6 mm i.d. 5 µm was used as the analytical column. All analysis was done at ambient temperature (25± 2 ºC). The mobile phase is a mixture of methanol-acetonitrile-water (80:5:20, v/v) and using orthophosphoric acid pH was adjusted to 3.2. The flow rate was 1.0 mL.min⁻¹ and volume of injection was 10 μL. Before using all solutions the mobile phase was sonicated for 30 min by WUC-A02H on-line degasser and filtered through 0.45-micron membrane filter, calibrated Pyrex glassware was used for the solution and mobile phase preparation. UV detection was performed at 275 nm for Ibuprofen and Sitagliptin.

**Preparation of Solution**

For the concentration of 1000 μg/mL⁻¹, 100 mg of the Ibuprofen and Sitagliptin was diluted to 100 mL using mobile phase. This solution was used for preparation of working solutions which were prepared by diluting the stock solutions using the same solvent to contain 31.25-1000 μg/mL⁻¹ for Ibuprofen and for Sitagliptin then filtered with 0.45-micron membrane filter. These solutions were ready to inject.

**Analysis in Formulation**

Twenty tablets of one brand of Ibuprofen and Sitagliptin were accurately weighed, grinded to create a fine powder. Calculated amount of powder of brand of ibuprofen was weighed, which was corresponding to 100 mg of Ibuprofen and transferred to a separate 100 mL volumetric flask and was dissolved in the mobile phase i.e. methanol-water, 80:20 v/v and filtered using a membrane filter of pore size 0.45 µ. The sample solution was further diluted to desire concentrations i.e. 31.25-1000 μg/mL⁻¹ and then used for the analysis.

**RESULTS AND DISCUSSION**

**Method development and optimization**

To select the optimal chromatographic conditions, different C18 stationary phases have been tried. Best separation, adequate resolution short retention time and symmetric peak of IBU and Internal Standard were achieved by Mediterranean Sea-18 of dimension 150 mm 4.6 mm i.d. 5 µm. To investigate appropriate wavelength for determination of Ibuprofen and Sitagliptin, we scanned solution of both drugs by UV-visible spectrophotometer. It was observed that the maximum absorbance of drug was obtained at 275 nm. Initially methanol and water were tried in different ratio o, at the ratio f 90:10 (v/v), the peak was not symmetrical. When mobile phase methanol-water, 80:20 v/v was used the drug showed typical peak nature and symmetry. To select the optimum mobile phase pH range 2.5 to 4.0 were investigated, excellent performance was achieved at pH 3.2 adjusted with orthophosphoric acid. Total run time was less than 7 min; short analysis times are essential for routine analysis.

**Method validation**

According to US Pharmacopeia and ICH guidelines, using a range of parameters (including system suitability, selectivity, specificity, accuracy test, linearity, precision, robustness, ruggedness, sensitivity, limit of detection and quantification) the developed method was validated.

**Specificity and selectivity**

To ascertain the specificity of the proposed method in presence of pharmacopoeial impurities, no peak of excipient was seen in
chromatogram, which authenticates that the method can be applied productively to dosage formulation and the method demonstrated excellent resolutions. Figure 5 represents typical chromatograms of drugs.

![Fig. 1. Structure of Sitagliptin](image_url)

![Fig. 2. Structure of Ibuprofen](image_url)

![Fig. 3. Linearity of Ibuprofen](image_url)

![Fig. 4. Linearity of Sitagliptin](image_url)

**Table 1. Regression Statistics**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc(µg mL⁻¹)</th>
<th>Regression Equations</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>31.25-1000</td>
<td>y = 1293.x + 39351</td>
<td>0.999</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>31.25-1000</td>
<td>y = 990.8.x + 4220</td>
<td>0.999</td>
</tr>
</tbody>
</table>

**Table 2. Accuracy of Ibuprofen and Sitagliptin in Formulation**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Conc injected µg mL⁻¹</th>
<th>Conc found µg mL⁻¹</th>
<th>% Recovery</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>250</td>
<td>249.99</td>
<td>99.99</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>499.99</td>
<td>99.99</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1000.0035</td>
<td>100.0035</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Drugs Conc injected µg mL⁻¹ Conc found µg mL⁻¹ % Recovery %RSD

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitagliptin</td>
<td>250</td>
<td>249.9</td>
<td>99.96</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>500.05</td>
<td>100.01</td>
<td>0.002</td>
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<tr>
<td></td>
<td>1000</td>
<td>1000.009</td>
<td>100.0009</td>
<td>0.32</td>
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</tbody>
</table>

Table 3. Interday Intraday Precision of Ibuprofen and Sitagliptin in API

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Conc injected µg mL⁻¹</th>
<th>% RSD</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>31.25</td>
<td>0.00082</td>
<td>100.002</td>
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<tr>
<td></td>
<td>62.5</td>
<td>0.00052</td>
<td>101.001</td>
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<td></td>
<td>125</td>
<td>0.00026</td>
<td>99.98</td>
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<td></td>
<td>250</td>
<td>0.00033</td>
<td>99.99</td>
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<tr>
<td></td>
<td>500</td>
<td>0.00008</td>
<td>99.99</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.00004</td>
<td>100.0035</td>
</tr>
<tr>
<td>Sitagliptin</td>
<td>31.25</td>
<td>0.0004</td>
<td>99.98</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>0.0002</td>
<td>100.002</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>0.0006</td>
<td>99.95</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.0001</td>
<td>99.96</td>
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<td></td>
<td>1000</td>
<td>0.00021</td>
<td>100.0009</td>
</tr>
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</table>

System suitability and Linearity
Parameter of system suitability are peak height, theoretical plates of the column, mass distribution ratio (capacity factor), retention time and resolution as summarized in table 1. Linearity was determined in the range 31.25-1000 µg mL⁻¹ for both the drugs. Concentration of Ibuprofen and Sitagliptin versus peak area was subjected to least square linear regression analysis. A linear regression line was obtained with correlation coefficient (R² > 0.999). The regression equations for active and serum (Figure 3 & 4) were shown in Table 2.

Accuracy and Precision
Method accuracy was evaluated as the percentage of recovery of known amounts of Ibuprofen and sitagliptin of the pharmaceutical formulation. It is performed at concentration that was 50%, 100% and 150%. Each sample was injected five times and result range was 99.99-100.003% for ibuprofen and 99.96-100.01, compiled in Table 3, high recovery specifies that the method has a high degree of accuracy. By repeatability i.e. intra-day precision and intermediate precision i.e.inter-day precision of the projected method was determined. It was expressed as relative standard deviation i.e. RSD. Six different concentrations of Ibuprofen and Sitagliptin in the linear range were analyzed in the same day (intra-day precision) and two consecutive days (inter-day precision); every sample was injected five times. Both intra- and inter-day RSD values were in the range 0.00004-0.00082 for Ibuprofen and 0.0001-0.0006 for Sitagliptin confirming excellent precision as shown in Table 4. The results were insignificant and indicated no noteworthy deference in interday and intraday precision.

Limit of detection and limit of quantification
The limits of detection i.e. LOD (calculated by
At least concentration of Ibuprofen and Sitagliptin) and quantification i.e. LOQ were determined from the calibration curve. The LOD and LOQ were 1.44 ng mL⁻¹ and 4.3 ng mL⁻¹ for the Ibuprofen and for Sitagliptin it was 1.2 ng mL⁻¹ and 2.2 ng mL⁻¹ respectively.

Robustness and Ruggedness
Robustness was performed by making minor variations in the percentage of mobile phase (methanol-water) wave length, pH and flow rate. Therefore, samples were injected repeatedly five times under small variations in each of the parameter. When a parameter was changed ± 0.2 % (in flow rate that was 1 ml min⁻¹), ± 0.2% (pH 3.2), and ± 5 % wave length from its optimum condition, the shifting in retention time of ± 0.2% was also observed that evaluated as insignificance. The method proved to be fairly stable (Table 5). Ruggedness of our proposed method was determined by two different operators and two consecutive days. All parameters were compared, the developed method did not show any remarkable difference in calculated results from acceptable limits in precision, but the area under curve of peak was affected with change of wavelength.

CONCLUSION
The method is accurate and precise as indicated from the recovery study and the reasonable retention times (< 10 minutes). It can be concluded that the proposed HPLC method has great promise for the simultaneous determination of Ibuprofen and Sitagliptin in pharmaceutical formulations.

REFERENCES


