EXTRACTIVE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF DIPEPTIDYL PEPTIDASE-4 INHIBITORS IN BULK AND THEIR PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A new economic, industrially acceptable and readily adaptable method has been developed following a complexation (acid-dye method) between Dipeptidyl peptidase-4 inhibitor drugs and dyes like Bromocresol green (BCG) and Bromothymol blue (BTB) and validated for determination of these drugs in bulk and their pharmaceutical dosage forms. Drugs used during analysis i.e. Sitagliptin phosphate (SGP) and Vildagliptin (VIL) were reacted with BCG and BTB in presence of acetate buffer pH 4.1. The colored complex formed was extracted with chloroform and the absorbance of the solutions were noted which followed a beer’s law in concentration range of 5-50μg/mL for Drug-BTB complex and 10-50μg/mL for Drug-BCG complex with correlation coefficient close to 0.998. The method was validated as per ICH guidelines for accuracy, precision, limit of detection and limit of quantification. The developed spectrophotometric method has the advantages of speed, simplicity, sensitivity and more affordable instrumentation and could find application as a rapid and sensitive analytical method for Sitagliptin phosphate and vildagliptin.

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INTRODUCTION

Background

Dipeptidyl peptidase-4 inhibitors are a newer category of oral hypoglycemic agents available in market. There are several USFDA approved drugs in this category but mainly sitagliptin phosphate and vildaglipitin are available in market all over the world as their tablet dosage forms. Sitagliptin Phosphate (SGP) and vildaglipitin (VIL) are chemically 7-[(3R)-3-Amino-1-oxo-4-(2,4,5-trifluorophenyl)butyl]-5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4 triazolo[4,3a]pyrazine-phosphate and (5)-1-[N-(3-hydroxy-1-adamantyl)glycyl]pyrrolidine-2-carbonitrile respectively. Both the drugs are not official in any of the pharmacopeias. Dipeptidyl peptidase 4 (DPP-4) inhibitors are a class of oral anti-hyperglycemic agents which improve glycemic control in patients with type 2 diabetes by enhancing the levels of active incretin hormones. Incretin hormones, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinoicropic polypeptide (GIP), are released by the intestine throughout the day, and levels are increased in response to a meal. The incretins are part of an endogenous system involved in the physiological regulation of glucose homeostasis. When blood glucose concentrations are normal or elevated, GLP-1 and GIP increase insulin synthesis and release from pancreatic beta cells by intracellular signaling pathways involving cyclic AMP. Treatment with GLP-1 or with DPP-4 inhibitors has been demonstrated to improve beta cell responsiveness to glucose and stimulate insulin biosynthesis and release. Several analytical methods like UV-spectrophotometry [1-3], Spectrofluorimetry [4], HPLC [5-8] and LC-MS [9] methods have been developed and validated for determination of these drugs in bulk, their pharmaceutical dosage forms and in biological fluids. All these methods require sophisticated instruments and complex sample preparation steps. Moreover due to absence of any conjugated double bonds in structure of vildaglipitin, it does not absorb in UV spectrum increasing the difficulties in its estimation by usual detectors. Thus it was thought of interest to develop new, simple, economic, accurate, precise and industrially applicable extractive spectroscopic methods for determination of these drugs in bulk and their pharmaceutical dosage form.

METHODS

Preparation of standard stock solutions

Accurately weighed SGP (20 mg) and VIL (20 mg) were transferred into two separate 10 mL volumetric flasks, dissolved and diluted up to the mark with distilled water. Aliquots (1mL) were transferred into another set of 10 mL volumetric flasks and diluted up to the mark with distilled water (Concentration= 200 μg/mL for SGP and VIL respectively).

Preparation of Dye solutions

Accurately weighed bromocresol green dye (BCG 100 mg) and bromothymol blue (BTB 100mg) were transferred into two separate 100 mL volumetric flasks. About 10 mL of methanol was added in both the flasks; flasks were sonicated for 10 min to assist dissolution, and diluted with distilled water up to the mark. (Concentration= 1mg/mL).

Preparation of buffer solutions

All the buffer solutions prepared were as per the formulas listed in USP NF 20.

Optimization of solvent for extraction of Drug-Dye Complex and selection of wavelength

Standard stock solution of SGP (1mL) was transferred into a series of different 25mL glass stoppered test-tubes. In each test-tube, 1mL of BCG dye solution was added along with 3 mL of 0.1N HCl. The solutions were mixed well and 10 mL of various organic solvents (chloroform, dichloromethane, ethyl acetate, toluene, diethyl ether and petroleum ether) were added to different tubes. The tubes were vortexed on cyclomixer for 2 min and were kept aside for layers to separate. From each test-tube, the organic layer was collected and filtered through anhydrous sodium sulphate (to remove traces of water) and was scanned for determination of wavelength maximum and its absorbance. The same procedure was adopted with another dye (BTB) and for another drug (i.e. VIL) for both the dyes.

Optimization of pH for Drug-Dye complex formation

Standard stock solution of SGP (1mL) was transferred into a series of different 25mL glass stoppered test-tubes. In each test-tube, 1mL of BCG dye solution was added along with 3 mL of different buffers (buffers of varying pH). The solutions were mixed well and 10 mL of chloroform was added in each tube. The tubes were vortexed on cyclomixer for 2 min and were kept aside for layers to separate. The organic layers were collected and filtered through anhydrous sodium sulphate (to remove traces of water) and the absorbances were measured at 423 nm. The same procedure was adopted for BTB dye and for another drug (i.e. VIL) and absorbances were measured at 415 nm.

Stoichiometric determination of Drug-Dye Complex (JOB’S Curve Method)

Equimolar solution of drugs and dyes (1.0 × 10⁻³) were prepared in distilled water. From this drug solution (0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4 and 2.7mL) were transferred into a series of 25 mL glass stoppered test tubes. To these tubes varying mL of dye solution (2.7, 2.4, 2.1, 1.8, 1.5, 1.2, 0.9, 0.6 and 0.3 mL respectively) were added in such a way that molar concentration of drug and dye remains constant while their mole fractions vary. To each test-tube were added 3 mL of acetate buffer pH 4.1 and 10 mL of chloroform. The tubes were vortexed on cyclomixer for 2 min and were kept aside for layers to separate. The organic layers were collected and filtered through anhydrous sodium sulphate (to remove traces of water) and the absorbances were measured at wavelength maximum (415 nm in case of BTB dye and 423 nm for BCG dye). Absorbance that is proportional to complex formation is plotted against the mole fractions of these two components.
Stability of formed Drug-Dye complex
Stability of formed drug-dye complex was ascertained by continuously monitoring the absorbance values of the colored complexes using UV-spectrophotometer. Absorbance values were noted at an interval of five minutes till there was deflection of more than 2% absorbance value than the initial. This time interval was termed as stability of the colored drug-dye complex.

Validation Studies
Calibration Curve (Linearity)
Stock solution of standard SGP (0.5, 1.0, 1.5, 2.0 and 2.5 mL) were transferred into a series of five 25 mL glass stoppered test-tubes. Each test-tube were added 1mL of BCG dye, 3mL of acetate buffer pH 4.1 and the volume was made upto 7 mL with distilled water. Chloroform (10 mL) was added in each tube and was vortexed on cyclomixer for 2 min. The tubes were kept aside for layers to separate and the organic layer was collected, filtered through anhydrous sodium sulphate, UV visible spectrum of each solution was obtained and absorbance of colored ion pair complex was measured at 423 nm. The methodology was adopted in triplicates. A plot of mean absorbance vs. concentration of drug was plotted and linear regression equation was computed. The same procedure was adopted with another dye (BTB) and with another drug (VIL) for both the dyes.

Intraday and inter day precision
Intraday precision was determined at three different levels of standard calibration curve (10, 30, 50 µg/mL) for three times in the same day. Inter day precision was determined by analyzing drug samples over a period of three different days.
Limit of detection and quantification
LOD and LOQ were determined using following equation as per ICH guidelines:

\[ \text{LOD} = 3.3 \frac{\sigma}{S} \]
\[ \text{LOQ} = 10 \frac{\sigma}{S} \]

Where \( \sigma = \text{S.D of } y\text{-intercept of calibration curves} \)
\( S = \text{Mean of slope of calibration curve} \)

Accuracy
Accuracy is a measure of the exactness of the analytical method. Accuracy was determined by spiking the known amount of standard solution into the pre-analyzed market formulation sample at three levels (50, 1000 and 150%). % recoveries were determined for every drug-dye complexes.

Assay of Market formulations
(Januvia-100 mg SGP, Galvus-50 mg VIL, Jalra-50 mg VIL)

For JANUVIA®
Ten tablets were accurately weighed and finely powdered. Powder equivalent to 100 mg SGP was transferred into 100 mL volumetric flask. To this, 50 mL distilled water was added and the flask was sonicated for 15 min, the solution was filtered using Whatman filter no.41 and volume was made upto the mark with distilled water. Aliquot (1 mL) was transferred into a 10 mL volumetric flask and was diluted with distilled water upto the mark. Two mL of this solution was transferred into a 25 mL glass stoppered test-tube and procedure was followed as per 4.7.

For JALRA® and GLAVUS®
Ten tablets were accurately weighed and finely powdered. Powder equivalent to 50 mg VIL was transferred into 50 mL volumetric flask. To this, 25 mL distilled water was added and the flask was sonicated for 15 min, the solution was filtered using Whatman filter no.41 and volume was made upto the mark with distilled water. Aliquot (1 mL) was transferred into a 10 mL volumetric flask and was diluted with distilled water upto the mark. Two mL of this solution was transferred into a 25 mL glass stoppered test-tube and procedure was followed as per 4.7.

RESULTS AND DISCUSSION
Chemistry of the Reaction
Both the drugs in presence of acidic pH (acetate buffer pH 4.1) undergo ionization and form a quaternary amine in solution whereas the dyes at this pH form an anion. Thus a complex is formed between cationic drug and anionic dye which could be easily extracted in organic solvent. The drug and dye forms a complex of a peculiar stochiometric ratio and excess of the components remain ionized in aqueous phase and could not be extracted. The reaction mechanism is depicted in Figure-1.
Figure 1: Chemistry of the Reaction
Optimization of solvent for extraction of Drug-Dye Complex

An organic solvent must be selected before the start of analysis in order to ensure maximum (complete) extraction of Drug-Dye ionic complex from aqueous phase. Amongst various organic solvents tried Chloroform and Dichloromethane gave almost similar results with slight higher absorbance values in case of chloroform (Figure 2). Moreover, dichloromethane is more volatile when compared to chloroform. Thus chloroform was selected for extraction of drug-dye complex throughout the analysis.

![Solvent Optimization](image)

**Figure 2: Solvent Optimization results for maximum extraction of Drug-Dye Complex**

Selection of optimum wavelength

The extracted drug-dye complex was scanned over a range of 400-800 nm for establishing wavelength maximum. It was found to be 423 nm when BCG dye was used for both the drugs and 415 nm in case of BTB dye. Thus the results showed that the wavelength maximum was specific to dye rather than a drug. Figure 3 displays the spectrum of extracted drug-dye complex.

![UV spectrum](image)

**Figure 3: UV spectrum for each Drug-Dye complex, A) SGP-BCG Complex B) SGP-BTB Complex C) VIL-BCG Complex D) VIL-BTB Complex**
Optimization of pH for Drug-Dye complex formation
pH of the solution plays a definite role in formation of Drug-Dye ionic complex. The results obtained (Figure 4) clearly indicated that acetate buffer pH 4.1 provided optimum environment for formation of Drug-Dye complex and hence was utilized throughout the analysis.

Figure 4: Optimization of pH for Drug-Dye complex formation

Stoichiometric determination of Drug-Dye Complex (JOB’S Curve Method)
The results indicates (Figure 5) that 1:1 (drug:dye) ion-pair is formed through the electrostatic attraction between the positive protonated drug and the anion of dye. The graph of the results obtained gave a maximum at a molar ratio of Xmax ~ 0.5 which indicated the formation of a 1:1 SGP-BCG, SGP-BTB, VIL-BCG and VIL-BTB complex.

Figure 5: Stoichiometric determination of Drug-Dye complex

Stability of formed drug-dye complex

<table>
<thead>
<tr>
<th>Complex</th>
<th>SGP-BCG</th>
<th>SGP-BTB</th>
<th>VIL-BCG</th>
<th>VIL-BTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability Time (min)</td>
<td>50</td>
<td>120</td>
<td>40</td>
<td>120</td>
</tr>
</tbody>
</table>

Validation Studies
The linearity was established in range of 10-50 µg/mL for SGP-BCG and VIL-BCG while 5-50 µg/mL for SGP-BTB and VIL-BTB respectively. Intra and Interday precision results clearly indicated that the % RSD values were less than 2% and the method was precise. Limit of detection and quantification were calculated as per ICH guidelines. Accuracy study was carried out at three levels (50, 100 and 150%) by standard addition method. The results of validation studies are summarized in Table 1. The assay results for various formulations are summarized in Table 2.
Table 1 Summary of validation parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>BCG DYE</th>
<th></th>
<th>BTB DYE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SGP</td>
<td>VIL</td>
<td>SGP</td>
<td>VIL</td>
</tr>
<tr>
<td>1</td>
<td>Linearity (µg/mL)</td>
<td>10-50</td>
<td>10-50</td>
<td>5-50</td>
<td>5-50</td>
</tr>
<tr>
<td>2</td>
<td>Regression (r²)</td>
<td>0.997</td>
<td>0.996</td>
<td>0.998</td>
<td>0.997</td>
</tr>
<tr>
<td>3</td>
<td>Intraday Precision (% RSD)</td>
<td>0.86-1.22</td>
<td>1.47-1.54</td>
<td>0.43-0.72</td>
<td>0.40-0.54</td>
</tr>
<tr>
<td>4</td>
<td>Interday Precision (% RSD)</td>
<td>1.30-2.33</td>
<td>1.58-1.93</td>
<td>0.87-1.36</td>
<td>0.42-0.51</td>
</tr>
<tr>
<td>5</td>
<td>Accuracy (% Recovery)</td>
<td>97.26 ± 1.46</td>
<td>97.61 ± 0.87</td>
<td>99.21 ± 0.62</td>
<td>99.97 ± 1.82</td>
</tr>
<tr>
<td>6</td>
<td>LOD (µg/mL)</td>
<td>3.43</td>
<td>1.90</td>
<td>1.45</td>
<td>1.65</td>
</tr>
<tr>
<td>7</td>
<td>LOQ (µg/mL)</td>
<td>10.40</td>
<td>5.77</td>
<td>4.41</td>
<td>5.00</td>
</tr>
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</table>

Table 2 Assay of Market Formulations

<table>
<thead>
<tr>
<th>Brand</th>
<th>Labeled Claim</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BCG</td>
</tr>
<tr>
<td>Januvia</td>
<td>Sitagliptin Phosphate -100mg</td>
<td>99.01</td>
</tr>
<tr>
<td>Jalra</td>
<td>Vildagliptin - 50 mg</td>
<td>105.49</td>
</tr>
<tr>
<td>Glavus</td>
<td>Vildagliptin - 50 mg</td>
<td>97.00</td>
</tr>
</tbody>
</table>

Comparison between developed methods

The comparisons made in Table 3 clearly indicated that both the developed methods could be utilized for analysis of Sitagliptin phosphate and Vildagliptin in bulk as well as their available market formulations, but more precise and accurate results would be obtained utilizing Bromothymol blue (BTB) dye.

Table 3 Comparison between developed methods

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Parameter</th>
<th>BCG Dye</th>
<th></th>
<th>BTB Dye</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SGP</td>
<td>VIL</td>
<td>SGP</td>
<td>VIL</td>
</tr>
<tr>
<td>1</td>
<td>Linearity Range (µg/mL)</td>
<td>10-50</td>
<td>10-50</td>
<td>5-50</td>
<td>5-50</td>
</tr>
<tr>
<td>2</td>
<td>Regression (r²)</td>
<td>0.997</td>
<td>0.996</td>
<td>0.998</td>
<td>0.997</td>
</tr>
<tr>
<td>3</td>
<td>Limit of Detection</td>
<td>3.43</td>
<td>1.90</td>
<td>1.45</td>
<td>1.65</td>
</tr>
<tr>
<td>4</td>
<td>Accuracy (% Recovery)</td>
<td>97.26</td>
<td>97.61</td>
<td>99.21</td>
<td>99.97</td>
</tr>
<tr>
<td>5</td>
<td>Stability of complex (min)</td>
<td>50</td>
<td>40</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>6</td>
<td>% Assay</td>
<td>99.01</td>
<td>97.00</td>
<td>100.60</td>
<td>100.50</td>
</tr>
</tbody>
</table>

CONCLUSION

The new colorimetric method is the first described method for the analysis of sitagliptin phosphate and vildagliptin by acid-dye complexation methodology. It has low concentrations of calibration ranges compared to the other existing photometric methods. There was no interference from common excipients encountered, and it is of equivalent accuracy with other reported HPLC method for their determinations. The method is simple, highly sensitive, accurate, precise, economical and suitable for routine analysis of Sitagliptin phosphate and vildagliptin in bulk and drug formulations.

REFERENCES

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