DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN AND METFORMIN IN BULK AND COMBINED PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: To develop simple, accurate, precise UV Spectrophotometric method for the simultaneous estimation of Sitagliptin and Metformin in tablet dosage form.

Methodology: The method is based on the determination of Sitagliptin and Metformin in tablet using simultaneous equation method. Sitagliptin exhibits maximum absorbance at 267 and Metformin exhibits maximum absorbance at 237 nm using distilled water as diluents.

Results: The calibration curve was linear in the range of 10-300 µg/ml for Sitagliptin and 4-14 µg/ml for Metformin. The %RSD were within the limit i.e., less than 2%. The % recovery of the proposed method was found to be 97.12-99.46% for Sitagliptin and 98.15-99.85% for Metformin. The LOD of the proposed method was 0.397 µg/ml for Sitagliptin and 0.8952 µg/ml for Metformin. The LOQ was 1.2951 µg/ml for Sitagliptin and 2.7159 µg/ml for Metformin.

Conclusion: A simple, accurate, precise UV Spectrophotometric method for the simultaneous estimation of Sitagliptin and Metformin in tablet dosage form.

Keywords: UV Spectrophotometric method, Sitagliptin, Metformin, simultaneous estimation

INTRODUCTION

Sitagliptin is chemically (R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine [1]. It is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor used in conjunction with diet and exercise to improve glycemic control in patients with type 2 diabetes mellitus. Sitagliptin inhibits DPP-4 which leads to increased levels of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), decreased levels of glucagon, and a stronger insulin response to glucose. This enzyme splits the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal [2].

Metformin is chemically, 1, 1-dimethyl biguanide hydrochloride [3]. Metformin is an antihyperglycemic agent of the biguanide class, used for the management of type II diabetes. It is a first line agent for the treatment of type 2 diabetes that can be used alone or in combination with sulfonylureas, thiazolidinediones, incretin-based drugs, sodium glucose cotransporter-2 inhibitors, or other hypoglycemic agents. Metformin is commonly described as an insulin sensitizer leading to a decrease in insulin resistance and a clinically significant reduction of plasma fasting insulin levels. Bio analytical, HPLC, HPTLC and UV-visible spectrophotometry methods have been reported for its individual determination of Metformin and in combination with other drugs [4-7].

Fig. 1: Sitagliptin phosphate

Fig. 2: Metformin hydrochloride

Sitagliptin Phosphate and Metformin Hydrochloride are available in combined dosage forms as film coated tablets (JANUMET). Each
tablet contains 50 mg of Sitagliptin Phosphate and 500 mg of Metformin Hydrochloride. The combination of Metformin and sitagliptin has been shown to be safe, effective and well-tolerated treatment for type II diabetes [8].

MATERIALS AND METHODS

Chemicals and reagents

Sitagliptin and Metformin hydrochloride API was gifted by pharma company, Hyderabad, Telangana, India. The marketed formulation, JANUMET 50 mg/500 mg was procured from the local market, distilled water.

Instruments

Double beam UV spectrophotometer; Model: SL 210; Make: ELICO. The output signal was checked and the acquisition and integration of data was performed using spectral treats Software on a computer. Contech electronic balance and labline sonicator was used during the experimentation.

Method development

Preparation of sitagliptin standard stock solution

10 mg of Sitagliptin was accurately weighed and transferred into 10 ml of volumetric flask and the volume was made up to the mark with distilled water to obtain the concentration of 1000µg/ml.

Preparation of metformin hydrochloride standard stock solution

10 mg of Metformin hydrochloride was accurately weighed and transferred into 10 ml of volumetric flask and the volume was made up to the mark with distilled water to obtain the concentration of 1000µg/mL.

Determination of \( \lambda_{max} \)

10µg/ml solution of Sitagliptin and Metformin hydrochloride were prepared from the standard stock solution and scanned in the range of 200-400 nm. Sitagliptin and Metformin hydrochloride showed maximum absorbance at 267 nm and 237 nm respectively.

RESULTS AND DISCUSSION

Validation parameters

Validation is the process of “establishing documented evidence” which provides high degree of assurance that a specific activity will consistently produce desired results or product meeting its predetermined specifications and quality specifications.

Linearity

From the standard stock solution of Sitagliptin pipette out 0.1-3µg/ml to obtain the concentration of 10-300 µg/ml.

From the standard stock solution of Metformin pipette out 0.04-0.14 µg/ml to obtain the concentration of 4-14 µg/ml.

| S. No. | Parameters | Results |
|-------|------------|---------|
| 1.    | Absorbance maximum (nm) | 267     |
| 2.    | Linearity and range (µg/ml) | 10-300µg/ml |
| 3.    | Slope | 0.0064 |
| 4.    | Correlation coefficient | 0.9991 |
| 5.    | Y-intercept | 0.0929 |

Fig. 4: Calibration curve of sitagliptin

Fig. 5: Calibration curve of metformin
Table 2: Results of quantitative determination of metformin

| S. No. | Parameters                      | Results     |
|--------|---------------------------------|-------------|
| 1.     | Absorbance maximum (nm)         | 237         |
| 2.     | Linearity and range (μg/ml)     | 4.1-14μg/ml |
| 3.     | Slope                           | 0.1308      |
| 4.     | Correlation coefficient         | 0.9992      |
| 5.     | Y-intercept                     | 0.0271      |

**Precision**

The precision of the proposed method was estimated in terms of inter-day and intra-day precision wherein the standard solution was observed for 6 times respectively. The results shown below indicating %RSD of less than 2% each level clearly indicate that the proposed method was precise enough for the analysis of drug.

\[ \text{% RSD} = \left( \frac{\text{SD of measurement}}{\text{mean value of measurement}} \right) \times 100 \]

**Table 3: Results of sitagliptin precision studies**

| Concentration | Intra-day precision (%RSD) | Inter-day precision (%RSD) Day 1, Day 2 |
|---------------|-----------------------------|-----------------------------------------|
| 100μg/ml      | 0.065459                    | 0.3935, 0.2165                           |

**Table 3a: Results of metformin precision studies**

| Concentration | Intra-day precision (%RSD) | Inter-day precision (%RSD) Day-1, Day-2 |
|---------------|-----------------------------|-----------------------------------------|
| 10μg/ml       | 0.0773                      | 0.1698, 0.3576                          |

**Accuracy**

The accuracy of the method was determined by performing recovery studies by spiking standard solution to that of sample solution at three different levels i.e., 50%, 100%, 150% was injected.

**Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

**Table 4: Results of sitagliptin accuracy studies**

| Level   | Amount of standard added (μg/ml) | Pre-analysed sample (μg/ml) | % Recovery |
|---------|----------------------------------|-----------------------------|------------|
| 50%     | 50                               | 100                         | 97.12%     |
| 100%    | 100                              | 100                         | 98.75%     |
| 150%    | 150                              | 100                         | 99.46%     |

**Table 4a: Results of metformin accuracy studies**

| Level   | Amount of standard added (μg/ml) | Pre-analysed sample (μg/ml) | % Recovery |
|---------|----------------------------------|-----------------------------|------------|
| 50%     | 5                                | 10                          | 98.15%     |
| 100%    | 10                               | 10                          | 99.38%     |
| 150%    | 15                               | 10                          | 99.85%     |

**Table 5: Results of sitagliptin robustness**

| Concentration | Wavelength | %RSD  |
|---------------|------------|-------|
| 100μg/ml      | 266        | 0.4580% |
|               | 268        | 0.1967% |

**Table 5a: Results of metformin robustness**

| Concentration | Wavelength | %RSD  |
|---------------|------------|-------|
| 10μg/ml       | 236        | 0.2925% |
|               | 238        | 0.1672% |

**Table 6: Results of sitagliptin ruggedness**

| Concentration | Analyst 1 | Analyst 2 | Instruments 1 (ELICO) | Instruments 2 (SYSTRONIC) |
|---------------|-----------|-----------|------------------------|---------------------------|
| 100μg/ml      | 0.04523%  | 0.02735%  | 0.0358%                | 0.04626%                  |
Table 6a: Results of metformin ruggedness

| Concentration | Analyst | %RSD | Instruments | %RSD |
|---------------|---------|------|-------------|------|
| 10μg/ml       | Analyst 1 | 0.0574% | Instrument 1 (ELICO) | 0.0596% |
|               | Analyst 2 | 0.0215% | Instrument 2 (SYSTRONIC) | 0.0453% |

Limit of detection: DL = 3.3σ/S

Where σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Table 7: Results of LOD

| Drug name | LOD   |
|-----------|-------|
| Sitagliptin | 0.397 μg/ml |
| Metformin  | 0.8952 μg/ml |

Limit of quantification

L. O. Q = 10σ/S

Where σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Table 8: Results of LOQ

| Drug name | LOQ    |
|-----------|--------|
| Sitagliptin | 1.2951 μg/ml |
| Metformin  | 2.7319 μg/ml |

Assay

Five tablets were accurately weighed and the average weight was determined. Then the tablets were grounded to a fine powder using clean and dry pestle and mortar. A quantity equivalent to 5 mg of Sitagliptin and 50 mg of Metformin was transferred to a 500 ml volumetric flask and dissolved in insufficient water. The contents were Ultra-Sonicated for 15 min and made up to the volume to obtain the concentration of 100 μg/ml and filtered through Whatmann filter paper. Absorbance of these solutions was measured at 267 nm and 237 nm, and concentration of Sitagliptin and Metformin in the sample solution was determined using the following equation:

\[ C_x = \frac{A_2 \times ay_2 - A_1 \times ay_2}{A_2 \times ay_1 - A_1 \times ay_2} \]

\[ C_y = \frac{A_1 \times ax_2 - A_2 \times ax_1}{A_2 \times ay_1 - A_1 \times ay_2} \]

Where \( C_x \) and \( C_y \) are the concentration of Sitagliptin and Metformin respectively, \( ax_1 \) and \( ax_2 \) are the absorptivity values of Sitagliptin at 267 and 237 nm respectively, \( ay_1 \) and \( ay_2 \) are the absorptivity values of Metformin at 267 and 237 nm respectively.

Table 9: Simultaneous data sitagliptin and metformin

| Drug name | Absorbance maxima λ 1 (267) | Absorbance maxima λ 2 (237) |
|-----------|-----------------------------|-----------------------------|
| Janumet 50 mg/500 mg | 0.5060(A1) | 0.5020(A2) |
| Absorbance of sitagliptin | 1.389 | 0.227 |
| Absorbance of metformin HCl | 0.0446 | 0.2491 |
| Absorptivity of sitagliptin | 0.1389(ax1) | 0.00227(ax2) |
| Absorptivity of metformin | 0.00249(ay1) | 0.00446(ay2) |

CONCLUSION

A simple and selective Spectrophotometric method was developed for the simultaneous estimation of Sitagliptin and Metformin in pharmaceutical dosage form. The developed method was validated as per ICH guidelines.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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