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# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF GLIMEPIRIDE AND SITAGLIPTIN PHOSPHATE IN BULK AND SYNTHETIC MIXTURE

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**ABSTRACT**

A simple, specific and accurate Reverse Phase High Performance Liquid Chromatography Method was developed for the simultaneous determination of Glimepiride and Sitagliptin Phosphate in Synthetic Mixture. The using Peerless $C_{18}$ (250 mm x 4.6 mm, 5 µm) column in Isocratic mode, with Mobile Phase containing ACN: Phosphate Buffer in the ratio of (75:25 %v/v) pH 3.6 adjusted with Orthophosphoric acid at detection wavelength 222 nm with flow rate is 1 ml/min and run time is 10 min. the average retention time was found to b 2.547 min and 4.950 min for Sitagliptin Phosphate and Glimepiride, respectively. The calibration was linear in concentration range of 1-6 µg/ml for Glimepiride and 50-300 µg/ml for Sitagliptin Phosphate. The low RSD (< 2%) Value indicates that the method is precise. The recoveries for Glimepiride and Sitagliptin Phosphate were found to be in the range of 98-99%. The proposed method was Validated and successfully applied for the estimation of Glimepiride and Sitagliptin Phosphate in Synthetic Mixture.

## INTRODUCTION

Glimepiride is chemically 3-ethyl-4-methyl-N-{2-[4-({[(4-methylcyclohexyl)\ carbamoyl]amino} sulfonyl) phenyl]ethyl}-2-oxo-2,5-dihydro-1H-pyrrole-1carboxamide. [1]It acts as an insulin secretagogue. It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. [2,3] Sitagliptin Phosphate is chemically(3R)-3-amino – 1-[3-(trifluoromethyl)-6,8-dihydro-5H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butan-1-one; phosphoric acid.[4]Sitagliptin Phosphate competitively inhibits dipeptidyl peptidase 4(DPP-4). This enzyme breakdown the incretins GLP-1, gastrointestinal hormones released in response to a meal. By preventing GLP-1 Inactivation, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of pancreas. This leads blood glucose level to normal. [4] Glimepiride and Sitagliptin Phosphate are commercially available in various dosage forms an individual formulation. Combination of Glimepiride and Sitagliptin Phosphate was studied under Clinical Trial phase. Identifier No: NCT01189890 by Merck Sharp & Dohme Corp. [6] Combination of Glimepiride with Sitagliptin Phosphate in "Efficacy of combination therapy with Sitagliptin and Low dose Glimepiride in Japanese patients with Type 2 Diabetes" was studied under clinical trial phase and was proved that the combination is effective in type -2 diabetes in such a way that it Synergistically stimulate insulin

secretion and also combination therapy leads to ameliorate hyperglycemia in type -2 Diabetes Mellitus.Also significant improvement in secretion of insulin by pancreatic alpha and beta cells which leads blood glucose level to normal. [7]. Glimepiride is official in IP [8]. Sitagliptin Phosphate is not official in any of the pharmacopoeia. From Literature Survey, various method (Spectroscopic method: UV, Mass Chromatographic method: HPLC, HPTLC) were reported for the analysis of individual drugs and also in combination with other drugs but no method were reported for simultaneous estimation of Glimepiride and Sitagliptin Phosphate. Hence, the purpose of the present work was to develop and validate RP-HPLC method for simultaneous estimation of Glimepiride and Sitagliptin Phosphate in synthetic mixture.

## Material and Methods

### Instruments and apparatus

- A Shimadzu RP-HPLC (LC-20-AD) (SPD-20 A) Instrument [Spinchrome]
- Column- Peerless $C_{18}$ (250×4.6 mm, 5 µm)
- Digital Analytical Balance – Wensar DA 13–220 (India)
- pH meter (Systronics, Naroda, Ahmedabad)
- Sonicator – Equitron (India)
- High vacuum pump (Parag Engineering)
- Volumetric flask – 10, 50 and 100 ml (Borosil)
- Pipettes – 1, 2, 5 and 10 ml (Borosil)
- Beaker –50,100 and 150 ml (Borosil)
- Hamilton syringe

### Chemicals and Materials

- Acetonitrile (HPLC grade) (Avantor Performance Material India Ltd)
- Methanol (HPLC grade) (Finar, Ahmedabad)
- Water (HPLC grade) (Astron Chemical India)
- Ortho Phosphoric Acid 75 % (AR Grade) (Astron Chemical India)
- Glimepiride and Sitagliptin Phosphate were supplied by West Coast Pharmaceuticals, Ahmedabad, India and Torrent Pharmaceuticals, Ahmedabad, India, respectively.

### Selection of Detection Wavelength:

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 222 nm both drug gave remarkable absorbance, good peak height and shape. So, 222 nm was selected for simultaneous estimation of GLIM and SITA in synthetic mixture.

### Mobile phase selection:

The composition and flow rate of mobile phase were changed to optimize the separation condition using combined solution. After number of trial experiments, it was established that theMobile phase ACN: Phosphate Buffer in the ratio of (75:25 %v/v) pH 3.6 adjusted with Orthophosphoric acid shows good peak shape and resolution.

### Chromatographic condition:

**Column:** Peerless $C_{18}$ (250 mm × 4.6 mm, 5 µm)

**Mobile phase:** ACN: Phosphate Buffer (pH 3.6 adjusts with 10% Ortho phosphoric acid) (75:25%v/v)

**Flow rate:** 1 ml/min
**Run time:** 10 min
**Detection wavelength:** 222 nm
**Detector:** U.V Detector
**Injection volume:** 20 µL
**Syringe:** Hamilton

### Preparation of Mobile phase:

**Preparation of 10% Orthophosphoric acid**:10% ortho phosphoric acid was prepared by diluting 1.3 ml of concentrated ortho phosphoric acid in 10 ml HPLC grade water.

**Preparation of buffer (10 mM phosphate buffer):**Accurately weighed 0.272 gm potassium dihydrogen phosphate ($KH_2PO_4$) was transferred it in 200 ml HPLC grade water and allowed it to dissolve. It was filtered through 0.45 µm membrane filter and sonicated for about 10 min. Buffer pH was adjusted to 3.6 with 10% ortho phosphoric acid.

**Preparation of Mobile phase:** ACN: Phosphate Buffer (75:25 %%v/v) (pH 3.6 adjusts with 10% Ortho phosphoric acid). Prepared Mobile phase was used after filtered it through 0.45 µm membrane filter and sonication.

**Preparation of standard stock solution:**

**Glimepiride (100 µg/ml):** Accurately weighed GLIM (10 mg) was transferred to a 100 ml volumetric flask, and diluted up to the mark with mobile phase to obtain a standard stock solution (100 µg/ml).

**Sitagliptin Phosphate (1000 µg/ml):**

Accurately weighed SITA (100 mg) was transferred to a 100 ml volumetric flask, and diluted up to the mark with mobile phase to obtain a standard stock solution (1000 µg/ml).

**Preparation and analysis of synthetic mixture [15, 54]**

The synthetic mixture of Glimepiride and Sitagliptin Phosphate was prepared in the ratio of 1:50. Common excipients such as Polivinyl Pyrolidine (10 mg), CCS [Cross Carmellose Sodium] (8 mg), Talc (4 mg), Magnesium Stearate (6 mg), MCC [Micro Crystalline Cellulose] (21 mg) were added in the motor pestle along with the drug Glimepiride (1 mg) and Sitagliptin Phosphate (50 mg). Accurately weighed equivalently weight of Glimepiride (1 mg) and Sitagliptin Phosphate (50 mg) which transferred in 100 ml volumetric flask and make up half mark with Methanol. This solution was sonicated till the drug dissolves and was made upto mark with methanol. This solution was filtered through Whatmann filter paper. The concentration of Glimepiride was 10 µg/ml and Sitagliptin Phosphate was 500 µg/ml. From above synthetic mixture solutions take 2 ml and transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with mobile phase to make final concentration of Glimepiride 2 µg/ml and Sitagliptin Phosphate 100 µg/ml.

**Method Validation:** The developed method was validated with respect to specificity,linearity, range, accuracy, and precision, limit of detection and limit of quantification in accordance with the ICH guideline.

- **Specificity**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.

- **Linearity & Range**

The linearity of Glimepiride and Sitagliptin Phosphate was found to be in the range of 1-6 µg/ml and 50-300 µg/ml respectively. Plot the calibration curve of Peak area Vs Concentration (µg/ml). Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

**Preparation of calibration curve**

Aliquots of stock solution of Glimepiride (100 µg/ml) 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml and Sitagliptin Phosphate (1000 µg/ml) 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml were pipette out in same six different 10 ml volumetric flasks and further diluted with mobile phase to obtain the concentration of about 1, 2, 3, 4, 5 and 6 µg/ml for Glimepiride and 50, 100, 150, 200, 250 and 300 for Sitagliptin Phosphate. 20 µL of each solution were injected into RP-HPLC system by Hamilton syringe and analyzed. Calibration curve was obtained by plotting respective Peak area Vs Concentration in µg/ml and Regression equation was obtained.

- **Precision**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, Reproducibility (Interday precision), Repeatability.

**1) Intraday Precision:** Solutions containing 1, 2, 3 µg/ml of GLIM and 50, 100, 150 µg/ml of SITA were analyzed three times on the same day and %R.S.D was calculated.

**2) Interday Precision:** Solutions containing 1, 2, 3 µg/ml of GLIM and 50, 100, 150 µg/ml of SITA were analyzed on three

different successive days and %R.S.D. was calculated.

**3) Repeatability:** Solutions containing 2 µg/ml of GLIM and 100 µg/ml of SITA were analyzed for six times and %R.S.D. was calculated.

- **Limit of Detection (LOD)**

Limit of detection can be calculated using following equation as per ICH guidelines.

**LOD = 3.3 x (σ/ S)**

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

- **Limit of Quantification (LOQ)**

Limit of quantification can be calculated using following equation as per ICH guidelines.

**LOQ = 10 x (σ/ S)**

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

- **Accuracy**

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50%, 100%, 150% and the values were measured for Glimepiride (**2 µg/ml**) and Sitagliptin Phosphate (**100µg/ml**). This performance was done in triplicate.

**Preparation of stock solution of Mixture (100 µg/ml): (Stock 1)**

The synthetic mixture of Glimepiride and Sitagliptin Phosphate was prepared in the ratio of 1:50. Common excipients such as Polivinyl Pyrolidine (10 mg), CCS [Cross Carmellose Sodium] (8 mg), Talc (4 mg), Magnesium Stearate (6 mg), MCC [Micro Crystalline Cellulose] (21 mg) were added in the motor pestle along with the drug Glimepiride (1 mg) and Sitagliptin Phosphate (50 mg).

Accurate weighedequivalently weight of Glimepiride (1mg) and Sitagliptin Phosphate (50 mg) which transferred in 100 ml volumetric flask and make up half mark with Methanol. This solution was sonicated till the drug dissolves and was made upto mark with methanol. This solution was filtered through Whatmann filter paper. The concentration of Glimepiride was 10 µg/ml and Sitagliptin Phosphate was 500 µg/ml.

**Preparation of Standard stock solution Glimepiride (100 µg/ml): (Stock 2)**

Accurately weighed GLIM (10 mg) was transferred to a 100 ml volumetric flask and was diluted to half and sonicated and made upto the mark with Methanol to obtain a standard stock solution.

**Preparation of Standard stock solution Sitagliptin Phosphate (1000 µg/ml): (Stock 3)**

Accurately weighed SITA (100 mg) was transferred to a 100 ml volumetric flask and was diluted to half and sonicated and made upto the mark with Methanol to obtain a standard stock solution. Each flask was made up to 10 ml with Methanol. Each procedure was carried out for 3 times (n=3).

- **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. It should show the reliability of an analysis with respect to deliberate variation in method parameter. In case of liquid chromatography, examples of typical variations are: Influence of variations of pH in mobile phase; Influence of variations in Mobile phase composition;

- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

**System suitability tests**

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test includes the

Resolution, Column efficiency, tailing factor and Theoretical plates.

**Selection detection wavelength:** The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 222 nm both drugs give good peak height and shape. So, 222 nm was selected for simultaneous estimation of GLIM and SITA in synthetic mixture.

**Optimization of chromatographic conditions:**

Various mobile phases, such as Methanol: Water, Acetonitrile: Water, ACN: Buffer in different proportion was tried. The combination of ACN: Phosphate buffer in the ratio of (75:25 %v/v) pH 3.6 adjusted with Orthophosphoric acid provided optimum polarity for proper migration, separation and resolution of Glimepiride and Sitagliptin Phosphate. Under these conditions, the eluted peaks were well defined and resolved.

**Result:** For RP- HPLC method various mobile phase compositions was tried to get adequate separation of eluted compound.

Separation of GLIM and SITA were performed by use of isocratic mobile phase prepared from ACN: Phosphate Buffer in the ratio of (75:25 %v/v) pH 3.6 adjusted with Orthophosphoric acid at detection wavelength 222 nm with flow rate is 1 ml/min and run time is 10 min. the average retention time was found to b 2.547 min and 4.950 min for SITA and GLIM respectively. The calibration was linear in concentration range of 1-6 µg/ml for GLIM and 50-300 µg/ml for SITA. The low RSD (< 2%) value indicates that the method is precise. The recoveries for GLIM and SITA were found to be in the range of 99.50-99.84%.

**Discussion:** The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in Synthetic Mixture with virtually no interference of additives. Hence the proposed method can be successfully applied in estimation of Glimepiride and Sitagliptin Phosphate in Synthetic Mixture.

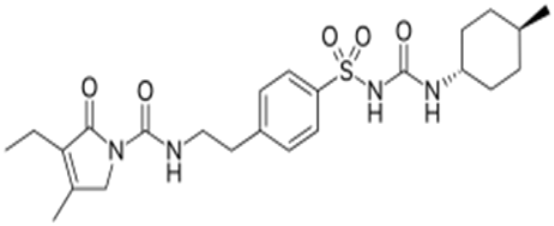

**Figure 1: Structure of Glimepiride [1]**

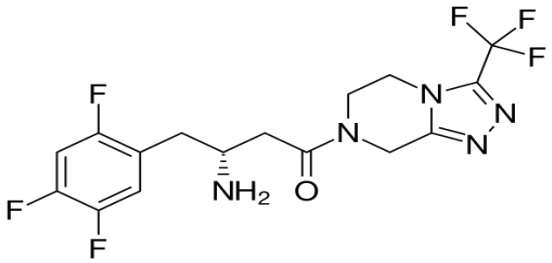

**Figure 2: Structure of Sitagliptin Phosphate [4]**

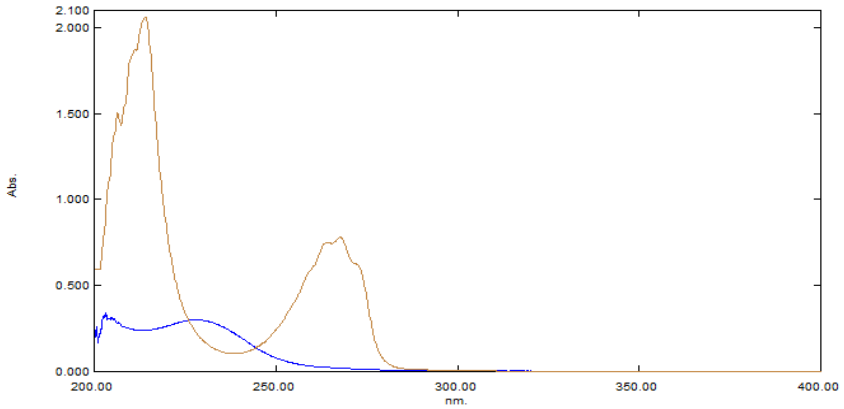


**Figure 3: Overlain spectra of GLIM (2 µg/ml) and SITA (100 µg/ml) in Methanol**

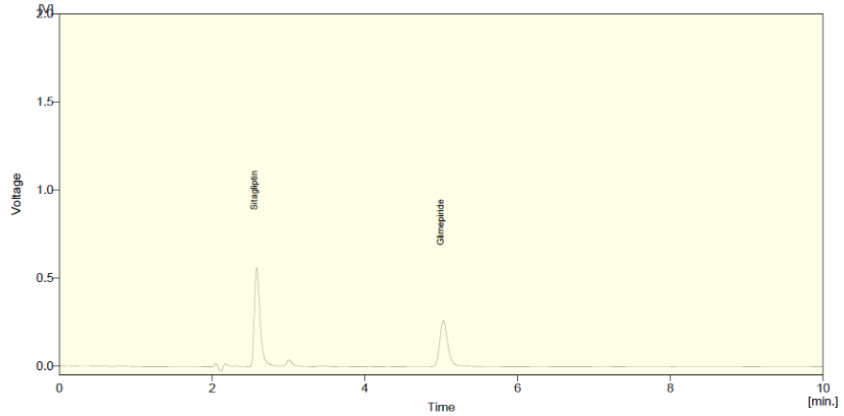


**Figure 4: Chromatogram of SITA (100 µg/ml) and GLIM (2 µg/ml) in ACN: Phosphate Buffer (pH 3.6 adjusts with Ortho phosphoric acid) (75:25 %%v/v)**

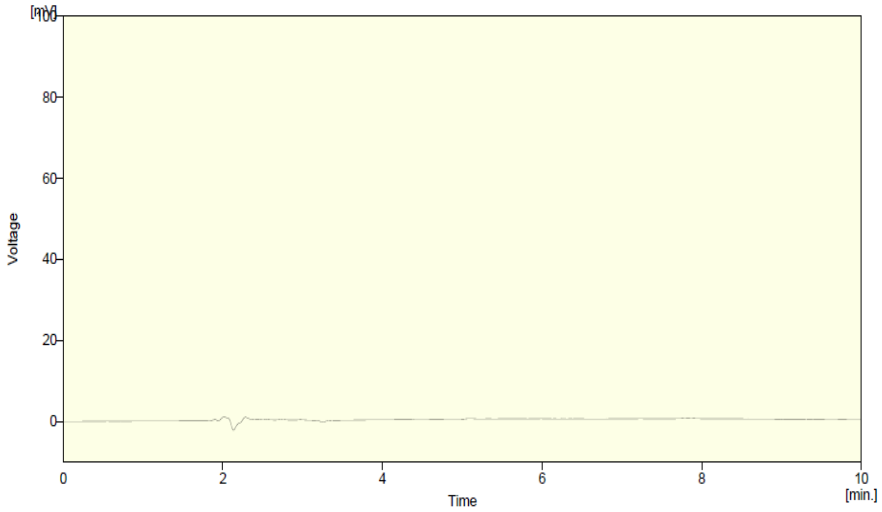


**Figure 5: Blank chromatogram**

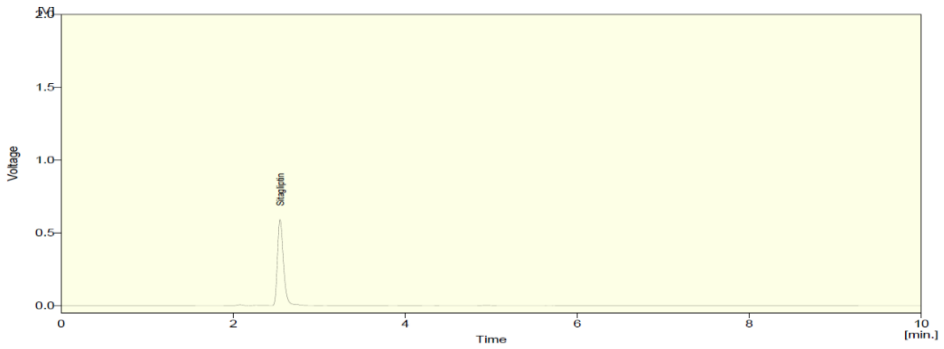


**Figure 6: Chromatogram of SITA (100 µg/ml) in in ACN: Phosphate Buffer (pH 3.6 adjusts with Ortho phosphoric acid) (75:25 %%v/v)**

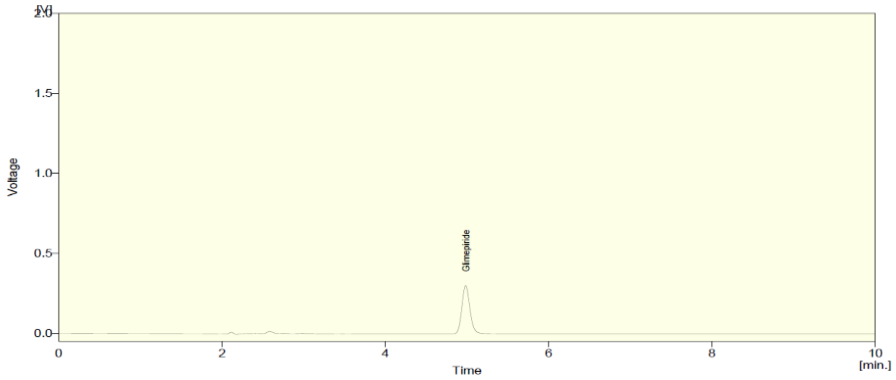


**Figure 7: Chromatogram of GLIM (2 µg/ml) in in ACN: Phosphate Buffer (pH 3.6 adjusts with Ortho phosphoric acid) (75:25 %%v/v)**

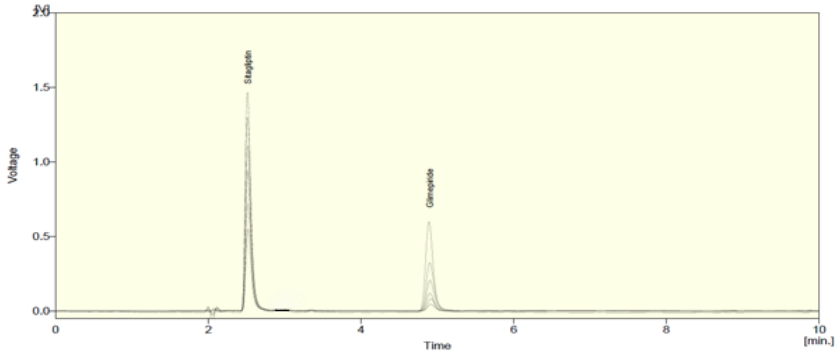


**Figure 8: Overlain chromatogram of SITA 50-300 (µg/ml) and GLIM (1-6µg/ml)**

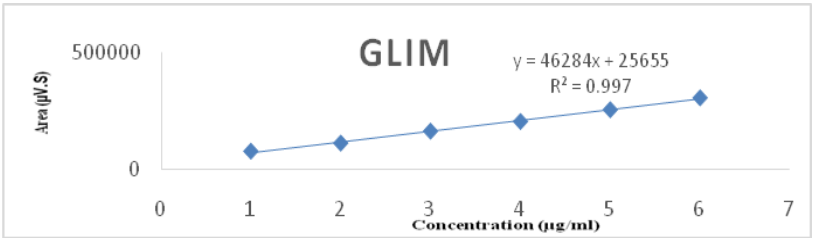


**Figure 9: Calibration curve of Glimepiride (1-6 µg/ml)**

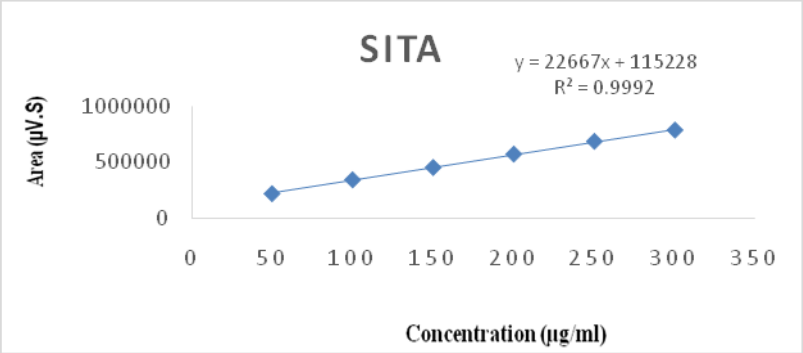


**Figure 10: Calibration curve of Sitagliptin Phosphate (50-300 µg/ml)**

**Table 1: Amount of drug taken in 10 ml volumetric flask for Glimepiride**

| Flask | % Level of Recovery | Stock 1 (µg/ml) | Stock 2 (µg/ml) | Total Amount(ml) |
|---|---|---|---|---|
| 1 | 50 | 2 | 0.1 | 2.1 |
| 2 | 100 | 2 | 0.2 | 2.2 |
| 3 | 150 | 2 | 0.3 | 2.3 |

**Table 2: Amount of drug taken in 10 ml volumetric flask for Sitagliptin Phosphate**

| Flask | % Level of Recovery | Stock 1 (µg/ml) | Stock 2 (µg/ml) | Total Amount(ml) |
|---|---|---|---|---|
| 1 | 50 | 2 | 0.5 | 2.5 |
| 2 | 100 | 2 | 1.0 | 3.0 |
| 3 | 150 | 2 | 1.5 | 3.5 |

**Table 3: Calibration data for GLIM (1-6µg/ml) and SITA 50-300 (µg/ml)**

| GLIMEPIRIDE | | | SITAGLIPTIN PHOSPHATE | | |
|---|---|---|---|---|---|
| Conc. (µg/ml) | Mean Peak Area (µV.s) ± S.D. (n=6) | % RSD | Conc. (µg/ml) | Mean Peak Area (µV.s) ± S.D. (n=6) | % RSD |
| 1 | 78138 ± 663.18 | 0.8487 | 50 | 222480 ± 1565.98 | 0.7038 |
| 2 | 113075 ± 922.79 | 0.8160 | 100 | 345625 ± 2026.31 | 0.5862 |
| 3 | 154385 ± 1087.42 | 0.7043 | 150 | 455142 ± 2298.55 | 0.5050 |
| 4 | 205994 ± 1414.77 | 0.6868 | 200 | 573805 ± 2716.07 | 0.4733 |
| 5 | 256620 ± 1631.36 | 0.6357 | 250 | 687189 ± 2765.61 | 0.4024 |
| 6 | 317674 ± 1750.73 | 0.5511 | 300 | 787150 ± 2775.62 | 0.3513 |

**Table 4: Precision study for Glimepiride**

| **Intraday precision of Glimepiride** | | |
|---|---|---|
| **Glimepiride** | | |
| **Conc. (µg/ml)** | **Mean peak area (µv*sec) ± S.D (n=3)** | **% RSD** |
| 1 | 78356 ± 597.16 | 0.7621 |
| 2 | 113804 ± 846.16 | 0.7435 |
| 3 | 154792 ± 1068.35 | 0.6901 |
| **Interday precision of Glimepiride** | | |
| **Conc. (µg/ml)** | **Mean peak area (µv*sec) ± S.D (n=3)** | **% RSD** |
| 1 | 78723 ± 707.18 | 0.8983 |
| 2 | 113708 ± 996.19 | 0.8760 |
| 3 | 154339 ± 1081.11 | 0.7004 |
| **Repeatability of Glimepiride** | | |
| **Conc. (µg/ml)** | **Mean peak area (µv*sec)±SD (n=6)** | **% RSD** |
| 2 | 113412 ± 718.88 | 0.6338 |

**Table 5: Precision study for Sitagliptin Phosphate**

| **Intraday precision of Sitagliptin Phosphate** | | |
|---|---|---|
| **Sitagliptin Phosphate** | | |
| **Conc. (µg/ml)** | **Mean peak area (µv*sec) ±SD (n=3)** | **% RSD** |
| 50 | 223502 ± 1688.35 | 0.7554 |
| 100 | 345080 ± 1969.70 | 0.5707 |
| 150 | 454754 ± 2238.18 | 0.4921 |
| **Interday precision of Sitagliptin Phosphate** | | |
| **Conc. (µg/ml)** | **Mean peak area (µv*sec) ±SD (n=3)** | **% RSD** |
| 50 | 223544 ± 1680.30 | 0.7516 |
| 100 | 344766 ± 1922.88 | 0.5577 |
| 150 | 454716 ± 2286.59 | 0.5028 |
| **Repeatability of Sitagliptin Phosphate** | | |
| **Conc. (µg/ml)** | **Mean peak area (µv*sec) ±SD (n=6)** | **% RSD** |
| 100 | 344550±1457.30 | 0.4229 |

**Table 6: Recovery study data**

| Name of Drug | % Level of recovery | Amount of drug Taken (µg/ml) | Spiked amt taken (µg/ml) | Total Std amount (µg/ml) | Total amount recovered (µg/ml) | % Recovery ±S.D. (n=3) |
|---|---|---|---|---|---|---|
| Glimepiride | 50 | 2 | 1 | 3 | 2.99 | 99.75 ± 0.2112 |
| | 100 | 2 | 2 | 4 | 3.98 | 99.50 ± 0.1569 |
| | 150 | 2 | 3 | 5 | 4.98 | 99.65 ± 0.5968 |
| Sitagliptin Phosphate | 50 | 100 | 50 | 150 | 148.76 | 99.84 ± 0.5885 |
| | 100 | 100 | 100 | 200 | 199.12 | 99.56 ± 0.5862 |
| | 150 | 100 | 150 | 250 | 249.46 | 99.78 ±0.6453 |

**Table 7: LOD and LOQ data**

| Drug Name | Glimepiride | Sitagliptin Phosphate |
|---|---|---|
| LOD (µg/ml) | 0.0472 | 0.2279 |
| LOQ (µg/ml) | 0.1432 | 0.6908 |

**Table 8: Analysis of synthetic mixture**

| Drug Name | Amount taken (µg/ml) | Mean Amount Found (µg/ml) | % Assay ± S.D. (n=3) | % R.S.D. |
|---|---|---|---|---|
| Glimepiride | 2 | 1.98 | 99.00 ± 0.2330 | 0.2353 |
| Sitagliptin Phosphate | 100 | 99.85 | 99.85 ± 0.1493 | 0.1495 |

**Table 9: Robustness data**

| Condition | Variation | Glimepiride | Sitagliptin Phosphate |
|---|---|---|---|
| | | %Assay ± SD (n=3) | %Assay ± SD (n=3) |
| Flow rate (1 ml ± 0.1 ml/ min) | 0.9 ml/min | 99.33 ± 0.6966 | 98.3 ± 0.5129 |
| | 1.0 ml/min | 99.66 ± 0.6634 | 100.33 ± 0.4921 |
| | 1.1 ml/min | 99 ± 0.6403 | 99.33 ± 0.4747 |
| Detection wavelength (222 nm ± 1 nm) | 221 | 98.66 ± 0.6957 | 99.00 ±0.5236 |
| | 222 | 100.33 ± 0.6634 | 99.66 ± 0.4921 |
| | 223 | 99.00 ± 0.6489 | 98.66 ± 0.4756 |
| Mobile Phase (ACN:Phosphate Buffer%v/v) | 74: 26 | 98.66 ± 0.6979 | 98.66 ± 0.5082 |
| | 75: 25 | 100.66 ± 0.6634 | 99.66 ± 0.4921 |

**Table 10: Summary of Validation Parameters**

| Sr. No. | Parameters | Glimepiride | Sitagliptin Phosphate |
|---|---|---|---|
| 1 | Wavelength (nm) | 222 nm | |
| 2 | Retention time (min) | 4.953 | 2.543 |
| 3 | Linearity Range (µg/ml) | 1-6 | 50-300 |
| 4 | Regression equation | y = 46284x + 25655 | y = 22667x + 115228 |

| | | | |
|---|---|---|---|
| | (y = mx +c) | | |
| 5 | Correlation Coefficient ($r^2$) | 0.9972 | 0.9992 |
| 6 | Repeatability (% RSD, n=6) | 0.6338 | 0.4229 |
| 7 | Intraday Precision (%RSD, n=3) | 0.7319 | 0.6060 |
| 8 | Interday Precision(% RSD, n=3) | 0.8249 | 0.6040 |
| 9 | Accuracy (% Recovery, n=3) | 99.50-99.75 | 99.56-99.84 |
| 10 | LOD (µg/ml) | 0.0472 | 0.2279 |
| 11 | LOQ (µg/ml) | 0.1432 | 0.6908 |
| 12 | % Assay (n=3) | 99.00% | 99.85% |

## CONCLUSION:

A simple, rapid, sensitive, accurate and precise RP-HPLC Method has been developed and Validated for routine analysis of Glimepiride and Sitagliptin Phosphate in Synthetic Mixture. The RP-HPLC method is suitable for simultaneous estimation ofGlimepiride and Sitagliptin Phosphate in Synthetic Mixture without interference of each other. The developed method was successfully applied in Synthetic Mixture. The proposed Method can be utilized for the routine analysis of Glimepiride and Sitagliptin Phosphate in Synthetic Mixture.

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