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# Simultaneous UV Spectrophotometric Method for Estimation of Sitagliptin phosphate and Metformin hydrochloride in Bulk and Tablet Dosage Form

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**ABSTRACT**

*Two simple, precise and economical UV methods have been developed for the simultaneous estimation of Sitagliptin phosphate and Metformin hydrochloride in bulk and pharmaceutical dosage form. Method A is Absorbance maxima method, which is based on measurement of absorption at maximum wavelength of 266 nm and 232 nm for Sitagliptin phosphate and Metformin hydrochloride respectively. Method B is area under curve (AUC), in the wavelength range of 244-279 nm for Sitagliptin phosphate and 222-240 nm for Metformin hydrochloride. Linearity for detector response was observed in the concentration range of 25-225µg/ml for Sitagliptin phosphate and 2-12 µg/ml for Metformin hydrochloride. The accuracy of the methods was assessed by recovery studies and was found to be99.64 % and 98.98% for Sitagliptin phosphate and Metformin hydrochloride. The developed method was validated with respect to linearity, accuracy (recovery), precision and specificity. The results were validated statistically as per ICH Q2 R1 guideline and were found to be satisfactory. The proposed methods were successfully applied for the determination of for Sitagliptin phosphate and Metformin hydrochloride in commercial pharmaceutical dosage form.*

**Keywords**: Sitagliptin phosphate, Metformin hydrochloride,Simultaneous estimation, Absorbance maxima method, Area under curve.

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## INTRODUCTION

Sitagliptin phosphate (STG) is 1,2,4-triazolo[4,3-a]pyrazine,7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluoro phenyl)butyl]-5,6,7,8- tetrahydro-3-(trifluoromethyl), phosphate (Fig. 1). It is used in the treatment of diabetes. It is an oral antihyperglycemic (anti-diabetic) drug of the dipeptidyl peptidase-4 (DPP-4) inhibitor class. Sitagliptin competitively inhibits dipeptidyl peptidase-4, an enzyme involved in the breakdown of incretins such as glucagon-like particle-1 (GLP-1) which potentiate insulin secretion in vivo. Inhibition of DPP-4 reduces the breakdown of GLP-1 and increases insulin secretion; this suppresses the release of glucagon from the pancreas and drives down blood sugar levels. This drug is not official in any pharmacopoeia.

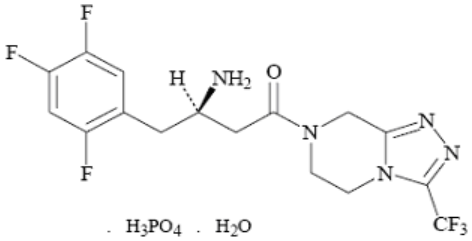

**Fig.1 Sitagliptin phosphate**

Metformin HCl (MET) is 3-(diaminomethylidene)-1, 1-dimethylguanidine (Fig. 2). It is an oral anti-diabetic drug which is the first line drug of choice for the treatment of type 2 diabetes, particularly in overweight or obese people and those with normal kidney function. Metformin improves hyperglycemia, primarily through its suppressive action on production of hepatic glucose (hepatic gluconeogenesis).

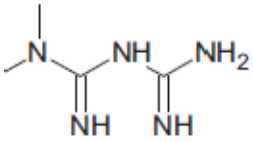

**Fig.2Metformin hydrochloride**

Several method were reported for the simultaneous estimation of Metformin HCl alone and in combination with other drugs vizUV-spectrophotometry [1,2,3,4,9,10,13],estimation in plasmaand urine using HPLC[7,14], assay using RP-HPLC[12].

A comprehensive literature research reveals the lack of a spectrophotometric analytical methodfor simultaneous estimation of Sitagliptin and Metformin HCl in pharmaceutical formulations.

Asuccessful attempt was made to developaccurate, precise and simple method of analysis for estimation of both the drugs in combined dosage form.

## MATERIALS AND METHODS

**Materials:**
Sitagliptin phosphate and Metformin hydrochloride was generous gift samples from Matrix Laboratory Limited (Hyderabad, India). Commercial Janumet tablets containing 50mg of Sitagliptin phosphate and 500mg of Metformin hydrochloride were purchased from local market and used within their shelf-life period. All other chemicals used were of analytical grade.

**Instrumentation:**
A Jasco double beam UV–visible spectrophotometer, Model: V-630, with a fixed bandwidth (2nm) and 1-cm quartz cell was used for Spectral and absorbance measurements. In addition, electronic balance, micropipette and sonicator were used in this study.

**Procedure:**
Preparation of standard stock solution-
Standard stock solutions ofeach STG and METwas prepared by dissolving 100 mg of standard MET and 10 mg of standard STG separately in 10 ml distilled water with vigorous shaking. Aliquot in the range of 25-225 µg/ml for STG and 2-12 µg/ml for MET was prepared using this stock solution.

**Method A: Absorption Maxima Method**
For the selection of analytical wavelength, standard solution of STG and MET were scanned in the spectrum mode from 400 nm to 200 nmseparately. From the spectra of drug $\lambda_{max}$ of STG, 266 nm [Fig.3], and $\lambda_{max}$ of MET, 232 nm

[Fig.4], were selected for the analysis. Aliquots of standard stock solution were made and calibration curve was plotted [Fig.5 and Fig.6].

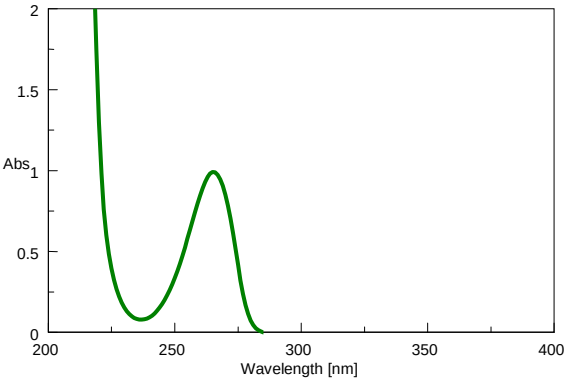


**Fig.3It shows $\lambda_{max}$ of STG**

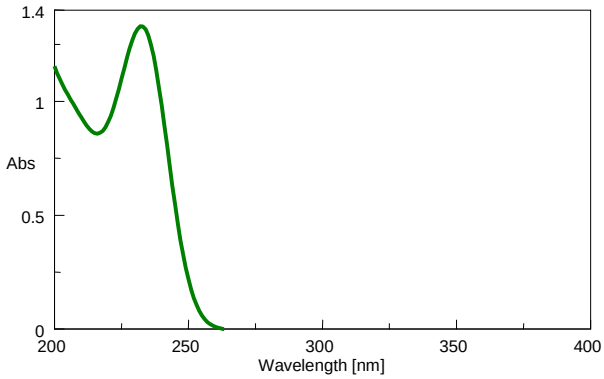


**Fig.4 It shows λmax of MET**

**Simultaneous estimation of Sitagliptin phosphate and Metformin hydrochloride:**

The wavelength maxima of Metformin HCl and Sitagliptin were determined and found to be 232 nm ($\lambda_1$) and 266nm ($\lambda_2$) respectively where there was no interference among the drugs. The overlain spectrum is shown in Fig.4.

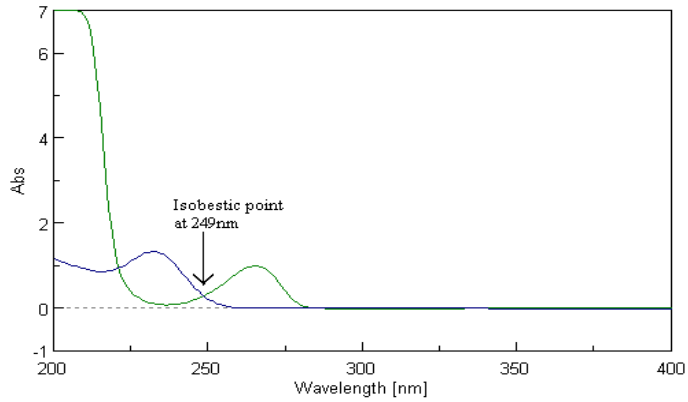


**Fig.4 Isobestic point of STG and MET**

**Method B: Area under Curve Method**

From the spectra of drug obtained after scanning of standard solution of STG and MET separately, area under the curve in the range of 244-279 nm and 222-240 nm was selected for the analysis. The calibration curve was prepared in the concentration range of 25-225 µg/mlfor STG and 2-12 µg/ml for MET at their respective AUC range.

Both drugs followed the Beer-Lambert's law in the above mentioned concentration range. The calibration curves were plotted as absorbance against concentration of STG and MET. The coefficient of correlation (r), slope and intercept values of this method are given in Table 1.

**Application of the proposed methods for the determination of STG and MET in tablet dosage form:**

For the estimation of drugs in the tablet formulation, 20 tablets were weighed and weight equivalent to 50mg of STG and 500mg of MET was transferred to 50 ml volumetric flask and ultrasonicated for 20 minutes and volume was made up to the mark with distilled water. The solution was then filtered through a Whatmann filter paper (No.42). The filtrate was appropriately diluted further. In Method-A, the concentration of STGand MET was determined by measuring the absorbance of the sample at 266nm and 232nm respectively in zero order spectrum mode. By using the calibration curve, the concentration of the sample solution was determined.

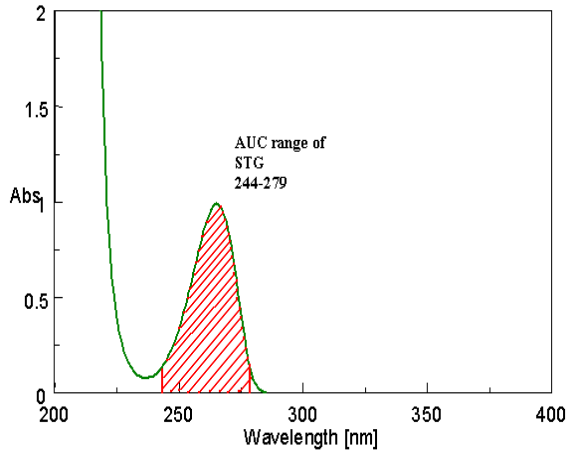


**Fig.5It shows AUC of STG**

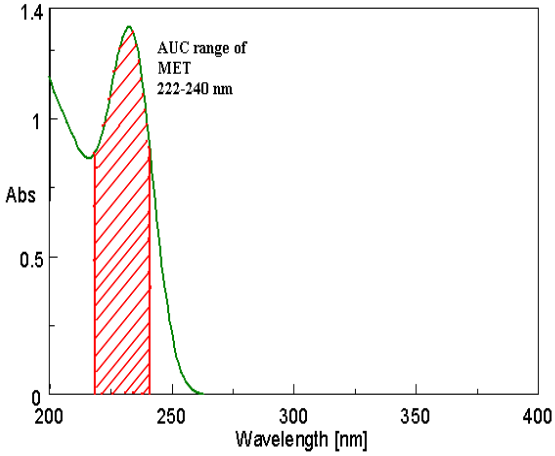


**Fig.6 It shows AUC of MET**

In Method-B, the concentration of STG and MET was determined by measuring area under curve in the range of244-279 nm and 222-240 nm. By using the calibration curve, the concentration of the sample solution was determined.

**Validation of the developed methods**[18]**:**
The methods were validated with respect to accuracy, linearity, precision and selectivity.

**Accuracy:**Accuracy of an analysis was determined by systemic error involved. Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. Recovery studies carried out for boththe methods by spiking standard drug in the powdered formulations 80%, 100%, 120% amount of each dosage content as per ICH guidelines**.**

**Linearity:** The linearity of measurement was evaluated by analyzing different concentration of the standard solution of STG and MET. Result should be expressed in terms of correlation co-efficient.

**Precision:** The reproducibility of the proposed method was determined by performing tablet assay at different time intervals (morning, afternoon and evening) on same day (Intra-day assay precision) and on three different days (Inter-day precision). Result of intra-day and inter-day precision is expressed in % RSD.

**Table 1: Table shows Optical characteristics and precision**

| Sr. No. | Parameter | STG | MET |
|---|---|---|---|
| 1. | λ- max | 266 | 232 |
| 2 | Beer's law limit (µg/ml) | 25-225 | 2-12 |
| 3. | Molar absorptivity (L/mol.cm) | 2357.975 | 16398.32 |
| 4. | Sandell's sensitivity (µg/Sq.cm/0.001) | 0.2219 | 0.010 |
| 5. | Correlation coefficient (r) | 0.999 | 0.999 |
| 6. | Slope (m) | 0.004206 | 0.0990 |
| 7. | Intercept | 0.03865 | 0.0760 |

**Table 2:Table showsResults of Analysis of Tablet Formulation (N*=6)**

| Method | Drug | Label Claim mg | Amount of drug estimated (mg/tab) | % Label Claim*± S.D. | % Recovery |
|---|---|---|---|---|---|
| **A** | **STG** | 50 | 99.58 | 99.58±0.6172 | 99.58 |
| **B** | | | 99.30 | 99.30±0.0852 | 99.30 |
| **A** | **MET** | 500 | 99.98 | 99.98±0.2727 | 99.98 |
| **B** | | | 99.65 | 99.65±0.0357 | 99.65 |

**Table 3: Table shows Result of Recovery studies**

| Excess drug added to the analyte (%) | Drug | % Recovery | | %RSD | | SE | |
|---|---|---|---|---|---|---|---|
| | | Method A | Method B | Method A | Method B | Method A | Method B |
| 80 | | 99.64 | 100.01 | 0.409 | 0.289 | 0.673 | |
| | | | | | | 0.154 | |
| 100 | **STG** | 99.64 | 100.06 | 0.367 | 0.194 | 0.417 | 0.128 |
| 120 | | 100.27 | 100.78 | 0.093 | 0.227 | 0.767 | 0.329 |
| 80 | | 98.98 | | 0.297 | | 0.813 | |
| | | 98.98 | | 0.288 | | 0.822 | |
| 100 | **MET** | 99.57 | | 0.437 | | 0.673 | |
| | | 100.01 | | 0.128 | | 0.987 | |
| 120 | | 100.38 | | 0.116 | | 0.994 | |
| | | 100.38 | | 0.124 | | 0.986 | |

*a) RSD: Relative Standard deviation b) SE: Standard error*

**Table 4: Table shows Result of Intra-day and Inter-day precision**

| Method | Drug | Intra-day precision | | | Inter-day precision | | |
|---|---|---|---|---|---|---|---|
| | | SD | %RSD | SE | SD | %RSD | SE |
| **A** | **STG** | 0.617 | 0.418 | 0.205 | 0.587 | 0.378 | 0.157 |
| **B** | | 0.543 | 0.358 | 0.125 | 0.356 | 0.237 | 0.115 |
| **A** | **MET** | 0.272 | 0.837 | 0.113 | 0.198 | 0.759 | 0.108 |
| **B** | | 0.183 | 0.765 | 0.104 | 0.167 | 0.658 | 0.098 |

## RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurateway for analysis of Sitagliptin phosphate and Metformin hydrochloride in its bulk and pharmaceutical dosage form. Absorbance maximaof STG at 266nm and MET at 232nm were selected for the analysis. Linearity for detector response was observed in the concentration range of 25-225 µg/ml for STG and 2-12µg/ml for MET. Percent label claim for STG and MET in tablet analysis was found in the range of 99.58% and 99.98% [Table 2]. Standard deviation and coefficient of variance for six determinations of tablet formulation, was found to be less than ± 2.0 indicating the precision of the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. % recovery for STG and METwas found in the range of 99.64% and 98.98% values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of all the methods. % RSD for Intraday assay precision for STGwas found to be 0.617 and0.543 for Method A and B, and for MET , 0.272 and 0.183 for Method A and B. Interday assay precision for STGwas found to be0.587 and 0.356 for Method A and B and for MET 0.198 and 0.167 for Method A and B. Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible & economical and can be employed for routine quality control of Sitagliptin phosphate and Metformin hydrochloridein bulk drug and its pharmaceutical dosage form.

## CONCLUSION

UV spectrophotometric methods for Sitagliptin phosphate and Metformin hydrochloridewere developed separately in bulk and tablet dosage form by, Absorbance maxima methodand Area under curve method. Further, UV spectrophotometric methods for the simultaneous estimation ofSitagliptin phosphate and Metformin hydrochloridewere in bulk and combined dosage form. The methods were validated as per ICH guidelines. The standard deviation and % RSD calculated for these methods are <2, indicating high degree of precision of the methods. The results of the recovery studies showed the high degree of accuracy of these methods. In conclusion, the developed methods are accurate, precise and selective and can be employed successfully for the estimation of STGand METin bulk and pharmaceutical dosage form.

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