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## Validated HPTLC Method for Simultaneous Estimation of Sitagliptin and Metformin Hydrochloride in Bulk Drug and Formulation.

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## Research Article

### ABSTRACT

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the simultaneous estimation of two anti-diabetic drugs, sitagliptin and metformin hydrochloride in bulk and tablet dosage form. Study was performed on TLC plates precoated with silica gel 60F254 using methanol: ammonia: glacial acetic acid (9.4:0.4:0.2 v/v/v)as the mobile phase. A TLC scanner set at 214 nm was used for direct evaluation of the chromatograms in the absorbance mode. Method was validated according to ICH guidelines. The correlation coefficients of calibration curves were found to be 0.999 and 0.998 in the concentration range of 100–1100 and 1000–11000 ng band-1for sitagliptin and metformin, respectively. The method had an accuracy of 99.70% for sitagliptin and 100.02% for metformin hydrochloride. Intra and inter day precision measured as coefficient of variation were less than 2% for both analytes. The limit of detection and quantitation were 7.08 ng band-1 and 21.82 ng band-1, respectively for sitagliptin and 19.31 ng band-1 and 58.51 ng band-1, respectively for metformin hydrochloride. The method had the potential to determine these drugs simultaneously from dosage form without any interference of the tablets excipients.

## INTRODUCTION

Sitagliptin(STG),(2R)-1- (2,4,5-trifluorophenyl)- 4-oxo-4- [3- (trifluoromethyl)- 5,6dihydro [1,2,4] triazolo [4,3-a] pyrazin -7 (8H)-yl] butan-2-amine (Fig. 1), is an orally active, potent and selective inhibitor of dipeptidyl peptidase-IV (DPP-IV), which has been ma0rketed in USA, Europe and other countries for the treatment of type 2 diabetes. DPP-IV inhibitors enhance levels of active glucagon-like peptide 1 (GLP-1) and other incretins, and facilitate glucose-dependent insulin secretion [1].

Metformin (MET) (N,N-dimethylbiguanide)(Fig. 1), originally marketed as Glucophage TM by Bristol-Myers Squibb, is now available in manygeneric formulations. Metformin is a biguanide type insulin sensitizing drug for the treatment of diabetes. The drugs mode of action is by activation of adenosine monophosphate activated protein kinase (AMPK) a liver enzyme that plays an important role in insulin signalling, whole body energy balance, and the metabolism of glucose and fats. Activation of AMPK exerts an inhibitory effecton the production of glucose by liver cells. Metformin is the most prescribed anti-diabetic drug in the world and forms the primary first line therapy for treatment of type II diabetes [2].

After a thorough literature survey, few reports for simultaneous estimation of STG and MET in pharmaceutical dosage form and biological fluids were found to be reported including UV-spectrophotometric [3,4,5], RP-HPLC [6,7,8], UPLC [9], and laser diode thermal desorption tandem mass spectrometry methods [10]. However, no HPTLC method was reported for the simultaneous estimation of STG and MET in combined dosage form yet. The present study describes a simple, sensitive and precise HPTLC method for the estimation of STG and MET from combined dosage form.

(a)



(b)

Figure 1: Molecular structure of (a) STG (b) MET

## EXPERIMENTAL

### Materials

Analytical pure samples of STG (Merck Private Ltd., Mumbai, Maharashtra, India) and MET (BriosiaPrivate Ltd., Jejuri, Maharashtra, India) were used in the study. The pharmaceutical dosage form used in this study was JANUMET(Merck Sharp and Dohme, MND Holland, Netherlands) procured from the local market and labelled to contain 50 mg of STG and 500 mg of MET per tablet. The solvents and chemicals used in the study were of AR grade (Research Lab., Nashik, Maharashtra, India).

### Instrumentation

Microsyringe (Linomat syringe 659.004, Hamilton-BonaduzSchweiz, Camag, Switzerland), pre-coated silica gel 60 F-254aluminium plates (10 ×10 cm, 250 µm thickness; Merck,Germany), Linomat 5 applicator (Camag, Muttenz, Switzerland),twin trough chamber (20 × 10 cm; Camag, Muttenz,Switzerland), saturation pad (Camag, Muttenz, Switzerland),UV chamber (Camag, Muttenz, Switzerland), TLC scannerIII (Camag, Muttenz, Switzerland), winCATS version 1.3.0software (Camag, Muttenz, Switzerland) were used in thisstudy. Microsoft excel was also used to treat data statistically.

### Preparation of Standard Solutions

Standard stock solutions were prepared by dissolving separately 10 mg of STG and 110 mg of MET in 10 ml of methanol to obtain a concentration of 1000 µg/ mlof STG and 11000 µg/ mlof MET, respectively. The standard stock solutions were suitably diluted with methanolto obtain the working standard solutions of both STG and MET.

### Preparation of Sample solutions

Twenty tablets (JANUMET, labelled to contain 50 mg of STGand 500 mg of MET per tablet, Merck Sharp and Dohme, MND Holland, Netherlands) were weighed and crushed to fine powder. An accurately weighed powder sample equivalent to 50 mg of STGwas weighed, transferred to a 20 ml volumetric flask and volume made up to about 10 ml with methanol. The solution was sonicated for about 20 min, then diluted to volume with the same solvent and filtered through What manfilter paper No. 42. Working sample solutions were freshly prepared by diluting suitable volumes of the stock sample solution with methanol.

### Optimized Chromatographic conditions

Suitable volumes of standard and sample solutions (µl) were applied to the HPTLC plates, 8 mm from the bottom and8 mm from the side edges in the form of bands or streaks with band length of 8 mm. The mobile phase consisting of methanol: ammonia: glacial acetic acid (9.4:0.4:0.2 v/v/v) was used in each chromatographic run. Ascending development technique was carried out in twin trough chambers. The optimized chamber saturation time for the mobile phase was 20 min at room temperature (25± 2ºC) that was assisted by saturation pads. The distance covered by the solvent front was 80 mm, which took

about 15 min. The spots were scanned using the TLC scanner3 in the absorbance mode at 214 nm and all measurements were operated by win CATS software. Concentrations of the separated compounds were determined from the intensity of absorbed light and peak areas were used for evaluation.

## Analysis of marketed formulation

The tablet sample solutions were prepared as discussed above. Suitable working sample solutions (1µl) containing STG and MET in the concentration ratio of 1:10 (500 ng: 5000 ng of STG and MET, respectively) were prepared, applied on HPTLC plate sand analysed under the optimized chromatographic conditions.

## Validation of HPTLC method [11,12]

### Linearity

Linear relationship between peak area and concentration of the drugs was evaluated over the concentration range expressed in ng band$^{-1}$by making three replicate measurements in the concentrations range of 100–1100 ng band$^{-1}$for STG and 1000–11000 ng band$^{-1}$for MET, respectively.

### Precision

Precision of the developed method was studied by performing repeatability and intermediate precision studies. The sample application and measurement of peak area was determined by performing six replicate measurements of the same band using a sample solution containing 900 ng band$^{-1}$of STG and 9000 ng band$^{-1}$of MET each.

### Recovery studies

Recovery studies were carried out by spiking three different known amounts of the standard substances to the drug product (standard addition method). Hence, 200, 250 and 300 ng band$^{-1}$ of STG and 2000, 2500 and 3000 ng band$^{-1}$ of MET were spiked to the dosage form that contained 250 and 2500 ng band$^{-1}$ of STG and MET, respectively, after sample dilution.

### Limit of detection (LOD) and limit of quantitation (LOQ)

The limits of detection and quantitation of the developed method were calculated from the standard deviation of they-intercepts and slope of the calibration curves of STG and MET using the formulae as given below.

$$\text{Limits of Detection} = 3.3 \times N/B$$
$$\text{Limits of Quantitation} = 10 \times N/B$$

Where N is the standard deviation of the y-intercepts and B is the slope of the calibration curve.

### Specificity

Peak purity of both STG and MET was assessed to evaluate the specificity of the method. The sample and standard bands were scanned at two different levels, i.e., peak start (S) and peak end (E) positions. Standard stock solutions (1000 and 11000µg/ml) of STG and MET were employed in the study, respectively.

## RESULTS AND DISCUSSION

### Analysis of marketed formulation

The marketed formulation, JANUMET was analysed using the developed method. The chromatogram of tablet sample showed only two peaks at R$_f$ value of 0.61 and 0.28 for STG and MET, respectively, indicating that there is no interference of the excipients present in the tablet formulation. The content of STG and MET was calculated by comparing peak areas of sample with that of the standard (Table 1). The densitogram of tablet formulation is shown in Fig. 3.
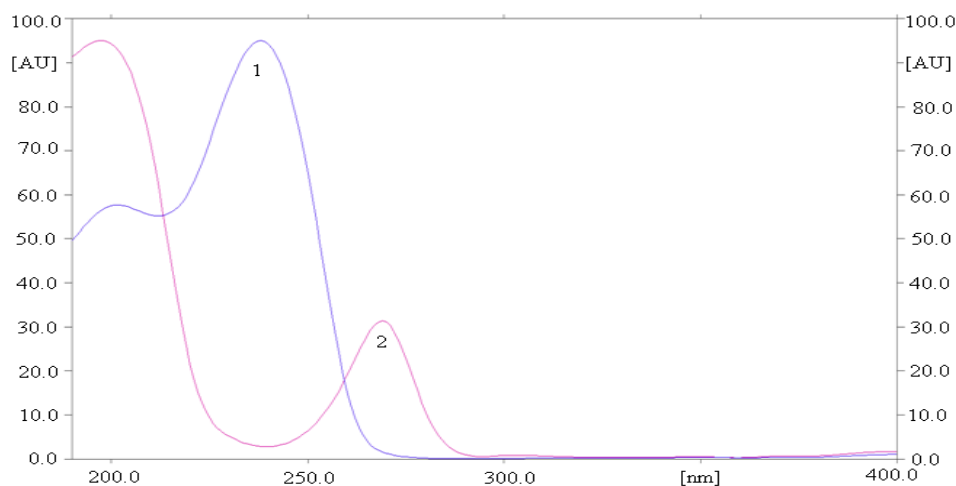
Figure 2: UV overlain spectra of the spots of the standards for MET (1) and STG (2)

## HPTLC method optimization

For the effective separation of STG and MET, several runs were made by using mobile phases containing solvents of varying polarity, at different concentration levels. Among the different mobile phase combinations employed, the mobile phase consisting of methanol: ammonia:glacial acetic acid in the ratio of 9.4:0.4:0.2 v/v/v gave the best resolution with sharp well defined peaks with $R_f$ values of 0.61 ± 0.02and 0.28 ±0.02 for STG and MET, respectively. For the selection of analytical wavelength for the quantification of the drugs, the standard spots applied on silica gel were scanned and their overlain spectra were obtained on the HPTLC instrument. From the overlain spectra (Fig. 2), it was observed that both STG and MET exhibited strong absorbance at about 214 nm which was selected as the analytical wavelength for further analysis.

### Table 1: Assay result of pharmaceutical dosage form

| Component | Amount present (mg per tablet) | % Amount found[a] | SD | % RSD[a] |
|---|---|---|---|---|
| STG | 50 | 100.29 | 0.2453 | 0.2445 |
| MET | 500 | 100.43 | 0.5229 | 0.5206 |

Tablet used for analysis – JANUMET (Merck Sharp and Dohme, MND Holland, Netherlands).
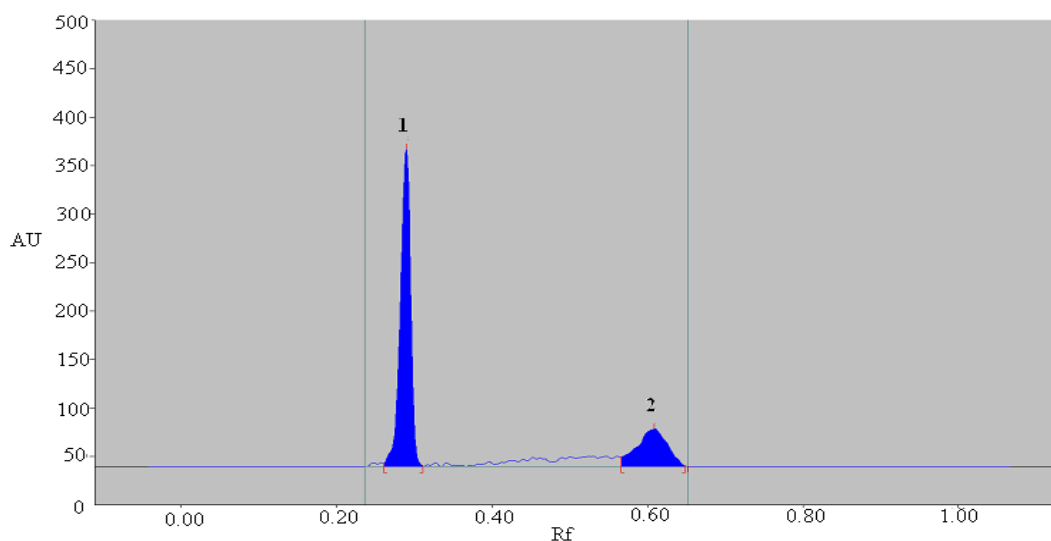[a] Denotes average of six estimation.



Figure 3: Typical densitogram of MET (1) and STG (2) in pharmaceutical dosage form

## Method validation

### Linearity

Peak areas were found to have better linear relationship with the concentration than the peak heights. For STG, the $r^2$ was found to be 0.999, and for MET the $r^2$ was 0.998. Calibration graphs were constructed in the concentration range of 100–1100 ng band$^{-1}$ for STG and 1000–11000ng band$^{-1}$ for MET. The correlation coefficients, y-intercepts and slopes of the regression lines of the two drugs were calculated and are presented in Table 2.

### Table 2: Summary of linear regression and validation data

| Parameters | STG | MET |
|---|---|---|
| Linearity range | 100-1100 ng band$^{-1}$ | 1000-11000 ng band$^{-1}$ |
| Linear regression equation | y = 0.857x + 209.3 | y = 0.271x + 1856 |
| Slope ± SD | 0.857 ± 0.0011 | 0.271 ± 0.0010 |
| Intercept ± SD | 209.3 ± 0.1000 | 1856 ± 0.2000 |
| Correlation coefficient ($r^2$) | 0.999 | 0.998 |
| Limit of detection (LOD) | 7.08 ng band$^{-1}$ | 19.31 ng band$^{-1}$ |
| Limit of quantification (LOQ) | 21.82 ng band$^{-1}$ | 58.51 ng band$^{-1}$ |
| Repeatability (RSD) | 0.600 | 0.850 |
| Intra-day (RSD) | 0.987 | 0.872 |
| Inter-day (RSD) | 0.936 | 0.496 |

### Precision

Repeatability and intermediate precision of the developed method were expressed in terms of relative standard deviation (RSD) of the peak area. The results showed that the repeatability, intra- and inter-day variation of the results at concentration of 900 ng band$^{-1}$ for STG and 9000 ng band$^{-1}$ forMET were within the acceptable range. The coefficients of variation for both the inter-day and intraday precision of the method was found to be less than 1% for both drugs (Table 2).

### Recovery studies

The recovery studies were carried out at 80%, 100% and 120%of the test concentration as per ICH guidelines. The percentage recovery of STG and MET at all the three levels was found to be satisfactory (Table 3). For STG, the % recovery was found between 99.66and 100.62 % and for MET between 99.12 and 101.67 %, respectively.

### Table 3: Recovery study of the method

| Drug | Recovery Level (%) | Initial amount (ng band$^{-1}$) | Amount added (ng band$^{-1}$) | % Mean Recovery* | % RSD |
|---|---|---|---|---|---|
| STG | 80 | 250 | 200 | 100.37 | 0.53 |
| | 100 | 250 | 250 | 99.66 | 0.60 |
| | 120 | 250 | 300 | 100.62 | 1.09 |
| MET | 80 | 2500 | 2000 | 99.12 | 0.22 |
| | 100 | 2500 | 2500 | 101.67 | 0.28 |
| | 120 | 2500 | 3000 | 99.83 | 0.29 |

* Denotes average of three estimations at each level of recovery.

## Limit of detection (LOD) and limit of quantification (LOQ)

The limits of detection and quantitation were found to be 7.08 and 21.82ng band$^{-1}$ for STG and 19.31 and58.51ng band$^{-1}$ for MET, respectively, indicating the sensitivity of the developed method.

## Specificity

The peak purity test of STG and MET spots were assessed by comparing their respective spectra at peak start and peak end positions of the spot and their spectra were overlaid to assess spectral matching.

## CONCLUSION

The developed HPTLC technique is precise, specific and accurate method for analysis of STG and MET in pharmaceutical preparations. The procedure can be readily used for selective analysis of drugs and repeatableresults are obtained without interference from auxiliary substances. It can be successfully applied for simultaneousestimation of STG and MET in tablet dosage forms without prior separation and any interference in qualitycontrol.

## ACKNOWLEDGMENTS

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