

VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND SITAGLIPTIN PHOSPHATE IN BULK DRUG AND FORMULATION

T. Raja¹ and A. Lakshmana Rao^{*}

¹Hindu College of Pharmacy, Guntur, A.P., India.

^{*}V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, A.P., India.

^{*}E-mail: dralrao@gmail.com

ABSTRACT

A new simple high performance thin layer chromatographic method for simultaneous determination of antidiabetic drugs, metformin hydrochloride and sitagliptin phosphate in bulk and tablet dosage form were investigated. Chromatographic separation of the drugs were performed on aluminum plates precoated with silica gel 60 F₂₅₄ as the stationary phase and the solvent system consisted of acetone:methanol:toluene:formic acid (4:3:2:1 v/v/v/v). Densitometric evaluation of the separated zones was performed at 220 nm and the method was validated. The R_f values and drug content of metformin hydrochloride and sitagliptin phosphate were 0.36±0.02, 0.63±0.02 and 100.1%, 99.84% respectively. The calibration curves of peak area versus concentration, which were linear from 2000-5000 ng per band for metformin hydrochloride, 200-500 ng per band for sitagliptin phosphate and regression coefficient (r²) was greater than 0.99. LOD for metformin hydrochloride and sitagliptin phosphate was 45 and 27 ng per band respectively, while LOQ was 150 and 87 ng per band respectively. The method was validated for linearity, accuracy, robustness and application for assay as per ICH guidelines. The study shows that the developed method is simple and accurate and it would be suitable for the simultaneous determination of metformin hydrochloride and sitagliptin phosphate in bulk drug and pharmaceutical formulations.

Keywords: Metformin, Sitagliptin, HPTLC, Estimation.

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INTRODUCTION

Metformin hydrochloride (MET) (Fig.-1) chemically, N,N-dimethylimidocarbonimidic diamide. It is a biguanide drug well known as antidiabetic drug, the mechanism of action of metformin is simulates glycolysis in peripheral tissue¹. Sitagliptin phosphate (STG) (Fig. 2) chemically, 7-[(3R)-3-amino-1-oxo-4-(2,4,5-trifluorophenyl)-5,6,7,8-tetrahydro-3-(trifluoromethyl)-1,2,4-triazole [4,3] pyrazoline phosphate(1:1) monohydrate. It is a novel hypoglycemic drug that belongs to dipeptidyl-peptidase 4 inhibitor class which stimulates glucose-dependent insulin release^{2,3}. Recently the combination of two drugs has been recommended in the treatment of diabetes mellitus to improve glycemic control⁴.

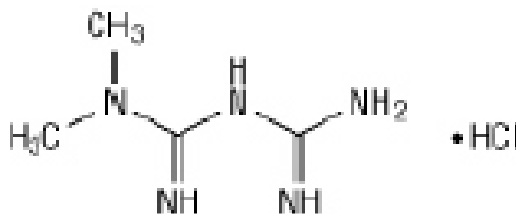


Fig.-1: Chemical structure of metformin hydrochloride

This combination proved to be effective in controlling the metabolic syndrome and resulted in significant weight loss, reversal of insulin resistance, islet and adipocyte hypertrophy and achieved hepatic steatosis. According to literature survey few spectrophotometric⁵⁻⁷, HPLC^{8,9} and HPTLC¹⁰ methods have been

reported for the determination of MET in single and in combination with other drugs. Analytical methods are reported for the determination of STG by UV¹¹, HPLC¹² have been reported. Simultaneous determination of MET and STG in bulk and tablet dosage form by using UV^{13,14}, HPLC¹⁵. However, there is no HPTLC method reported for the simultaneous estimation of MET and STG in tablet dosage form. The aim of present work was to develop and validate a sensitive HPTLC method that can be applied for simultaneous estimation of MET and STG.

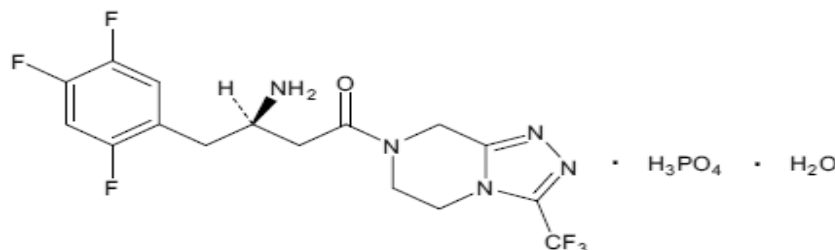


Fig.-2: Chemical structure of sitagliptin phosphate

EXPERIMENTAL

Instrumentation

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample syringe (Hamilton, Bonded, Switzerland) on silica gel precoated aluminum plate 60F- 254 plates, [10 cm X 10 cm with 250 μ m thickness; E. Merck, Darmstadt, Germany] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. The slit dimension was kept at 5 mm X 0.45 mm and the scanning speed was 10 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of acetone:methanol:toluene:formic acid (4:3:2:1 v/v/v/v) and 10 mL of mobile phase was used per chromatography run. Linear ascending development was carried out in a 10 cm X 10 cm twin rough glass chamber (Camag, Muttens, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 40 min at room temperature (25°C $\pm$ 2) at relative humidity of 60% $\pm$ 5. The saturation time was kept more than an ideal time (30 min) because of less amount of organic solvent present in the mobile phase used for chromatography run. Each chromatogram was developed over a distance of 8 cm. Following the development the TLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow rate in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance absorbance mode at 220 nm and operated by CATS software (V 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographer were determined from the intensity of the diffused light. Evaluation was performed by linear regression of peak areas determined by UV absorption as a function of sample amounts.

Materials

Working standards of pharmaceutical grade MET (Batch No.: 3489/201), STG (Batch No.: 5436/501) were obtained as generous gifts from Merck Sharp Dohme, USA. They were used without further purification and certified to contain 99.96% and 99.99% (w/w) on dry weight basis for MET and STG respectively. Fixed dose combination tablets (Brand Name: Janumet) containing 500 mg of MET and 50 mg of STG and procured from Merck Sharp Dohme, India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Optimization of the HPTLC method

Initially, ethylacetate and methanol in the ratio of 5:5 (v/v) was tried for both drugs simultaneously. The spots were not developed properly and dragging was observed. Then, acetone, methanol and toluene in the ratio of 4:2:4 (v/v/v) were tried. The developed spots were diffused. To the above mobile phase, 0.5

mL formic acid was added. Both the peaks were symmetrical in nature and tailing was observed. To improve resolution, the volume of formic acid was increased. Ultimately, mobile phase consisting of acetone:methanol:toluene:formic acid (4:3:2:1 v/v/v/v) gave good resolution. Both the peaks were symmetrical in nature and no tailing was observed when plate was scanned at 220 nm. The chamber was saturated with the mobile phase for 20 min at room temperature and plates were activated at 110⁰ C for 5 min to obtain well-defined.

Preparation of standard stock solution

Accurately weigh and transfer 1 mg of MET and 10 mg of STG working standards into a 100 mL clean dry volumetric flask, add about 10 mL of diluent and make volume up to the mark with the same diluent. Further pipette out 1 mL from above stock solution into 100 mL volumetric flask and dilute up to the mark with diluent. Calibration was done by mixed standard solutions ranging from 2 to 5 µL.

Preparation of sample solution

Twenty tablets were accurately weighed and crushed into a fine powder in a mortar. An amount of powder equivalent to 50 mg of MET and 5 mg of STG transferred in to 100 mL volumetric flask and 10 mL of diluent was added to it. The mixture was sonicated to dissolve and then made volume up to the mark with diluent and the solution filtered through 0.45 µm filter paper. 1 mL of solution was pipette out and transferred to 100 mL volumetric flask, made volume up to the mark by using diluent. From the above stock solution pipette out 1 mL of the solution into a 10 mL volumetric flask made up to volume with mobile phase to yield concentration of MET (500 ng per band) and STG (50 ng per band). A 4 µL sample was spotted six times under optimized chromatographic conditions. The peak areas were measured at 220 nm.

Method validation

The method was validated in accordance with ICH guidelines¹⁶. The parameters assessed were linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, reproducibility and robustness.

Linearity

Six different concentrations of the mixed standard drugs of MET and STG were prepared for linearity studies and injected into system (n=6). The response was measured as peak areas. Each concentration was prepared from individual stock solution. The plate was then developed by using mobile phase by keeping the injection volume constant. The peak areas were plotted against concentrations to obtain the calibration curve.

Accuracy

The accuracy was carried out by adding known amounts of each analyte corresponding to three concentration levels (80, 100, 120%) of the labeled claim to the excipients. At each level, six determinations were performed and the results were recorded. Accuracy was expressed as percent analyte recovered by the proposed method.

Precision

The precision of analytical method is the degree of agreement among the individual test results, when the method is applied repeatedly to multiple sampling of homologous samples. The precision of the method was checked by repeatability of injection, repeatability (intra-day), inter- mediate precision (inter-day) and reproducibility. Injection repeatability was studied by calculating the percentage relative standard deviation (%RSD) for six determination of peak areas of MET (3500 ng per band) and STG (350 ng per band), performed on the same day. For both intra-day and inter-day variations standard solutions of MET (2000, 2500, 3000 ng per band) and STG (200, 250, 300 ng per band) were injected six times for each concentration.

Detection limit and quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) were calculated according to Equation 1 and 2, respectively.

$$\text{LOD} = 3.3 \times \text{SD}/S \quad (1)$$

$$\text{LOQ} = 10 \times \text{SD}/S \quad (2)$$

Where, SD is the standard deviation of response (peak area) and S is the average of the slope of the calibration curve.

Robustness

Robustness was assessed by introducing small changes in the mobile phase composition and measuring the effects of result, mobile phase. The amount of mobile phase was varied by ± 0.5 mL, the plates were pre-washed with methanol and activated at $60 \pm 5^\circ\text{C}$ for 2, 5 and 7 min before chromatography. Time from application to chromatography and from chromatography to scanning was also varied (20, 40 and 60 min). The robustness of the method was measured 2000 ng per band for MET and 200 ng per band for STG.

Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation products and related substances. In the present work, the densitograms of the samples were checked for the appearance of any extra peaks.

RESULTS AND DISCUSSION

A typical densitogram recorded at 220 nm is shown in Figure-3. The R_f values of MET 0.36 and STG 0.63 respectively. The analytic peaks were well resolved.

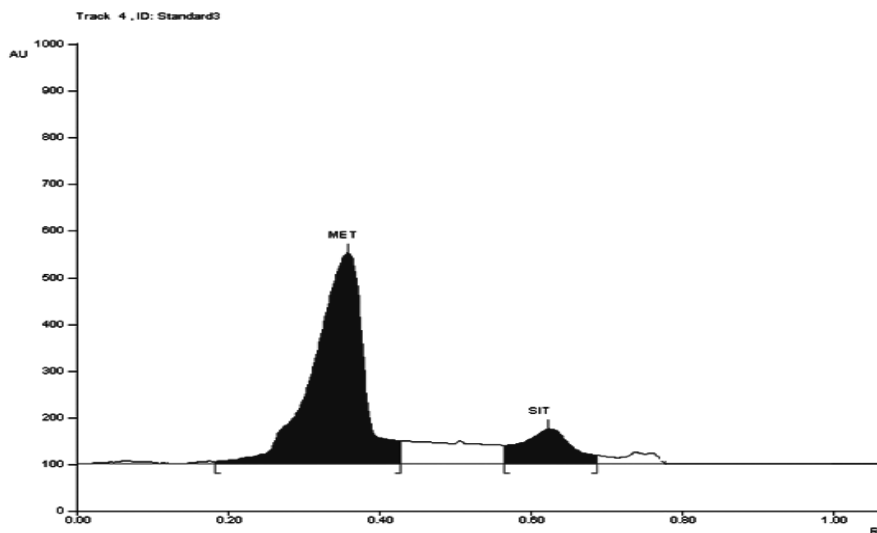


Fig.-3: Typical densitogram of metformin hydrochloride and sitagliptin phosphate

Method validation

Linearity

The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 2000-5000 ng per band and 200-500 ng per band for MET and STG respectively. Linear regression data for the calibration curves are given in Table-1.

Table-1: Linear regression data for the calibration curves^a

Parameter	MET	STG
Linear range (ng per band)	2000-5000	200-500
Correlation coefficient	0.997	0.995
Slope	3.21	5.106
Intercept	7697.7	146.1
LOD (ng per band)	45	27
LOQ (ng per band)	150	80

^an = 6**Accuracy**

The mean recovery obtained for MET and STG was 100.19 and 100.47 % respectively. The %RSD is less than 2, results were given in Table-2.

Table-2: Results of accuracy for proposed method^a

Spiked level of drug (%)	Amount of drug added (ng/spot)		Mean recovery (%) (n=6)		RSD (%)	
	MET	STG	MET	STG	MET	STG
80	4000	400	98.7	99.54	0.30	0.82
100	5000	500	101.14	101.29	0.43	0.37
120	6000	600	100.85	100.58	0.60	0.17

^an = 6**Precision**

Results for repeatability and intermediate precision, expressed as %RSD, results were given in Table-3. The low values of %RSD indicate that the method is precise. Reproducibility was checked by analyzing the samples by another analyst using same instrument and same laboratory. There was no significant difference between the %RSD values, which indicates that the proposed method was reproducible.

Table-3: Results of precision for proposed method^a

Drug name	Amount (ng per band)	Intra-day precision			Inter-day precision		
		Mean area	SD	%RSD	Mean area	SD	%RSD
MET	2000	13894.96	30.56	0.21	13987.26	32.0	0.23
	2500	15718.39	51.87	0.33	15818.18	53.78	0.34
	3000	17560.74	41.74	0.27	17229.70	128.18	0.74
STG	200	1105.64	37.57	0.34	1177.84	42.4	0.38
	250	1484.64	40.08	0.27	560.66	46.8	0.30
	300	167.22	67.16	0.40	1610.88	64.4	0.40

^an = 6**Detection limit and quantification limit**

LOD for MET and STG was 45 and 27 ng per band respectively, while LOQ was 150 and 87 ng per band.

Robustness

There was no significant change in the peak areas and R_f values of MET and STG when the composition of mobile phase was varied by ± 0.5 mL, variation of time for activation of plates before chromatography and chromatography scanning also varied. The results are showed in Table-4.

Table-4. Results of robustness for proposed method^a

Chromatographic conditions	%RSD
Mobile phase composition	1.21
Plate pretreatment	0.99
Time from application to chromatography	0.38
Time from chromatography to scanning	0.33

^a*n* = 6**Specificity**

No interference from any of the excipients was found at *R_f* values of the examined drugs. In addition, the densitogram of each drug in the sample solution was found identical to the densitogram received by the standard solution at the wavelengths applied. These results demonstrate the absence of interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the proposed method.

Quantification of MET and STG in tablet dosage form

The proposed method was applied to the simultaneous determination of MET and STG in tablets. The results of the assay yielded 100.14±0.33% for MET and 99.84±0.24% for STG, of label claim of the tablets. The assay results show that the method was selective for the simultaneous determination of MET and STG without interference from the excipients used in the tablet dosage form. The results are shown in the Table-5.

Table-5: Results of sample analysis for proposed method^a

Brand name	Analyte	Label claim per tablet (mg)	% Analyte estimated (mean±SD)	%RSD
Janumet	Metformin	500	100.1±0.33	0.3302
	Sitagliptin	50	99.84±0.24	0.2395

^a*n* = 6

In order to achieve simultaneous estimation of the two components, initial trials were performed with the objective of selecting adequate and optimum chromatographic conditions. Parameters, such as ideal mobile phase and their proportions, detection wave length and concentrations of the standard solutions were carefully studied. Several solvents were tested in varying proportions. Finally, a mixture of acetone:methanol:toluene:formic acid (4:3:2:1 v/v/v/v) was selected as the optimum mobile phase. The optimized chromatographic conditions were selected based on sensitivity, *R_f* values, peak shape and baseline drifts. The method was validated in terms of linearity, accuracy, precision, LOD, LOQ, robustness and specificity as per ICH guidelines. The accuracy data shows that the method is accurate within desired range. The LOD and LOQ values were low which indicates that the method is sensitive. The method was robust as minor changes in the chromatographic parameters did not bring about any significant changes in peak area and *R_f* value.

CONCLUSION

The developed method for the simultaneous determination of MET and STG has advantage of sensitivity, accuracy, precision and low cost. The non-interference of tablet excipients make the method suitable for the simultaneous estimation of these drugs in tablets and hence can be used for routine quality control of MET and STG in pharmaceutical dosage form.

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