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Research article

HPTLC method for simultaneous estimation of Metformin HCl and Sitagliptin in pharmaceutical dosage form

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Abstract

An accurate, precise and derivatized HPTLC method has been developed for the simultaneous estimation of Metformin and Sitagliptin in tablet formulation. In this method standard and sample solutions of Metformin and Sitagliptin were applied on pre-coated silica gel $60F_{254}$ TLC plate, and developed using Ammonium sulphate (0.5%)-2-Propanol-Methanol (8:1.6 :1.6v/v), as mobile phase[1] and derivatized using CAMAG-REPROSTAR-3. A camag HPTLC system comprising of Camag Linomat-5-applicator, camag twin trough chamber, camag reprostar-3, camag TLC -3- scanner was used for the analysis. The drugs on the plate were scanned at 254 nm. The dynamic linearity range was 700-1500 ng/spot for Sitagliptin and 7-15µg/spot for Metformin. The method was validated for precision, accuracy and reproducibility.

Key words: Simultaneous estimation, HPTLC, Metformin and Sitagliptin.

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1. Introduction

Sitagliptin [SGT] (figure 1) is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor, which improves glycaemic control by inhibiting DPP-4 inactivation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). This increases active incretin and insulin levels and decreases glucagon levels and post-glucose-load glucose excursion [2]. Metformin [MET] (Metformin HCl, *N*,*N*-dimethylimidodicarbonimidic diamide hydrochloride), is an oral antidiabetic drug [3] (figure 2). Literature survey revealed that various analytical methods like spectrophotometric [4], HPLC [5,6] , LC-MS [7,8] and UPLC [9] methods, have been reported for the determination of Metformin and Sitagliptin , individually and combination with some other drugs. A very few HPTLC methods for simultaneous estimation of Metformin and Sitagliptin in combined dosage forms has so far been reported. The review of literature prompted us to develop an accurate, precise and derivatized simultaneous method for the estimation of

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Sitagliptin and Metformin in combined dosage forms.

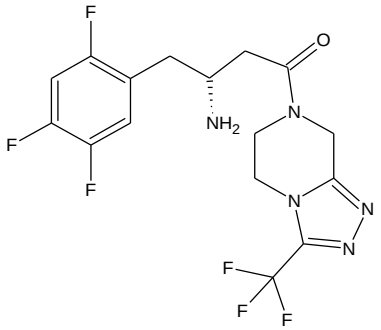

Figure 1. Sitagliptin

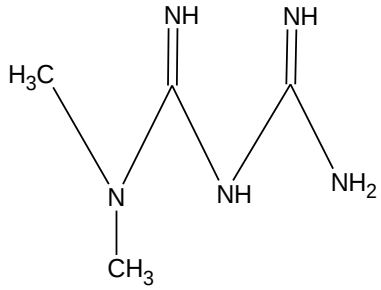

Figure 2. Metformin

2. Materials and Methods

Chemicals and Equipment

JANUMET Tablet used for the formulation analysis contains Metformin (500 mg) and Sitagliptin (50 mg) and it is manufactured and marketed by MSD Pharmaceuticals Pvt. Ltd, Maharashtra. Pure samples were procured from, Metformin– Orchid Pharmaceuticals, Chennai and Sitagliptin – Micro Labs, Bangalore. All the chemicals and reagents used were of analytical grade. A Camag HPTLC system comprising of Camag Linomat-5-applicator, Hamilton syringe, Camag twin trough chamber, Camag TLC scanner, and stationary phase pre coated with Silica gel $60F_{254}$ were used.

Preparation of Standard Solutions

The given standard Sitagliptin 1 mg was dissolved in 1 ml Methanol, this solution used as working standard solution (1µg/1µl) for the analysis. The given standard Metformin 1 mg was dissolved in 1 ml Methanol, this solution used as working standard solution (1µg/1µl) for the analysis. Standard solutions having concentration ranging from 700-1500 ng/spot for Sitagliptin and 7-15µg/spot for Metformin were applied on TLC plates.

Analysis of Tablet Formulation

The given Janumet 14 tablets were powdered using Pestle & Mortar to fine powder. From this, powder 2 mg was weighed in an Electronic balance (Afcoset) and dissolved in 1 ml of Methanol, centrifuged and the supernatant liquid was taken for the HPTLC studies. This solution contains 1.41 µg of Sitagliptin and 14.1 µg of Metformin drug sample as test solution per µl. From this 1µl of Sample solution was applied on the pre-coated silica gel $60F_{254}$ plate and from the peak area obtained, the amount of Sitagliptin and Metformin in formulation was simultaneously calculated using the respective calibration graph.

Development of Chromatograms

The TLC plates were pre washed with methanol and activated by keeping at 115˚ for about 30 min. The samples were spotted in the form of bands of width 4 mm with 100 µl Hamilton syringe on the pre-coated silica gel $60F_{254}$ plate (12×10cm) and the slit dimension was kept at 15 min respectively. The mobile phase Ammonium sulphate (0.5%)-2-Propanol-Methanol (8 : 1.6 : 1.6 v/v) used was in chamber and the plate saturation time was 15 min, migration distance was allowed up to 90 mm, linear ascending development was carried out in (20×10cm) twin trough glass chamber. Subsequent to the development, TLC plates were dried in current of air and kept in photo documentation chamber. The images of developed plate were captured at white light, UV 254 nm and UV 366 nm using Camag - Reprostar - 3 instrument. The developed plate was derivatized with iodine

vapor and the images were done in white light using Camag - Reprostar -3 instrument. The derivatized plate was scanned at 254 nm using Camag-TLC-scanner-3 instrument.

Validation Parameters

The method was validated in accordance with WHO guidelines [10] for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra-day assay precision, repeatability of measurement and repeatability of sample application. Samples applied on the plate were developed with the mobile phase and the peak areas were noted. The mobile phase, Ammonium sulphate (0.5%)-2-Propanol-Methanol (8: 1.6:1.6v/v) gave R_f value of 0.35 ± 0.02 for Sitagliptin and 0.63 ± 0.04 for Metformin.

Linearity and Regression

A good linear relationship was obtained over the concentration range700-1500 ng/spot for Sitagliptin and 7-15µg/spot for Metformin respectively. The linear regression data showed a regression coefficient of 0.999 for Sitagliptin and 0.999 for Metformin.

LOD and LOQ

The LOD with signal/ noise ratio were found to be 25 ng and 500 ng/spot for Sitagliptin and Metformin respectively. The LOQ with signal/ noise ratio was found to be 900 ng and 1500 ng /spot for Sitagliptin and Metformin respectively.

Precision

Intra-day assay precision was found by analysis of standard drug at three times on the same day. Inter-day assay precision was carried out using at three different days, and percentage relative standard deviation (%RSD) was calculated. The RSD was found to be less than 2 for both intra-day and inter-day precision. Repeatability of sample application was assessed by spotting 1 µl of drug solution, six times. From the peak areas, the percentage RSD was determined. The complete validation parameters are shown in Table 1.

Table 1. Validation Parameters

S. No.	Sitagliptin	Metformin
Rf	0.35	0.63
LOD	25 ng/spot	500 ng/spot
LOQ	900 ng/spot	1500 ng/spot
Recovery	99.5%	97.7%
Repeatability (%RSD)	1.4	1.02
Linearity range	700-1500 ng	7-15µg
Regression coefficient	0.999	0.999

R_f - resolution factor, RSD- relative standard deviation, LOD – limit of detection, LOQ – limit of quantification

Recovery Studies

The recovery study was carried out at two levels, 50%, 100 %. To the powdered formulation, the standard drugs of Sitagliptin and Metformin were added at 50 % and 100 % levels, dilutions were made and analyzed by the method. The % recovery and % RSD were calculated and found to be within the limit, as listed in Table 2.

Table 2. Result f Analysis f Formulation

Formulation	Label Claim		Amount Obtained (mg)*		% Assay		Standard Deviation		% RSD*	
	MET	STG	MET	STG	MET	STG	MET	STG	MET	STG
Janumet	500	50	495.8	48.6	99.16	97.8	0.4743	0.8456	0.095	1.73

*An average value ± relative standard deviation of 5 observations.

3. Result and Discussion

During the stage of method development different mobile phases were tried and the mobile phase comprising of Ammonium sulphate (0.5%)-2-Propanol-Methanol (8:1. 6:1.6v/v) was confirmed. The R_f value was found to be 0.35 & 0.63 for Sitagliptin and Metformin respectively. Linearity of the drug was determined by the calibration curves and the linearity based on the peak area was in the range of 700-1500 ng & 7-15µg respectively (table 1). The regression coefficient value for Sitagliptin and Metformin are 0.999 (figure 3) and 0.999 (figure 4) respectively. The limit of quantification was determined by injecting minimum concentration of the drugs. The limit of quantification was found as 1000 ng/spot, & 50 ng/spot. The recovery was 99.5%, and 97.7% for sitagliptin and metformin (table 1) and the repeatability showed excellent % RSD less than 1.4 (table 2, table 3). The method passes all the validation parameter limits and proves to be selective, sensitive and precise. Hence the proposed method can be used for the routine assay of Metformin and Sitagliptin using HPTLC.

Table 3. Recovery Data

Level	Amount added (mg)		Amount Obtained (mg)*		% Recovery*		% RSD*	
	MET	SIT	MET	SIT	MET	SIT	MET	SIT
50%	250	25	252.42	25.5	100.96	102	0.94	1.03
100%	500	50	505	51.6	101	103.2	1.02	1.33

*An average value ± relative standard deviation of 5 observations.

Table 4. Peak Table

Track	Peak	Rf	Area	Assigned substance
S1	1	0.35	480.6	Sitagliptin standard
S2	1	0.35	658.8	Sitagliptin standard
S3	1	0.35	879.6	Sitagliptin standard
S4	1	0.35	1087.8	Sitagliptin standard
S5	1	0.35	1300.4	Sitagliptin standard
F	1	0.63	4074.9	Metformin
F	2	0.35	1170.5	Sitagliptin
M1	1	0.63	2351.9	Metformin standard
M2	1	0.63	2889.7	Metformin standard
M3	1	0.63	3445.9	Metformin standard
M4	1	0.63	3505.9	Metformin standard
M5	1	0.63	4435.3	Metformin standard

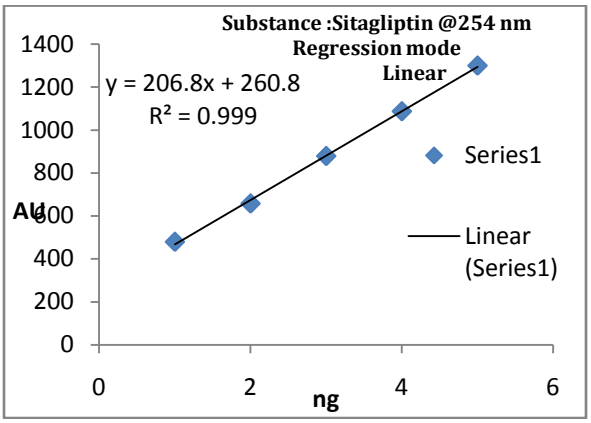

Figure 3. Linearity graph Sitagliptin Based on Area

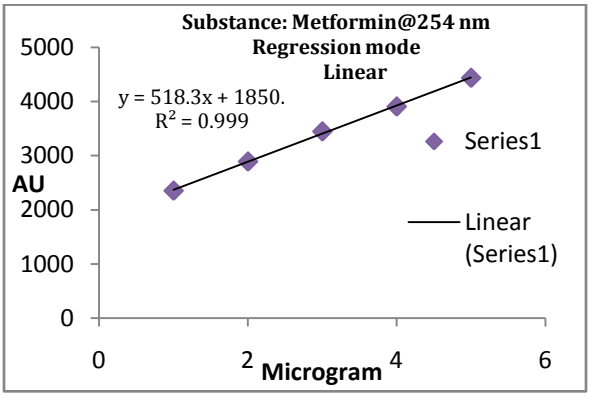

Figure 4. Linearity Graph Metformin Based on Area

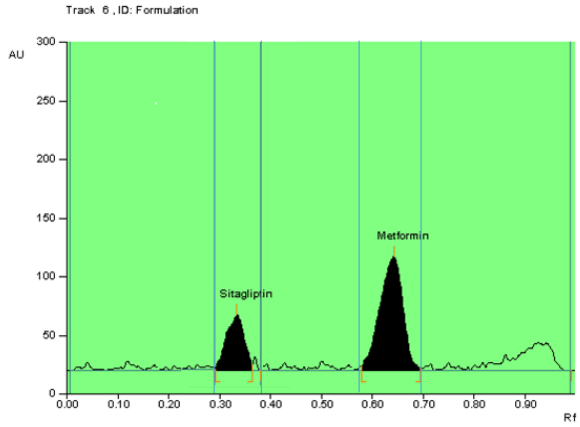

Figure 5. Chromatogram of Metformin HCl and Stagliptin Phosphate. Chromatogram showing resolution of Metformin (R_f = 0.63), Stagliptin (R_f = 0.35) .

Conclusion

Hence, the developed HPTLC method is precise, specific and accurate; the statistical analysis proved that the method is repeatable and selective for the simultaneous analysis of Metformin and Sitagliptin in bulk drugs and in pharmaceutical dosage forms without any interference from the excipients.

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