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RESEARCH ARTICLE

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Sitagliptin Phosphate from Bulk and Combined Dosage Form

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ABSTRACT

A simple, accurate, precise and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of Metformin Hydrochloride and Sitagliptin Phosphate in pure and tablet formulation. The proposed method is based on the separation of the two drugs in reversed-phase mode using BDS HYPERSIL C18 (4.6mmø×250mm) analytical column. The optimised mobile phase consisted of phosphate buffer (pH adjusted to 4 using o-phosphoric acid): Methanol: Acetonitrile in the ratio of 50:30:20 v/v/v. Flow rate was kept at 0.8 ml/min. The simultaneous estimation was carried out at detection wavelength of 253 nm using variable wavelength detector. Both drugs- Metformin Hydrochloride and Sitagliptin Phosphate were well resolved and retained at 3.15 minutes and 6.05 minutes respectively. The method was statistically validated as per ICH guideline for analytical method validation. The validated method was used for simultaneous estimation of Metformin Hydrochloride and Sitagliptin Phosphate from their marketed tablet formulation.

KEYWORDS

RP-HPLC, Sitagliptin Phosphate, Metformin Hydrochloride, Tablet Formulation, Validation

INTRODUCTION

Metformin Hydrochloride (MET) is glucose lowering agent that is widely used for management for type II diabetes.[1] Sitagliptin phosphate (STG) is an oral anti-hypoglycemic drug which is highly selective dipeptidyl peptidase-4(DPP-4) inhibitor that prolongs the action of incretins, hormones that stimulate postprandial insulin secretion via direct action on pancreatic β-cells and suppress glucagon secretion by the α-cells.[1] These two drugs are generally co-administered to diabetic patients. They are marketed in combined tablet dosage form. Hence, the proposed experimental work was aimed to develop and validate RP-HPLC method for simultaneous estimation of MET and STG.

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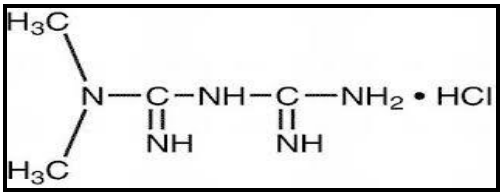

Figure 1: Chemical structure of Metformin Hydrochloride

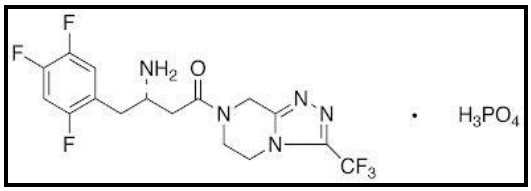

Figure 2: Chemical structure of Sitagliptin Phosphate

MATERIALS AND METHODS

Materials and Reagents

Working standards of pharmaceutical grade MET, STG were obtained as generous gifts from Glenmark generics, Mumbai. Fixed dose combination tablets (Brand Name: Janumet) containing 500 mg of MET and 50 mg of STG were procured from Glenmark Generics, India.

Methanol, Acetonitrile were purchased from SD Fine Chemicals, Mumbai.

Instrument

The HPLC system used was Agilent 1200 series equipped with variable wavelength detector. The chromatogram was recorded using EZChrom software.

Experimental

Analytical Method Development

Preparation of Standard Stock and Working Solution

100 mg STG and MET each were accurately weighed and transferred into 100 ml volumetric flask separately and volume was made upto 100 ml with distilled water.

Working solution was prepared from standard solution. 1ml from each of stock solutions were pipetted out and transferred to 10ml volumetric flask and volume was made upto the mark with mobile phase.

Preparation of Sample Solution for Simultaneous Estimation from Marketed Tablet Formulation

Twenty tablets were accurately weighed and crushed into a fine powder. The weight of powder equivalent to 500 mg of MET and 50mg of STG was transferred into 100ml volumetric flask and dissolved in water. The mixture was sonicated to dissolve drugs and then volume was made up to the mark with distilled water. The solution was filtered through 0.45µm filter paper. 1ml was pipetted out from this resulting solution and transferred into 100ml volumetric flask. Volume was made upto the mark with mobile phase to yield concentration of MET (500 µg/ml) and STG (50 µg/ml).

Selection of Detection Wavelength

UV absorption spectra for 10 ppm solution of each MET, STG individually and their mixture were generated by scanning over the range of 200-400 nm.

Optimisation of Chromatographic Conditions

Many preliminary trials were carried out for selection and optimisation of stationary phase, mobile phase, flow rate, injection volume and column temperature.

Analytical Method Validation

Performance characteristics of analytical HPLC method were statistically validated as per ICH guideline for analytical method validation.[3]

Table 1: Analytical Method Validation: Parameters and their determination

Parameter	Method / Procedure followed
Specificity	As per ICH, Specificity should be carried out to ensure identity of an analyte. To determine specificity chromatograms were obtained for blank, MET, STG individually and their mixture.
Accuracy	Accuracy was established across the specified range of analytical procedure by adding known added quantities of analyte to the synthetic mixture of drug product components and to the combined dosage form. As per ICH, Accuracy should be assessed using a minimum of 9 determinations over a minimum of three concentration levels covering the specified range i.e. 3 concentrations levels in triplicate. (e.g., 3 concentrations/ 3 replicates each).

<table>
<tr><td></td><td colspan="2">Accuracy of the method is reported as percent recovery of known added amount of analyte in sample. The percent recovery was calculated by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% of 10ppm solution of synthetic mixture of MET and STG Recovery studies were also performed on tablets containing MET and STG.</td></tr>
<tr><td rowspan="3">Precision</td><td colspan="2">Precision was carried out at two levels.</td></tr>
<tr><td>Repeatability</td><td>Intermediate Precision</td></tr>
<tr><td>Repeatability was assessed by using minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/ 3 replicates each)</td><td>Intermediate Precision was established to study the effects of random events i.e. days, on the precision of the analytical procedure.
Intraday and interday precision studies were performed by taking 9 determinations of 3 concentrations/3 replicates each, at 3 times in a same day and on 3 different days, respectively.</td></tr>
<tr><td colspan="2">Precision is reported as standard deviation and relative standard deviation (coefficient of variation) for each type of precision investigated.</td></tr>
<tr><td>Detection Limit and Quantification Limit</td><td colspan="2">Detection limit and quantification limit is determined based on the standard deviation of the response and the slope
<table><tr><th>DL (LOD)</th><th>QL (LOQ)</th></tr><tr><td>$LOD = \frac{3.3\,\sigma}{S}$</td><td>$LOQ = \frac{10\,\sigma}{S}$</td></tr></table>$\sigma$ = Standard deviation of response estimated based on the calibration curve.
S = Slope of the calibration curve.</td></tr>
<tr><td>Linearity</td><td colspan="2">A linear relationship was evaluated across the range of 10 to 60 mg for both drugs namely MET and STG.
As per ICH, for the establishment of linearity, a minimum of 5 concentrations are recommended.
Linearity is reported by the value of the correlation coefficient, y-intercept, and slope of the regression line along with a plot of the data.</td></tr>
<tr><td>Robustness</td><td colspan="2">Robustness was evaluated for proving the reliability of an analytical method with respect to deliberate variations in method parameters.
To establish robustness of analytical method following factors were studied
1. Influence of variations of pH in a mobile phase
2. Influence of variations in mobile phase composition
3. Temperature
4. Flow rate</td></tr>
</table>

Validation parameters and procedures followed for their determination are tabulated in table 1.

RESULTS AND DISCUSSION

Analytical Method Development

Selection of Wavelength

UV absorption spectra for 10 ppm solution of each MET, STG individually and their mixture were overlaid (Figure 3) and 253 nm (an Isobestic wavelength) was selected as a detection wavelength for simultaneous chromatographic determination of MET and STG.

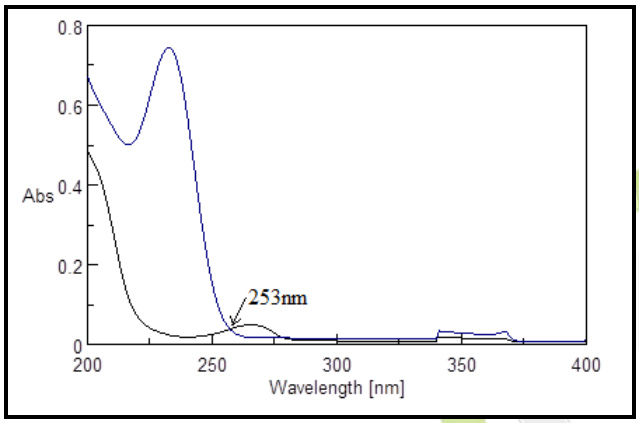

Figure 3: An overlay of UV spectra of MET and STG

Optimization of Chromatographic Conditions

According to the literature survey, it was observed that both the drugs MET and STG were well retained on C18 column respectively. Thus, in order to get optimum resolution simultaneously C18 column was selected. Many preliminary trials were carried out for selection of mobile phase; some are tabulated in table 2.

Table 2: Optimization trials for mobile phase composition

Mobile phase components	Compositions
Methanol : Water	(60:40),(80:20)
ACN : Water	(50:50),(70:30)
100mM Ammonium acetate buffer(pH 5): ACN	(50:50)
20mM Ammonium acetate buffer(pH 5.5) : ACN	(30:70)
20 mM Phosphate Buffer (pH 4) : Methanol :ACN	(50:40:10)

Different flow rate in the range of 0.5 to 1.5 ml/min and different injection volumes in the range of 20 µl to 100 µl were tried. Optimized mobile phase selected was composed of 20 mM Phosphate Buffer (pH 4): Methanol: Acetonitrile (50:30:20).

Optimized chromatographic conditions are tabulated in table 3.

Table 3: Optimized chromatographic conditions

Mobile Phase	20 mM Phosphate Buffer (pH 4) : Methanol : Acetonitrile (50:30:20)
Stationary Phase	BDS HYPERSIL C18 (4.6mmø×250mm) analytical column
Flow rate	0.8ml/min
Detection wavelength	253nm
Injection volume	50 µl

Chromatogram obtained using these optimised chromatographic conditions showed that both drugs namely MET and STG were well resolved and retained at 3.15 minutes and 6.05 minutes respectively. Representative chromatogram of MET and STG is shown in figure 4.

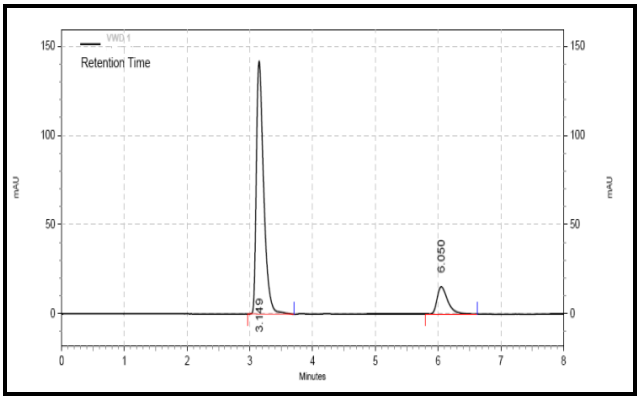

Figure 4: Representative chromatogram of MET and STG

Analytical Method Validation

Specificity

Separate chromatograms were obtained for blank, MET, STG individually and their mixture to ensure the identity of both analytes under study namely, MET and STG. The labelled overlay of chromatograms of blank, MET, STG individually and their mixture is shown in figure 5.

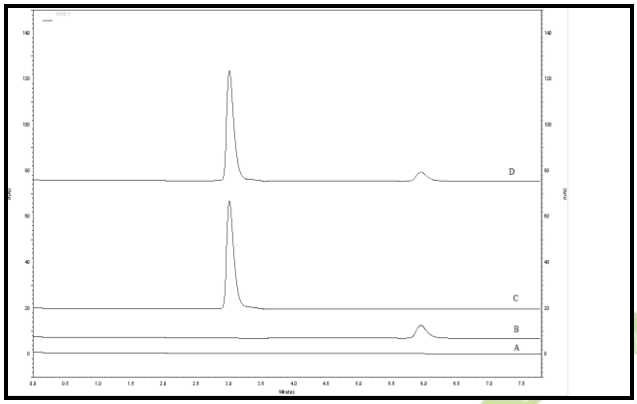

Figure 5: An overlay of chromatograms of blank, MET, STG individually and their mixture.

A: Blank run, B: STG, C: MET, D: Mixture of MET and STG

Linearity

Six serial dilutions of MET and STG were prepared using a standard stock solution and dilution were made with mobile phase. [20 mM Phosphate Buffer (pH 4): Methanol: Acetonitrile (50:30:20)]. Responses were recorded as peak area. The peak areas were plotted against concentrations to obtain the calibration curve. Both MET and STG were found linear in the range of 10-60 ppm. The linearity plots of MET and STG are given in figure 6 and 7. The values of correlation coefficient, y intercept and slope of regression line are shown in table 4.

Table 4: Values for linearity

Drug	R^2	y – intercept	Slope
MET	0.9972	70215	51568
STG	0.9908	9022.2	8328.3

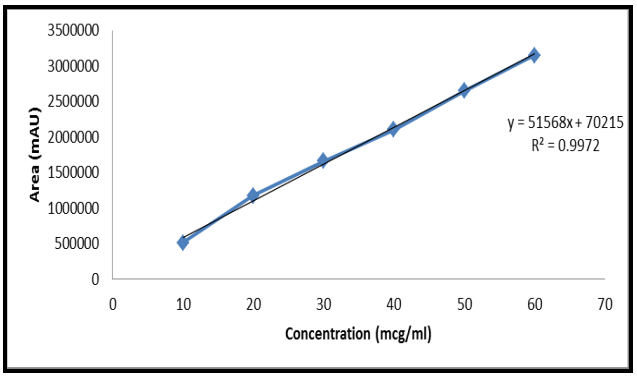

Figure 6: Linearity: Calibration plot for MET

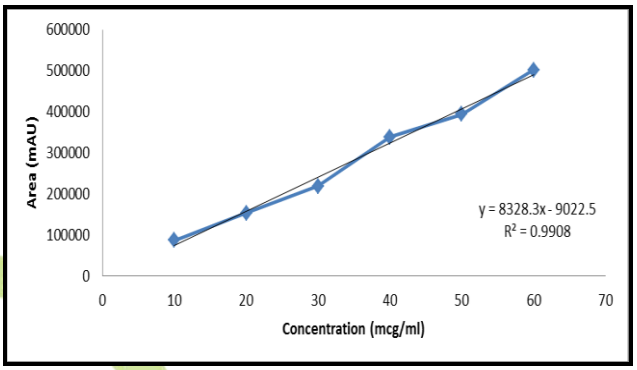

Figure 7: Linearity: Calibration plot for STG

Limit of Detection and limit of Quantitation

Values for detection limit and quantification limit were determined based on the standard deviation of the response and the slope of regression line. The calculated values of limit of detection (LOD) and limit of quantitation (LOQ) for MET and STG are shown in table 5.

Table 5: LOD and LOQ

	MET	STG
LOD	0.342µg/ml	0.373354µg/ml
LOQ	1.036µg/ml	1.131375µg/ml

Accuracy

Accuracy of the method is reported as percent recovery of known added amount of analyte in sample. The percent recovery was calculated by performing recovery studies in triplicates of three concentration levels viz. 80%, 100%, 120% of 10 ppm solution of synthetic mixture of MET and STG. Results are tabulated in table 6.

Table 6: Accuracy: Recovery studies on bulk drugs

	Observations					Inference
Drug	% Level	Concentration before spiking (µg/ml)	Total Concentration after spiking (µg/ml)	Amount Recovered	% Recovery	Acceptable recovery hence accurate
MET	80	10	18	18.25	101.43%	
	100	10	20	20.48	102.40%	
	120	10	22	21.58	98.10%	
STG	80	10	18	17.28	96.02%	
	100	10	20	19.4	97%	
	120	10	22	22.5	102.70%	

Table 7: Accuracy: Recovery studies for tablet formulation

	Observations					Inference
Drug	**% Level**	**Concentration before spiking (µg/ml)**	**Total Concentration after spiking (µg/ml)**	**Concentration injected after dilution with mobile phase (µg/ml)**	**% Recovery Analyte (Back calculated from response obtained for dilute solution)**	**Acceptable recovery hence accurate**
MET	80	500	900	45	98.33%	
	100	500	1000	50	102.21%	
	120	500	1100	55	97.43%	
STG	80	50	90	4.5	100.32%	
	100	50	100	5	99.32%	
	120	50	110	5.5	101.44%	

Table 8: Intraday precision studies

		Observations						Inference
		MET			STG			
Level		LQC	MQC	HQC	LQC	MQC	HQC	
Amount (µg/ml)		20	40	50	20	40	50	
Peak Area	1	1175188	2105675	2624947	154925.3	339984.3	2656068	Acceptable % RSD, hence precise
	2	1172229	2099021.6	2640415.3	154129.7	339124.6	2669216	
	3	1174433	2096852	2637262	153930.3	339221	2650060	
Average Peak Area		117394	2100516	2634208	155216	342400.4	2658448	
S.D.		2675	9408	35675	973.6532	1367	19826.3	
%RSD		0.2	0.4	1.3	0.6	0.4	0.7	

Recovery studies were also performed on tablets containing MET and STG. The marketed tablets of MET and STG were triturated and sample solution was prepared which yield a concentration of MET (500 µg/ml) and STG (50 µg/ml). To this solution known amount of MET and STG were added at three concentration levels viz. 80%, 100%, 120%.

Then these samples were diluted with mobile phase with a dilution factor of 20 and injected for HPLC analysis.

% Recovery values for both analytes-MET and STG were back calculated from response obtained for dilute solution. Results are tabulated in table 7.

Table 9: Interday precision studies

		Observations						**Inference**
		MET			**STG**			
Level		LQC	MQC	HQC	LQC	MQC	HQC	
Amount (µg/ml)		20	40	50	20	40	50	
Peak Area	Day 1	1185163	2123112	2647801	154593.7	344414.3	2663120	Acceptable % RSD, hence Precise.
	Day 2	1197320.6	2103062.6	2650105	155252.3	341355	2655455.3	
	Day 3	1187287	2100635	2679326	155802.3	341432	2670253	
Average Peak Area		1189923.3	2108936.5	2659077.4	155216.1	342400.4	2662942.6	
S.D.		6493.708	12336.08	17573.66	605.3184	1744.311	7400.594	
%RSD		0.5	0.5	0.6	0.3	0.5	0.2	

Table 10: Robustness: Effect on retention time and response by variation in mobile phase composition and its pH, column temperature and flow rate

Method Parameters and variations	**Level of variations**	**MET**		**STG**	
		%RSD	**Retention time (Min)**	**%RSD**	**Retention time (Min)**
Proportion of organic phase in mobile phase 50:30(±2):20	-2	0.24	0.17	0.89	1.04
	+2	0.54	0.41	0.91	0.62
Flow Rate (0.8± 0.2)	-0.2	1.24	1.0	0.77	0.43
	+0.2	0.88	0.55	0.33	1.07
Column Temperature 29 ℃± 5 ℃	-5℃	0.66	1.45	1.23	0.43
	+5℃	0.56	0.33	0.66	0.87
pH	-2	1.1	0.81	0.65	1.34
	+2	0.9	0.80	1.2	0.22

Precision

The results of intraday and interday precision studies are tabulated in table 8 and 9 respectively. Percent RSD values for both intraday and interday precision were found within acceptable limit.

Robustness

To determine robustness of analytical HPLC method changes observed in retention time and response were recorded. Method was found to be reliable and robust as method performance (retention time and response) is not much affected by deliberate variations in mobile phase composition and its pH, column temperature and flow rate. The results obtained are tabulated in table 10.

CONCLUSION

The developed RP HPLC method has been statistically validated following the recommendations of ICH guidelines and it is found to be specific, accurate, precise and robust. Validation studies indicated that the proposed method is suitable for the simultaneous estimation of MET and STG in bulk and in pharmaceutical formulation. The method can be conveniently adopted for routine analysis of the formulations containing MET and STG.

ACKNOWLEDGMENTS

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