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Research article

Development and validation of a new simple RP-HPLC method for estimation of Metformin HCl and Sitagliptin phosphate simultaneously in bulk and dosage forms

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ABSTRACT

A new, 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 sitagliptin phosphate and metformin hydrochloride in pure and dosage forms. The proposed method is based on the separation of the two drugs in reversed-phase mode using Inertsil ODS column (250×4.6 mm I.D., 5µm particle size). The optimized mobile phase consisted of ammonium di-hydrogen phosphate buffer (pH 4.3): acetonitrile in the ratio 74:26 v/v, flow rate was set at 1.0 ml/min and UV detection was set at 246 nm. The retention times were 2.443 and 4.293 for metformin hydrochloride and sitagliptin phosphate respectively. The method was validated according to ICH guidelines. It was found to be accurate and reproducible. Linearity was obtained in the concentration range of 75-175µg/ml for metformin hydrochloride and 7.5-17.5µg/ml for sitagliptin phosphate. Mean percent recovery of samples at each level for both drugs were found to be 100.24% for metformin hydrochloride and 100.35% for sitagliptin phosphate. The proposed method can be successfully applied in the quality control of bulk and pharmaceutical dosage forms.

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Key Words
Sitagliptin Phosphate,
RP- HPLC,
metformin HCl,
Validation Quality control.

INTRODUCTION

Metformin hydrochloride (MET) is chemically N,N-dimethylimidodicarbonimidic diamide and sitagliptin phosphate monohydrate (SPM) is chemically 7-[(3R) - 3- amino-1- oxo-4- (2, 4, 5-trifluorophenyl) butyl] - 5, 6, 7, 8 tetrahydro - 3- (trifluoromethyl)-1, 2, 4triazolo [4, 3-a] pyrazine phosphate are oral anti-diabetic drugs. Sitagliptin phosphate blocks dipeptidylpeptidase-4 (DPP-4) activity and increases incretin levels (GLP-1 and GIP) which inhibit glucagon release[1-3] and more significantly increases insulin secretion, in case of metformin improves hyperglycemia, primarily through its suppressive action on production of hepatic glucose. This combination recommended in the treatment of diabetes mellitus and also 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 steatasis.

According to literature survey, a few spectrophotometric[4,5] HPLC[6-8] and HPTLC[9] methods have been reported for the determination of metformin in single and in combination with other drugs. Analytical methods are reported for the determination of (SPM) by spectrophotometric[10-12] and HPLC[13] have been reported. Simultaneous determination of SPM and MET in bulk and tablet dosage form were reported[14-16] by using spectrophotometric, spectroflourometric and HPLC methods. The aim of the present work was to develop simple and accurate sensitive RP-HPLC method and validate that can be applied for simultaneous estimation of sitagliptin and metformin.

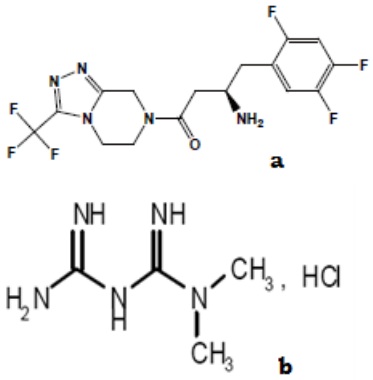

Fig. 1 Chemical structure of a) sitagliptin phosphate monohydrate and b) metformin HCl

MATERIALS AND REAGENTS

Pure Metformin HCl (MET) & sitagliptin phosphate monohydrate (SPM) were obtained as gift samples from Chandra Labs, Hyderabad, India. Commericial tablet JANUMET manufactured by Merck Sharp and Dhome were purchased from local medical shop. All chemicals and reagents used of AR grade were

purchased from Merck Chemicals, Mumbai, India.

Chromatographic system and conditions

Separation was performed with Shimadzu HPLC equipped with model SPD-10A equipped and UV detector source of deuterium lamp. The chromatogram was recorded and peaks quantified by means of PC based Spinchrome software.

The separation was achieved on an Inertsil ODS C18 (250 x 4.6mm, 5µ) analytical column. The mobile phase consisted of ammonium dihydrogen phosphate buffer (pH 4.3): ACN in the ratio of 74:26 v/v (pH 4.3 was adjusted with orthophosphoric acid). The flow rate was 1.0 ml/min and UV detection was performed at 246 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45 µ membrane filter. The injection volume was 20 µl and all the experiments were performed at ambient temperature.

Preparation of Standard Stock Solution

Accurately weigh 125mg of MET and 12.5mg of SPM and transfer into a clean and dry 100ml volumetric flask, dissolve with sufficient volume of mobile phase (10ml) and make up to 100ml with mobile phase. 1ml of above stock solution was further diluted in a 10ml volumetric flask with mobile phase to get a concentration of 125µg/ml of MET and 12.5µg/ml of SPM.

Preparation of Sample Solution

Twenty tablets (Janumet) were accurately weighed and crushed into a fine powder. An amount of powder equivalent to 125 mg of MET and 12.5mg of SPM transferred into a 100 ml volumetric flask and 10 ml of mobile phase was added to it. The mixture was sonicated 15 min with intermittent shaking. Filter through whatmann filter paper. 1ml of the sample stock solution was further diluted in a 10ml volumetric flask with mobile phase.

METHOD DEVELOPMENT

Lots of mobile phase and their different proportions and different columns were tried and finally an ammonium dihydrogen phosphate buffer (pH 4.3): ACN in the ratio of 74:26 v/v (pH 4.3 was adjusted with orthophosphoric acid) appropriate mobile phase and INERTSIL column which gave good resolution and acceptable system suitability parameters the results are shown in table 1.

METHOD VALIDATION

The method was validated in accordance with ICH guidelines[17-19]. The parameters assessed were Linearity, Accuracy, Precision, Robustness, Ruggedness, Assay, Specificity, Limit of Detection (LOD), Limit of Quantification (LOQ), and Stability (forced degradation).

Linearity

The standard solutions are to be prepared at five different concentration levels ranging from 60 % to 140 % of working concentration and finding the response at each concentration level for assay. Regression analysis was done on the peak areas of the two drugs (y axis) v/s concentration (x axis). The linear ranges of MET and SPM are presented in table 2 and figure 4, 5& 6.

Accuracy (recovery)

Accuracy was carried out by adding known amounts of the analyte in the sample corresponding to three concentration levels (80, 100, and 120%) of the labelled claim to the excipients. At each level, three determinations were performed and the accuracy results were expressed as percent analyte recovered by the proposed method. The results are shown in table 3.

Precision

Precision is the degree of closeness of agreement among individual test results when the method is applied to multiple samplings of a homogenous sample. It is a measure of either the degree of reproducibility (agreement under different conditions) or repeatability (agreement under same conditions) of the method. Six replicate working standard solution of the sample were injected and the response was measured. The results were shown in table 4. Percentage RSD for the areas of sample injection results should NMT 2.0%.

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters. Introduced small changes in flow rate by ± 0.2ml/min and in wavelength by ± 2nm.The results are presented in table 5 & 6.

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It was checked that the results were reproducible under differences in conditions, analysts and instruments. The standard solution and sample solution were injected by different

analysts and the area for injections in HPLC was measured. The results are shown in table 7.

Assay of formulation

20 µl of the standard and sample solutions were injected five times into the chromatographic system, chromatograms were recorded and peak areas were measured and the percentage assay was calculated. The results are presented in Table 8.

Specificity

The method was determined as specific by comparing test results obtained from analyses of sample solution containing placebo ingredients with that of test results obtained from analyses of standard solution.

Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The detection limit (LOD) may be expressed as:

$$LOD = \frac{3.3\sigma}{S} \quad \text{---- (1)}$$

Where σ =Relative standard deviation of the response.

S =the slope of the calibration curve (of the analyte).

Quantitation Limit

The Quantitation limit of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantitation Limit (LOQ) may be expressed as:

$$LOQ = \frac{10\sigma}{S} \quad \text{------ (2)}$$

Where σ = Relative standard deviation of the response.

S= the slope of the calibration curve (of the analyte).

Stability (forced degradation) study [17]

Forced degradation were carried out at various stress conditions like acidic (adding 0.1ml of 0.1 N HCl to drug solution), alkaline (adding 0.1ml of 0.1 N NaOH to drug solution), oxidative (adding 0.1ml of 50% H_2O_2 to drug solution), thermal (exposing drug solution to heat by placing in a water bath) and photolytic (placing drug solution under UV lamp) conditions. Stability was checked for 24h at room temperature.

RESULT & DISCUSSION

Tab 1. System Performance for Metformin HCl and Sitagliptin phosphate

Sample	Retention time (min)	Area (mV.s)	Height (mV)	Resolution	Efficiency (th.pl)	Asymmetry
Metformin HCl	2.443	5652.398	716.061	7.869	2174	1.414
Sitagliptin phosphate	4.293	909.428	95.600		4343	1.211

Method Development

Different columns were tried for the separation and resolution. It was found that INERTSIL column offered more advantages. Individual drug solution was injected into column and elution pattern of all the drugs and resolution parameters were studied. In addition to this, UV spectra of individual drugs were recorded at the wavelength from 200 to 400nm and the response for optimization was compared (Fig 3). The choice of wavelength 246nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity.

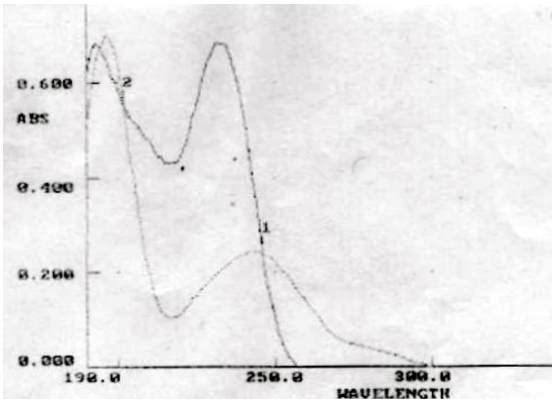

Fig 2. Comparison of UV Spectra of Sitagliptin phosphate and Metformin HCl

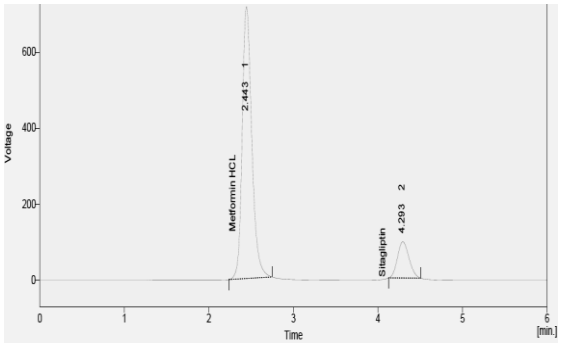

Fig 3. Typical chromatogram of Metformin HCl and Sitagliptin phosphate

Method Validation

Linearity

The linear ranges of MET & SPM are 75-175 µgml^{-1}and 7.5-17.5µg ml^{-1} respectively.

Tab. 2 Linear regression analysis data

Parameters	Metformin HCl	Sitagliptin Phosphate
Slope	967.1	200.79
Y-Intercept	2790.1	393.33
Correlation Coefficient, R^2	0.9994	0.9992

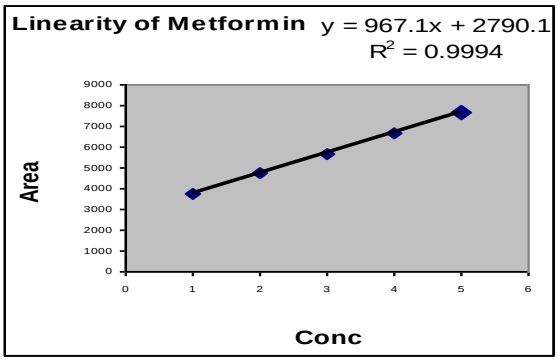

Fig 4. Calibration curve of Metformin hydrochloride

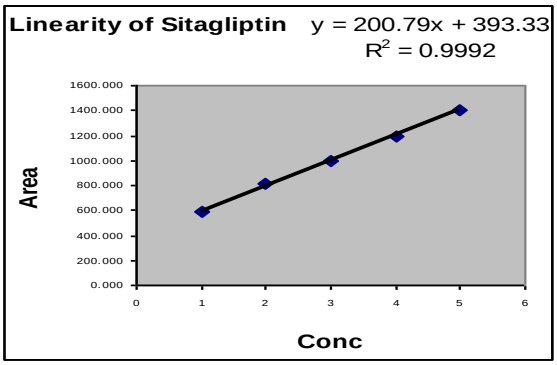

Fig 5. Calibration curve of Sitagliptin phosphate

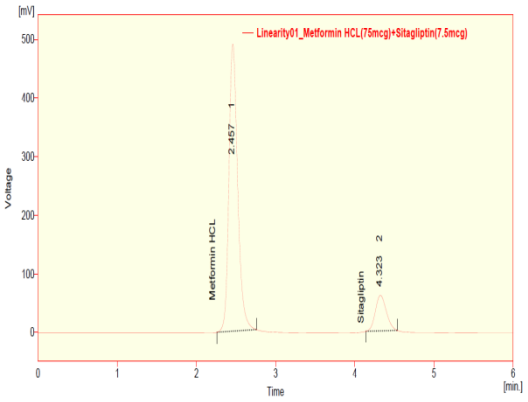

Fig 6. Representative Chromatogram of Metformin HCl and Sitagliptin phosphate for 60% linearity range

Accuracy (recovery)

The % mean recovery obtained for SPM and MET were 102% and 101% respectively as shown in table 3.

Precision

Percentage RSD for the areas of six sample injection results were found to be in range between 0.36 and 0.89 and not found more than 2.0%.

Robustness

There was no significant change in the peak areas and retention times of MET and SPM when the flow rate was varied by ±0.2 ml and wavelength by ±2nm.

Ruggedness

Reproducibility of the results obtained under a variety of conditions (Tab.7)

Assay of formulation

The proposed method was applied to the simultaneous estimation of MET and SPM in dosage form. The results of the assay yielded 99.79 ±0.33% for MET and 100.11±1.25% for SPM, of label claim of the dosage form (500 mg of Metformin and 50 mg of Sitagliptin). The assay results showed that the method was selective for the simultaneous estimation of MET and SPM without interference from the excipients used in the dosage form.

Tab 3. Accuracy data for proposed method

Sample	Accuracy (%)	Peak area	% recovery	Mean % recovery (SD)
Metformin HCl	80	4905.111	98.42	99.01±0.51
	80	4953.301	99.39	
	80	4944.058	99.20	
	100	5860.427	100.07	100.44±0.34
	100	5900.297	100.75	
	100	5884.402	100.48	
	120	7006.618	101.32	101.27±0.11
	120	6993.3	101.13	
	120	7007.916	101.34	
Sitagliptin phosphate	80	867.76	102.31	100.95±1.22
	80	853.18	100.59	
	80	847.807	99.95	
	100	1067.703	102.85	99.00±3.33
	100	1005.762	96.89	
	100	1009.627	97.26	
	120	1251.873	101.97	101.10 ±2.50
	120	1199.287	97.69	
	120	1272.483	103.65	

Tab.4 Precision of purposed HPLC method

Inj. No	**Metformin**		**Sitagliptin**	
	Retention time (min)	Peak area	Retention time (min)	Peak area
1	2.443	5652.398	4.293	909.398
2	2.417	5691.563	4.257	913.747
3	2.423	5677.261	4.270	920.902
4	2.423	5674.826	4.263	932.619
5	2.447	5659.658	4.293	921.662
6	2.423	5628.584	4.267	924.785
Mean	2.4293	5664.048	4.274	920.519
SD	0.0124	22.183	0.015	8.193
%RSD	0.51	0.39	0.36	0.89

Tab 5. Robustness data for effect of flow rate variation

Flow rate (mL/min)		**Metformin**			**Sitagliptin**		
	Injection No.	Rt (min)	Efficiency (th.pl)	Asymmetry	Rt (min)	Efficiency (th.pl)	Asymmetry
0.8	1.	3.040	2380	1.429	5.343	4706	1.244
	2.	3.057	2406	1.457	5.367	4579	1.261
1.2	1.	2.043	2033	1.400	3.593	3830	1.194
	2.	2.043	2033	1.400	3.593	3830	1.194

Rt= retention time; Th.pl= Theoretical plate

Tab 6. Robustness data for effect of different wavelength

Wave length (nm)		**Metformin**			**Sitagliptin**		
	Injection No.	Rt (min)	Efficiency (th.pl)	Asymmetry	Rt (min)	Efficiency (th.pl)	Asymmetry
244	1	2.450	2309	1.379	4.293	4343	1.270
	2	2.453	2316	1.414	4.303	4364	1.263
248	1	2.453	2192	1.448	4.300	4173	1.214
	2	2.443	2174	1.414	4.297	4167	1.231

Tab 7. Ruggedness Data

	Analyst -1		**Analyst -2**	
	Metformin	**Sitagliptin**	**Metformin**	**Sitagliptin**
Standard Area	5617.833	969.994	5655.657	913.448
Sample Area	5610.897	961.837	5637.704	907.301

Tab 8. Shows the results of standard and sample analysis

Injection No.	**Metformin HCl**			**Sitagliptin Phosphate**		
	Peak Area of Standard	Peak Area of Sample	% Assay	Peak Area of Std	Peak Area of Sample	% Assay
1	5682.593	5658.385	99.34	938.025	954.559	101.12
2	5658.803	5664.536	99.86	936.88	951.458	100.91
3	5672.981	5655.622	99.46	937.682	933.505	98.92
4	5667.187	5673.243	99.87	962.132	954.493	98.57
5	5654.355	5693.498	100.45	944.103	960.158	101.05
Mean	5667.18	5669.05	99.79± 0.435	943.76	950.83	100.11 ± 1.25

Specificity

No interference from any of the excipients was found at retention times of the examined drugs.(fig.) In addition, the chromatogram of each drug in the sample solution was found identical to the chromatogram 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.

Detection Limit and Quantitation Limit

LOD was found to be 0.13ppm for MET and 0.06ppm for SPM and LOQ was found to be 0.41ppm for metformin and 0.20ppm for SPM indicating high sensitivity of the method, the results are depicted in table 9.

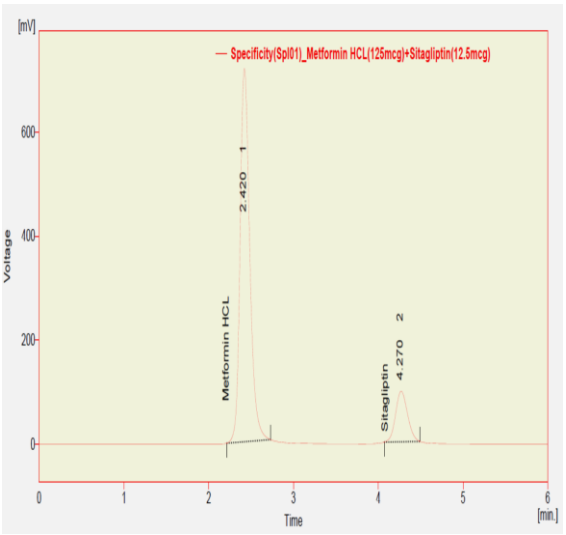

Fig 7.Specificity Chromatogram shows no interference from excipients of dosage form

Tab 9. Linearity Data (LOD, LOQ)

Levels	**Metformin HCl**		**Sitagliptin phosphate**	
	Conc. (ppm)	**Peak area**	**Conc. (ppm)**	**Peak area**
Level 1 (60%)	75	3762.552	7.5	585.374
Level 2 (80%)	100	4746.249	10	807.768
Level 3 (100%)	125	5630.868	12.5	998.120
Level 4 (120%)	150	6691.961	15	1188.007
Level 5 (140%)	175	7625.193	17.5	1399.190
SD(σ)	39.5	1529.6	3.95	317.6
Slope(S)	967.1		200.79	
Y-Intercept	2790.1		393.33	
LOD	0.13ppm		0.06ppm	
LOQ	0.41ppm		0.20ppm	

Tab 10.Comparison of the validation report with a reported method

Parameters	**Limit**	**Proposed method**	**Reported method[16]**
	%RSD - NMT 2	MET: TF-1.414	MET: TF-1.52
	TF - NMT 2	TP-2174	
	TP - NLT 2000	STG: TF-1.211	STG: TF-1.57
		TP-4343	
System suitability		Resolution: 7.869	Resolution: 4.626
Specificity	No Interferences at retention time of the analyte peak.	Interference at retention time of the analyte peak not observed	Interference at retention time of the analyte peak not observed
		MET: %RSD- 0.39%	MET: %RSD- 0.1799
Precision	RSD NMT 2.0%	STG: %RSD- 0.89%	STG: %RSD- 0.9947
		MET: 0.9994	MET: 0.9996
Linearity of detector response	Correlation co-efficient NLT 0.999	STG: 0.9992	STG: 0.9991
	% Recovery range	MET: 99.01- 101.27%	MET: 99.53- 99.89%
Accuracy	98-102%	STG: 99.00- 101.11%	STG: 99.16- 99.59%
Robustness	Should comply with system suitability parameters	Within limits	Within limits
		MET: 99.79%	
Assay	100% ± 2%	STG: 100.11%	**Not available**

TF – Tailing Factor; TP – Theoritical Plates

Stability (forced degradation) study

Comparing the data obtained in forced degradation conditions with the normal condition, the drugs were found to be stable**.**

CONCLUSION

The developed RP-HPLC method for the simultaneous determination of Sitagliptin and Metformin was found to be accurate, precise and reliable with good sensitivity and specificity. The results of both Ruggedness and Robustness were found to be satisfactory. Thus, this method can be successfully employed for the simultaneous determination and quality control of Sitagliptin and Metformin in both bulk drug and pharmaceutical formulation.

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