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Development and Validation of RP-HPLC and HPTLC Methods for Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride in Bulk and Dosage form

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ABSTRACT

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Two simple, specific, accurate and precise methods, namely, reverse phase high performance liquid chromatography and high performance thin layer chromatography were developed for simultaneous estimation of Sitagliptin phosphate and Metformin HCl in tablet dosage forms. For the HPLC method, Phenomenex C_{18} column (250 mm × 4.6 mm, 5µm) in gradient mode, with mobile phase containing acetonitrile: phosphate buffer 0.03M in proportion of 70:30 v/v, pH 3.5 adjusted with orthophosphoric acid at a flow rate of 1mL/min was used, and effluent was monitored at 218 nm. The retention time of Sitagliptin phosphate and Metformin HCl was 5.27 and 1.93 min respectively. For the high performance thin layer chromatographic method separation was achieved on silica gel 60F_{254} HPTLC plates with water-methanol-ammonium sulphate, 4.5 + 4.5 + 1.5 (v/v/v) as mobile phase. The detection of spot was carried out at 254 nm. The R_f value was found to be 0.68 and 0.59 for Sitagliptin phosphate and Metformin HCl respectively. The methods were validated in terms of linearity, accuracy and precision. The proposed methods were successfully used for estimation of Sitagliptin phosphate and Metformin HCl in tablet dosage form.

Keywords – RP-HPLC; HPTLC; Sitagliptin Phosphate; Metformin Hydrochloride; Validation

INTRODUCTION

Metformin HCl (MET) is chemically N,N-dimethylimidodicarbonimidic diamide hydrochloride has antidiabetic activity.[1-3] Sitagliptin phosphate is 7-[(3R)-3-amino-1-oxo-4-(2,4,5trifluorophenyl) butyl] 5,6,7,8 tetrahydro-3-(trifluoromethyl)-1,2,4-triazolo [4,3-a]pyrazine phosphate has hypoglycaemic activity.[4,5] Metformin is official in IP and USP, while Sitagliptin is not official in any pharmacopoeias.

Many methods have been described in the literature for the determination of Sitagliptin phosphate and Metformin HCl individually, and in combination with other drugs.[6-12] However, there is no RP-HPLC and HPTLC method reported for the simultaneous estimation of these drugs in combined dosage forms. But only one method was reported for the estimation of Metformin HCl alone.[13] The aim of this work was to develop an RP-HPLC and HPTLC method with ultraviolet detection for the simultaneous determination of Sitagliptin phosphate and Metformin HCl in tablet dosage form. The performance of the RP-HPLC and HPTLC method was determined by studying different system suitability parameters.

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MATERIAL AND METHODS

Reagents and Chemicals

Active pharmaceutical ingredient (API) working standards of Sitagliptin phosphate and Metformin HCl were received as gift samples from Matrix Pharmaceuticals Ltd., Nasik and Sun Pharmaceuticals Ltd., Mumbai respectively. The Pharmaceutical dosage form used in this study was Janumet tablets manufactured by Merck Co. Ltd. which were purchased from local market. HPLC grade acetonitrile, methanol and water were procured from Qualigens Fine Chemicals, Mumbai. AR grade orthophosphoric acid and ammonium sulphate was procured from Qualigens Fine Chemicals, Mumbai. All dilutions were performed in standard volumetric flasks.

Chromatographic condition

RP-HPLC method

The analysis was carried on a Cyberlab LC-100B HPLC (binary gradient system) equipped with a Cyberlab LC-100 HPLC solvent delivery pump, Cyberlab UV-100 UV-VIS detector, Cyberlab DS-100 HPLC control data software, Cyberlab GM-100 gradient mixer and rheodyne injector with 20µl loop volume. A Phenomenex C_{18} column (5 µm, 250 mm × 4.6 mm) was used for separation. The mobile phase was acetonitrile: phosphate buffer 0.03M in proportion of 70:30 v/v, pH 3.5 adjusted with orthophosphoric acid at a flow rate of 1.0 mL/min with detection at 218nm.

HPTLC method

The HPTLC was performed on aluminium backed silica gel $60F_{254}$ HPTLC plates (Merck) with water-methanol-ammonium sulphate, 4.5 + 4.5 + 1.5 (v/v/v), as mobile phase. Samples were applied to the plates with Linomat 5 sample applicator. The plates were developed in a 20 cm x 10 cm Camag twin-trough chamber previously saturated with mobile phase vapour 10 min. The development distance was 8 cm. Plates were then removed from chamber, dried on chromatography plate heater III at 40° C for 20 min, and densitometric scanning at λ = 254 nm was performed with a Camag TLC Scanner III with CATS 1.4.4 software.

Preparation of standard stock solutions

In RP-HPLC method, the standard stock solutions (100µg/ml) of Sitagliptin phosphate and Metformin HCl were prepared separately, by accurately dissolving 10 mg of each drug in 50 ml of mobile phase and sonicated for 10 min., and the volume was made up to the mark (100 ml) with mobile phase. The stock solutions were filtered through 0.45 µ membrane filter. In HPTLC method, the standard stock solutions (100µg/ml) of Sitagliptin phosphate and Metformin HCl were prepared separately by accurately dissolving 10 mg of drug in 10 ml ammonia and then the volume was made up to the mark (100 ml) with methanol.

Preparation of sample solutions

Twenty tablets were weighed and finely powdered. A quantity of powder equivalent to 50 mg of Sitagliptin phosphate and 500 mg of Metformin HCl of the tablet formulation was weighed accurately and transferred to a 100 ml volumetric flask. In RP-HPLC, to this solution 50 ml mobile phase was added and in HPTLC, 10 ml ammonia was added to the solution and dissolved in 80 ml of methanol. The solution was sonicated for 10 min and then final volume was made up to the mark with mobile phase and methanol for RP-HPLC and HPTLC respectively. The resulting solution was filtered through Whatmann filter paper no 41, and the filtrate was appropriately diluted to get desired concentration of Sitagliptin phosphate and Metformin HCl which was required for analysis.

Assay method

RP-HPLC

A 20µl each of the standard and sample solutions were injected separately into the stabilised HPLC system. Detection was carried out at 218 nm. The retention time for Sitagliptin phosphate and Metformin HCl were found to be 5.27 and 1.93 min respectively. The area of each solution peak was measured at 218 nm. The amount of each drug present in sample solution was determined using prepared calibration curves of standard solutions. The result obtained is given in Table No. 1.

HPTLC

The stock solution thus contains 100µg mL^{-1} SITA and 1000 µg mL^{-1} MET (Solution A), 1 ml of this solution is diluted with 10 ml Methanol (Solution B). 10 µL of Solution A was spotted for Sitagliptin and 2 µl of Solution B was spotted for Metformin HCl solution, and the plate was developed and scanned under the optimized conditions as described above for analysis of tablet formulation. Densitograms were recorded and the amounts of Sitagliptin and Metformin HCl in the formulation were estimated using calibration plots.The result obtained is given in Table No. 1.

Method validation [14]

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection and limit of quantification. To ascertain effectiveness of HPLC method, the result obtained is given in Table No. 2 and Table No. 3.

Recovery studies

Recovery experiments were conducted to confirm the accuracy and suitability of the method and to check the presence of interferences from excipients present in the formulation by standard addition method. The standards were added to the formulation at three different levels 80, 100 and 120% and analysis was performed. At each level of recovery six determinations were performed. Results of the recovery studies are shown in Table No. 2

Table 1: Results of analysis of commercially available tablets containing Sitagliptin phosphate and Metformin HCl by RP-HPLC and HPTLC

Method	RP-HPLC		HPTLC	
	Sitagliptin phosphate	Metformin HCl	Sitagliptin phosphate	Metformin HCl
Label claim (mg/tablet)*	50	500	50	500
Amount found (mg)*	49.78	500.16	49.98	500.33
Assay (%)*	99.63	100.07	99.94	100.07
RSD (%)*	0.39	0.090	0.68	0.088

*n = 6

RESULT AND DISCUSSION

Typical chromatogram obtained from sample preparation is shown in Fig No.1 and Fig No.2 for RP-HPLC and HPTLC respectively.

RP-HPLC A satisfactory separation was obtained when using acetonitrile: phosphate buffer 0.03M in proportion of 70:30 v/v, pH 3.5 adjusted with orthophosphoric acid under gradient mode and a flow rate of 1.0mL/min. Peaks were well defined, symmetrical and resolved. Retention times for Sitagliptin phosphate and Metformin HCl were observed at 5.27 min and 1.93 min. respectively (Fig. 1) and the optimum wavelength was determined to be 218 nm. Method was found to be linear in the range of 5-50 µg/ml for Sitagliptin phosphate and 2-20 µg/ml for Metformin HCl with regression coefficients of 0.9991 and 0.9994 for Sitagliptin phosphate and Metformin HCl respectively. Limits of detection for Sitagliptin phosphate and Metformin HCl were 1.70 µg/ml and 0.14 µg/ml, and limit of quantification were 2.13 µg /ml and 0.41 µg /ml respectively. Average recovery for Sitagliptin phosphate and Metformin HCl was 99.61% and 100.3% respectively, which shows that the method is free from interference from excipients present in the formulation. The low values (below 2%) of standard deviation and coefficient of variation for recovery at each level are indicative of the high precision of the method.

Fig. 1: RP-HPLC Chromatogram of tablet dosage form

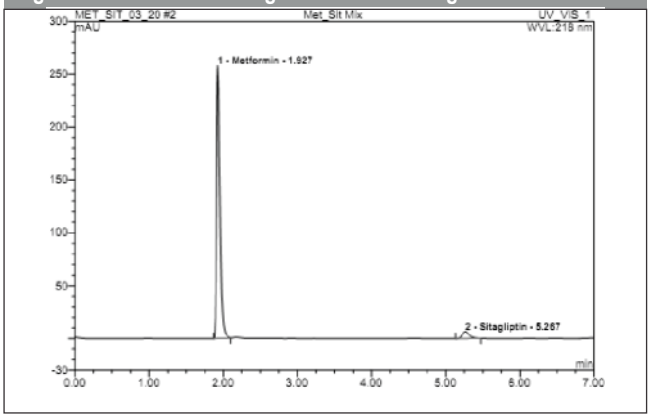

Fig. 2: HPTLC Chromatogram of tablet dosage form

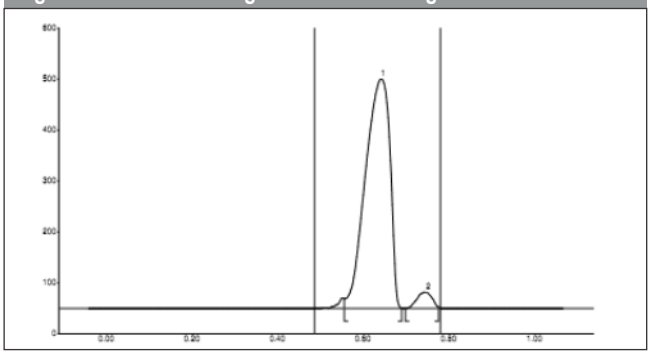

Table 2: Present recovery study

Level of % Recovery	% Recovery*			
	RP-HPLC		HPTLC	
	Sitagliptin phosphate	Metformin HCl	Sitagliptin phosphate	Metformin HCl
80	99.46	100.23	99.16	99.94
100	99.96	100.15	99.77	99.66
120	99.43	100.52	98.41	100.50

*n = 6

Table 3: Method validation and system suitability parameters

Parameter	RP-HPLC		HPTLC	
	Sitagliptin phosphate	Metformin HCl	Sitagliptin phosphate	Metformin HCl
Linearity range	5-50 µg/ml	2-20 µg/ml	3000-24000 ng/spot	50-400 ng/spot
Regression coefficients (r^2)	0.9991	0.9994	0.9983	0.9986
Limit of detection	1.70 µg/ml	0.14 µg/ml	1.10 ng/spot	0.28 ng/spot
Limit of quantitation	2.13 µg/ml	0.41 µg/ml	3.32 ng/spot	0.80 ng/spot
Precision				
Intra-day (%RSD)	0.7669	0.8441	0.48	0.71
Inter-day (%RSD)	0.315	0.784	0.15	0.49
Rf value	-		0.68	0.59
Retention time (min)	5.27	1.93	-	-
Tailing factor	1.023	1.25	-	-
Theoretical plates	10,884	16,081	-	-

HPTLC

Sitagliptin phosphate and Metformin HCl were separated using silica gel $60F_{254}$ HPTLC plates with water-methanol-ammonium sulphate, 4.5 + 4.5 + 1.5 (v/v/v), as mobile phase with good resolution. Sitagliptin phosphate shows (R_f 0.68) and Metformin HCl shows (R_f 0.59). Method was found to be linear in the range of 3000-24000 ng $spot^{-1}$ for Sitagliptin phosphate and 50-400 ng $spot^{-1}$ for Metformin HCl with regression coefficients of 0.9983 and 0.9986 for Sitagliptin phosphate and Metformin HCl respectively. Limits of detection for Sitagliptin phosphate and Metformin HCl were 1.10 ng $spot^{-1}$ and 0.28 ng $spot^{-1}$ and limit of quantification were 3.32 ng $spot^{-1}$ and 0.80 ng $spot^{-1}$respectively. Average recovery for Sitagliptin and Metformin HCl was 99.16% and 100.03% respectively, which shows that the method is free from interference from excipients present in the formulation. The low values (below 2%) of standard deviation and coefficient of variation for recovery at each level are indicative of the high precision of the method.

CONCLUSION

The developed HPLC and HPTLC techniques are precise, specific, accurate, stability indicating and robust. Validation studies indicated that the proposed methods are suitable for the simultaneous estimation of Metformin HCl and Sitagliptin phosphate in bulk and in pharmaceutical formulation without any interference from the excipients. A RP-HPLC and HPTLC methods have been validated following the recommendations of ICH guidelines. Hence they can be conveniently adopted for routine quality analysis of the tablet formulation.

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REFERENCES

1. The British Pharmacopeia. London: Her Majesty's Stationary Office; 2009.
2. The Indian Pharmacopeia. New Delhi; 2007.
3. Mubeen G, Khalikha N. Spectrophotometric method for analysis of metformin hydrochloride; Ind. J. Pharm. Sci. 2009; 71(1):100–2.
4. The Merck Index, 14th edn, pp. 1447 and 5939.
5. Sekaran BC, Rani P. Development and validation of spectrophotometric method for the determination of dpp-4 inhibitor, sitagliptin in its pharmaceutical dosage forms; Int. J. Pharm. Pharm. Sci. 2010; 2(4): 138-42.
6. Pulla PR, Sastry BS, Prasad YR, Raju NA. Simultaneous estimation of metformin HCl and sitagliptin phosphate in tablet dosage forms by RP-HPLC; Res. J. Pharm. Techn. 2011; 04(04):646-50.
7. Ramzia I, Bagary E, Elkady EF, Ayoub BM. Spectroflourometric and spectrophotometric methods for the determination of sitagliptin in binary mixture with metformin and ternary mixture with metformin and sitagliptin alkaline degradation product; Int. J. Biomed. sci. 2011; 7(1): 62-9.
8. Khan G, Sahu D, Agrawal YP, Sabarwal N, Jain A, Gupta AK. Simultaneous estimation of metformin and sitagliptin in tablet dosage form; Asian. J. Biochem. Pharm. Res. 2011; 1(2): 352-8.
9. Sahoo PK, Sharma R, Chaturvedi SC. Simultaneous estimation of metformin hydrochloride and pioglitazone hydrochloride by RP-HPLC method from combined tablet dosage form; Ind. J. Pharm. Sci. 2008; 70(3): 383–6.
10. Havaldar FH, Vairal DL. Simultaneous estimation of metformin hydrochloride, rosiglitazone and pioglitazone hydrochloride in the tablets dosage form; Int. J. Appl. Biol. Pharm. Tech. 2010; 1(3):1000-5.
11. Rashid SH, Munjewar R, Farooqui M. Simultaneous determination of atenolol and metformin hydrochloride by reverse phase high performance liquid chromatography; Current Pharma. Res. 2010; 1(1): 44-8.
12. Bhamare PC, Bari SB, Natarajan S, Patil AA, Patil SH, Shirode PT. A new analytical method development and validation of metformin hydrochloride and fenofibrate by absorbance ratio UV spectrophotometric method; Asian. J. Biochem. Pharm. Res. 2011; 2 (1): 115-28.
13 Havele S, Dhaneshwar S. Estimation of metformin in bulk drug and in formulation by HPTLC; J. Nanomed. Nanotech. (2010); 1(1): 100-2.
14. Validation of Analytical Procedures, ICH Harmonised Tripartite Guidelines, Q2B, 1997.