INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.09.7.2021.11(28)

RP- HPLC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF SITAGLIPTIN PHOSPHATE AND METFORMIN HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Reversed phase high performance liquid chromatography procedure was made and affirmed for the simultaneous estimation of Sitagliptin phosphate (SGP) and Metformin hydrochloride (MFH) in united estimation structure by using UV identifier. Picked adaptable stage was a blend of ACN: Methanol: Phosphate cradle (30:25:45 % v/v) at pH 3 and recurrence picked was 252 nm. Maintenance time of MFH and SGP is 2.36 and 3.39 min. Linearity of the strategy was viewed as 80% to 120% of test obsession with backslide coefficient of 0.9997. This technique was endorsed by ICH rules. Estimation was done by registering zone of the apex and beyond what many would consider possible and quantitation limit (S/N) were 0.16 and 0.46 µg/ml. Present strategy can be applied for the finishes of SGP and MFH in quality control tests, subtleties without impedance of the excipients present and in the breaking down examinations of the medicine.

Keywords: Sitagliptin phosphate, Metformin hydrochloride and Liquid Chromatography.

INTRODUCTION[1,2,3,4,26,35]

Hypoglycemia is a condition wherein your (glucose) level is lower than ordinary. Glucose is your body's principle vitality source. Diabetes treatment and different conditions can cause hypoglycaemia. Disarray, heart palpitations, instability and tension are side effects. Sitagliptin attempts to seriously repress the compound dipeptidyl peptidase 4 (DPP-4). This chemical separates the incretins GLP-1 and GIP, gastrointestinal hormones discharged in light of a dinner. It is used as hypoglycemic specialists [2]. Metformin hydrochloride diminishing the retention from the gastrointestinal tract. Also they can decrase gluconneogensis while expanding glucose take-up by muscle and fat cells [1, 2]. Composing study reveals that single not many RP-HPLC and HPTLC procedures are represented the affirmation of MFH and SGP in Stability-indicating test[3].

The essential inspiration driving this assessment is to make and support pivoted stage HPLC procedure which is clear, accurate, budgetary, sensitive and specific for quantitation of MFH and SGP in mass and combined portion structures. The made system can be successfully used in routine quality control and for crumbling learns at low centralization of MFG and SGP. Sensible quantifiable test were performed on endorsement data (Fig. 1: Structure of MFH and SGP).

MATERIALS AND METHODS[5,6,7,8,9,26,35]

MATERIALS

Pure sample of Sitagliptin phosphate and Metformin hydrochloride was gotten from Meditab Specialities Pvt. Ltd, (Daman) as a gift test. Methanol was of HPLC grade and purchased from spectrochem Mumbai and other pad salts little of logical assessment. Twofold refined water was used for arranging

of pad. Potassium Dihydrogen phosphate pad plan (25 mM) was organized and was isolated anyway 0.22 channel (Millipore). Tablet meaning of 50 mg SGP and 500 mg MFH were made from MSD Pharmaceuticals {JANUMET}.

Equipment

HPLC gear (Waters Corporation) Consisted of model waters - 510 twofold reacting siphons, Water's 486 tunable absorbance UV Detector. Chromatograms were destitute down using Borwin programming outfitted with the structure.

Chromatographic condition

Separation was performed on a reversed stage Phenomenex C18 section (250 × 4.6 mm, 5 µm) portion. Versatile stage contained a mixture of ACN: Methanol: Phosphate support (30:25:45 % v/v) at pH 3. Stream rate was changed as per 1ml/min and the wavelength was set to 252 nm, showed up in fig. No. 03.

Calibration Curve

Independently precisely gauged amount 2.5 mg of MFH and SGP were broken down in Methanol and volume was made up to 25 ml imprint to make stock arrangement (0.1 mg/ml). The stock standard arrangement was weakened further with versatile stage to get adjustment guidelines in a focus extend 10-60 µg/ml of MFH and 1-6 µg/ml of SGP. Adjustment bend was plotted between top territories of MFH and SGP against convergence of medication (Fig. 2: Calibration curve obtained by MFH and SGP).

Analytical Validation[34,35]

Procedure was affirmed by ICH rules. Identity and selectivity of the procedure was assessed by preparing medicine assemblies of MFH and SGP 1000 µg/ml from unadulterated prescription stock and business test stock in picked flexible stage and explored. To choose the accuracy of the proposed system, different degrees of drug obsessions. Different assemblies of unadulterated medicine courses of action of MFH and SGP (15, 25 and half) were added to a known pre-dissected definition were explored. The recovery of the extra unadulterated prescription was settled. As a bit of precision, repeatability was controlled by taking different degrees of medicine center (same as exactness) organized from new stock game plan and were bankrupt down. Cover day and intra day assortment and bury master assortment inspects were finished to choose widely appealing precision of the procedure. To set up linearity of the proposed technique, six separate game plan of course of action of the OLZ and FLX were set up from the stock game plan and analyzed. ANOVA test (single heading) was performed to choose the assortment between the copies and it relied upon the district of the apex looked for unadulterated prescription concentration during the mimic estimation of the standard plans. Sign to upheaval extent of 3 were taken as cutoff of disclosure (LOD) and sign to noise extent of 10 were taken as limit of quantitation (LOQ). The LOQ trial of MFH and SGP were set up in reproduces (n =5) using same strategy clung to for arrangement gauges and dismembered. Intensity of the procedure was constrained by changing bit of flexible stage by 1%, by changing the pH of potassium dihydrogen phosphate pad by 0.1 units and by working up the seat top, stock game plan reliability of MFH and SGP at room temperature (Fig. 3: Chromatogram obtained by MFH and SGP, showing retention time for MFH – 2.36 min. and SGP –3.39 min).

RESULTS AND DISCUSSION

Analysis of combined dosages Form (Marketed formulation)

Substance of 10 tablets of MFH and SGP were discharged and material proportional to 1 mg of medication (MFH and SGP) were taken to set up an answer of 50 µg/ml in methanol. Last weakening was made with portable stage to acquire focus inside the linearity go. Six duplicates were set up for all definitions.

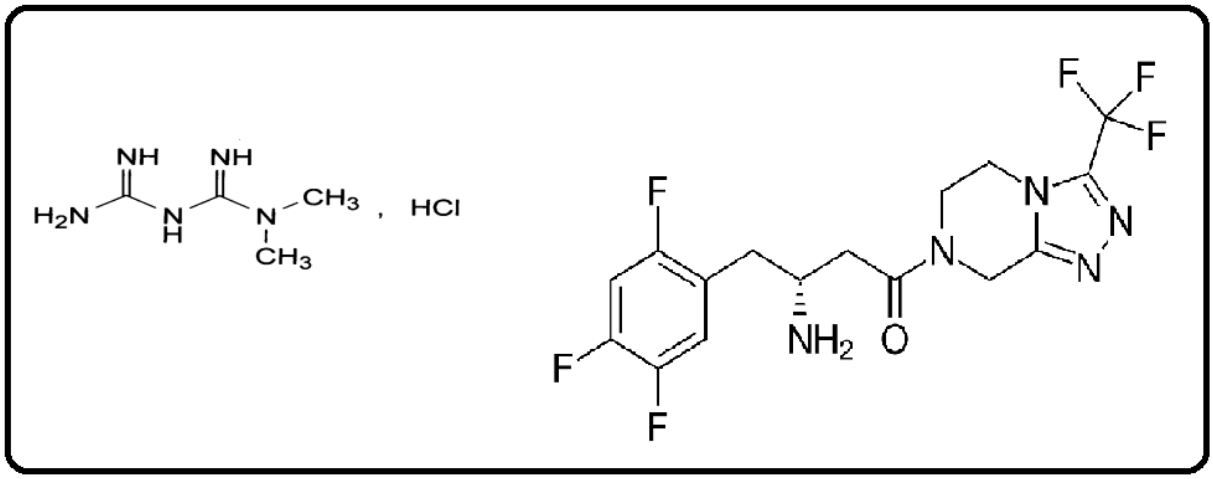

Fig. 1: Structure of MFH and SGP

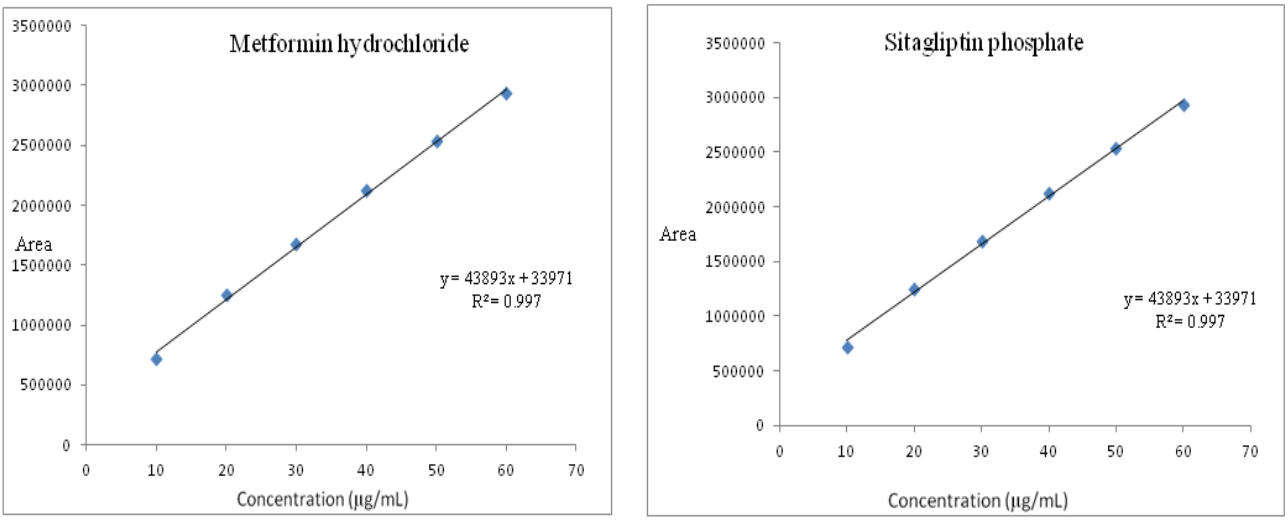

Fig. 2: Calibration curve obtained by MFH and SGP

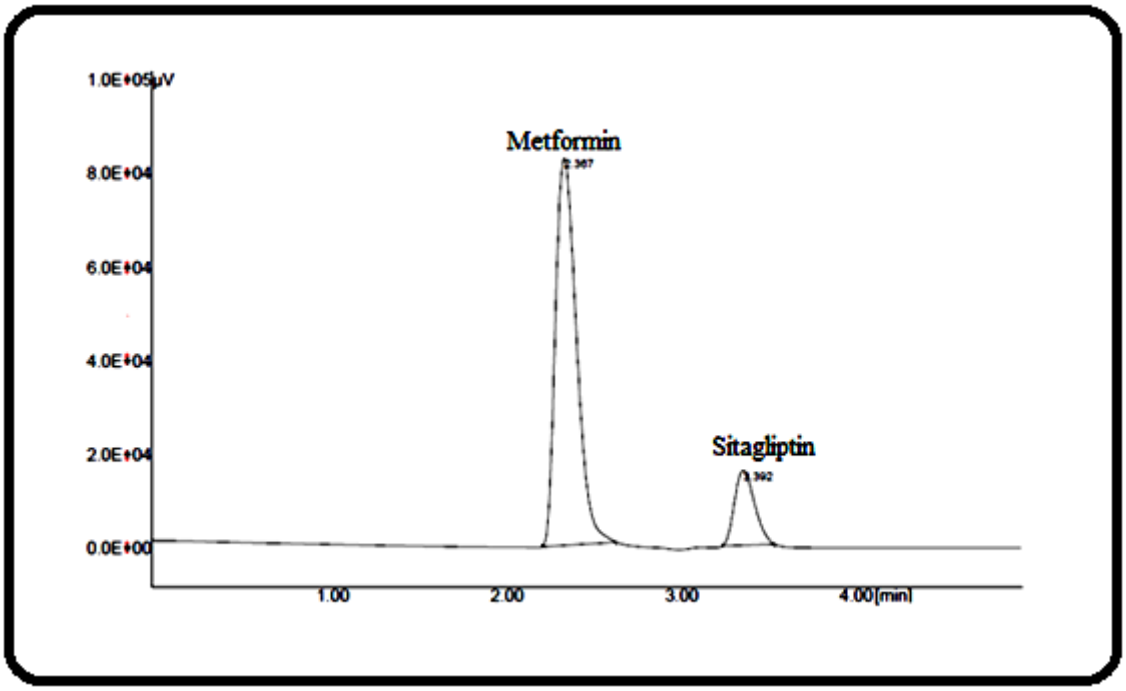

Fig. 3: Chromatogram obtained by MFH and SGP, showing retention time for MFH – 2.36 min. and SGP –3.39 min

Table 1: SPH and MFH calibration curve data

Conc. of SPH (µg/ml)	Conc. of MFH (µg/ml)	Area of SPH	Area of MFH	SD of SPH	SD of MFH
1	10	28243.33	721754	338.85	10468.9
2	20	55489.66	1254589	661.27	38566.3
3	30	82478	1681775	2155.13	1307.02
4	40	113223.33	2123666.66	1774.25	4316.92
5	50	143222	2538375.33	4760.94	926.116
6	60	171473.33	2935590.66	4417.43	2932.17

Each value is average of six determinations

Table 2: Precision data for MFH and SGP developed method

S. No.	Sample	Statistical data	% Estimation		% Recovery	
			MFH	SGP	MFH	SGP
1.	Standard lab. Mixture	**Mean**	99.95	99.91	628.12	63.11
		S.D.	0.3124	0.3282	0.3644	0.3858
		C.V.	0.1816	0.1949	0.1798	0.2357
2.	Marketed Formulation	**Mean**	99.98	99.95	626.21	62.45
		S.D.	0.4512	0.5569	0.6147	0.6826
		C.V.	0.2839	0.2402	0.3040	0.3484

Each value is average of three determinations

Table 3: Repeatability Study for MFH and SGP developed method

Parameter	Statistical data	% Estimation by RP-HPLC method	
		MFH	SGP
Interday	Mean	100.01	99.99
	S.D.	0.1744	0.2757
	C. V.	0.1090	0.1688
Intraday	Mean	99.94	99.91
	S.D.	0.3702	0.2304
	C. V.	0.8118	0.1946
Different analyst	Mean	99.97	100.03
	S.D.	0.3792	0.3631
	C. V.	0.1553	0.2195

Each value is average of three determinations

Table 4: Accuracy and Recovery data for MFH and SGP developed method

Amount Added	Amount Found (mg) of SGP								
	Day 1			Day 2			Day 3		
	Mean	S.D.	%R.S.D	Mean	S.D.	%R.S.D	Mean	S.D.	%R.S.D
Lable claim + 15% = 57.5 mg	57.65	0.32	0.19	57.44	0.55	0.24	57.59	0.27	0.16
Lable claim + 25% = 62.5 mg	63.11	0.38	0.23	62.45	0.68	0.34	62.41	0.74	0.94
Lable claim + 50% = 75.0 mg	75.84	0.36	0.21	75.05	0.38	0.23	75.48	0.27	0.14
Amount Added	**Amount Found (mg) of MFH**								
	Day 1			Day 2			Day 3		
	Mean	S.D.	%R.S.D	Mean	S.D.	%R.S.D	Mean	S.D.	%R.S.D
Lable claim + 15% = 575 mg	576.08	0.3	0.18	574.5	0.45	0.28	576.8	0.17	0.10
Lable claim + 25% = 625 mg	628.12	0.36	0.17	626.21	0.611	0.304	623.41	0.37	0.81
Lable claim + 50% = 750 mg	756.71	0.26	0.11	751.08	0.3214	0.13	757.12	0.37	0.15

Each value is average of three determinations

Table 5: System suitability parameters for MFH and SGP developed method

S.No	MFH		SGP	
	Parameter	Value	Parameter	Value
1	Theoretical plates	4500	Theoretical plates	2500
2	Retention time (min)	2.35	Retention time (min)	3.37
3	Asymmetry	1.05	Asymmetry	1.10

Each value is average of three determinations

CONCLUSION

For compact stage smoothing out various supports of different pH like ammonium acidic corrosive induction pads, acidic corrosive determination bolsters and different blends of the regular stage (acetonitrile and methanol) close by pad were explored. Change the pad, pH and extension of common dissolvable in various degrees changed the support time of the medicine and uniformity of the zenith. An official decision of using ACN: Methanol: Phosphate cradle pH-3 (30:25:45 %, v/v) (25 mM) as a convenient stage relied upon explicit estimates like unbalanced factor, support time of the OLZ and FLX drugs, affectability of the procedure and cost. Affectability of the procedure was not actually recognized at 271 nm, however at 252 nm affectability was extended stunningly. Upkeep time of MFH and SPG was 2.36 and 3.39 min.

ACKNOWLEDGEMENTS

The makers are thankful to Meditab Specialties Pvt. Ltd, (Daman) for giving Sitagliptin phosphate and Metformin hydrochloride as gift samples.

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