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Research Article

Analytical method development and validation for simultaneous estimation of sitagliptin and etruglifloxin in bulk and pharmaceutical dosage form by RP-HPLC

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination Sitagliptin and Etruglifloxin in pharmaceutical dosage form. The column used was Discovery C_{18}(250mm x 4.6 mm, 5µm) in isocratic mode, with mobile phase containing phosphate buffer and acetonitrile (45:55 v/v). The buffer is prepared by adding accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 5.4 with dil. Orthophosphoric acid solution. The flow rate was 1.0ml/ min and effluents were monitored at 260 nm. The retention times of Sitagliptin and Etruglifloxin were found to be 2.381 min and 3.429 min, respectively. The linearity for Sitagliptin and Etruglifloxin were in the range of 25-150 µg/ml and 3.75-22.5 µg/ml respectively. The recoveries of Sitagliptin and Etruglifloxin were found to be 99.46 to 101.19% and 99.36 to 100.99%, respectively. The proposed method was validated and successfully applied to the estimation of Sitagliptin and Etruglifloxin in combined tablet dosage forms.

Keywords: Sitagliptin; Etruglifloxin; Validation; Buffer; ICH Guidelines.

INTRODUCTION

Sitagliptin belongs to the class of potent and selective inhibitors of the sodium-dependent glucose co-transporters (SGLT), more specifically the type 2 which is responsible for about 90% of the glucose reabsorption from glomerulus. This drug was developed under the collaboration of Merck and Pfizer. It was FDA approved as monotherapy and in combination with Ertugliflozin or metformin hydrochloride on December 22, 2017. Ertugliflozin is a new oral hypoglycemic (anti-diabetic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. This enzyme-inhibiting drug is to be used either alone or in combination with metformin or a thiazolidinedione for control of type 2 diabetes mellitus. The drug works to competitively inhibit a

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protein/enzyme, dipeptidyl peptidase 4 (DPP-4), that results in an increased amount of active incretins (GLP-1 and GIP), reduced amount of release of glucagon (diminishes its release) and increased release of insulin.

Different analytical methods have been reported in the literature for the assay of Sitagliptin and Etruglifloxin in pharmaceuticals and include spectrophotometry, HPLC and HPTLC [1–13]. The present study was to establish a simple, sensitive and low-cost RP-HPLC method for simultaneous estimation of Sitagliptin and Etruglifloxin in bulk as well as in other dosage forms. The developed method was validated as per ICH guidelines[14, 15].

EXPERIMENTAL

Reagents: Sitagliptin and Etruglifloxin were kindly supplied by Ranbaxy. Acetonitrile, water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. A tablet STORVAS-EZ(Ranbaxy) containing 10mg of Sitagliptin and 10mg of Etruglifloxin were used.

Instrumentation: The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals were monitored and integrated using Empower 2 software.

Chromatographic conditions: The elution was isocratic and the mobile phase consisted of a mixture of buffer (accurately weighed 1.41gm of sodium dihyrogen ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 5 with dil. orthophosphoric acid solution), acetonitrile and methanol (30:60:10 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A BDS C_{18} (250mm x 4.6 mm, 5µm) was used for determination. The flow rate was 1.0 ml/min and the column was operated at ambient temperature (~30ºC). The volume of sample injected was 10 µL. Prior to injection of the solutions, column was equilibrated for at least 30 min with mobile phase flowing through the system. The UV detector was set at wavelength of 244nm. The mixture of Water and acetonitrile (50:50%v/v) was used as a diluent. A typical RP-HPLC chromatogram of Sitagliptin and Etruglifloxin is shown in (Fig. 1).

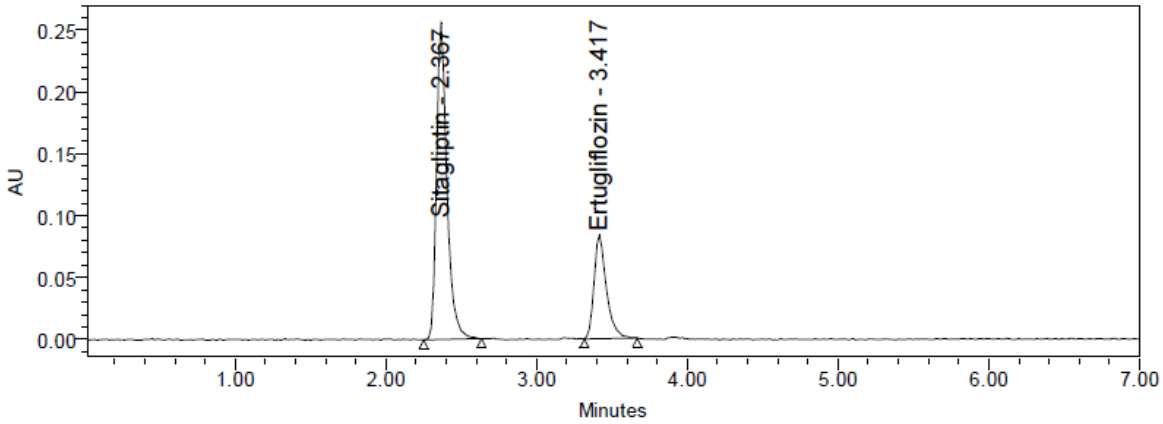

Fig - 1: HPLC chromatogram of Sitagliptin and Etruglifloxin in optimized chromatographic condition.

Standard Preparation: Accurately weighed and transferred 10mg of Sitagliptin and 10mg of Etruglifloxin working Standards into a 100 ml clean dry volumetric flask, add 70ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 2ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Sample Preparation:
20 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and drug equivalent to 10mg were transferred to a volumetric flask and dissolved in solvent. Transfer 2ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter to get concentration of 20µg/ml and 20µg/ml for Sitagliptin and Etruglifloxin.

Method Validation: The developed method was validated as per ICH guidelines for its accuracy, linearity, precision, specificity, robustness, ruggedness, limit of detection and limit of quantification.

RESULTS AND DISCUSSION

A reverse-phase column procedure was proposed as a suitable method for the simultaneous estimation of Sitagliptin and Etruglifloxin dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, buffer and acetonitrile in the ratio 45:55v/v was used as mobile phase, which showed good resolution of Sitagliptin and Etruglifloxin peak. The wavelength of detection selected was 244nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of Sitagliptin and Etruglifloxin were about 2.367mins and 3.417mins and none of the impurities were interfering in its assay.

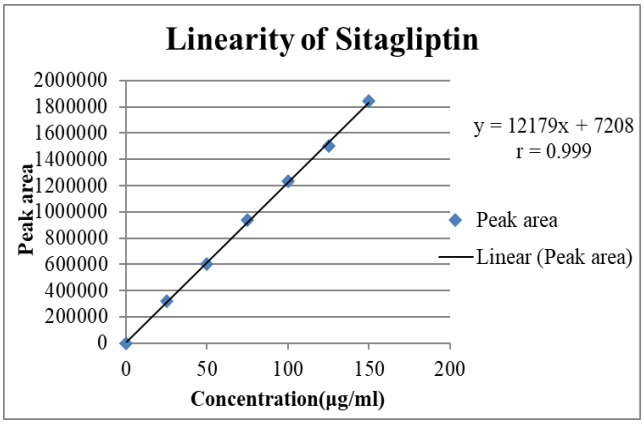

Fig – 2: Linearity of Sitagliptin in the range 25 to 150µg/ml.

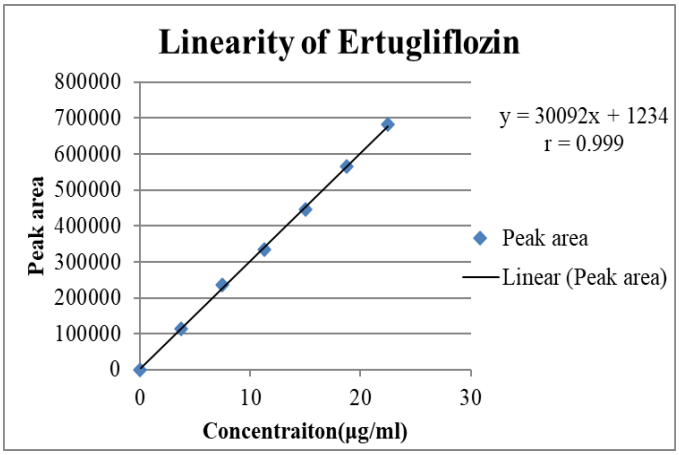

Fig – 3: Linearity of Etruglifloxin in the range 3.75 to 22.5µg/ml.

Method validation:

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Sitagliptin and Etruglifloxin at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug (Figure: 2 & 3). The response was found to be linear in the range 5-30µg/ml & 5-30µg/ml for Sitagliptin and Etruglifloxin. The data was given in table-1.

Table 1: Linearity data of Sitagliptin and Etruglifloxin

S.No	Sitagliptin			Etruglifloxin		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	25	2.367	321715	3.75	3.336	114461
2	50	2.343	604208	7.5	3.346	235418
3	75	2.354	935882	11.25	3.333	334186
4	100	2.365	1234676	15	3.347	446830
5	125	2.355	1505358	18.75	3.407	565355
6	150	2.354	1842453	22.5	3.344	682167
	r = 0.9994			r = 0.9999		
	y = 12179x + 7208			y = 30092x + 1234		

Accuracy

Accuracy was performed in triplicate for various concentrations of Sitagliptin and Etruglifloxin equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. The average % recovery was calculated. The data was given in table-2.

Table 2: Accuracy data

S.No		Sitagliptin			Etruglifloxin		
	Spiked level	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD
1(n=6)	50%	25.00	24.95	99.99 ± 0.43	3.75	3.73	100.99 ± 0.46
2(n=6)	100%	50.00	50.03	100.21 ± 0.28	7.5	7.48	100.94 ± 0.55
3(n=6)	150%	75.00	55.06	101.19 ± 0.40	11.25	11.27	99.84 ± 0.59

*n=6 (Average of 6 determinations)

Precision

A) Method Repeatability

Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table-3.

Table 3: Precision data of Sitagliptin and Etruglifloxin

S.No	Sitagliptin			Etruglifloxin		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	50	2.543	609720	7.5	3.542	235147
2	50	2.545	603435	7.5	3.545	236404
3	50	2.55	605412	7.5	3.546	240156
4	50	2.556	610050	7.5	3.554	242466
5	50	2.558	611439	7.5	3.555	233861
6	50	2.561	606954	7.5	3.563	237924
Mean			607835			237660
Std.dev			3081			3216
%RSD			0.51			1.35

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. The LOD and LOQ of Sitagliptin were found to be 0.523µg/ml and 1.586µg/ml respectively. The LOD and LOQ of Etruglifloxin were found to be 0.442µg/ml and 1.340µg/ml respectively.

Robustness and Ruggedness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Sitagliptin and Etruglifloxin were noted. The factors selected were flow rate and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters as shown in table-4 and 5. Ruggedness of the method was checked by using different days and instruments. The relative standard deviation of the results obtained from different days and instruments was <2.0%. The results were given in table-6 and 7.

Table 4: Robustness data relating to change in flow rate (1.0ml/min)

S.No		Sitagliptin			Etruglifloxin		
	Flow rate (ml/min)	Average Peak Area*	Std.dev	%RSD	Average Peak Area*	Std.dev	%RSD
1	0.9ml/min	606364	1453	0.36	236606	3411	0.73
2	1.0ml/min	606108	1087	0.27	238575	1400	0.30
3	1.1ml/min	606214	1233	0.30	236866	2723	0.58

*n=3 (Average of 3 determinations)

Table 5: Robustness data relating to change in mobile phase composition

S.No		Sitagliptin			Etruglifloxin		
	Mobile phase variation (%)	Average peak area*	Std.dev	%RSD	Average peak area*	Std.dev	%RSD
1	M.P-1-(BUFFER:ACN:44:56)	606072	3048	0.75	235789	1720	0.37
2	M.P-2-(BUFFER:ACN::45:55)	606995	1237	0.30	236045	1356	0.29
3	M.P-3-(BUFFER:ACN::46:54)	606451	1751	0.43	235058	3622	0.78

*n=3 (Average of 3 determinations)

Table 6: Ruggedness data relating to change of day

S.No	Inter-day precision					
	Sitagliptin			Etruglifloxin		
	Peak area			Peak area		
	Conc (µg/ml)	Day-1	Day-2	Conc (µg/ml)	Day-1	Day-2
1	50	606934	599202	7.5	235638	235929
2	50	607283	600292	7.5	232833	234633
3	50	599474	603848	7.5	234383	234182
4	50	605849	604122	7.5	235132	232901
5	50	603938	605393	7.5	236293	230322
6	50	602384	603033	7.5	234842	233932
Mean		604310	602648		234854	233650
SD		3011	2397		1189	1906
%RSD		0.50	0.40		0.51	0.82

Table 7: Ruggedness data relating to change of instrument

S.No	Instrument to Instrument					
	Sitagliptin			Etruglifloxin		
		Peak area			Peak area	
	Conc (µg/ml)	Day-1	Day-2	Conc (µg/ml)	Day-1	Day-2
1	50	604847	604283	7.5	256282	263832
2	50	605838	608822	7.5	262837	262922
3	50	601934	602838	7.5	267927	261973
4	50	602931	603848	7.5	261983	260283
5	50	602482	601344	7.5	257282	262833
6	50	603013	603939	7.5	260484	265752
Mean		603508	604179		460183	262933
Std.dev		1505	2513		4209	1828
%RSD		0.25	0.42		0.91	0.70

Assay

The assay and % purity were calculated for brands Storvas-EZ(Ranbaxy) and Atofast-EZ(Intralabs) with label claim 10mg and 10mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form. The results were given in table-8.

Table-8: Results of analysis of laboratory samples (Assay)

S.No	Sample		Sitagliptin		Etruglifloxin	
		Label	Amount found	%Purity + RSD*	Amount found	%Purity + RSD*
1	Brand-1 (STEGLUJAN)	5mg/100mg	9.99	99.48 ± 0.30	9.96	99.25 ± 0.73

*n=3 (Average of 3 determinations)

System suitability: System suitability and chromatographic parameters were validated such as asymmetry factor, tailing factor and number of theoretical plates were calculated and shown in table-9.

Table 9: System suitability parameters

Validation parameter	Results	
	Sitagliptin	Etruglifloxin
Linearity range (µg/ml)	25 – 150	7.5 – 22.5
Regression equation	y = 12179x + 7208	y = 30092x + 1234
Correlation Coefficient(r)	0.9996	0.9996
Accuracy	98.58% to 100.71%	98.94% to 100.58%
Precision (%RSD)	0.70	0.66
Robustness (%RSD)		
Flow rate: (0.9ml/min & 1.1ml/min)	NMT 0.36	NMT 0.73

Mobile phase: Buffer : ACN::45:55	NMT 0.75	NMT 0.78
Ruggedness (%RSD) Interday – (Day 1 & Day 2)	NMT 0.74	NMT 0.55
Instrument to Instrument (Inst-1 & Inst-2)	NMT 0.62	NMT 0.91

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in simultaneous estimation of Sitagliptin and Etruglifloxin in marketed formulation.

Conclusion

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of Sitagliptin and Etruglifloxin in bulk and its pharmaceutical dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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