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The RP-UPLC method for simultaneous quantification of Sitagliptin and Ertugliflozin

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ABSTRACT

In order to develop a newer or improved analytical method, the analyst has to set some goals. The method should be precise to the drug under study. It is necessary to determine the analyte at trace levels accurately.The UPLC techniques have now become extremely reliable and indispensable.Sitagliptin works to competitively inhibit the enzymedipeptidyl peptidase 4 (DPP-4).Ertugliflozin is a small inhibitor of the SGLT2 and its activity increases glucose excretion, reducing hyperglycemia without the requirement of excessive insulin secretion.The percent recovery of Sitagliptin and Ertugliflozinwas found to be in between 98.0 to 102.0%. The analytical method was found to be linear over the range 25-150 µg/mL of Sitagliptin and 3.75-22.5 µg/mL Ertugliflozinof the target concentration.

Keywords: RP-UPLC, Sitagliptin,Ertugliflozin, Precision, Accuracy

INTRODUCTION

Ultra Performance Liquid Chromatography spectroscopic detection is a powerful hyphenated technique for the analysis of drugs. Its sensitivity, accuracy and short analysis time make it ideal for determination of many drugs in dosage forms. Further, with the development of more sophisticated instrumentation, efficient column materials, sensitive detectors and moderate pricing, the UPLC techniques have now become extremely reliable and indispensable. In view of these advantages, the author has chosen to develop UPLC methods in this investigation for determination of some of selected drugs [1].

Sitagliptin is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor used in conjunction with diet and exercise to improve glycemic control in patients with type 2 diabetes mellitus. The effect of this medication leads to glucose dependent increases in insulin and decreases in glucagon to improve control of blood sugar. Sitagliptin was granted FDA approval on October 16, 2006.Sitagliptin works to competitively inhibit the enzyme dipeptidyl peptidase 4 (DPP-4). This enzyme breaks down the incretins GLP-1 and GIP, gastrointestinal hormones released in response to a meal. By preventing breakdown of GLP-1 and GIP, they are able to increase the secretion of insulin and suppress the release of glucagon by the alpha cells of the pancreas. This drives blood glucose levels towards normal [2].

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 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.Ertugliflozin is a small inhibitor of the SGLT2 and its activity increases glucose excretion, reducing hyperglycemia without the requirement of excessive insulin secretion [3].

MATERIALS AND METHODS

The chromatographic system consisted of a Waters Acquity H-Class UPLC (Model 2695) chromatograph equipped with BEH C18 100 X 2.1mm 1.8m, LC-20AD pumps and an SPD-20A photo diode array (PDA) detector. Samples were injected into the system through a Rheodyne7725 injector valve via a 2 µL loop. The output signal was monitored and integrated by Empower-2 software. Solubility of the compound was enhanced by sonication on an ultrasonicator (PCI Analytics PCI81).

Preparation of the mixed working standard solution of Sitagliptin and Ertugliflozin

Accurately Weighed and transferred 25 mg of Sitagliptin and 10 mg of Ertugliflozin working Standards into a 25 mL clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 min and make up to the final volume with diluents [4].

Optimization of chromatographic conditions and method development

Under the below mentioned Table 1, the optimized conditions, the retention times obtained for Sitagliptin and Ertugliflozin were 0.755 and 1.005 min respectively.

Table 1: Optimized chromatographic conditions of the proposed method

S. No.	Parameter	Value
1	Stationary phase	BEH C18 100 x 2.1mm; 1.8µ
2	Mobile phase	0.1% OPA: Acetonitrile (50:50 %v/v)
3	Flow rate	0.3 mL/min
4	Column temperature	30ºC
5	Volume of injection	2uL
6	Detection wavelength (λmax)	220 nm
7	Run time (min)	2 min

System suitability

System suitability was assessed by analyzing the mixed standard drug solution (100 ppm of Sitagliptin and 15 ppm of Ertugliflozin) and calculating the chromatographic parameters such as resolution, theoretical plates, and tailing factor [5].

Specificity

Specificity is the extent to which the procedure applies to the analyte of interest and is checked by examining the formulation samples for any interfering peaks. The specificity of the method was evaluated with regard to interference due to presence of excipients. The excipients used in formulation did not interfere with the drug peaks and thus the method is specific [6].

Linearity

To establish linearity, a stock solution containing 1000 µg/mL of Sitagliptin and 150 µg/mL of Ertugliflozin were prepared using diluent and further diluted to yield solutions in the concentration range of 25-150 µg/mL of Sitagliptin and 3.75-22.5 µg/mL Ertugliflozin. The solutions were prepared and analyzed in triplicate. The experiment was repeated thrice by preparing different solution and analyzed by injecting 2 µL in UPLC [7].

Accuracy

To determine the accuracy of the proposed method, different amounts of Sitagliptin and Ertugliflozin within linearity limits were taken and analyzed by the proposed method [8]. Accuracy for Sitagliptin and Ertugliflozinwas conducted by spiking the drug to the pre-analyzed drug solutions at three different levels of the test concentration (i.e. 50%,

100%, and 150%) and three times at each level). The mean % Recovery and % RSD values were calculated [9].

Precision

To ascertain the effectiveness of method system suitability tests were carried out on freshly prepared standard stock solution containing 100 µg/mL of Sitagliptin and 15 µg/mL of Ertugliflozin. 2 µL of solution was injected into the optimized chromatographic system. For system suitability 6 replicates of working standard samples were injected and the peak responses of sample were calculated [10].

Limit of detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ values were calculated from the average standard deviation and slope from the calibration curve as per ICH guideline [11].

Robustness

Robustness study was done by applying small deliberate changes in the chromatographic conditions and studying the system suitability parameters of both the drugs. The conditions selected for testing were the flow rate, column oven temperature and composition of the mobile phase. The study was conducted on a mixed standard solution containing 100 µg/mL of Sitagliptin and 15 µg/mL of Ertugliflozin [12].

RESULTS AND DISCUSSION

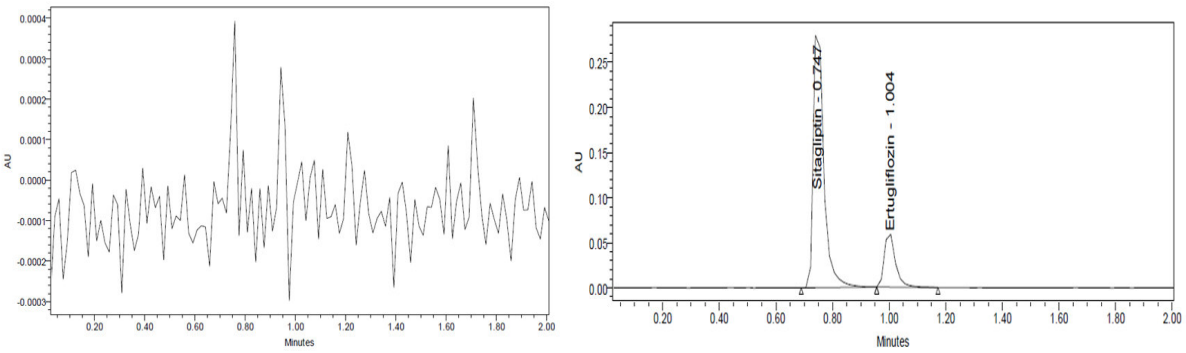
System suitability: It was represented in Table 2.

Table 2: System suitability values for the present method

	Parameter	Sitagliptin	Ertugliflozin
1	Retention time (min)	0.747	0.998
2	Peak area	660704	141262
3	Resolution	-	3.7
4	Theoretical Plates	2395	3437
5	Tailing Factor	1.52	1.26

Specificity

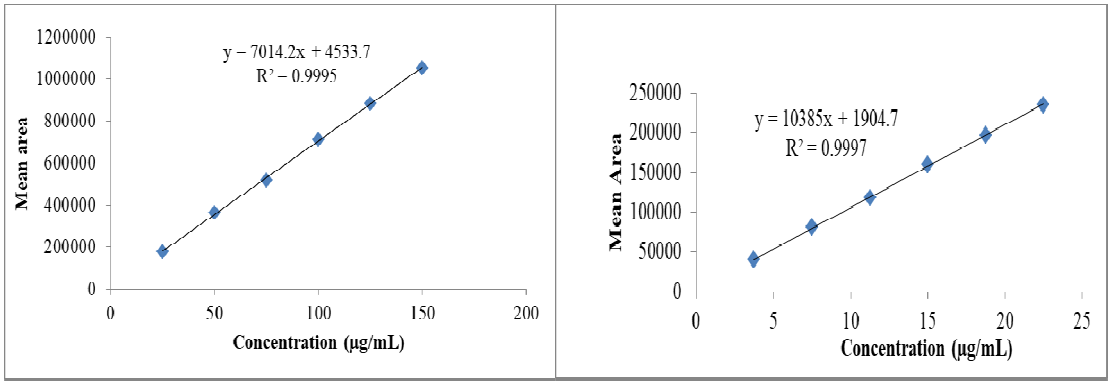
The UPLC chromatograms recorded for the drug matrix (mixture of the drug and the excipients) showed almost no interfering peaks within retention time ranges.Figure 1a and Figure 1b show the representative chromatograms for standard and the formulation. The figures show that the selected drugs were clearly separated. Thus the proposed UPLC method is selective.



(a) (b)

Figure 1: (a) A Typical Chromatogram of Placebo (b) A Typical Chromatogram of Sitagliptin and Ertugliflozin in mixed standard solution

Linearity: Linearity data for Sitagliptin and Ertugliflozin are given in the Figure 2a and 2b respectively.



(a) (b)

Figure 2: Linearity plot of (a) Sitagliptin (b) Ertugliflozin

Accuracy

The results are present in Table 3 and 4.The %Recovery value was found to be in between 98.0 % to 102.0 %.

Table 3: Recovery of Sitagliptin

Accuracy Level	Peak area difference	Amount added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean % Assay
50%	346850	50	50.479	100.96	99.90
	337108	50	49.093	98.19	
	345437	50	50.278	100.56	
100%	690644	100	99.362	99.36	99.97
	698072	100	100.420	100.42	
	695968	100	100.120	100.12	
150%	1037264	150	148.65	99.10	99.34
	1040630	150	149.12	99.42	
	1041414	150	149.24	99.49	

Table 4: Recovery ofErtugliflozin

Accuracy Level	Peak area difference	Amount added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean % Assay
50%	76841	7.5	7.5122	100.16	100.3
	77021	7.5	7.5294	100.39	
	77003	7.5	7.5277	100.37	
100%	154650	15	14.965	99.77	99.49
	154120	15	14.914	99.43	
	153894	15	14.893	99.28	
150%	232920	22.5	22.462	99.83	99.92
	232914	22.5	22.462	99.83	
	233560	22.5	22.524	100.10	

Precision

The inter-day precisions were determined by analyzing a mixed solution containing 100µg/mL of Sitagliptin and 15µg/mL of Ertugliflozin. The intermediate precision was determined on two consecutive days different instrument. The results are depicted in the Table 5.

Table 5:Inter-day precision data

S.No	Injection	Sitagliptin		Ertugliflozin	
		Day-1	Day-2	Day-1	Day-2
1.	Injection-1	524622	514622	155420	151420
2.	Injection-2	522093	512093	155128	153710
3.	Injection-3	522996	522996	154883	150240
4.	Injection-4	526012	521012	154106	151720
5.	Injection-5	522371	521371	154545	152470
6.	Injection-6	529315	520315	152149	151179
Mean		521652		153079	
SD		4611.72		1756.72	
% RSD		0.88		1.15	

LOD and LOQ: The LOD and LOQ values for Sitagliptin andErtugliflozin were represented in Table 6.

Table 6: LOD and LOQ values of the method

S. No	Parameter	Sitagliptin	Ertugliflozin
1	LOD	1.140	0.324
2	LOQ	3.450	0.981

Robustness

The results remained unaffected by small variations in these conditions. The results were represented in tables 7 and 8.

Table 7: Robustness data of Sitagliptin

Chromatographic conditions	Sitagliptin			
	% assay	Theoretical Plates	Asymmetry	Retention time
Water: Acetonitrile (60:40% v/v)	98.81	3104	1.69	0.742
Water: Acetonitrile (40:60% v/v)	99.12	2378	1.57	0.768
0.2 mL/min	99.02	2359	1.59	0.780
0.4 mL/min	99.18	2114	1.58	0.733
28°C	98.02	2014	1.47	0.761
32°C	98.87	2039	1.51	0.747

Table 8: Robustness data of Ertugliflozin

Chromatographic conditions	Ertugliflozin				
	% assay	Theoretical Plates	Asymmetry	Resolution	Retention time
Water: Methanol (60:40% v/v)	99.07	3435	1.29	3.9	0.985
Water: Methanol (40:60% v/v)	98.99	2982	1.35	3.7	3.8
0.2 mL/min	101.0	3284	1.37	4.0	1.036
0.4 mL/min	99.7	2583	1.29	3.8	0.971
28°C	99.2	3160	1.31	3.9	1.010
32°C	100.1	3292	1.26	3.9	0.997

CONCLUSION

The present analytical method was developed by studying different parameters. BEH C18 100 X 2.1 mm 1.8µ column because it gave good separation and peak shapes.Ideal λ max for both the drugs was found to be at 220 nm as the peak purity was good.

Injection volume was selected to be 2µL which gave a good peak area. The flow rate was fixed at 0.3 mL/min for giving satisfactory retention times. A mixture of 0.1% OPA and Acetonitrile (50:50% v/v)was found to be ideal for the proposed study as it resulted in good resolution of the drugs. Run time was selected to be 3 min because the analysis gave peaks around 1.009 and 1.284 ±0.02min of Sitagliptin and Ertugliflozinrespectively.The percent recovery was found to be in between 98.0 to 102.0%. The analytical method was found to be linear over the range 25-150 µg/mL of Sitagliptin and 3.75-22.5 µg/mL Ertugliflozinof the target concentration. The analytical method passed both the robustness and ruggedness tests. In both the cases, relative standard deviation was below 2.0.

REFERENCES

[1]. ICH, Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, IFPMA , Geneva, 1996

[2]. https://www.rxlist.com/consumer_sitagliptin_januvia/drugs-condition.htm

[3]. P.Harshalatha, A novel RP-HPLC method for simultaneous determination of Ertugliflozin and Sitagliptin in bulk and tablet dosage form, *Internationl Journal of Research in Pharmaceutical Sciences*, 9(3), 2018, 1042-1050.

[4]. D.ChinaBabu, Novel Stress Indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Ertugliflozin and Sitagliptin in Bulk and its Formulation, Oriental journal of chemistry, 34(5),2018, 2554-2561.

[5]. PasamVenkateswaraRao, Development and Validation of New Stability Indicating Reversed-Phase High-Performance Liquid Chromatography Method for Simultaneous Determination of Metformin Hydrochloride and Ertugliflozin in Bulk and Pharmaceutical Dosage Form,.*Asian Journal of pharrmacutical and science research,*12(1), 2019, 235-240.

[6]. P.Ramalingam,V. UdayaBhaskar,Stability-indicating RP-HPLC Method for the Simultaneous Determination of Sitagliptin and Simvastatin in Tablets*, Indian journal of pharmaceutical sciences,*76(5),2014, 407–414.

[7]. Arshiya sultana, Analytical method development and validation for the determination of sitagliptin and metformin using RP-HPLC method in bulk and tablet dosage form, *Journal Bioanal Biomed,*06(02), 2014, 8785-8794.

[8]. S. K. Sahoo, Development and validation of RP HPLC method for determination of metformin and sitagliptin in bulk and pharmaceutical dosage form, *Journal of applied pharmaceutical*

[9]. XiangjunQiu., UPLC-MS/MS method for the quantification of ertugliflozin and sitagliptin in rat plasma, *Anal. Biochem.,*567, 2018, 112-116.

[10]. Dayyih WA, Hamad M, Mallah E, Dayyih AA, Awad R, Zakaria Z and Arafat T: Method development and validation of vildagliptin and metformin HCl in pharmaceutical dosage form by reverse Phase–High-Performance Liquid Chromatography (RP-HPLC). International J of PharmaSci and Res 9(7), 2018, 2965-72.

[11]. Patil MD, Bapna M, Shah P and Khoja SS: Development and validation of analytical method for simultaneous estimation of metformin hydrochloride and teneligliptinhydrobromide hydrate in the pharmaceutical dosage form. Journal Pharmal Science Bioscie Res 7(2), 2017, 200-08.

[12]. Harshalatha P, Chandrasekhar KB and Chandrasekhar MV: A novel RP-HPLC method for simultaneous determination of ertugliflozin and sitagliptin in bulk and tablet dosage form. International Journal of Research in Pharmaceutical Sciences 9(3), 2018, 1042-50.