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DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF ERTUGLIFLOZIN AND SITAGLIPTIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

Raju M[1], Hanzala Fathima[2], Hafsa Siddiqua[3], Sunil Kumar Chaitanya.P[4]*

[1,3,4] Dept of Pharmaceutical Analysis,
[2]Dept of Pharmaceutical Chemistry, St. Pauls college of Pharmacy Turkhyamzal -510510,
RR – Dist, Telangana, India.

*Corresponding Author E-mail: sunilpadavala@gmail.com

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ABSTRACT

A rapid and a precise Reverse Phase High Performance Liquid Chromatographic method has been developed and validated for the simultaneous estimation of ertugliflozin and sitagliptin, in bulk as well as in tablet dosage form. Separation was carried out on HPLC –Waters Model NO.2695with Phenomenex Luna C18 (4.6mm×150mm, 5µm) column using a mixture of methanol: Buffer (35:65% v/v) as mobile phase at a flow rate of 1.0ml/min. The detection was carried out at 261nm.The retention times of ertugliflozin and sitagliptin were found to be 2.256, 5.427respectively. The method produces linear response in the concentration range of 60ppm to 140 ppm for ertugliflozin and 100ppm to 600 ppm for sitagliptin with respect to target concentration. The method was precise since the %RSD values of peak areas for five duplicate injection was found to be below " 2". The % recovery values for ertugliflozin and sitagliptin were found to be "100.35% &100.51%" respectively indicating the method was accurate. The LOD & LOQ of ertugliflozin and sitagliptin were found to be 2.63µg/ml, 3.02µg/ml &7.92µg/ml, 9.06. µg/ml. The method was found to be rapid, linear, precise, accurate, sensitive and suitable to adopt for routine quality control analysis.

INTRODUCTION

Ertugliflozin belongs to the class of potent and selective inhibitors of the sodium-dependent glucose cotransporters (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 Sitagliptin or Metformin hydrochloride on December 22, 2017. Ertugliflozin is very slightly soluble in water,Soluble in DMSO,soluble in ethyl alcohol and acetone, slightly soluble in ethyl acetate and Acetonitrile. The molecular formula is $C_{22}H_{25}ClO_7$ and molecular weight is436.89. 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. It is soluble in water and N, N-dimethyl formamide, slightly soluble in methanol, soluble in ethanol, acetone and Acetonitrile and insoluble in isopropanol and isopropyl acetate. The molecular formula is

$C_{16}H_{15}F_6N_5O$and molecular weight is 407.31.Literature revealed a few reported analytical methods on this drug individually and combination with some other drugs. A few methods were also reported on the development and validation of ertugliflozin and sitagliptin. Literature showed a few colorimetric methods were also reported for the estimation in biological fluids with LC-MS. But there is avery few methods reported for the estimation of these two drugs by RP-HPLC. Hence the present work is aimed to develop an RP-HPLC method for the simultaneous estimation of ertugliflozin and sitagliptin in bulk and pharmaceutical dosage form.

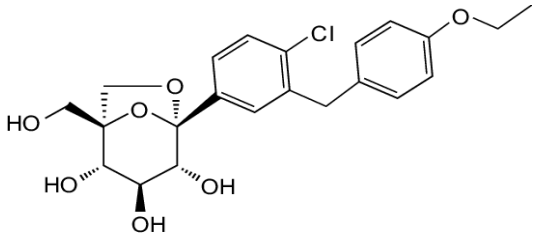

Fig.1: Chemical structure of ertugliflozin

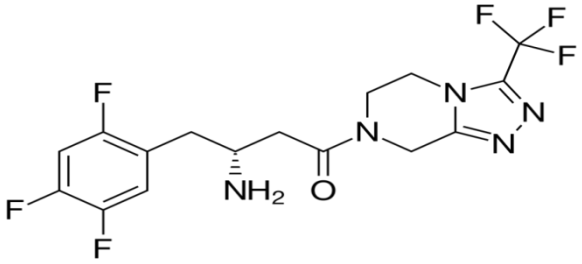

Fig.2: Chemical structure of sitagliptin

II. Materials and Methods

A. Instruments used: The liquid chromatographic system used was WATERS, software: Empower 2, Alliance 2695 with Phenomenex Luna C18 (4.6mm×150mm, 5µm) column**.**

B. Chemicals used: Samples of ertugliflozin and sitagliptin were received from Optus labs as gift samples. HPLC grade water, methanol and Acetonitrile and Potassium dihydrogen phosphate ($K_2H_2PO_4$) were purchased from MERCK laboratories, Mumbai.

Method Development:

1. Preparation of standard solution: 10mg's of ertugliflozin and sitagliptin samples were accurately weighed and dissolved in 10ml of Mobile phase and sonicated for 20 minutes to get 1000ppms. 1 ml was taken from each solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase.

2. Preparation of Sample Solution: Equivalent weight of one tablet was taken and crushed. From this 10 mg equivalent weight of ertugliflozin and sitagliptin was transferred into 10ml clean dry volumetric flasks. About 7mL of diluent was added and sonicated to dissolve it completely. The volume was made up to the mark with the same solvent. The method development was started with liquid chromatographic system WATERS, software: Empower 2, Alliance 2695, with Phenomenex Luna C18 (4.6mm×150mm, 5µm) column. Several compositions of mobile phase were utilized to optimize the method. The method was finally optimised by mixture of methanol: Buffer (35:65% v/v) as mobile phase at a flow rate of 1.0ml/min. The detection was carried out at 261nm.The retention times of ertugliflozin and sitagliptin were found to be 2.256, 5.427 respectively. Both the analytes were eluted with good resolution and satisfactory number of theoretical plates. The Chromatogram is shown in figure-3**.**The developed method was validated for specificity, accuracy, precision, linearity, LOD & LOQ as per the ICH guidelines.

B. Method Validation:

1. System suitability: Standard solutions of ertugliflozin and sitagliptin were prepared from working standards as per test method and injected in replicates for five times into the HPLC system. The system suitability parameters like theoretical plates, tailing factor and resolution were observed from standard chromatograms. The results were given in table-III& IV.

2. Specificity: To ensure no interference from mobile phase and excipients specificity studies were carried out by injecting sample, standard, and blank solutions into the HPLC system. The results were given in figures 4- 6.

3. Precision: System precision: Standard solutions were prepared as per test method and injected five times in replicates.

Method precision: Sample solutions were prepared as per the test procedure and six injections were given in replicates. Intermediate Precision: Studies were performed on different days under same experimental conditions. The peak areas for all six injections

were recorded and the %RSD for the same was calculated and reported. The results were given in tables V to VII.

4. Accuracy: Recovery studies at three different levels equivalent to 50,100&150% of ertugliflozin and sitagliptin. At each level the target concentration was spiked in triplicates and the amount recovered was observed and the percentage recovery at each level was calculated and reported in table VIII and IX.

5. Linearity: Solutions were prepared using ertugliflozin and sitagliptin working standards at60ppm to 140 ppm for ertugliflozin and 100ppm to 500 ppm for sitagliptin with respect to target concentration. Each sample solution was injected into HPLC system in replicates and the peak areas were measured. A graph was plotted with peak areas vs concentrations and the r2 values were calculated. The results were shown in fig 7&8, table – X and XI.

6. Limit of Detection and Limit of Quantification:

Limit of detection and quantification were calculated using the following formulae.

$$LOD = \frac{3.3\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

$$LOQ = \frac{10\ \sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte. The values were given in table-XII.

7. Robustness: Standard solutions prepared as per the test method were injected into the HPLC system using flow rates, 1.0ml/min and 1.2ml/min. The same studies were also performed by varying mobile phase composition and detection wavelength. The system suitability parameters were evaluated and reported in table XIII and XIV

III. RESULTS AND DISCUSSION

A. Method development: A series of trials have been conducted and finally the method was optimized by the following conditions.

B. Method Validation:

1. System suitability: **S**ystem suitability studies were performed by evaluating the parameters like theoretical plates and tailing factor. The theoretical plates were more than 2000 and the tailing factor was less than 2 in each injection for both the analytes. The observed values were in agreement with the acceptance criteria.

2. Specificity: **S**pecificity studies were carried out by injecting sample, standard, and blank solutions into the HPLC system. No interference was observed in blank chromatogram. The sample and standard chromatograms identical with same retention times

3. *Precision:* The % RSD for the peak areas of six standard injections for system precision were 0.212 and 0.064and intermediate precision 0.611 and 0.296for ertugliflozin& sitagliptin respectively which were within the limits.The results are given in tables V to VII.

4. Accuracy: Three target concentrations 50%, 100%, 150% were prepared and injected into HPLC system in triplicates. The mean recovery values of ertugliflozin and sitagliptin were found to be 100.36 & 100.15 % respectively which satisfies the acceptance criteria. The recovery values indicate the method is accurate. The results are observed in table VIII&IX.

5. ***Linearity***. A graph was plotted with peak area versus concentration and the correlation coefficient was calculated. The r^2 values of both the drugs were found to 0.999 which were within the limits. The r^2 values confirmed the method was linear and the results were shown in table 9 & 10 and figures 7&8.

6. ***Limit of Detection and Limit of Quantification***

7. Robustness: A study was carried out with variation in flow rate to evaluate the robustness of the method. The standard solutions were injected in the selected robust conditions and the system suitability parameters like theoretical plates, tailing factor and resolution were observed. The results showed that the theoretical plate count was more than 2000, tailing factor was less than 2 and resolution was found more than 2. The results of the study indicated that the method was robust and the results were shown in table 11 &12

Table I: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Phenomenex Luna C18 (4.6mm×150mm, 5µm)
Mobile Phase	methanol: Buffer (35:65% v/v)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop	20
Detection wavelength	261nm

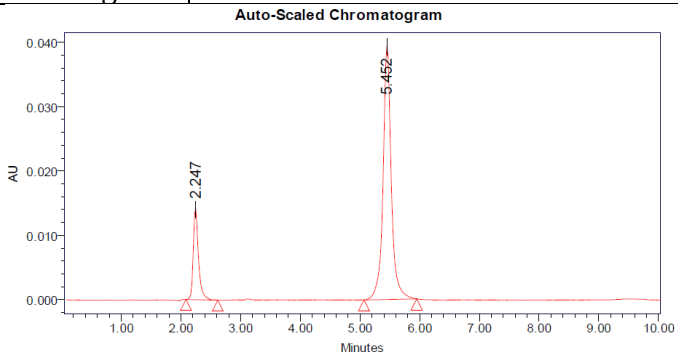

Figure 3 Optimized Chromatogram

Table II Peak Characteristics of ertugliflogin and sitagliptin

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ertugliflozin	2.247	106532	19865		1.09	7698
2	Sitagliptin	5.452	1869582	645265	5.89	1.05	6452

Table-III: Data of System Suitability for Ertugliflozin

S.No.	Name	Rt	Peak Area	Height	USP plate Count	USP Tailing
1	Ertugliflozin	2.247	105698	18652	7592	1.08
2	Ertugliflozin	2.246	105874	18754	7584	1.09
3	Ertugliflozin	2.248	105698	18698	7562	1.08
4	Ertugliflozin	2.252	105465	18689	7549	1.08
5	Ertugliflozin	2.248	105236	18695	7591	1.09
Mean			105594.2			
Std. Dev			247.4049			
% RSD			0.234298			

Table-IV: Data of System Suitability forSitagliptin

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing	USP Resolution
1	Sitagliptin	5.452	1856985	63659	6359	1.05	5.86
2	Sitagliptin	5.484	1856754	63598	6384	1.04	5.85
3	Sitagliptin	5.491	1856985	63845	6395	1.05	5.86
4	Sitagliptin	5.482	1856574	63989	6345	1.04	5.86
5	Sitagliptin	5.491	1854735	63895	6395	1.05	5.85
Mean			1856407				
Std. Dev			950.2696				
% RSD			0.051189				

The theoretical plates for each drug are more than 2000 and the tailing factor is less than **2.**

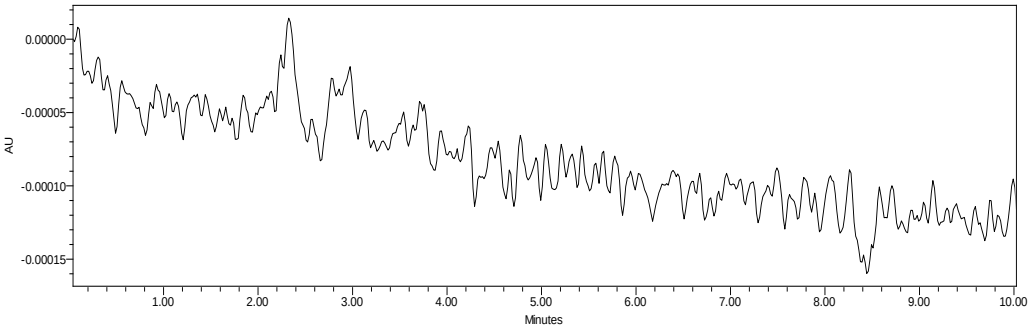

Figure 4 Chromatogram of blank

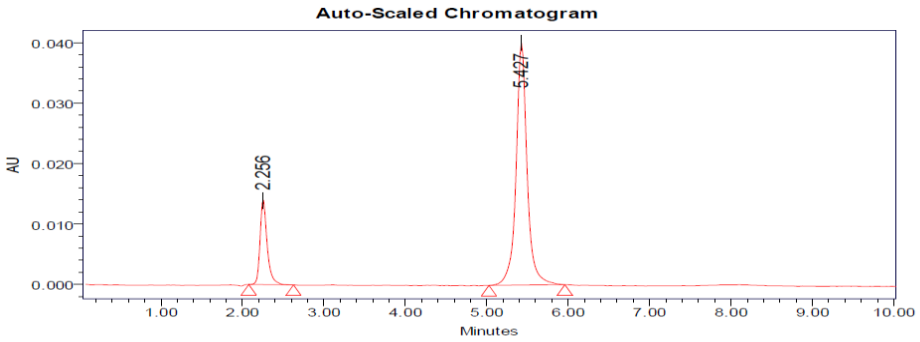

Figure 5 Chromatogram of Standard

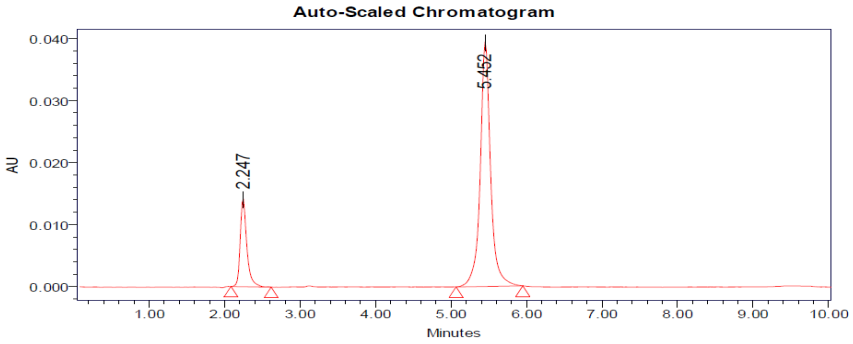

Figure 6 Chromatogram of Sample

Table-V : Data of Repeatability (System precision) forertugliflozin&sitagliptin

	Ertugliflozin		Sitagliptin	
Injection	**Peak Areas**	**Assay**	**Peak Areas**	**Assay**
1	105698	99.74	1856985	99.98
2	105684	99.14	1857458	99.38
3	105421	99.62	1854795	99.60
4	105879	99.72	1857469	99.84
5	105326	99.42	1857685	99.72
Mean	105601.6		1856878	
SD	224.5023		1192.4	
% RSD	0.212594		0.064215	

Table-VI: Intermediate Precision for ertugliflozin&sitagliptin (day-1)

	Ertugliflozin		Sitagliptin	
Injection	**Peak Areas**	**Assay**	**Peak Areas**	**Assay**
1	115246	99.25	1948592	99.54
2	116985	99.14	1958245	99.72
3	115847	99.12	1947584	99.31
4	116985	99.52	1948675	99.84
5	115848	99.84	1959854	99.72
6	116582	99.54	1958246	99.65
Mean	116248.8		1953533	
SD	710.3091		5792.661	
% RSD	0.611025		0.296522	

Table-VII: Intermediate Precision for ertugliflozin&sitagliptin(day-2)

	Ertugliflozin		Sitagliptin	
Injection	**Peak Areas**	**Assay**	**Peak Areas**	**Assay**
1	102658	99.45	1798952	99.92
2	102856	99.87	1789854	99.87
3	102658	99.84	1798659	99.54
4	102698	99.28	1789898	99.74
5	102451	99.87	1796856	99.81
6	102368	99.27	1798568	99.37
Mean	102614.8		1795465	
SD	176.9592		4390.879	
% RSD	**0.17245**		**0.244554**	

Table VIII-: The Accuracy Results for Ertugliflozin

%Concentration	**Area**	**Amount Added (ppm)**	**Amount Found (ppm)**	**% Recovery**	**Mean Recovery**
50%	539070	50	50.373	100.746%	100.36%
100%	**1063578**	**100**	**100.274**	**100.274%**	
150%	**1587149**	**150**	**150.085**	**100.056%**	

Table-IX: The accuracy results for Sitagliptin

%Concentration	**Area**	**Amount Added (ppm)**	**Amount Found (ppm)**	**% Recovery**	**Mean Recovery**
50%	949127	150	150.328	100.218%	100.15%
100%	1867824	300	300.441	100.147%	
150%	2785321	450	450.359	100.079%	

Table: X Linearity Data of ertugliflozin

Concentration (ppm)	**Average Area**	**Statistical Analysis**	
0	0	Slope	10511
60	648743	y-Intercept	9597
80	856982	Correlation Coefficient	0.999
100	1068542		
120	1268984		
140	1469853		

Table: XI Linearity Data of sitagliptin

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	6120
100	667564	y-Intercept	29119
200	1268547	Correlation Coefficient	0.999
300	1868598		
400	2465487		
500	3085864		

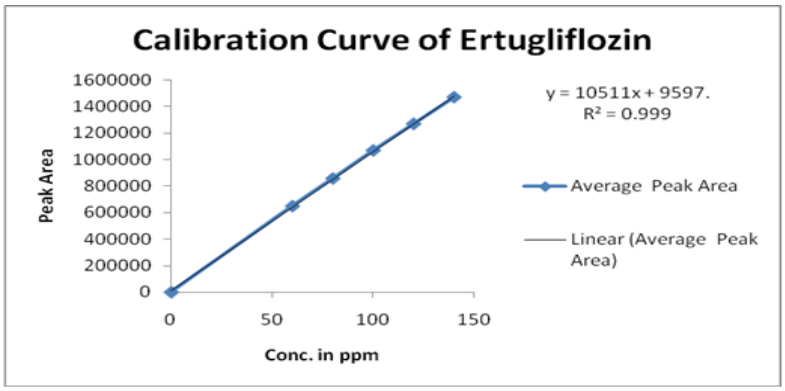

Figure: 7 Linearity Plot of ertugliflozin

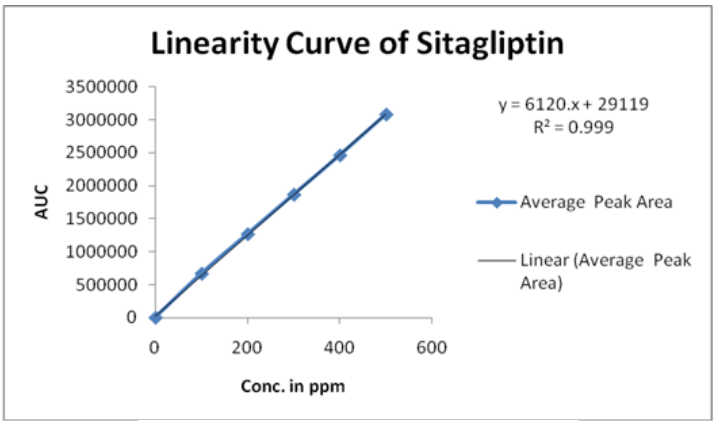

Figure: 8 Linearity Plot of sitagliptin

Table XII : LOD & LOQ of Artemether &Lumafantrine

Drug	LOD	LOQ
Ertugliflozin	2.63µg/ml	7.92µg/ml
Sitagliptin	3.02µg/ml	9.06. µg/ml

Table: XIII Data for Effect of variation in flow rate and mobile phase of ertugliflozin:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	105265	2.256	7589	1.08
Less Flow rate of 0.9 mL/min	109898	2.505	7256	1.05
More Flow rate of 1.1 mL/min	102365	2.046	7469	1.07
Less organic phase	101548	2.505	7358	1.06
More organic phase	104645	2.046	7659	1.02

Table: XIV Data for Effect of variation in flow rate Artemether

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1858475	5.427	6354	1.04
Less Flow rate of 0.9 mL/min	1925684	5.599	6253	1.05
More Flow rate of 1.1 mL/min	1863525	4.576	6248	1.03
Less organic phase	1825471	5.599	6415	1.02
More organic phase	1836594	4.576	6529	1.06

IV. CONCLUSION

A Rapid and Precise Reverse Phase High Performance Liquid Chromatographic method has been developed and validated for the estimation of ertugliflozin and sitagliptin in its pure form as well as in tablet dosage form. Chromatography was carried out on Inertsil-ODS C18 (250 x 4.6mm, 5µm) column. The method was optimized using a mixture of methanol: Buffer (35:65% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 261nm.The retention times of the ertugliflozin and sitagliptin were 2.247& 5.452 respectively. The method produced linear responses for ertugliflozin and sitagliptin in the concentration range of 60-140& 100-500µg/ml respectively. The method was precise since the %RSD values of peak areas were found to be below "2". The % recovery values for both the analytes were found to be "100.36% &100.16%" indicating the method was accurate. The specificity of the method was assessed by injections of standard, sample and blank solutions separately and the chromatograms were recovered. The LOD values of ertugliflozin and sitagliptin were found to be 2.63µg/ml, 3.02µg/ml & LOQ values were found to be 7.96µg/ml, 9.02µg/ml respectively.

When compared to the previous methods reported the present method is rapid due to less Rt and economical since the present method consumes a minimum organic phase for elution. The accountability of the method was assessed and documented by validation as per ICH guidelines. The results of validation were in agreement with acceptance criteria. This indicates that the method is suitable and can be adopted for routine analysis of these analytes as a part of regular quality control analysis of ertugliflozin and sitagliptin in bulk and tablet dosage form.

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REFERENCES

1. D. China Babu, C. Madhusudan Chetty and Sk. Mastanamma, 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, 2018; 34 (5): 2554-2561.
2. P. Venkateswara Rao, A. Lakshmana Rao, and S.V.U.M Prasad, A New Stability Indicating RP-HPLC Method for Simultaneous Estimation of Ertugliflozin and Sitagliptin in Bulk and Pharmaceutical Dosage Form Its Validation as Per ICH Guidelines, Indo American Journal Of Pharmaceutical Sciences, 2018; 05 (04): 2616-2627.
3. Harshalatha P, Kothapalli Bannoth Chandrasekhar 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), 1042-1050.
4. AmtulHadi Hadiya and Mohammad Yunoos. A New Validated Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Sitagliptin and Ertugliflozin in Bulk and Tablet Dosage Form, Indo American Journal Of Pharmaceutical Sciences, 2018;05 (09): 8785-8794.
5. Syed Wajahat Shafaa ,Aejaz Ahmed , G. J. Khan , Shaikh Anas and Absar A. Qureshi .Analytical method development and Validation for the simultaneous estimation of ertugliflozin and metformin in bulk and

pharmaceutical dosage form by HPLC. IJPSR, 2020; Vol. 11(1): 226-232.

6. Anjali,Manaaz,M.Shreshta ,R.Prasanna , T.Shrisha , M.Sathish Kumar. Method Development and Validation of Ertugliflozin and Sitagliptin by Using Simultaneous Equation Method. Journal of Innovation in Pharmaceutical Sciences ,2019; 3(1): 22-28.
7. Rao P, V., L. Rao A, and P. Svum. "Development and Validation of new stability indicating RP-HPLC method for simultaneous determination of Metformin HCl and Ertugliflozin in bulk and pharmaceutical dosage form. *Asian Journal of Pharmaceutical and Clinical Research*, 2019,12(1): 235-40.
8. Novel RP-HPLC Method for Simultanious Determination of Sitagliptin and Simvastation in Bulk and Tablet Dosage Form. P. Ravisankar, SK. Hassain1, Shaik Mohammed Neeha. IOSR Journal of Pharmacy, 2015, 5(8): 34-40.
9. Tahir Nizami, Birendra Shrivastava and Pankaj Sharma. Analytical method development and validation for simultaneous estimation of Ertugliflozin and Metformin in tablet dosage form by RP-HPLC method. Int. J. of Pharm. Life Sci, 2018;9(5):5854-5859.
10. Ashutosh KS, Manidipa D, Seshagiri RJVLN, Gowri SD (2015) New Validated Stability Indicating RP-HPLC Method for Simultaneous Estimation of Metformin and Alogliptin in Human Plasma. J Chromatogr Sep Tech 6: 293. doi:10.4172/2157-7064.1000293