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STUDIES A NEW STABILITY-INDICATING RP-HPLC METHOD FOR THE COMBINED ESTIMATION OF ERTUGLIFLOZIN AND SITAGLIPTIN IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

The objective of the current study was to develop a simple, sensitive, rapid, accurate, and precise stability-indicating RP-HPLC method for the simultaneous estimation of sitagliptin and ertugliflozin. Chromatographic separation was carried out using a Standard Fortis C18 column (4.6 × 100 µm, 2.5 µm particle size) under ambient conditions. The mobile phase consisted of methanol and buffer solution in a 75:25 (v/v) ratio, with a flow rate of 0.8 mL/min. The wavelength of maximum absorption for both sitagliptin and ertugliflozin was determined to be 215.0 nm. Chromatograms of the prepared standard stock solutions of the two drugs were recorded under optimized chromatographic conditions. Retention times for sitagliptin and ertugliflozin were observed to be 3.02 minutes and 6.58 minutes, respectively.Validation of the method was conducted as per ICH guidelines Q2(R1), and stability-indicating studies followed the guidelines outlined in ICH Q1A(R2). Intra-day and inter-day precision values were within acceptable limits. Linearity was assessed over a concentration range of 66–396 µg/mL for sitagliptin and 10–60 µg/mL for ertugliflozin, with linear regression coefficients of 0.9996 and 0.9995, respectively. The limits of detection (LOD) and quantitation (LOQ) for ertugliflozin were found to be 0.55 µg/mL and 1.674 µg/mL, while those for sitagliptin were 2.80 µg/mL and 8.485 µg/mL. These values were determined using the slope and standard deviation. To confirm the accuracy of the proposed method, recovery studies were performed using the standard addition method, as per ICH guidelines. The mean percentage recovery for sitagliptin and ertugliflozin ranged between 99.97% and 100.86%. The developed method was successfully applied for stability-indicating studies and routine analysis of sitagliptin and ertugliflozin in pharmaceutical formulations.

Keywords: Validation, Sitagliptin, HPLC, ICH guidelines, Ertugliflozin, Forced degradation studies

INTRODUCTION:

The rising global incidence of T2DM, together with accompanying microvascular and macrovascular

consequences, is a serious public health concern that places an increasing strain on health-care systems worldwide. [1,2] Because type 2 diabetes mellitus (T2DM) is a progressive condition, many patients require combination medication to maintain glycemic levels over time. [3] Efficacy and safety of adding Ertugliflozin to metformin and sitagliptin-treated individuals with Type 2 diabetes mellitus. [4] Ertugliflozin is a sodium-glucose transporter 2 inhibitor used orally. The study compared the efficacy and safety of ertugliflozin (ERTU) and sitagliptin (STG) co- administration to placebo in patients with T2DM who were adequately controlled on diet and exercise. [5]

Sitagliptin is a DPP-4 inhibitor, which inhibits the enzyme that degrades the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (GIP). Sitagliptin is made up of three amino acids. -1- [3(trifluoromethyl)6,8dihydro5H[1,2,4] triazolo[4,3-a]pyrazin-7-yl] -4-(2,4,5trifluorophenyl) butan1-one; phosphoric acid; hydrate. It has the molecular formula C16H15F6N5O•H3PO4•H2O and the atomic weight 523.324. [7,8]

Ertugliflozin is an oral, selective inhibitor of sodium glucose co-transporter-2 (SGLT2) that results in urine glucose excretion (UGE) and decreases in plasma glucose and haemoglobin A1c (A1C) in individuals with type 2 diabetes mellitus (T2DM). Ertugliflozin is a newly discovered chemical entity with the chemical name of (1S,2S,3S,4R,5S) [4-Chloro-3- (4ethoxybenzyl)phenyl] -5-[4-Chloro-3- (4ethoxybenzyl)phenyl] -1-hydroxymethyl-6,8-dioxabicyclo[3.2.1]octane-2,3,4-triol.[9,10]

Fig 1: Chemical structure of Ertugliflozin

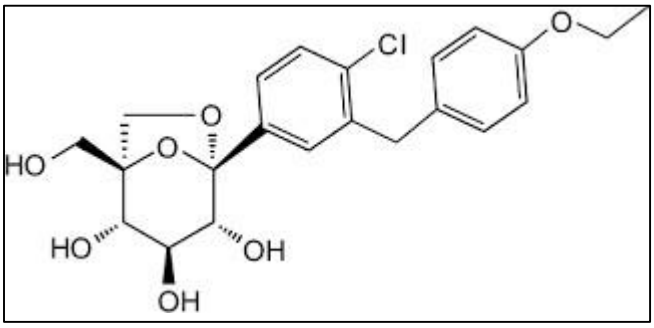
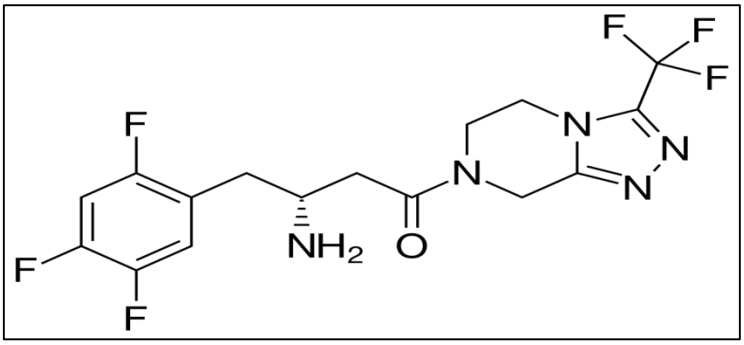


Fig2:Chemical structure of Sitagliptin



MATERIALS & METHODS

Chemicals and Solvent:

Sitagliptin and Ertugliflozin is obatained as gift sample from Ajanta Pharma Ltd, Mumbai, methanol (HPLC grade), double distilled water, ortho phosphoric acid, sodium hydroxide, hydrochloric acid, hydrogen peroxide, marketed formulation. All the chemicals and reagents are used in this experiment were analytical grade

Instrumentation:

The HPLC was Agilent Technologies Gradient system with auto injector with UV(DAD) detector, binary pump, model no 1260, software chemostation 10.1, pH meter, electronic weighing balance, ultrasonic bath.

Preparation of Buffer and Mobile phase:

0.1% OPA Buffer: 1ml of ortho phosphoric acid was diluted to 1000ml with double distilled water becomes 0.1% OPA buffer.

Mobile phase: 750ml of methanol and 250ml of 0.1% OPA buffer were mixed and degassed in an ultrasonic water bath for 30minutes and then filtered.

Stock solution of Ertugliflozin:

Standard stock solution of Ertugliflozin was prepared by dissolving 10mg of Ertugliflozin in 10ml of methanol in a 10ml clean dry volumetric flask separately and the standard solutions was filtered and degassed by sonicator to get the concentration of 1000µg/ml of Ertugliflozin.[11]

Stock solution of Sitagliptin:

Standard stock solution of Sitagliptin was prepared by dissolving 66mg Sitagliptin in 10ml of methanol in a 10ml clean dry volumetric flask separately and the standard solutions was filtered and degassed by sonicator to get the concentration of 1000µg/ml of Sitagliptin.

Working standard of solution of Ertugliflozin and Sitagliptin:

Working standard solution of ertugliflozin and sitagliptin was prepared by taking out 10ml from stock solution and dilute to 100ml of mobile phase (Methanol:0.1%OPA v/v) to get 10ug/ml and 66ug/ml respectively.

Preparation of sample solutions of Ertugliflozin and Sitagliptin:

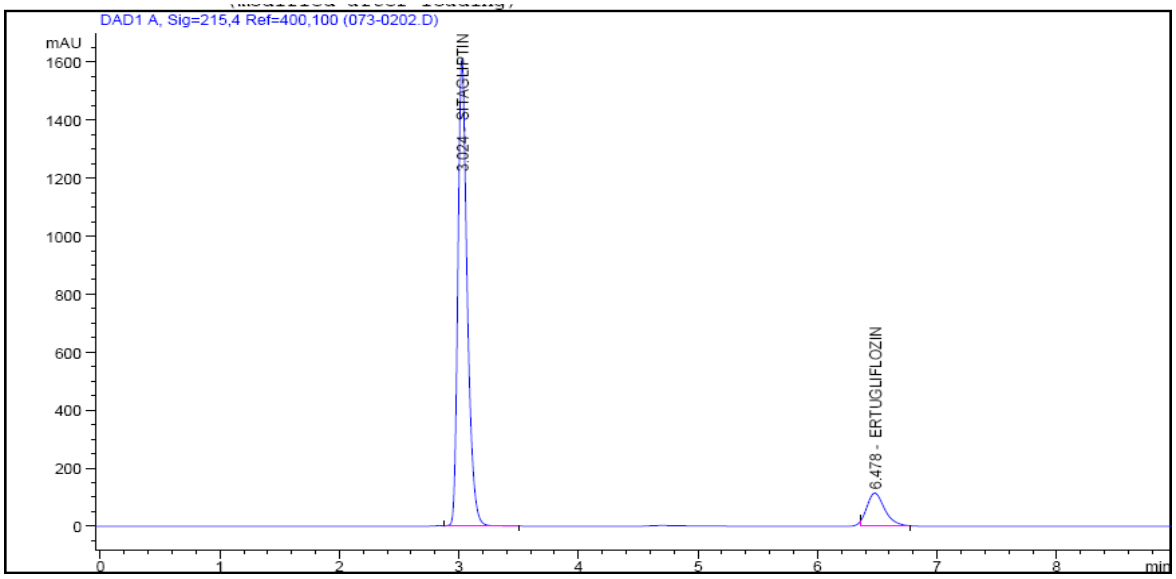
Take ERTU 10mg AND STG 66mg in 10ml Methanol = 1000µg/ml ERTU and 6600µg/ml STG. Take out 1.0ml from stock I and make volume with mobile phase 100ml = 10µg/ml ERTU AND 66 µg/ml STG.[12]

Optimization of chromatographic conditions:

The chromatographic separation was performed on standard fortis C18 (4.6×100µm with 2.5µm particle size) at an ambient temperature. The samples were eluted using Methanol : Buffer solution (75:25v/v) as a mobile phase at a flow rate 0.8ml/minute. The common wavelength of absorption of Sitagliptin and Ertugliflozin was found to be 215.0nm. The chromatograms of the prepared standard stock solutions of

Sitagliptin and Ertugliflozin were recorded under optimized chromatographic conditions.[13]

Fig 3: Optimized chromatograph.



RESULTS AND DISCUSIONS

Validation of developed method:

Linearity: The linearity was evaluated by determining six standard working solution drug, over range 66-396ug/ml and 10-60ug/ml and found to be linear with linear regression value 0.9996 and 0.9995 respectively(Table 1, Figure 4 and 5).

Table No. 1 Linearity for STG and ERTU

Sr.No.	STG		ERTU	
	Conc. in ug/ml	Peak area	Conc. in ug/ml	Peak area
1	66	3327.02	10	398.76
2	132	5871.61	20	794.80
3	198	8731.47	30	1134.33
4	264	11139.20	40	1481.06
5	330	13634.20	50	1873.87
6	396	16359.46	60	2240

Fig 4: Calibration curve of STG

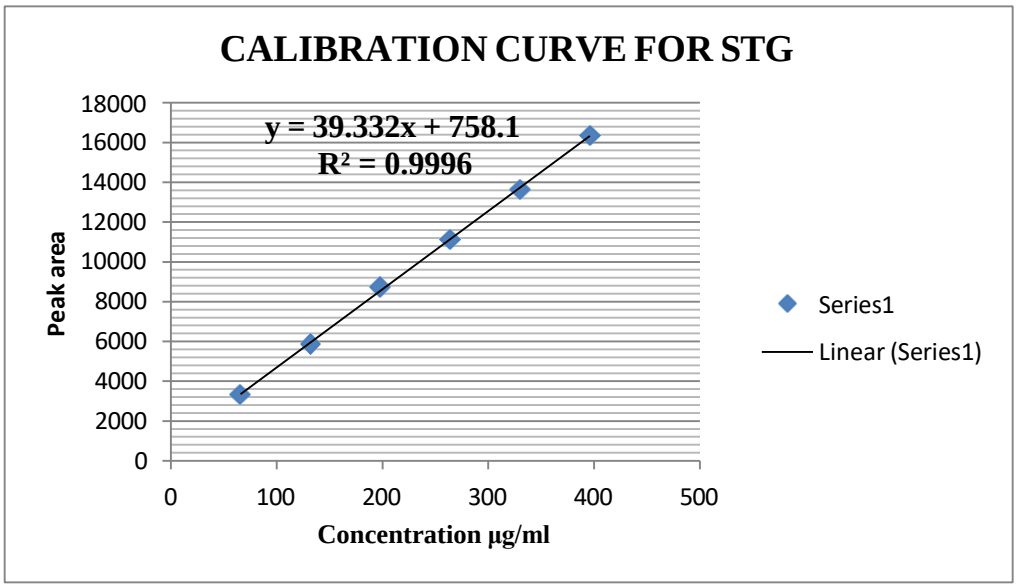
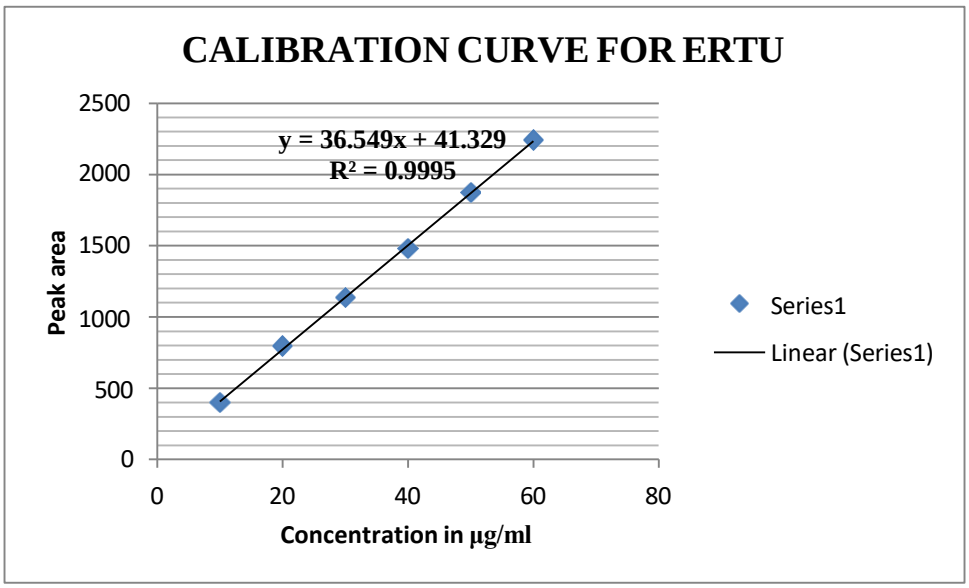


Fig 5: Calibration curve for ERTU



System suitability:

The retention time of STG and ERTU using optimum conditions was 3.02 and 6.58 minutes respectively. For two of them the peak symmetries were <1.5 and theoretical plates were >2000 and %RSD were less than 2 (Table 2).

Precision: the within run precision and between run precision of the developed HPLC method were determined by analysis of STG and ERTU on same day. Intermediate precision of the method was checked by repeating the studies on two different days and expressed in terms of %RSD (Table 3).

Table 2: System suitability Parameters

STG						**ERTU**				
Inj.	**RT**	**Plates**	**Tailing factor**	**Peak area**	**Peak height**	**RT**	**Plates**	**Tailing factor**	**Peak area**	**Peak height**
1	3.033	7962	1.02	3328.70	548.18	6.68	8902	1.25	398.70	35.53
2	3.02	8203	1.00	5866.82	1091	6.58	9191	1.22	794.41	73.94
3	3.02	7913	1.04	8722.13	1612.33	6.50	9746	1.11	1127.73	110.94
4	3.02	7550	1.05	11114.60	2036.73	6.47	9656	1.18	1485.22	147.89
5	3.02	7299	1.08	13700.64	2464.55	6.49	9731	1.17	1884.22	186.47
6	3.02	7378	1.06	16342.06	2954.21	6.52	9237	1.20	2236.51	222.42

Table 3: Precision

STG					**ERTU**			
Sr. No.	**Conc.**	**Peak area**	**% Assay**	**Statastical analysis**	**Conc.**	**Peak area**	**% Assay**	**Statastical analysis**
Interday								
1	132	5997.93	100.88	SD= 4.21 %RSD= 0.33	20	791.18	102.24	SD= 5.64 %RSD= 0.51
2	198	8764.80	102.89		30	1136.26	99.98	
3	264	11195.75	100.65		40	1486.05	99.03	
Intraday								
1	132	5976.94	100.48	SD= 8.24 %RSD= 0.38	20	785.04	101.68	SD= 7.45 %RSD= 0.70
2	198	8570.80	100.39		30	1129.44	99.36	
3	264	11128.45	100.00		40	1487.75	99.15	

Recovery studies: To ascertain the accuracy of proposed method, recovery studies were carried out by standard addition method as per ICH guidelines and the mean percentage recovery of STG and ERTU was achieved between 99.97% and 100.86% respectively (Table 4).

Robustness: Each factor selected to examine were changed at two levels (-1, +1) shown in table no 5. Table 4: Recovery study for STG and ERTU

	STG				**ERTU**			
Sr.No.	**Accuracy level**	**Amount spiked**	**% Recovery**	**Statistical Analysis**	**Accuracy level**	**Amount spiked**	**% Recovery**	**Statistical Analysis**
	80%				**80%**			
1	66	52.8	99.78	Mean=99.46 SD=0.31 %RSD=0.31	10	8	99.33	Mean= 99.59 SD= 0.23 %RSD=0.23
2	66	52.8	99.16		10	8	99.77	
3	66	52.8	99.43		10	8	99.66	
	100%				**100%**			

1	66	66	101.07	Mean=100.81 SD=0.34 %RSD=0.33	10	10	100.70	Mean=100.46 SD=0.32 %RSD=0.31
2	66	66	100.43		10	10	100.57	
3	66	66	100.93		10	10	100.10	
	120%				**120%**			
1	66	79.2	100.63	Mean=100.64	10	12	99.51	Mean=99.91
2	66	79.2	100.81	SD=0.17 %RSD=0.17	10	12	99.98	SD=0.37 %RSD=0.37
3	66	79.2	100.47		10	12	100.25	

Table 5: Robustness of STG & ERTU

Sr. No.	**Condition/Parameters**	**%RSD for STG**	**%RSD for ERTU**
1	Flow rate(-) 0.7ml/min	0.29	0.71
2	Flow rate(+) 0.9ml/min	0.05	0.16
3	Mobile Phase (-) 74+26 v/v	0.16	0.52
4	Mobile Phase (+) 76+24 v/v	0.12	0.07
5	Wavelength (-) 214nm	0.38	0.71
6	Wavelength (+) 216nm	0.20	0.70
7	Temperature 30˚C	0.28	0.19
8	Temperature 40˚C	0.37	0.23

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

The LOD and LOQ were estimated 0.55µg/ml & 1.674µg/ml for Ertugliflozin and 2.80µg/ml and 8.485µg/ml for Sitagliptin. The limit of detection and quantitation limits performed based on the slope and standard deviation.

Assay: Assay of different formulations available in the market was carried by injecting sample corresponding to equivalent weight into HPLC system and recovery studies were carried out (Table 6).

Force degradation studies:

The assay method was used to test the drug stability by conducting forced degradation studies for the drug substances under various stress conditions. Stress degradation studies were carried out for acid hydrolysis (0.1N HCl heated for 1hr at 60°C), alkali hydrolysis (0.1N NaOH heated for 1hr at 60°C), oxidative degradation (3%H2O2 heated at 60°C for 1hr) and thermal degradation (samples placed in an oven at 105°C for 3hr) and further results are shown in Table 7**.**

Table 6: Assay data for STG and ERTU combination marketed formulation

Drug	Labeled claim (mg)	Drug found (mg)	% Purity
Sitagliptin	66	65.97	99.97
Ertugliflozin	10	10.26	100.86

Table 7: Force degradation studies

Stress condition	% Assay of active moiety			
	STG	% Degradation	ERTU	% Degradation
Acid(0.1NHCL)	92.65	7.32	93.74	7.12
Alkali(0.1NNaOH)	93.32	6.65	97.74	6.12
Oxidation(3% H2O2)	91.59	8.38	91.97	8.89
UV light	96.76	3.21	96.61	3.36
Thermal(105°C)	96.42	3.55	96.01	3.96
Water	97.84	2.13	98.51	2.35

CONCLUSIONS

For the simultaneous measurement of Sitagliptin (STG) and Ertugliflozin (ERTU) in bulk and tablet dosage form, a new stability indicating High performance liquid chromatographic (RP-HPLC) approach was developed.. Linearity, accuracy, precision, robustness, LOD, LOQ, and stability experiments were used to verify the suggested technique. The new approach demonstrated good linearity concentration ranges of 66-330g/ml for Sitagliptin (STG) and 10-50g/ml for Ertugliflozin (ERTU), respectively, with linear regression coefficients (r2= 0.9996 and 0.9995). The LOD and LOQ for Ertugliflozin were calculated to be 0.55g/ml and 1.674g/ml, respectively, and 2.80g/ml and 8.485g/ml for Sitagliptin. The low %RSD figures imply that the approach is precise and accurate. The stability tests were carried out in accordance with ICH recommendations in acidic, alkaline, oxidative, and thermal environments for varying lengths of time. Because the developed RP- HPLC method was found to be linear over a wider concentration range, it can be used for routine qualitative and quantitative analysis of Sitagliptin and Ertugliflozin in bulk and tablet dosage form and validated according to ICH guidelines. As a result, the proposed method is easily applicable for routine analysis and stability studies for monitoring the assay in the pharmaceutical industry.

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