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# A novel RP-HPLC method for simultaneous determination of Ertugliflozin and Sitagliptin in bulk and tablet dosage form

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ABSTRACT

A simple, rapid, accurate, precise and novel Reverse-Phase High-Performance Liquid Chromatographic method for simultaneous analysis of Ertugliflozin and Sitagliptin in pharmaceutical dosage form has been developed and validated. The chromatographic separation was accomplished on Waters RP-C18 Column (250 mm X 4.6 mm; 5µm), Waters (Waters, Milford, MA, USA) Liquid Chromatograph and with a mixture of OPA (Ortho Phosphoric acid) 0.2%v/v: Acetonitrile (60:40 v/v). The flow rate was fixed at 1 mL/min and the analysis was performed using Waters, model 2998 UV-detection was performed at 250 nm. Ertugliflozin and Sitagliptin were separated within six minutes. The retention time for Ertugliflozin and Sitagliptin was found to be 2.375 minutes and 3.955 minutes respectively. The calibration plots were linear over the concentration range of 32.50 – 97.50 µg/ml for Ertugliflozin (r2 = 0.9992) and 216.50 – 649.50 µg/ml for Sitagliptin (r2 = 0.9992). There was no interference due to commonly used excipients. The relative standard deviation for inter-day precision was lower than 2.0% which indicates that the present method was said to be highly precise. Regarding the accuracy of the developed method the mean % recoveries were found to be 100.67 and 100.69 % for Ertugliflozin and Sitagliptin respectively, which shows the method is completely accurate. The LOD and LOQ for Ertugliflozin of the proposed method were found to be 0.0068µg/ml and 0.029 µg/ml for Sitagliptin 0.104 µg/ml and 0.347 µg/ml respectively. The mean assay values for Ertugliflozin and Sitagliptin were determined in tablet dosage form were found to be within limits. The developed RP HPLC method was found to be simple, rapid, sensitive, highly precise and accurate highly suitable for routine analysis of drug samples containing Ertugliflozin and Sitagliptin.

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## INTRODUCTION

Type 2 diabetes (T2DM) involves alterations in some metabolic pathways. In addition to alteration in glucose metabolism, patients with T2DM are more likely to have concomitant/secondary complications such as adverse cardiac events (Cinti F *et al.,* 2017), hypertension (Ghazala K *et al.,* 2011) and dyslipidemia (C.D. Agardh *et al.,* 1997) that may lead to microvascular and macrovascular complications.

As the number of individuals affected by diabetes is continuing to increase worldwide, the need for

effective management assumes ever greater urgency. Newer classes of medications, particularly those which work via the incretin pathway, achieve glucose lowering and minimizing risks associated with more traditional therapies. Ideally, combination therapies should be well tolerated, convenient to take, have few contraindications, have a low risk of hypoglycemia and weight gain, and be reasonably effective over both the short and long term such as the combination of Ertugliflozin (ERT) and Sitagliptin (SIT). The chemical structure of the drugs was shown in Figures 1 & 2 respectively.

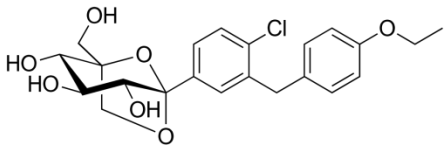


**Figure 1: Chemical structure of Ertugliflozin**

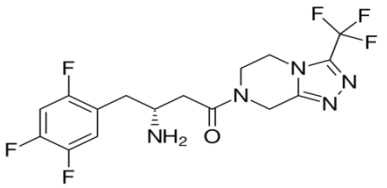


**Figure 2: Chemical structure of Sitagliptin**

Ertugliflozin is chemically known as ertugliflozin L-pyroglutamic acid is (1S,2S,3S,4R,5S)-5-[4-chloro-3-[(4-methoxyphenyl) methyl] phenyl]-1-(hydroxymethyl)-6,8-dioxabicyclo [3.2.1]octane-2,3,4-triol. The molecular formula is $C_{22}H_{25}ClO_7$and the molecular weight is 566.0gm/mol. Ertugliflozin 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. Administration of ertugliflozin increases urinary glucose excretion which leads to a negative balance and osmotic diuresis. Thus, this antidiabetic agent has been reported to significantly reduce the body weight and blood pressure of diabetic patients (S. Bianchi *et al.,* 1995).

Sitagliptin chemically (3R)-3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5H-[1,2,4] triazolo [4,3-a] pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)butane-1-one. SIT is a compound with molecular formula $C_{16}H_{15}F_6N_5O$, molecular weight of 407.314 gms/mol and it is soluble in water. Sitagliptin is a highly selective DPP-4 inhibitor, which is believed to exert its actions in patients with type 2 diabetes by slowing the inactivation of incretin hormones, thereby increasing the concentration and prolonging the action of these hormones (Herman GA *et al.,* 2005). Soluble in water and N, N-diethyl formamide, slightly soluble in methanol, very slightly soluble in ethanol, acetone and acetonitrile, insoluble in isopropanol and Isopropyl acetate (Plosker GL 2014). The present work describes a simple, sensitive and accurate RP-HPLC method for simultaneous estimation of the two drugs in their combined tablet dosage form.

Though several methods are reported in literature for the estimation of SIT with other drugs combination (Meher Vijay Dalawai *et al.,* 2015; Vasanth PM *et al.,* 2013; Hanan A *et al.,* 2017; S.N. Konari 2015; R. Lavanya 2013; Mohamed Karam Qassas 2015; Hitesh P. Inamdar 2012) and individually, no methods are reported for estimation of ERT and SIT in combination. The objective of the present study is to develop a novel, simple, accurate, precise, economical method for the simultaneous estimation of ERT and SIT and validate the method with forced degradation studies according to ICH guidelines (ICH 1996).

## Experimental

### Chemicals

All reagents and solvents were of analytical and HPLC grade and included orthophosphoric acid and acetonitrile. Ertugliflozin was supplied by Torrent, India and Sitagliptin was supplied by Dr. Reddy's Lab (Hyderabad, India). All other chemicals were commercial analytical reagent grade. In-house double distilled water was used for preparing solutions.

### Instrumentation

The HPLC system consisted of an LC Waters (Waters, Milford, MA, USA) using a Water's C18 250 x 4.6 mm, 5µ column, a quaternary gradient system (600 Controller), in line degasser (Waters, model AF24). The system was equipped with a photodiode array detector (Waters, 2998 model) and autosampler (Waters, model 717 plus). Data was processed using the Empower 2 software (Waters, Milford, MA, USA). The mobile phase was pumped at a flow rate of 1.0 mL min−1. Injection volume was 20 µL and the column temperature was ambient. The detection wavelength (Isobestic point) for ERT and SIT was 250 nm. Mobile phase was used as diluent.

### Preparation of Mobile Phase

600 mL (60%) of 0.2% orthophosphoric acid and 400 mL (40%) of ACN was mixed in a 1000 mL volumetric flask and filtered through a 0.45µ filter under vacuum filtration. The solution was kept for sonication and degassing in an ultrasonic water bath for 10 mins. The mobile phase was used as diluent.

### Preparation of Primary ERT & SIT Standard Solution

100 mg of ERT and SIT were accurately weighed and transferred to individual 100 mL volumetric

flask, diluent was added to dissolve and final volume was made up to the mark with the same to get the final concentration of 1 mg/mL (1000 µg/mL) of ERT and SIT.

**Preparation of working standard solution of ERT and SIT**

0.65 mL of ERT and 4.33 mL of SIT primary stock solution was diluted to 10 mL with diluent in a 10 mL volumetric flask. The diluent was added up to the mark to get the final concentrations of 65 µg/mL and 433 µg/mL of ERT and SIT respectively.

**Assay of tablet dosage forms**

Twenty Steglujan Tablets were accurately weighed and the average weight was determined. Tablets were ground and the tablet powder equivalent to 10 mg (370.41 mg) of ERT was weighed accurately and transferred to a 10 mL volumetric flask. 6 mL of mobile phase was added and sonicated for 10 mins. The final volume was made up to the mark with the same to get the sample stock solution of 1000 µg/mL. The resulting solution was filtered through the membrane filter of 0.45 µ. 0.65 mL of the above solution was transferred to a 10 mL volumetric flask and diluted up to the mark with mobile phase. 20 µL of the resulting solution was injected into the chromatograph. Peak area and RT were determined from chromatogram and the amount of ERT and SIT were calculated.

**Validation criteria**

**Selectivity**

The selectivity is defined as the ability of the method to measure the analyte accurately and specifically in the presence of components present in the sample matrix, was determined by analysis of chromatograms of drug-free blank and formulation.

**Linearity**

Five-point standard curves for both compounds were constructed by drawing peak area versus ERT and SIT concentration at 32.50 – 97.50 µg/ml and 216.50 – 649.50 µg/ml respectively. The concentration ranges were selected based on optimized drug concentration. Calibration curves were generated using weighted linear regression analysis with a weighting factor of 1/x over the respective standard concentration range.

**Accuracy**

For the determination of the accuracy of the method, recovery study was carried out by analyzing the samples at three different concentrations at 50, 100 and 150%. The percentage of recoveries at three concentrations was calculated.

**Precision**

Repeatability of the method was checked by analyzing six replicate samples of ERT and SIT at 100% concentration. The %RSD was calculated in terms of % area. Intra-day and inter-day variations were studied to establish intermediate precision of the proposed method. Intraday precision was determined by analyzing six replicate samples of optimized concentration. The same procedure was followed for three different days to study inter-day variation (n = 18). The precision of the assay was evaluated by performing six independent assays of test samples of ERT and SIT. The %RSD of six results was calculated.

**Sensitivity**

The sensitivity of the method was proved by establishing the limit of detection (LOD) and the limit of quantitation (LOQ) for ERT and SIT with a signal-to-noise ratio of 3:1 and 10:1, respectively. LOD and LOQ were determined by injecting a series of diluted solutions having known concentrations of drugs. The precision study was also carried out at the LOQ level by injecting six individual preparations of ERT and SIT at LOQ concentration and by calculating the %RSD for the areas of each peak. Accuracy at LOQ level was verified by injecting three individual preparations of ERT and SIT at LOQ level and by calculating % recoveries of each analyte.

**Robustness**

The robustness study was carried out to evaluate the influence of small but deliberate variation in the chromatographic conditions. The factors chosen for this study which were critical sources of variability in the operating procedures such as a temperature of the column (±5°C), mobile phase and flow rate (±0.2 mL/min) were identified. Resolution between ERT and SIT was evaluated in the deliberately altered experimental conditions.

**Stress studies**

Specificity is the ability of the method to measure the analyte in the presence of its potential degraded products. Specificity of the developed HPLC method for ERT and SIT was performed in the presence of degradation products. Stress studies were performed at concentration 65 µg mL−1 of both drug substance to indicate the stability indicating property and specificity of the proposed method.

For acid degradation condition, drug solution was treated with 1 ml of 0.5 N HCl and heated for 90°C for 6 H, cooled and added 1ml of 0.5 N NaOH to neutralize any excess acid present in the sample. 20 µl of the sample solution was injected into HPLC. For base degradation, drug solution was

**Table 1: Optimized chromatographic conditions for determination of ERT and SIT**

| S. No. | Parameter | Description/Value |
|---|---|---|
| 1 | Stationary Phase | Water's C18 (250X4.6X5) |
| 2 | Mobile Phase | OPA (Ortho Phosphoric acid) 0.2%v/v: Acetonitrile (60:40) |
| 3 | Flow rate | 1 ml/min |
| 4 | Detection Wavelength (Isosbestic Point) | 250 nm |
| 5 | Detector | Photodiode array |
| 6 | Injection | Autosampler -Waters, model 717 plus |
| 7 | Injection volume | 20 µl |
| 8 | Column Temperature | Ambient |
| 9 | Runtime | 6 mins. |
| 10 | Diluent | Mobile Phase |

**Table 2: System suitability data of ERT and SIT**

| S. No | Parameter* | ERT | SIT |
|---|---|---|---|
| 1 | Theoretical Plate Count | 8496 | 6482 |
| 2 | Average Peak Area | 1050919 | 2065980 |
| 3 | Peak Height | 181936 | 232994 |
| 4 | RT | 2.375 | 3.955 |
| 5 | Tailing | 1.4 | 1.2 |
| 6 | Resolution | - | 15.63 |
| 7 | S/N | 1148 | 1850 |

* Average of 6 replicates

**Table 3: Linearity data of ERT and SIT**

| S. No. | Linearity Level | Ertugliflozin | | Sitagliptin | |
|---|---|---|---|---|---|
| | | Concentration (µg/ml) | Peak Area | Concentration (µg/ml) | Peak Area |
| 1 | 50 | 32.50 | 550052 | 216.50 | 1077379 |
| 2 | 75 | 48.75 | 814045 | 324.75 | 1606706 |
| 3 | 100 | 65.00 | 1093430 | 433.00 | 2163781 |
| 4 | 125 | 81.25 | 1365524 | 541.25 | 2685789 |
| 5 | 150 | 97.50 | 1674294 | 649.50 | 3304006 |
| Reg. Equation | | y = 17231x - 2051( | | y = 5110.7x - 45403 | |
| Slope | | 17231 | | 5110.7 | |
| Y-Intercept | | 20516 | | 45403 | |
| $R^2$ | | 0.9992 | | 0.9992 | |

treated with 1 ml of 0.5 N NaOH and heated for 80°C for 5 H, cooled and added 1ml of 0.5 N HCl to neutralize any excess base present in the sample. 20 µl of the sample solution was injected into HPLC. For peroxide degradation, drug solution was treated with 3 ml of 3 % $H_2O_2$ and heated for 80°C for 5 H, cooled and 20 µl of the sample solution was injected into HPLC. For UV degradation, drug sample was exposed to UV light in a UV chamber at 250 nm for 24 H. 20 µl of the sample solution was injected into HPLC. For heat degradation, drug solution was refluxed at 80°C for 24 H. 20 µl of the sample solution was injected into HPLC.

## RESULTS AND DISCUSSION

### Method development

For HPLC analysis, initially, various mobile phases and stationary phases were tried in attempts to obtain the best separation and resolution between ERT and SIT. The mobile phase consisting of OPA (Ortho Phosphoric acid) 0.2%v/v: Acetonitrile (60:40 v/v) was found to be an appropriate mobile phase allowing adequate separation of all the compounds using a waters C18 250 cm column at a flow rate of 1.0 ml/min. Typical chromatograms of standard and sample compounds were shown in Figure 3 and 4 respectively. The summary of optimized chromatographic conditions was shown in table 1. The retention time of ERT and SIT was found to be 2.375 and 3.955 mins respectively. The total time of analysis was less than 6 minutes.

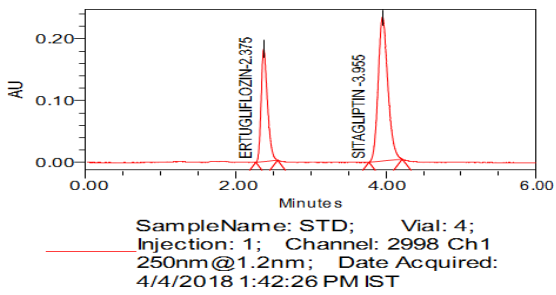


**Figure 3: Representative chromatogram of Standard of ERT and SIT**

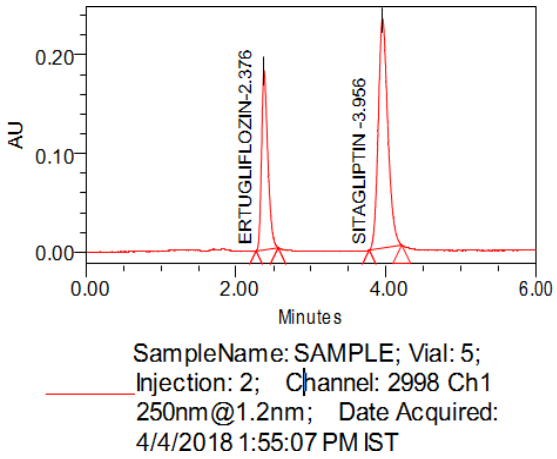


**Figure 4: Representative chromatogram of Sample of ERT and SIT**

As ERT and SIT exhibit significant absorbance at wavelength 250 nm, it was selected as detection wavelength for the simultaneous determination of ERT and SIT in pharmaceutical dosage forms. All system-suitability results of the method were in acceptance limits and are presented in Table 2.

### Method validation

After method development, validation of the current test method for ERT and SIT was performed in accordance with ICH guidelines which include accuracy, precision, specificity, linearity etc.

### Specificity

The specificity of the HPLC method is illustrated in figure 3 and 4 where complete separation of ERT and SIT were noticed in the presence of tablet excipients. In addition, there was no any interference at the retention time of ERT and SIT in the chromatogram of the blank solution. In peak purity analysis with a photodiode detector, the purity angle was less than purity threshold for both the analytes. This shows that the peak of analytes was pure and excipients in the formulation did not interfere the analytes.

### Linearity

Linearity was constructed with five concentration at the level of 50-150% (32.50, 48.75, 65.00, 81.25, 97.50 µg/ml of ERT and 216.50, 324.75, 433.00, 541.25, 649.50 µg/ml of SIT). The peak areas of the analytes were found to be linear in the studied concentration range the correlation coefficient was found to be 0.9992 for both ERT and SIT. The linearity data and curve was shown in table 3 and figure 5 and 6 respectively and the Chromatograms of linearity was shown in Figure 7.

### Accuracy

The accuracy of the method was determined by % recovery method at three concentration levels (50%, 100% and 150%) of the test solution. Six replicates were analyzed for 50 % and 150 %, 3 replicates were tested for 100%. The mean recovery of ERT and SIT was found to be in between 99-100 %. Table 4 shows the results of accuracy.

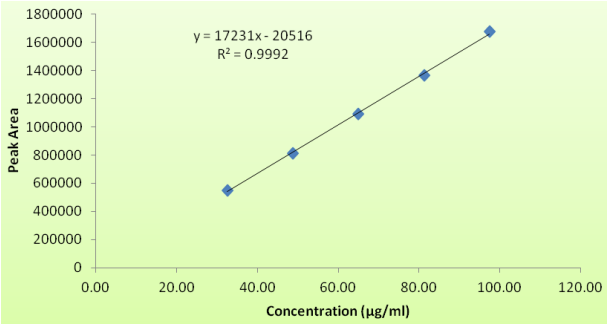


**Figure 5: Linearity Plot of Ertugliflozin**

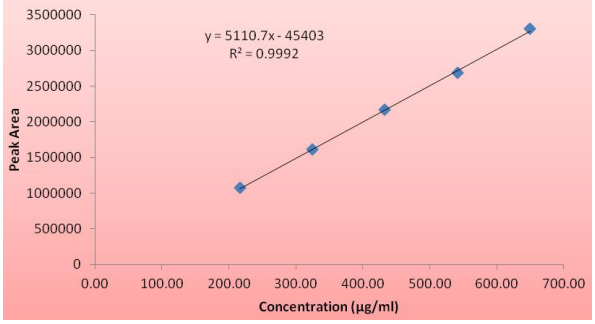


**Figure 6: Linearity Plot of Sitagliptin**

### Precision

Inter and Intra-day precision of the method was determined by performing precision for three times in the same day and followed by three consequent days. % RSD was calculated and found to be within the specified limits (<2%), which proves that the developed method was precise. Table 5 shows the precision results.

### Sensitivity

LOD and LOQ were determined by using the standard deviation of response and slope of calibration curves. The LOD and LOQ for ERT of the proposed method were found to be 0.0068 µg/ml and 0.029 µg/ml for SIT 0.104 µg/ml and 0.347 µg/ml respectively. Figure 8 shows the chromatograms of LOD and LOQ.

### Robustness

The robustness of the analytical method was evaluated by assaying the test solutions after slight but deliberate changes in the conditions like flow rate (± 0.2 ml/min) and the column temperature (± 5°C). System suitability data was found to be satisfactory during variations of the analytical conditions. System suitability results were also remained unaffected by slight changes in the analytical conditions. Table 6 shows the results of the robustness of ERT and SIT and chromatograms were shown in figure 11.

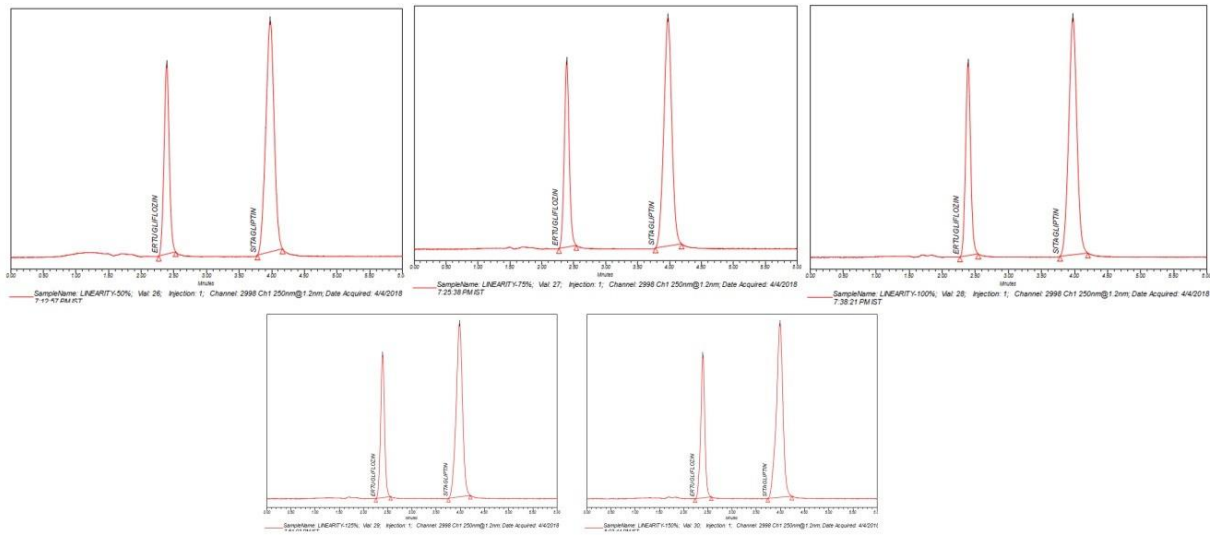


**Figure 7: Linearity Chromatograms of ERT and SIT**

**Table 4: Accuracy data of ERT and SIT**

| Level (%) | Sample Wt. (mg) | Mean Sample area | Amount added (µg/ml) | Amount found (µg/ml) | % Re-covery | Mean % Re-covery |
|---|---|---|---|---|---|---|
| Accuracy of Ertugliflozin | | | | | | |
| 50 | 185.205 | 532660.50* | 32.74 | 32.95 | 100.64 | 100.67 |
| 100 | 370.41 | 1064090.66# | 65.47 | 65.81 | 100.52 | |
| 150 | 555.615 | 1601702.66* | 98.21 | 99.07 | 100.87 | |
| Accuracy of Sitagliptin | | | | | | |
| 50 | 185.205 | 1045947.33* | 218.33 | 219.22 | 100.41 | 100.69 |
| 100 | 370.41 | 2102495.33# | 436.65 | 440.65 | 100.92 | |
| 150 | 555.615 | 3148420.83* | 654.98 | 659.86 | 100.75 | |

*n=6; #n=3

**Table 5: Precision results of ERT and SIT**

| S. No | Peak Areas | | | |
|---|---|---|---|---|
| | Intraday precision | | Interday precision | |
| | Ertugliflozin | Sitagliptin | Ertugliflozin | Sitagliptin |
| 1 | 1062793 | 2091053 | 1046008 | 2057670 |
| 2 | 1060064 | 2069649 | 1062826 | 2087405 |
| 3 | 1037767 | 2041950 | 1058149 | 2090952 |
| 4 | 1044516 | 2058798 | 1056888 | 2086398 |
| 5 | 1049454 | 2068453 | 1062511 | 2080369 |
| 6 | 1050919 | 2065980 | 1065187 | 2097663 |
| Average | 1050918.83 | 2065980.50 | 1058594.83 | 2083409.50 |
| STDEV | 9388.74 | 15982.83 | 6902.70 | 13832.67 |
| % RSD | 0.89 | 0.77 | 0.65 | 0.66 |

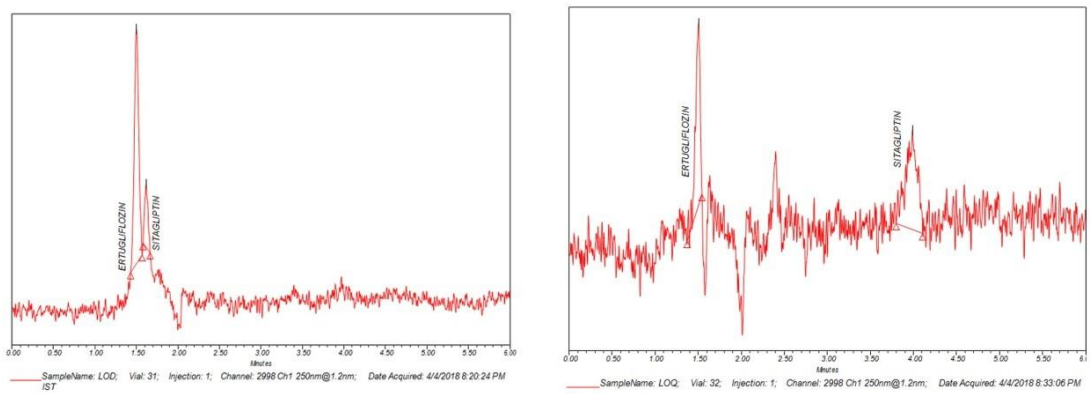


**Figure 8: LOD and LOQ Chromatograms of ERT and SIT**

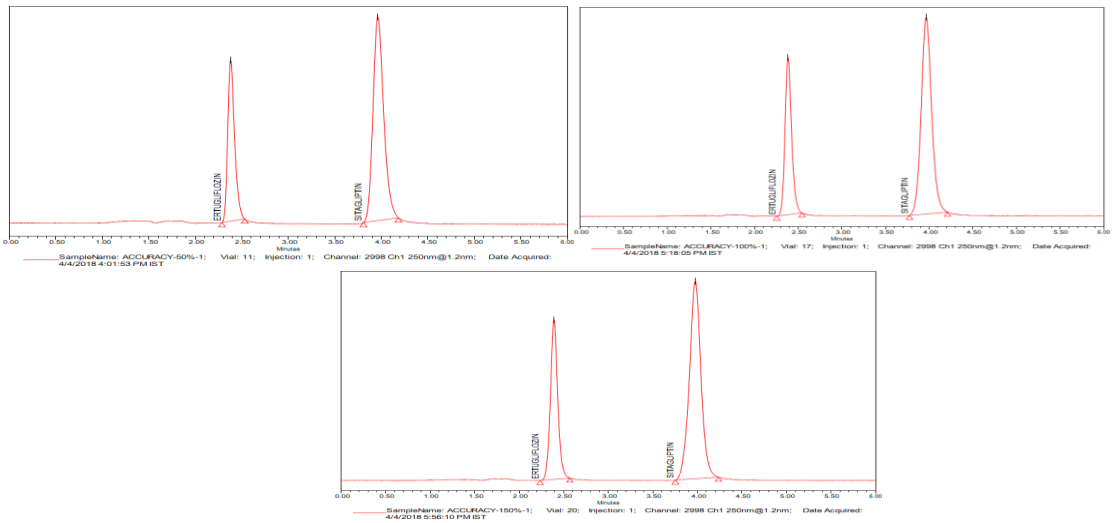


**Figure 9: Accuracy chromatograms of ERT and SIT**

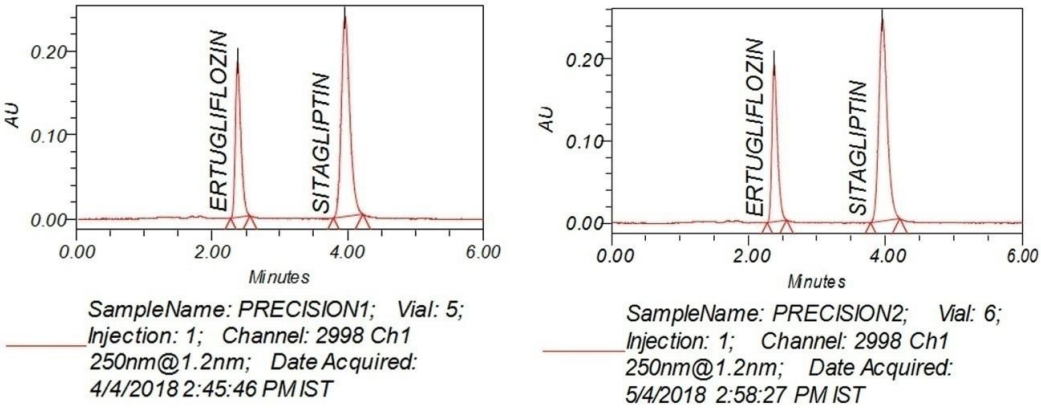


**Figure 10: Intra and Inter day precision chromatograms of ERT and SIT**

**Table 6: Robustness results of ERT and SIT**

| Parameter | Condition | Ertugliflozin | | | Sitagliptin | | |
|---|---|---|---|---|---|---|---|
| | | RT | Peak Area | % Assay | RT | Peak Area | % Assay |
| Flow | 0.8 ml/min | 3.085 | 1040160 | 98.98 | 4.938 | 2122354 | 99.26 |
| | 1 ml/min | 2.375 | 1049454 | 99.86 | 3.955 | 2069649 | 100.10 |
| | 1.2 ml/min | 2.011 | 1057635 | 100.64 | 3.460 | 2055872 | 99.44 |
| Temp | 25 °C | 2.408 | 1071014 | 101.91 | 4.026 | 2108495 | 101.98 |
| | 30 °C | 2.375 | 1049454 | 99.86 | 3.955 | 2069649 | 100.10 |
| | 35 °C | 2.431 | 1070644 | 101.88 | 4.051 | 2116491 | 99.47 |
| | Average | 2.43 | 1056394 | 100.52 | 4.064 | 2068751.6 | 100.06 |

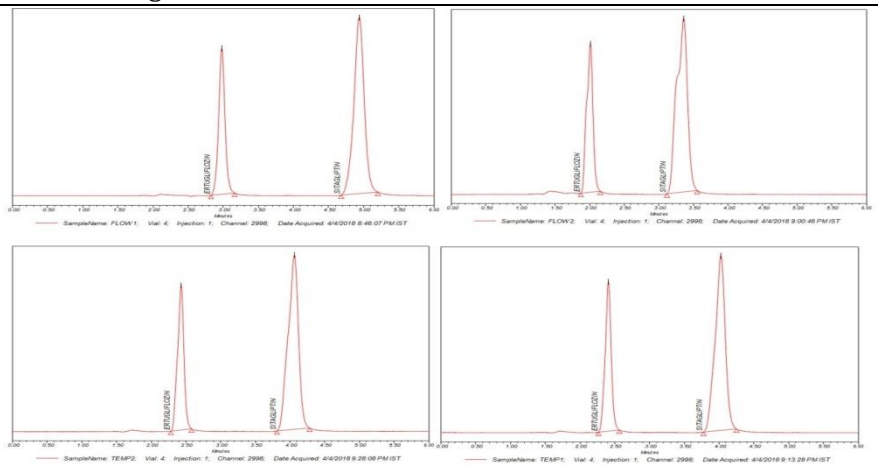

**Figure 11: Robustness chromatograms of ERT and SIT**

**Table 7: Assay results of ERT and SIT**

| S. No | Sample Wt. (mg) | Ertugliflozin | | Sitagliptin | |
|---|---|---|---|---|---|
| | | Peak area | % Assay | Peak area | % Assay |
| 1 | 370.41 | 1046008 | 99.53 | 2057670 | 99.52 |
| 2 | 370.41 | 1062826 | 101.13 | 2087405 | 100.96 |
| 3 | 370.41 | 1058149 | 100.69 | 2090952 | 101.13 |
| 4 | 370.41 | 1056888 | 100.57 | 2086398 | 100.91 |
| 5 | 370.41 | 1062511 | 101.10 | 2080369 | 100.62 |
| 6 | 370.41 | 1065187 | 101.36 | 2097663 | 101.46 |
| Average | | 1058594.83 | 100.73 | 2083409.50 | 100.77 |
| STDEV | | 6902.70 | 0.66 | 13832.67 | 0.67 |
| % RSD | | 0.65 | 0.65 | 0.66 | 0.66 |

**Table 8: Forced degradation results of ERT and SIT**

| S. No. | Condition | Ertugliflozin | | | Sitagliptin | | |
|---|---|---|---|---|---|---|---|
| | | Peak Area | % Assay | % Degradation | Peak Area | % Assay | % Degradation |
| 1 | Acid | 952793 | 90.66 | 9.34 | 1891053 | 91.46 | 8.54 |
| 2 | Base | 960064 | 91.36 | 8.64 | 1799649 | 87.04 | 12.96 |
| 3 | $H_2O_2$ | 927767 | 88.28 | 11.72 | 1841950 | 89.09 | 10.91 |
| 4 | UV | 944516 | 89.88 | 10.12 | 1858798 | 89.90 | 10.10 |
| 5 | HEAT | 935687 | 89.04 | 10.96 | 1842587 | 89.12 | 10.88 |

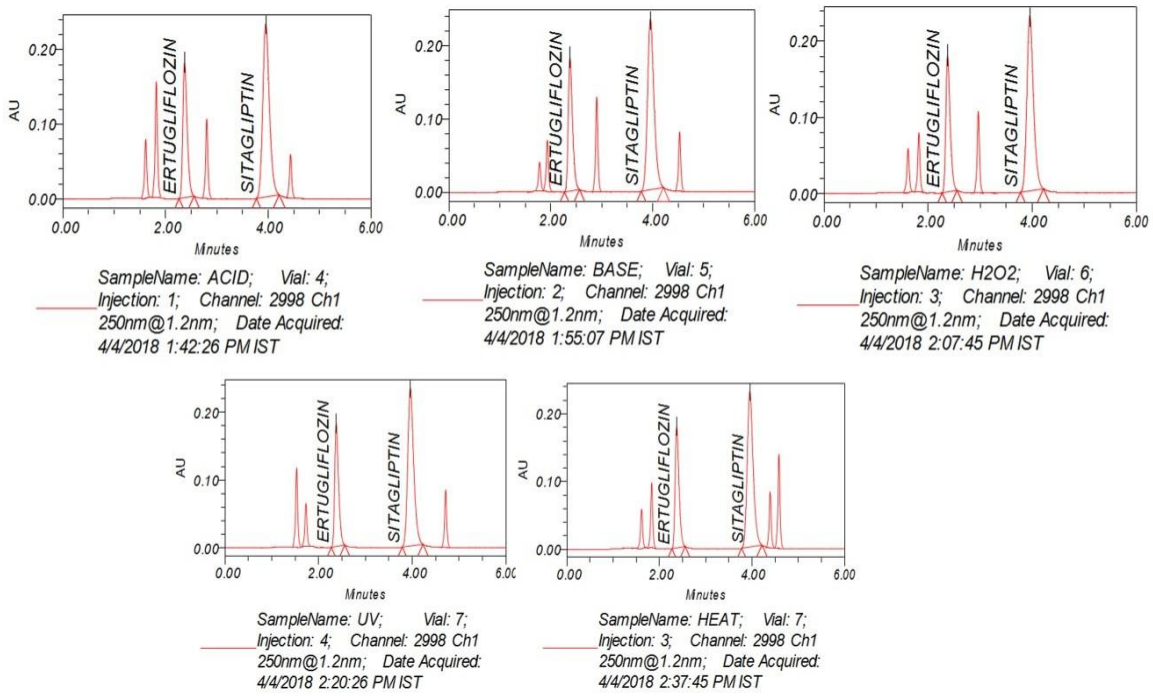


**Figure 12: Degradation studies of ERT and SIT**

**Assay**

The proposed method was applied to the tablets of ERT and SIT. The mean % assay was found to be 100.73 % and 100.77 % for ERT and SIT respectively. Results were given in table 7.

**Stress studies**

Stress studies were performed to evaluate the stability indicating an ability of the developed analytical method by exposing the sample solution to different the stress conditions viz., acid, base, peroxide, UV and heat. Assay studies were carried out for stress samples at 65µg/ml against a reference standard. The proposed analytical method can able to detect the analyte even in the presence of degraded products and thus confirms the stability indicating the power of the developed method. Results of stress studies were shown in table 8 and figure 12 shows chromatograms of Stress studies of ERT and SIT.

**CONCLUSION**

A simple, accurate and precise stability-indicating RP-HPLC analytical method was developed and validated for the simultaneous analysis of ERT and SIT in tablet formulations. Low LOD and LOQ of

this method enable the detection and quantification of this impurity at low concentration. The method is very simple and specific as both peaks are well separated from one another and excipients peaks with a total runtime of 6 min, which makes it especially suitable for routine quality control analysis work.

**REFERENCES**

C.D. Agardh, E. Agardh, O. TorffvitThe association between retinopathy, nephropathy, cardiovascular disease and long-term metabolic control in type 1 diabetes mellitus Diabetes Res. Clin. Pract., 35 (1997), pp. 113-121.

Cinti F, Moffa S, Impronta F, Cefalo CM, Sun VA, Sorice GP, Mezza T, Giaccari A: Spotlight on ertugliflozin and its potential in the treatment of type 2 diabetes: evidence to date. Drug Des Devel Ther. 2017Oct3; 11:2905-2919.

Ghazala K, Dinesh S, Agrawal YP, Neetu S, Avnish J, Gupta AK. Simultaneous estimation of metformin and sitagliptin in tablet dosage form. Asian J Biochem Pham Res. 2011; 2:223–9.

Hanan A. Merey, Nesrin K. Ramadan, Sherine S. Diab, Azza A. Moustafa Chromatographic methods for the simultaneous determination of a binary mixture of Saxagliptin HCl and Metformin HCl, Bulletin of Faculty of Pharmacy, Cairo University, Volume 55, Issue 2, 2017, 311-317.

Herman GA, Stevens C, Van Dyck K, Bergman A, Yi B, De Smet M, et al. Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: Results from two randomized, double-blind, placebo-controlled studies with single oral doses. Clin Pharmacol Ther. 2005 Dec; 78(6):675-88.

Hitesh P. Inamdar, RP-HPLC method for simultaneous determination of metformin hydrochloride, rosiglitazone and sitagliptin – application to commercially available drug products, IJPSR, 2012; Vol. 3(9): 3267-3276.

ICH (1996) Proceedings of the International Conference on Harmonisation of Technical Requirement of Registration of Pharmaceuticals for Human Use (ICH Harmonised Tripartite Guidelines). Validation of Analytical Procedures: Methodology, Q2B, Geneva, Switzerland.

J.R. Sowers, M. EpsteinDiabetes mellitus and associated hypertension, vascular disease, and nephropathy: an update. Hypertension, 26 (1995), pp. 869-879

Meher Vijay Dalawai et al., Development and validation of stability indicating assay method by HPLC for the analysis of sitagliptin phosphate in bulk drug substances, Journal of Chemical and Pharmaceutical Research, 2015, 7(10):781-787.

Mohamed Karam Qassas, A Validated HPLC Stability Indicating Method for the Determination of Sitagliptin in Bulk Drug Substance and Tablets, Int. J. Pharm. Sci. Rev. Res., 32(1), 2015; 33, 194-19.

Plosker GL, Sitagliptin: A review of its use in patients with type 2 diabetes mellitus, Drugs.2014Feb;74(2):22342.

R. Lavanya MD. Yunoos, Development and Validation of RP-HPLC method for the estimation of Sitagliptin Phosphate in Bulk and itsTablet Dosage Form; J. Adv. Pharm. Edu. & Res. 2013: 3(4):47 5-479.

S. Bianchi, R. Bigazzi, A. Galvan, E. Muscelli, G. Baldari, N. PecoriInsulin resistance in microalbuminuric hypertension: sites and mechanisms. Hypertension, 26 (1995), pp. 189-195.

S.N. Konari, Stability indicating validated RP-HPLC technique for the analysis of multicomponent anti-diabetic drug combos in pharmaceutical dosage forms, Karbala International Journal of Modern Science 1 (2015) 39-48.

Vasanth PM et al., Method development and validation of sitagliptin and metformin using reverse phase HPLC method in bulk and tablet dosage form, Der Pharmacia Lettre, 2013, 5 (5):168-174.