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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ERTUGLIFLIZIN AND SIGLIPTIN IN BULK AND DOSAGE FORM

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ABSTRACT:
Several spectrophotometric and HPLC methods have been reported for determination of Ertugliflozin and Sitagliptin in drugs and in pharmaceutical dosage forms. Hence, in the present study, a new, sensitive, suitable and robust reversed-phase high performance liquid chromatography method was developed and validated for the determination of Ertugliflozin and Sitagliptin in bulk drug and in tablet formulation. In RP-HPLC method, Methanol and Water (75:25 % v/v) was used as mobile phase, at a flow rate of 1.0 ml/min, on HPLC system containing UV- detector with Openlab EZchrome software and Kromasil C18, 250 mm X 4.6 mm, 5 µm. The detection was carried out at 258 nm. The method gave suitable retention time i.e. 3.60 min for Ertugliflozin and Sitagliptin. The results of analysis in the method were validated in terms of Filter study, Solution stability, specificity, Linearity, accuracy, precision (Repeatability and intermediate precision), limit of detection, limit of quantification and robustness. A simple and precise method was developed for the assay of Ertugliflozin and Sitagliptin in bulk drug and in tablet formulation.
KEYWORDS: RP-HPLC, Ertugliflozin, Sitagliptin Methanol, Validation.

INTRODUCTION

In pharmaceutical industries, the validation of analytical method is used to demonstrate that the method is fitted for its purpose; it must follow a plan which includes scopes, Performance characteristics, and acceptance limits. Analytical methods need to be validated or revalidated prior to their introduction into routine analyses. Chromatography is an analytical techniques based on the separation of molecules due to differences in their structure and/or composition. In general, Chromatography involves moving a sample through the system over a stationary phase. The molecules in the samples will have different affinities and interaction with the stationary support, leading to separation of Molecules. Samples components that display stronger interaction with the stationary phase will move more slowly through the column than components with weaker interaction. Different compounds can be separated from each other as they move through the column. Chromatographic separation can be carried out using a variety of stationary phases. High-Performance liquid chromatography (HPLC) is types of liquid Chromatography used to separate and quantify compounds that have been dissolved in Solution. HPLC can be used to determine the amount of a specific compound in a solution.

METHODS

Determination of solubility

The solubility was determined in Water and methanol at a concentration of 3 mg/mL as follows and are given in results**.**

Water:

Sitagliptin: Weighed approx 38.56 mg of Sitagliptin phosphate (Equivalent to 30 mg of Sitagliptin) and sonicated for 5-10 minutes to dissolve in 10 ml of Water.

Ertugliflozin: Weighed approx 38.86 mg of Ertugliflozin L-pyroglutamic acid (Equivalent to 30 mg of Ertugliflozin) and sonicated for 5-10 minutes to dissolve in 10 ml of Water.

Methanol:

Sitagliptin: Weighed approx 38.56 mg of Sitagliptin phosphate (Equivalent to 30 mg of Sitagliptin) and sonicated for 5-10 minutes to dissolve in 10 ml of Methanol.

Ertugliflozin: Weighed approx 38.86 mg of Ertugliflozin L-pyroglutamic acid (Equivalent to 30 mg of Ertugliflozin) and sonicated for 5-10 minutes to dissolve in 10 ml of Methanol. **Selection of analytical wavelength**
Selection of solvent
Methanol was selected as the solvent for dissolving Sitagliptin and Ertugliflozin.
Preparation of standard stock solutions
Sitagliptin: In order to prepare stock solution, weighed accurately 25.71 mg Sitagliptin phosphate (Equivalent to 20 mg of Sitagliptin) and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (1000 PPM).
Further diluted 0.5 mL to 25 mL with methanol. (20 PPM)
Ertugliflozin: In order to prepare stock solution, weighed accurately 25.91 mg Ertugliflozin L-pyroglutamic acid (Equivalent to 20 mg of Ertugliflozin) and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (1000 PPM).
Further diluted 0.5 mL to 25 mL with methanol. (20 PPM)
Selection of analytical wavelength
Methanol as a blank and Sitagliptin and Ertugliflozin standard solution (20 PPM each) was scanned from 400 nm to 200 nm. Absorption maxima was determined for both drug. Sitagliptin and Ertugliflozin showed Q-point at 212 nm shown in results.

METHOD DEVELOPMENT BY RP – HPLC
Sitagliptin: In order to prepare stock solution, weighed accurately 25.71 mg Sitagliptin phosphate (Equivalent to 20 mg of Sitagliptin) and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (1000 PPM).
Further diluted 1 mL to 10 mL with Mobile phase (100 PPM). It was prepared in mobile phase of each trial and injected in development trials.
Ertugliflozin: In order to prepare stock solution, weighed accurately 25.91 mg Ertugliflozin L-pyroglutamic acid (Equivalent to 20 mg of Ertugliflozin) and transferred into 20 ml volumetric flask, added 15 ml of methanol and sonicated to dissolve the standard completely and diluted up to the mark with methanol (1000 PPM).
Further diluted 1 mL to 10 mL with Mobile phase (100 PPM). It was prepared in mobile phase of each trial and injected in development trials.

Selection of analytical wavelength for HPLC method development: Analytical wavelength for the examination was selected from the Q-point from the spectrophotometric analysis and it was 212 nm**.**
Preparation of System suitability stock solutions:
Sitagliptin: Weighed 64.27 mg Sitagliptin phosphate (Equivalent to 50 mg of Sitagliptin) and transferred in 50 mL volumetric flask, added 35 mL of methanol, sonicated to dissolve it, made volume up to the mark with methanol. (1000 PPM)
Ertugliflozin: Weighed 12.95 mg Ertugliflozin L-pyroglutamic acid (Equivalent to 10 mg of Ertugliflozin) and transferred in 50 mL volumetric flask, added 35 mL of methanol, sonicated to dissolve it, made volume up to the mark with methanol. (200 PPM)
System suitability standard mixture solution:
Pipette out 2 mL of Sitagliptin standard stock solution and 1.5 mL of Ertugliflozin standard stock solution and transferred in 10 mL volumetric flask, made volume up to the mark with diluent.
(Sitagliptin = 200 ppm)
(Ertugliflozin = 30 ppm)
100 PPM of Sitagliptin and 15 PPM of Ertugliflozin are the working concentration.
Marketed formulation contains Ertugliflozin (15 mg) and Sitagliptin (100 mg) in the ratio of 1:6.67, hence concentration is selected in this ratio.
System suitability is a Pharmacopoeial requirement and is used to verify, whether the chromatographic system is adequate for analysis to be done. The tests were performed by collecting data from Five replicate injection of standard drug solution and the results are recorded.
Acceptance criteria
1. RSD should not be more than 2.0 % for five replicate injections of standard.
2. USP Tailing Factor/ Asymmetry Factor is not more than 2.0.
3. The column efficiency as determined for Plate Count should be more than 2000.
Analysis of marketed Test sample:
Marketed tablet formulation was not available, hence physically lab mixture of tablet prepared at lab level and used for analysis and for doing validation.

Tablet Preparation:
Physical lab mixture of Tablet prepared at lab level by following formula:
300 mg as average weight considered for preparing tablet mixture.

Sr. No.	Ingredients	Role	Qty (mg)

1	Sitagliptin phosphate (equivalent to 100 mg of Sitagliptin)	Active ingredient	128.53
2	Ertugliflozin L-pyroglutamic acid (equivalent to 15 mg of Ertugliflozin)	Active ingredient	19.43
3	Placebo	NA	152.04
Total			300.0

Placebo prepared at lab level by using formula as follows:

Sr. No.	Ingredients	Role	Qty (mg)
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5
4	Talc	Glidant	5
5	Crospovidone	Disintegrants	5
Total			100 mg

Total 30 gm of placebo prepared:

Sample preparation of Marketed test sample:
Weighed the powder material equivalent to 200 mg of Sitagliptin and 30 mg of Ertugliflozin (600 mg of powder material) from physical lab mixture. Transfer it in a clean and dried 100 mL of volumetric flask, added 70 ml of methanol sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with methanol. Filter the solution through suitable 0.45 µ syring filter discarding 3-5 mL of filtrate. Further diluted 1 ml of filtrate to 10 ml with mobile phase. (200 PPM of Sitagliptin and 30 PPM of Ertugliflozin).

Sample Prepared in duplicate. Summary of sample preparation as follows:

Sample	Sample (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
Sample 1	600.4	100	1	10
Sample 2	600.7	100	1	10

Formula for % Assay calculation:

$$\%\ Assay\ of\ Sitagliptin = \frac{Sitagliptin\ Spl\ area}{Sitagliptin\ Std\ avg\ area} X \frac{Sitagliptin\ STD\ wt\ (mg)}{50} X \frac{2}{10} X \frac{100}{Tablet\ sample\ weight\ (mg)}$$

$$X \quad \frac{10}{1} X \frac{Avg\ wt\ of\ tablet\ (mg)}{Label\ claim\ of\ Sitagliptin\ (mg)} X\ F X\ 100$$

$$\%\ Assay\ of\ \text{Ertugliflozin} = \frac{\text{Ertugliflozin}\ Spl\ area}{\text{Ertugliflozin}\ Std\ avg\ area} X \frac{\text{Ertugliflozin}\ STD\ wt\ (mg)}{50} X \frac{1.5}{10} X \frac{100}{Tablet\ sample\ weight\ (mg)}$$

$$X \quad \frac{10}{1} X \frac{Avg\ wt\ of\ tablet\ (mg)}{Label\ claim\ of\ \text{Ertugliflozin}\ (mg)} X\ F X\ 100$$

VALIDATION OF RP-HPLC METHOD
The developed method for estimation of Ertugliflozin and Sitagliptin was validated as per ICH guidelines for following parameters.

FILTRATION STUDY:
Filtration study of an analytical procedure checks the interference of extraneous components from filter, deposition on filter bed and compatibility of filter with sample. This study was conducted with Test sample solution. (Physical Lab Mixture). Filtration study carried out with unfiltered (Centrifuged at 3000 RPM for 5 minutes) and filtered test solution. During filtration activity 0.45 µm PVDF and 0.45 µm Nylon syringe filters used by discarding 5 mL of aliquot sample.

STABILITY OF ANALYTICAL SOLUTION

Stability study was conducted for standard and test sample solution. Stability study was performed at normal laboratory conditions. The solution was stored at normal illuminated laboratory conditions and analyzed after 12 hours and 24 hours. Standard and Test solution stability study was performed by calculating the difference between results of test solution at each stability time point to that of initial.

SPECIFICITY:

Specificity is the ability to access unequivocally the analyte in the presence of components which may be expected to be present. Following solution shall be prepared and injected to prove the specificity nature of the method. (Checked peak purity for standard and test sample solution)

I. Blank (Mobile phase)
II. Placebo
III. Sitagliptin and Ertugliflozin Standard solution mixture
IV. Tablet test sample solution

Analyzing marketed test sample contains excipients (additives) which are totally unknown. So Placebo prepared at lab level by using formula as follows:

Sr. No.	Ingredients	Role	Qty (mg)
1	Lactose	Filler	80
2	Starch	Binder	5
3	Magnesium stearate	Lubricant	5
4	Talc	Glidant	5
5	Crospovidone	Disintegrants	5
Total			100 mg

Total 10 gm of placebo prepared:

Placebo Sample solution preparation:

Weighed 304.07 mg of placebo material (Which is equivalent to 200 mg of Sitagliptin and 30 mg of Ertugliflozin) and transferred to clean and dried 100 mL of volumetric flask. Added 70 mL of methanol, sonicated for 15 minutes with intermittent shaking. After 15 minutes allow to cool the solution to room temperature and made volume up to the mark with methanol. Filtered the solution through suitable 0.45 µ syringe filter discarding 3-5 mL of initial filtrate. Further dilute 1 ml of filtered stock solution to 10 ml with mobile phase, injected the resultant solution and chromatograms were recorded

1) LINEARITY AND RANGE

Preparation of linearity solution

5 levels of Linearity was performed from 50% to 150% of working concentration

Linearity stock solutions:

Sitagliptin: Weighed 64.27 mg Sitagliptin phosphate (Equivalent to 50 mg of Sitagliptin) and transferred in 50 mL volumetric flask, added 30 mL of methanol, sonicated to dissolve it completely, made volume up to the mark with methanol. (1000 PPM)

Ertugliflozin: Weighed 12.95 mg Ertugliflozin L-pyroglutamic acid (Equivalent to 10 mg of Ertugliflozin) and transferred in 50 mL volumetric flask, added 30 mL of methanol, sonicated to dissolve it completely, made volume up to the mark with methanol. (200 PPM)

Linearity levels prepared as follows:

Level	Sitagliptin Stock solution (mL)	Ertugliflozin Stock solution (mL)	Diluted to with Mobile phase	Sitagliptin Conc (µg/mL)	Ertugliflozin Conc (µg/mL)
50%	2.00	1.50	20	100.0	15.0
75%	3.00	2.25	20	150.0	22.5
100%	4.00	3.00	20	200.0	30.0
125%	5.00	3.75	20	250.0	37.5
150%	6.00	4.50	20	300.0	45.0

Acceptance criteria

Correlation Coefficient: NLT 0.98
Intercept: To be reported
Slope: To be reported

Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Detection limit:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation limit:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

As per ICH Q2R1 guidelines LOD and LOQ was determined by using the approach Based on the Calibration Curve in which residual standard deviation of a regression line was calculated and determined the LOD and LOQ by using following formula:

LOD = 3.3 σ / S

LOQ = 10 σ / S

Where,

σ = residual standard deviation of a regression line

S = Slope of regression line

ACCURACY (% RECOVERY)

Accuracy will be conducted in the range from 50 % to 150 % of working concentration. Solution of each accuracy level was prepared in triplicate. Calculated % Recovery for each sample, Mean % recovery for each level and overall recovery and also calculated % RSD for each level and % RSD for overall recovery.

Accuracy levels details: Refer Following table for each sample:

Level (%)	Sitagliptin Phosphate API (mg)	Ertugliflozin L-pyroglutamic acid API (mg)	Wt of Placebo (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)	Sitagliptin Added Conc (µg/mL)	Ertugliflozin Added Conc (µg/mL)
50	128.6	19.6	304.6	100	1.00	10	100.05	15.13
	128.7	19.5	303.9	100	1.00	10	100.13	15.05
	128.6	19.7	304.8	100	1.00	10	100.05	15.21
100	257.1	38.9	304.2	100	1.00	10	200.02	30.03
	257.3	39.1	303.7	100	1.00	10	200.18	30.19
	257.2	38.9	304.5	100	1.00	10	200.10	30.03
150	385.8	58.4	304.3	100	1.00	10	300.15	45.08
	385.7	58.6	303.9	100	1.00	10	300.07	45.24
	385.7	58.5	305.1	100	1.00	10	300.07	45.16

Acceptance criteria

1. % Recovery for each sample and Mean recovery and overall recovery should be in the range of 98-102%.
2. The Relative Standard Deviation should not be more than 2.0%.

PRECISION

Precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous test under the prescribed conditions. Precision is of two types, Repeatability and Intermediate precision. It is performed on tablet test sample.

Repeatibility:

Preparation of sample solution (6 Samples prepared):

Weighed the powder material equivalent to 200 mg of Sitagliptin and 30 mg of Ertugliflozin (600 mg of powder material) from physical lab mixture. Transfer it in a clean and dried 100 mL of volumetric flask, added 70 ml of methanol sonicated it for 15 minutes with intermittent shaking. Made the volume up to the mark with methanol. Filter the solution through suitable 0.45 µ syring filter discarding 3-5 mL of filtrate. Further diluted 1.0 ml of filtrate to 10 ml with mobile phase. (200 PPM of Sitagliptin and 30 PPM of Ertugliflozin)

Six samples prepared.

Precision (Repeatability) Sample details are as follows:

Sample No.	Test powder material (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
1	600.3	100	1	10
2	599.7	100	1	10
3	600.2	100	1	10
4	601.2	100	1	10

5	599.8	100	1	10
6	600.4	100	1	10

Acceptance criteria:
% Assay: 90-110% for each sample and mean assay value
% RSD for % assay value of 6 samples: NMT 2%

Intermediate precision

It is performed by doing analysis on another day to check reproducibility of results. Samples prepared in same manner as that of Repeatability parameter (6 Samples prepared).

Intermediate Precision Sample details are as follows:

Sample No.	Test powder material (mg)	Diluted to (mL)	Volume taken (mL)	Diluted to (mL)
1	601.2	100	1	10
2	600.8	100	1	10
3	600.4	100	1	10
4	600.9	100	1	10
5	599.9	100	1	10
6	600.5	100	1	10

Acceptance criteria:
% Assay: 90-110% for each sample and mean assay value
% RSD for % assay of 6 samples of Intermediate precision**:** NMT 2
% RSD for Total 12 samples: NMT 2% for test results (6 of Repeatability and 6 of Intermediate precisio

ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Determination: Blank and Standard solution were injected under different chromatographic conditions as shown below.

a). Changes in flow rate by ±10%. (± 0.1ml/min)
b). Change in column oven temperature. (± 2ºC)
c) Change in wavelength (± 3 nm)

RESULT AND DISSCUSION

Method Development by RP – HPLC

Optimization of HPLC method

Trial 1: Chromatogram:

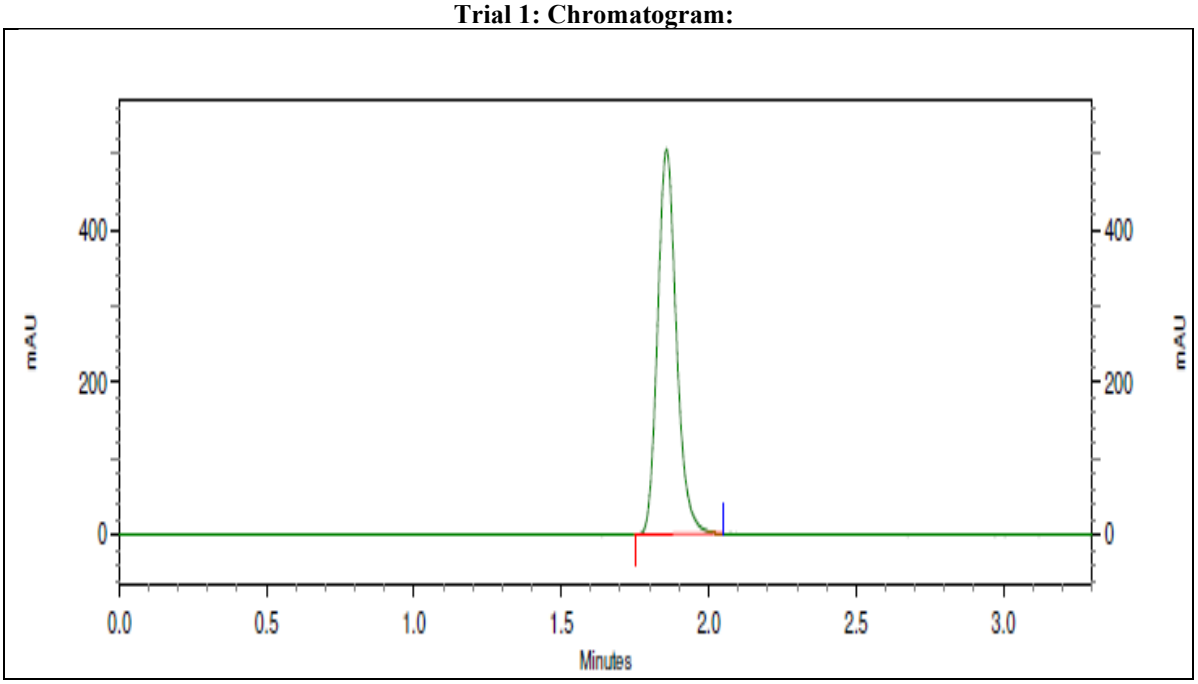

Figure 1: Typical chromatogram of Sitagliptin of Trial 5

Chromatogram:

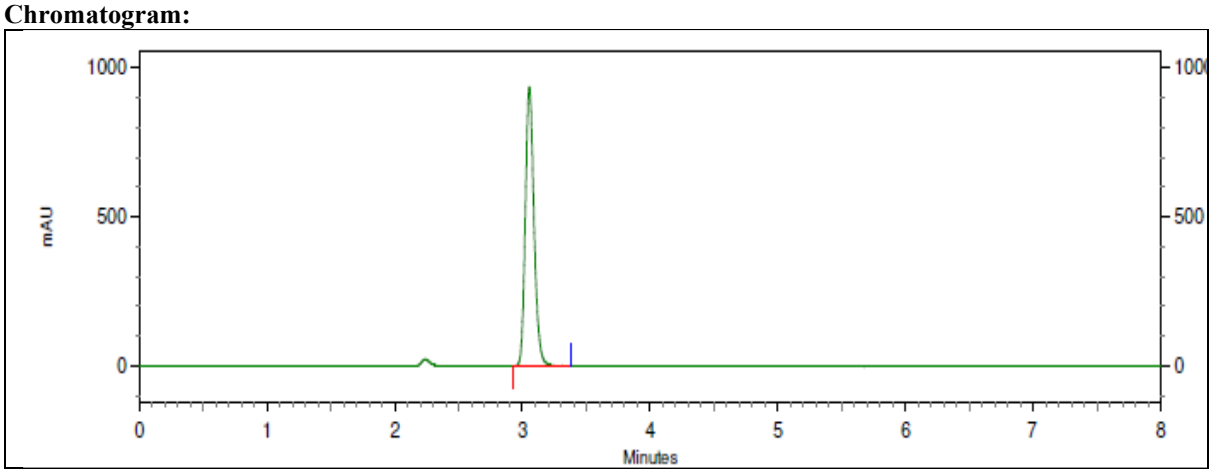

Figure 2: Typical chromatogram of Ertugliflozin of Trial 5

Observation: Sitagliptin and Ertugliflozin eluted with acceptable chromatography**.**
Conclusion: Method Accepted
Mixture (each 100 ppm)

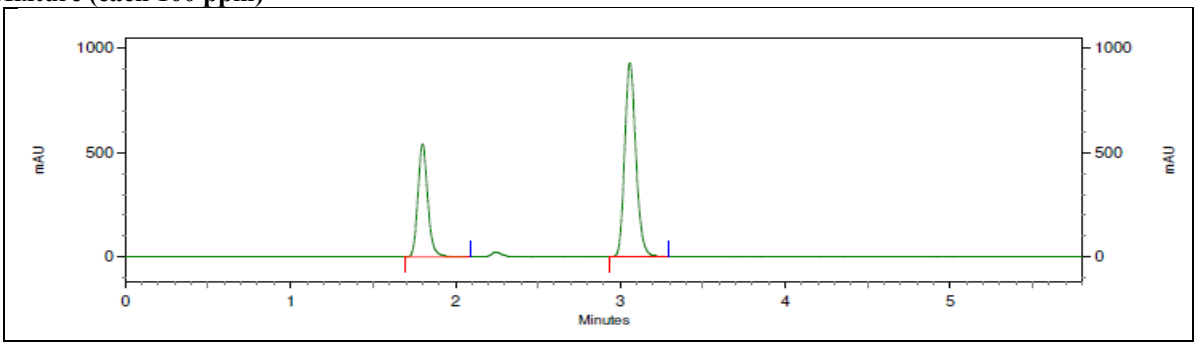

Figure 3: Typical chromatogram of Mixture of Trial 5 (Sitagliptin and Ertugliflozin)

Observation: Both drugs eluted with good chromatography with good resolution.
Conclusion: From the observations of trials first to five, it was concluded that chromatographic conditions in trial five gives better peak, good retention time, good tailing factor, Theoretical plates and good resolution therefore chromatographic conditions in trial five was subjected for method validation

System suitability test

Results for System Suitability Test of Sitagliptin:

Sr No.	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard_1	72923517	1.06	3725
2	Standard_2	72425036	1.06	3717
3	Standard_3	72629145	1.06	3749
4	Standard_4	72602361	1.07	3732
5	Standard_5	72964780	1.06	3741
Mean		**72708968**	**1.06**	**3733**
STD Dev		**229033.40212**		
% RSD		**0.32**		

Results for System Suitability Test of Ertugliflozin:

Sr No.	Standard solution	Area	Asymmetry	Theoretical plates
1	Standard_1	21394658	1.20	9776

2	Standard_2	21332563	1.19	9764
3	Standard_3	21418014	1.20	9759
4	Standard_4	21456943	1.20	9772
5	Standard_5	21296958	1.21	9782
Mean		**21379827**	**1.20**	**9771**
STD Dev		**64664.98249**		
% RSD		**0.30**		

System Suitability Acceptance Criteria:

1. Relative standard deviation of the area of analyte peaks in standard chromatograms should not be more than 2.0 %.
2. Theoretical plates of analyte peak in standard chromatograms should not be less than 2000.
3. Tailing Factor (Asymmetry) of analyte peaks in Standard Chromatograms should be less than 2.0

CONCLUSION

The present work involved the development of simple, accurate, precise and suitable RP- HPLC. In developed RP-HPLC method, the analyte were resolved by using isocratic program and mobile phase was used Methanol : Water (75:25 % v/v) at a flow rate of 1.0 ml/min, on HPLC system containing UV- visible detector with Openlab EZ-Chrome Software and Kromasil C18, 250 mm X 4.6 mm, 5 µm. The detection was carried out at 258 nm. The results of analysis in the developed method were validated in terms of linearity, accuracy, precision, robustness, limit of detection and limit of quantification. The developed method has several advantages, including reproducibility of results, rapid analysis, simple sample preparation and improved selectivity as well as sensitivity. The regression coefficient (r2) for each analyte is not less than 0.999 which shows good linearity. The % recovery was in the acceptable range in tablet dosage form. The %RSD was also less than 2% showing high degree of precision of the proposed method. Since the developed method is robust and reproducible and also less time consuming, it can be performed for routine analysis in pharmaceutical industry for bulk drug of Ertugliflozin and Sitagliptin and also in pharmaceutical dosage form.

REFERENCES

1. Beckett A.H, Analytical Chemistry and Practical Chemistry. 1996; 14th edi. –Vol-2.: 275-95
2. Skoog D.A, Holler F.J, Timothy A, Nieman N.W, Principle of instrumental Analysis. Eastern Press, Bangalore 2004; 5th edi.: 1-4,729-35
3. Willard H.H, Merritt L.L, Dean J.A, Settle Jr. F.A, Instrumental Methods of analysis. CBS Publishers and distribution, Delhi, 2001; 7th edi.: 118-19
4. Christan G.D, Analytical chemistry. John Wiley and sons. 2003; 6th Edn:1-3
5. Chatwal G.R, Sharma A, Instrumental Method of chemical Analysis. Himalaya Publishing House Delhi 2004; 5th edi: 1.1-1.5
6. Jeffery G.H, Bassat J, Mendham J, Denny R.C., Vogel's textbook of Quantitative Chemical Analysis. Elbs with Longman Publication Harlow,1989; 5th edi:1-6
7. Sharma B.K, Instrumental Methods of Chemical Analysis. Goal Publishing House, 2005; 24th edi.:3-12
8. Snyder, L.R, Joseph J.K, Joseph L.G, Practical HPLC Method Development. A Wiley-Interscience publication, New York; 2nd edi: 3-35
9. Ojeda C.B, Rojas F.S, Analytical Chemistry Actta. 2004;1-24
10. ICH, Q2A, Text on validation of analytical Procedures International Conference on harmonization, Geneva, 1994 October,1-5
11. ICH, Q_2B, Validation of analytical Procedure: Methodology, International conference on Harmonization, Geneva, 1996 November,1-8
12. ICH, Q2R1, Text on validation of analytical Procedures International Conference on harmonization, Geneva, 1994 October,1-13
13. www.drugbank.com