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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF ERTUGLIFLOZIN AND SITAGLIPTIN IN BULK DRUG AND DOSAGE FORM

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ABSTRACT

Several spectrophotometric and HPLC methods have been reported for the determination of Ertugliflozin and Sitagliptin in drugs and pharmaceutical dosage forms. In the present study, a new, sensitive, suitable, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the determination of Ertugliflozin and Sitagliptin in bulk drug and tablet formulation. The RP-HPLC method utilized Methanol and Water (75:25 % v/v) as the mobile phase at a flow rate of 1.0 ml/min on an HPLC system with a UV detector, using Openlab EZchrome software and a Kromasil C18 column (250 mm x 4.6 mm, 5 µm). Detection was carried out at 258 nm, providing suitable retention times of 3.60 min for both Ertugliflozin and Sitagliptin.

The method was validated according to ICH guidelines, assessing filter study, solution stability, specificity, linearity, accuracy, precision (repeatability and intermediate precision), limit of detection, limit of quantification, and robustness. The developed method demonstrated simplicity and precision for the assay of Ertugliflozin and Sitagliptin in bulk drug and tablet formulations. It requires regular reagents and is less time-consuming, making it suitable for routine analysis in the pharmaceutical industry.

This research article will discuss the method development process, optimization of chromatographic conditions, detailed validation results, and the applicability of the method for routine quality control of Ertugliflozin and Sitagliptin in pharmaceutical formulations.

Keywords: RP-HPLC, Ertugliflozin, Sitagliptin, Method Development, Validation, Bulk Drug, Dosage Form

INTRODUCTION

Ertugliflozin and Sitagliptin are widely used medications for the treatment of type 2 diabetes mellitus. Ertugliflozin, a sodium-glucose co-transporter-2 (SGLT2) inhibitor, functions by reducing glucose reabsorption in the kidneys, thus lowering blood glucose levels. Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, enhances the body's incretin levels, which in turn increases insulin secretion and decreases glucagon levels in a glucose-dependent manner. These drugs are often used in combination therapies to improve glycemic control in diabetic patients **[1-3]**.

Accurate estimation of Ertugliflozin and Sitagliptin in bulk drug and dosage forms is crucial for ensuring the efficacy, safety, and quality of these pharmaceutical products. The precision of analytical methods directly impacts the quality control processes, influencing the pharmacological performance and therapeutic outcomes of the drugs. Therefore, it is essential to develop reliable and validated analytical methods for their estimation **[4-6]**.

Several spectrophotometric and HPLC methods have been reported for the determination of Ertugliflozin and Sitagliptin. However, there remains a need for a new, sensitive, suitable, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method. RP-HPLC is renowned for its high resolution, accuracy, and reproducibility, making it an ideal choice for the simultaneous estimation of multiple components in complex matrices **[7]**.

The objective of this study is to develop and validate a novel RP-HPLC method for the simultaneous estimation of Ertugliflozin and Sitagliptin in bulk drug and tablet formulations. This method aims to be simple, time-efficient, and cost-effective while adhering to the stringent guidelines for analytical method validation **[8]**.

MATERIALS & METHODS

Materials: Ertugliflozin and sitagliptin was procured from Simson Pharma Ltd (Mumbai), Januvia Pharmaceuticals Pvt. Ltd. respectively, to perform this project.

Methods:

1. Preliminary Characterization of Drug

- Color, Odor, and Appearance: Evaluated for Sitagliptin and Ertugliflozin (Table 1).

- Factor Calculation:

- Sitagliptin Phosphate:

- Molecular weight: 523.32 (phosphate), 407.314 (base).

- Calculation example: 10 mg Sitagliptin = 12.85 mg Sitagliptin phosphate.

- Ertugliflozin L-pyroglutamic acid:

- Molecular weight: 566 (L-pyroglutamic acid), 436.89 (base).

- Calculation example: 10 mg Ertugliflozin = 12.95 mg Ertugliflozin L-pyroglutamic acid **[9, 10]**.

2. Determination of Solubility

- Solvents: Water and methanol, concentration 3 mg/mL **(Tables 2 & 3)**.

- Sitagliptin: 38.56 mg Sitagliptin phosphate in 10 mL.

- Ertugliflozin: 38.86 mg Ertugliflozin L-pyroglutamic acid in 10 Ml **[12]**

3. Selection of Analytical Wavelength

- Solvent: Methanol.

- Standard Stock Solutions:

- Sitagliptin: 25.71 mg Sitagliptin phosphate in 20 mL, diluted to 20 PPM.

- Ertugliflozin: 25.91 mg Ertugliflozin L-pyroglutamic acid in 20 mL, diluted to 20 PPM.

- Absorption Maxima: 212 nm for both drugs **[13]**.

4. RP-HPLC Method Development

- Preparation of Stock Solutions:

- Sitagliptin: 25.71 mg Sitagliptin phosphate in 20 mL, diluted to 100 PPM.

- Ertugliflozin: 25.91 mg Ertugliflozin L-pyroglutamic acid in 20 mL, diluted to 100 PPM.

- Chromatographic Conditions:

- Detector: UV

- Column: Phenomenex C18 (250 mm x 4.6 mm, 5µm)

- Temperature: 40°C

- Injection Volume: 20 µl

- Wavelength: 212 nm

- Mobile Phase: Methanol : 0.05% TFAA in water (70:30)

- Flow Rate: 1.0 ml/min

5. Optimization of HPLC Method

- Trials: Various mobile phase compositions tested (Methanol:Water, Acetonitrile:Water).

- Optimized Conditions: **Trial 4** deemed optimal (Methanol: 0.05% TFAA in water (70:30)) **[14]**.

6. System Suitability

- Stock Solutions:

- Sitagliptin: 25.71 mg in 20 mL (1000 PPM).

- Ertugliflozin: 12.95 mg in 20 mL (500 PPM).

- Standard Mixture: 2 mL Sitagliptin and 0.6 mL Ertugliflozin in 20 mL.

- Criteria:

- RSD ≤ 2.0%

- Tailing Factor ≤ 2.0

- Plate Count > 2000

7. Analysis of Marketed Sample

- Preparation: Lab mixture of tablet ingredients (Sitagliptin 128.53 mg, Ertugliflozin 19.43 mg, placebo 202.44 mg).
- Sample Solution: 350.4 mg of lab mixture in 100 mL methanol, sonicated, filtered, and diluted **[15]**.

8. Validation of RP-HPLC Method

- Filtration Study: Analyzed with 0.45 µm PVDF and Nylon filters.
- Solution Stability: Checked at 12 and 24 hours under lab conditions.
- Specificity: Ensured using blank, placebo, standard, and sample solutions.
- Linearity and Range: Evaluated across 10-150% of working concentration **[16]**.

9. Linearity Solution Preparation

- Sitagliptin: 64.27 mg in 50 mL methanol (1000 PPM).
- Ertugliflozin: 19.43 mg in 50 mL methanol (1000 PPM).

These methods outline the critical steps and materials for the development and validation of an RP-HPLC method for Sitagliptin and Ertugliflozin, ensuring accuracy, precision, and reliability in the analysis **[17, 18]**.

RESULT AND DISCUSSION

Preliminary Characterization and Identification of Drug

Color, Odour, and Appearance

The preliminary characterization involved observing the physical properties of Sitagliptin Phosphate and Ertugliflozin L-pyroglutamic Acid **(Table 1)**.

Both drugs were found to be white, odourless, and crystalline in appearance.

Solubility Study

The solubility of Sitagliptin and Ertugliflozin was tested in different solvents to identify a suitable solvent for further analysis **(Table 2)**.

Ertugliflozin was not soluble in water, making water an unsuitable solvent for it.

Both drugs were found to be soluble in methanol, making it a suitable solvent for dissolving Sitagliptin and Ertugliflozin.

Selection of Analytical Wavelength

UV Spectra Analysis

UV spectra were obtained for methanol, standard solutions of Sitagliptin and Ertugliflozin, and their overlay **(Figure 1)**.

Table 1: Color, Odour, and Appearance of Drugs

Sr. No	Name	Colour, Odour, and Appearance
1	Sitagliptin Phosphate	White, odourless, crystalline powder
2	Ertugliflozin L-pyroglutamic Acid	White, odourless, crystalline powder

Table 2: Solubility Study of Sitagliptin and Ertugliflozin in Water

Sr. No	Name of Drug	Observation	Conclusion Summary
1	Sitagliptin	No drug particles seen after sonication	Drug was found soluble in water
2	Ertugliflozin	Particles seen after sonication	Drug was not found soluble in water

Table 3: Solubility Study of Sitagliptin and Ertugliflozin in Methanol

Sr. No	Name of Drug	Observation	Conclusion Summary
1	Sitagliptin	No drug particles seen after sonication	Drug was found soluble in methanol
2	Ertugliflozin	No drug particles seen after sonication	Drug was found soluble in methanol

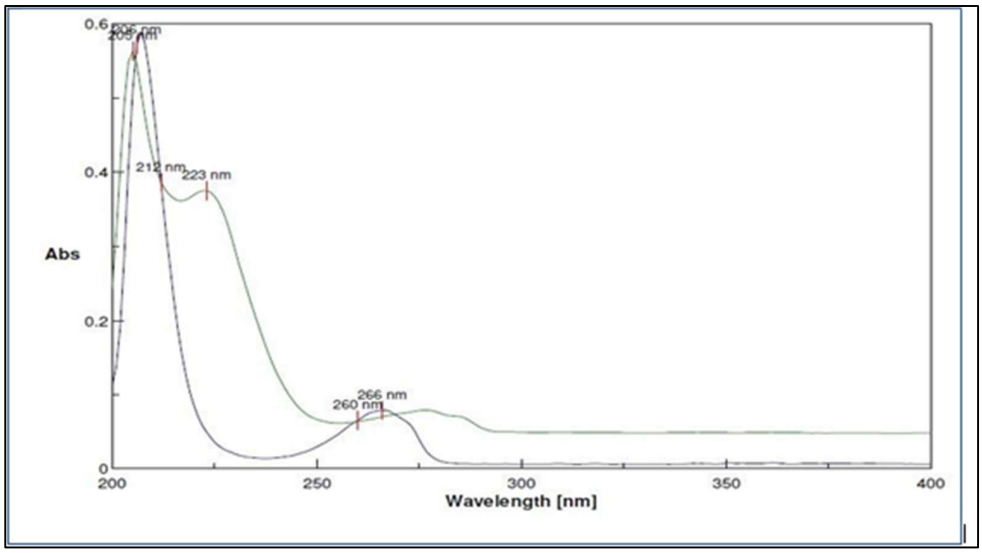


Figure 1: Overlay UV Spectrum of Sitagliptin & Ertugliflozin

Observation: Both standard solutions were scanned between 200 nm to 400 nm. The Q-absorption point was determined for both drugs, found at 212 nm.

Method Development by RP-HPLC

Optimization of HPLC Method

Trial 1:

Observation: Sitagliptin eluted with unacceptable chromatography and Ertugliflozin eluted but fronting was observed (Asymmetry: 0.78).

Conclusion: Method rejected.

Trial 2:

Observation: Sitagliptin eluted with unacceptable chromatography and Ertugliflozin eluted with good chromatography.

Conclusion: Method rejected.

Trial 3:

Observation: Sitagliptin eluted with unacceptable chromatography and Ertugliflozin eluted with good chromatography.

Conclusion: Method rejected.

Trial 4:

Observation: Sitagliptin and Ertugliflozin eluted with acceptable chromatography.

Conclusion: Method accepted.

Mixture (Each 100 ppm):

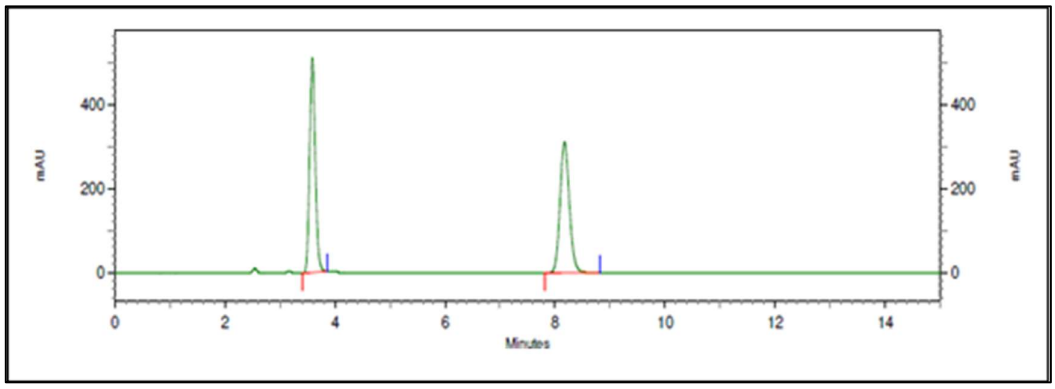


Figure 2: Typical Chromatogram of Mixture of Sitagliptin and Ertugliflozin (Trial 4)

Observation: Both drugs eluted with good chromatography and resolution.

Conclusion: Chromatographic conditions in trial four give better peak, good retention time, good tailing factor, theoretical plates, and resolution. Therefore, these conditions were used for method validation.

Table 2: Optimized Chromatographic Conditions

Parameter	Description
Mode	Isocratic
Detector	UV Detector
Column Name	Phenomenex C18, 250 mm X 4.6 mm ID, 5 µm
Column Oven Temp	40°C
Injection Volume	20 µl
Wavelength	212 nm
Mobile Phase	Methanol : 0.05% TFAA in water (70:30)
Flow Rate	1.0 ml/min
Diluents	Mobile phase
Run time	12 Minutes

System Suitability Test

Table 3: Results for System Suitability Test of Sitagliptin

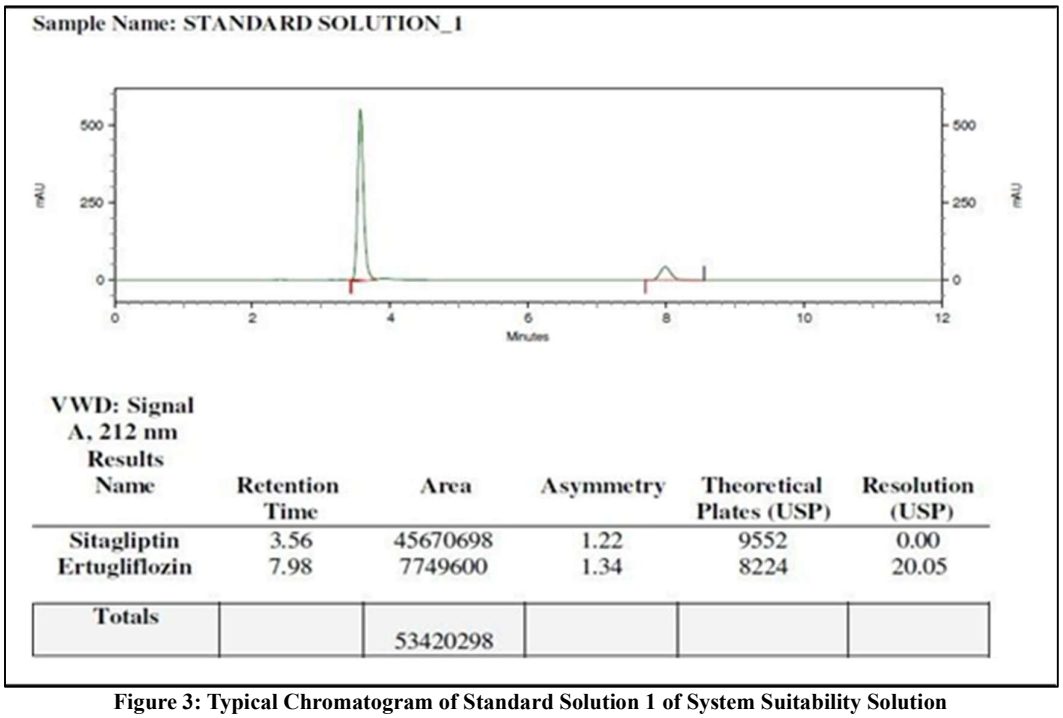
Sr No	Standard Solution	Area	Asymmetry	Theoretical Plates
1	Standard_1	45670698	1.22	9552
2	Standard_2	45436921	1.22	9539
3	Standard_3	45325703	1.21	9550
4	Standard_4	45790458	1.22	9563
5	Standard_5	45621490	1.22	9547
Mean		45569054	1.22	9550
STD Dev		186265.68		
% RSD		0.41		

Table 4: Results for System Suitability Test of Ertugliflozin

Sr No	Standard Solution	Area	Asymmetry	Theoretical Plates
1	Standard_1	7749600	1.34	8224
2	Standard_2	7756932	1.35	8231
3	Standard_3	7736904	1.35	8245
4	Standard_4	7739878	1.35	8236
5	Standard_5	7769817	1.34	8228
Mean		7750626	1.35	8233
STD Dev		13350.33		
% RSD		0.17		

System Suitability Acceptance Criteria:

- Relative standard deviation of the area of analyte peaks in standard chromatograms should not be more than 2.0%.
- Theoretical plates of analyte peak in standard chromatograms should not be less than 2000.
- Tailing Factor (Asymmetry) of analyte peaks in Standard Chromatograms should be less than 2.0.
- Data Interpretation: It was observed from the data tabulated above; the method complies with system suitability parameters. Hence, it can be concluded that the chromatographic method is adequate for the intended analysis.



VWD: Signal A, 212 nm Results

Name	Retention Time	Area	Asymmetry	Theoretical Plates (USP)	Resolution (USP)
Sitagliptin	3.56	45670698	1.22	9552	0.00
Ertugliflozin	7.98	7749600	1.34	8224	20.05
Totals		53420298			

Figure 3: Typical Chromatogram of Standard Solution 1 of System Suitability Solution

Assay of Physical Lab Mixture

The physical lab mixture's average tablet weight was considered to be 350.4 mg.

Table 5: Assay Results of Physical Lab Mixture

Drug	Sample	Area	% Assay	Mean Assay
Sitagliptin	Sample 1	45236901	99.23	98.36
	Sample 2	44396520	97.49	
Ertugliflozin	Sample 1	7666921	98.84	99.15

Validation of RP-HPLC Method

Filtration Study

The filtration study of an analytical procedure is crucial for assessing the interference of extraneous components from the filter, the deposition on the filter bed, and the compatibility of the filter with the sample. This study was performed on a

tablet test sample to ensure that the filter does not introduce any artifacts or contaminants that could affect the accuracy of the analytical results.

Table 6: Results of Filter Study

Drug	Sample Description	Area	% Absolute Difference
Sitagliptin	Unfiltered	45,596,356	NA
	0.45 µ PVDF Filter	45,325,807	0.59
	0.45 µ Nylon Filter	45,426,930	0.37
Ertugliflozin	Unfiltered	7,762,581	NA
	0.45 µ PVDF Filter	7,726,980	0.46
	0.45 µ Nylon Filter	7,739,604	0.30

Acceptance Criteria: The % Absolute Difference of filtered samples relative to unfiltered samples should not exceed 2.0%.

Data Interpretation: Both filters (PVDF and Nylon) met the acceptance criteria for the filter study, as the % Absolute Difference was within the acceptable range. However, the Nylon filter showed a smaller absolute difference compared to the PVDF filter for both drugs. Therefore, the Nylon filter was selected for further analyses to ensure minimal interference and better results.

Solution Stability

The solution stability study was performed to ensure that the standard and test samples remain stable under normal laboratory conditions over time. The stability of the solutions was assessed by analyzing the samples at different time points to confirm that there were no significant changes in the results.

Table 7: Solution Stability Study

Drug	Time Point	Area	% Change in Area
Sitagliptin	Initial	45,596,356	NA
	After 12 hours	45,679,892	0.18%
	After 24 hours	45,716,587	0.26%
Ertugliflozin	Initial	7,762,581	NA
	After 12 hours	7,765,732	0.04%
	After 24 hours	7,770,080	0.10%

Data Interpretation: The % Change in Area for both Sitagliptin and Ertugliflozin was within the acceptable range of less than 2.0% at 12 and 24 hours, indicating that the solutions were stable under normal laboratory conditions. These results confirm that the analytical method is reliable for use over an extended period.

CONCLUSION

The present work successfully developed and validated a simple, accurate, and precise RP-HPLC method for the determination of Ertugliflozin and Sitagliptin in bulk drug and pharmaceutical dosage form. Utilizing an isocratic program with a Methanol:Water (70:30 % v/v) mobile phase at a flow rate of 1.0 ml/min and detection at 212 nm, the method demonstrated excellent linearity ($r^2 \geq 0.999$), accuracy, and precision, with %RSD less than 2%. The robustness, reproducibility, and rapid analysis capabilities of this method make it suitable

for routine pharmaceutical industry applications. Future work may expand its use to other formulations and biological matrices, enhancing its applicability in pharmacokinetic studies and stability testing.

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