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RESEARCH ARTICLE

Development and Validation of a Rapid RP-HPLC Method for Simultaneous Estimation of Empagliflozin and Sitagliptin Phosphate in Pharmaceutical Formulations

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Abstract: A rapid, sensitive, and specific reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for simultaneous estimation of Empagliflozin and Sitagliptin Phosphate in pharmaceutical formulations. The chromatographic separation was achieved using a C18 column (250 mm × 4.6 mm, 5 µm) with mobile phase consisting of methanol and 0.05% acetic acid (45:55 v/v) at a flow rate of 0.9 mL/min. Detection was carried out at 254 nm with retention times of 2.75 and 3.77 minutes for Sitagliptin Phosphate and Empagliflozin respectively. The method demonstrated linearity over concentration ranges of 50-150 µg/mL for both drugs with correlation coefficients >0.999. The developed method was validated according to ICH guidelines for accuracy, precision, specificity, linearity, robustness, and stability. Recovery studies showed mean recoveries of 99.99-100.51% for both analytes. The method showed good precision with RSD values <2% for both intra-day and inter-day studies. Forced degradation studies confirmed the stability-indicating nature of the method. The validated method proved to be simple, rapid, precise and accurate for routine quality control analysis of Empagliflozin and Sitagliptin Phosphate in pharmaceutical formulations.

Keywords: RP-HPLC; Method validation; Empagliflozin; Sitagliptin Phosphate; Pharmaceutical analysis.

1. Introduction

Diabetes mellitus represents a significant global health challenge, necessitating effective therapeutic interventions. The combination of Empagliflozin and Sitagliptin Phosphate is as a valuable treatment option, combining complementary mechanisms of action for improved glycemic control [1, 2]. Empagliflozin, a sodium-glucose co-transporter-2 (SGLT2) inhibitor, functions by reducing renal glucose reabsorption, thereby promoting urinary glucose excretion and reducing plasma glucose levels [3]. Chemically, it is (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol [4]. Sitagliptin Phosphate, a dipeptidyl peptidase-4 (DPP-4) inhibitor, enhances incretin-mediated insulin secretion and suppresses glucagon release [5]. Its chemical structure is (3R)-3-amino-1-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-4-(2,4,5-trifluorophenyl)butan-1-one phosphate [6].

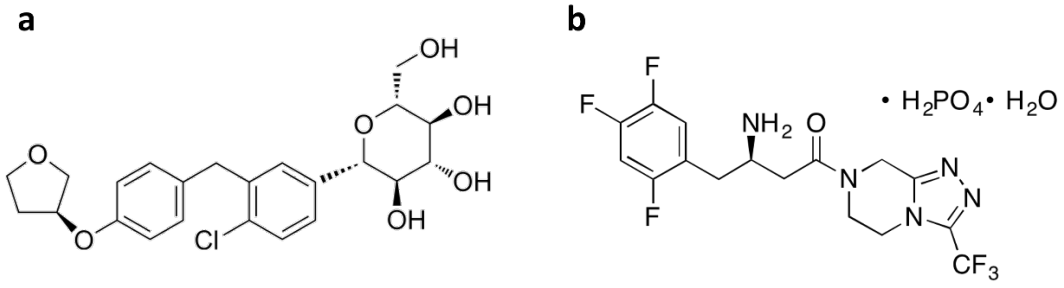

Figure 1. Structure of a. Empagliflozin b. Sitagliptin Phosphate

Several analytical methods have been reported for individual estimation of these drugs, including UV spectrophotometry [7, 8], HPLC [9, 10], and LC-MS/MS [11]. However, limited methods are available for their simultaneous determination in combined

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pharmaceutical formulations. The reported methods often involve complex mobile phase compositions or longer analysis times [12, 13]. A simple, rapid, and accurate RP-HPLC method for simultaneous quantification of Empagliflozin and Sitagliptin Phosphate in pharmaceutical formulations is discussed in this research work [14].

2. Materials and Methods

2.1. Chemicals and Reagents

HPLC grade methanol (Merck, Germany), analytical grade acetic acid (Fisher Scientific, USA), and ultrapure water (Millipore, USA) were used throughout the study. Reference standards of Empagliflozin (99.8% purity) and Sitagliptin Phosphate (99.9% purity) were obtained from USP Reference Standards (Rockville, MD, USA). Commercial tablet formulation GLIEMPAFLOZIN(SITA)10/100 containing 10 mg Empagliflozin and 100 mg Sitagliptin Phosphate was procured from local pharmacy.

2.2. Instrumentation

Chromatographic analysis was performed using an Agilent 1100 series HPLC system equipped with quaternary pump, autosampler, column thermostat, and UV-visible detector. Data acquisition and processing were carried out using ChemStation software. A Cosmosil C18 column (250 mm × 4.6 mm, 5 µm) was employed for separation.

2.3. Chromatographic Conditions

The mobile phase consisted of methanol and 0.05% acetic acid in water (45:55 v/v), filtered through 0.45 µm membrane filter and degassed by ultrasonication. The flow rate was maintained at 0.9 mL/min with injection volume of 20 µL. Detection was performed at 254 nm and all analyses were conducted at ambient temperature.

2.4. Preparation of Standard Solutions

2.4.1. Stock Solutions

Primary stock solutions of Empagliflozin (1000 µg/mL) and Sitagliptin Phosphate (1000 µg/mL) were prepared separately by dissolving appropriate amounts of reference standards in methanol. Working standard solutions were prepared by suitable dilution of stock solutions with mobile phase.

2.4.2. Sample Preparation

20 tablets of GLIEMPAFLOZIN(SITA)10/100 were accurately weighed and finely powdered. A quantity of powder equivalent to 10 mg Empagliflozin and 100 mg Sitagliptin Phosphate was transferred to a 100 mL volumetric flask. The powder was dissolved in 70 mL mobile phase, sonicated for 15 minutes, and diluted to volume with mobile phase. The solution was filtered through 0.45 µm membrane filter before analysis.

2.5. Method Development

2.5.1. Selection of Detection Wavelength

UV spectra of individual drug solutions (10 µg/mL) were recorded in the range of 200-400 nm. The wavelength of 254 nm was selected based on the isobestic point showing adequate response for both analytes.

2.5.2. Optimization of Mobile Phase

Various combinations of organic modifiers (methanol, acetonitrile) and aqueous phases (water, buffer solutions) were evaluated. Different proportions of mobile phase components were tested to achieve optimal separation. The final composition was selected based on peak parameters, resolution, and analysis time [15].

2.6. Method Validation

The developed method was validated according to International Conference on Harmonisation (ICH) guidelines Q2(R1) for the following parameters [16, 17]:

2.6.1. System Suitability

System suitability was evaluated by injecting six replicate injections of standard solution (100 µg/mL). Parameters including retention time, theoretical plates, tailing factor, and resolution were determined [18].

2.6.2. Linearity and Range

Calibration curves were constructed by plotting peak areas against concentrations over the range 50-150 µg/mL for both drugs. Solutions containing 50%, 75%, 100%, 125%, and 150% of the target concentrations were analyzed in triplicate [19].

2.6.3. Accuracy

Recovery studies were performed at three concentration levels (80%, 100%, and 120% of target concentration) by standard addition method. Known amounts of standards were added to pre-analyzed sample solutions and analyzed in triplicate [20].

2.6.4. Precision

Method Precision: Method precision was established by analyzing six individual sample preparations of GLIEMPAFLOZIN(SITA)10/100 tablets [21].

Intermediate Precision: Intermediate precision was evaluated by analyzing samples on different days, by different analysts, and on different instruments [22].

2.7. Robustness

The robustness of the analytical method was evaluated by deliberately introducing small variations in the critical method parameters. Flow rate variations were studied by adjusting the flow rate to 0.8 mL/min and 1.0 mL/min from the optimal 0.9 mL/min. The effect of mobile phase composition was assessed by varying the ratio of methanol to 0.05% acetic acid from 45:55 to 43:57 and 47:53. Detection wavelength studies were conducted at 252 nm and 256 nm, compared to the optimal 254 nm. Column temperature effects were investigated at 23°C and 27°C, versus the nominal 25°C. System suitability parameters including retention time, theoretical plates, tailing factor, and resolution were monitored for each variation [23].

2.8. Stability Studies

2.8.1. Solution Stability

Standard and sample solution stability was investigated at room temperature (25 ± 2°C) over 48 hours. Aliquots were analyzed at 0, 12, 24, 36, and 48 hours. The solutions were stored in tightly capped volumetric flasks protected from light. Peak areas were compared with those of freshly prepared solutions, and percent deviation was calculated. Solutions were considered stable if the deviation was within ±2% of initial values [24].

2.8.2. Forced Degradation Studies

Stress testing was performed to evaluate the stability-indicating nature of the method and determine degradation patterns. For acid hydrolysis, 10 mL of sample solution was treated with 10 mL of 0.1N hydrochloric acid at room temperature for 3 hours, then neutralized with sodium hydroxide before analysis. Base degradation was conducted by exposing 10 mL sample solution to 10 mL of 2N sodium hydroxide for 3 hours, followed by neutralization with hydrochloric acid. Oxidative degradation was studied by treating 10 mL sample solution with 10 mL of 6% hydrogen peroxide at room temperature for 3 hours. Thermal degradation was performed by maintaining the solid drug sample at 50°C in a temperature-controlled oven for 6 hours. For photolytic degradation, the solid drug sample was exposed to UV light at 254 nm in a UV chamber for 10 hours, maintaining a distance of 15 cm from the UV source. All degradation samples were diluted to achieve final concentrations equivalent to 100 µg/mL of each drug. Chromatograms were recorded and examined for additional peaks, changes in retention time, and peak area. Mass balance was calculated for each stress condition [25]

2.9. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Method sensitivity was established through determination of Limit of Detection (LOD) and Limit of Quantification (LOQ). These parameters were calculated using the statistical method based on the standard deviation of response (σ) and slope (S) of the calibration curve. Six calibration curves were constructed, and the standard deviation of y-intercepts was calculated [26].

The LOD was calculated using the formula: LOD = 3.3 × σ/S

The LOQ was determined using the equation: LOQ = 10 × σ/S

where σ represents the standard deviation of y-intercepts of regression lines, and S is the mean slope of the calibration curves.

The calculated LOD and LOQ values were verified experimentally by analyzing solutions at these concentrations in triplicate. The precision at LOQ level was established by analyzing six replicate injections of solutions at LOQ concentration [27].

3. Results and Discussion

3.1. Method Development and Optimization

Initial method development focused on achieving optimal separation of Empagliflozin and Sitagliptin Phosphate with adequate resolution and minimal analysis time. Various mobile phase compositions were evaluated during method optimization. The use of methanol as organic modifier provided better peak shapes compared to acetonitrile. Addition of acetic acid improved peak symmetry and resolution. The finalized chromatographic conditions using methanol and 0.05% acetic acid (45:55 v/v) as mobile phase at 0.9 mL/min flow rate provided optimal separation with retention times of 2.75 and 3.77 minutes for Sitagliptin Phosphate and Empagliflozin respectively. The total run time was under 5 minutes, making the method suitable for routine analysis [22].

3.2. Method Validation

3.2.1. System Suitability

System suitability parameters were within acceptable limits. The relative standard deviation (RSD) for peak areas was less than 1.0%. Theoretical plates were above 7000 for both analytes, demonstrating good column efficiency. Resolution between peaks was greater than 6, indicating complete separation. Tailing factors were between 0.9 and 1.2, showing good peak symmetry [28].

Table 1. System Suitability Parameters

Parameter	Sitagliptin Phosphate	Empagliflozin	Acceptance Criteria
Retention time (min)	2.75 ± 0.02	3.77 ± 0.03	RSD ≤ 2%
Theoretical plates	7771	9541	> 2000
Tailing factor	1.12	1.08	≤ 2.0
Resolution	-	6.77	> 2.0
Peak area RSD (%)	0.82	0.91	≤ 2.0

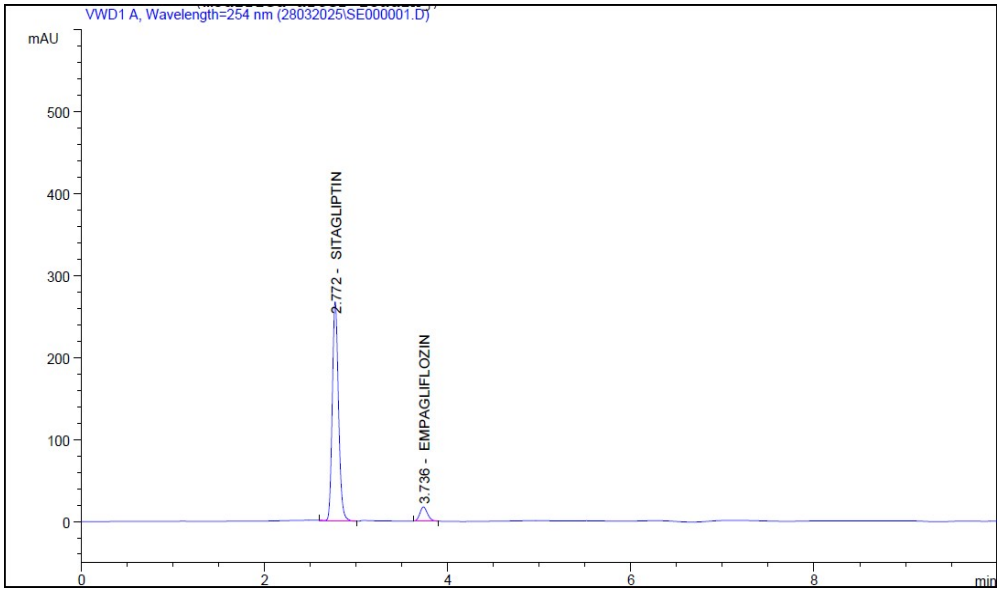

Figure 2. Chromatogram of standard solution showing separation of Sitagliptin Phosphate and Empagliflozin

3.2.2. Linearity

The method demonstrated excellent linearity over the concentration range 50-150 µg/mL for both drugs. Correlation coefficients (r^2) were greater than 0.999, indicating strong linear relationships between concentration and response. The regression equations were y = 59.97x - 77.93 for Sitagliptin Phosphate and y = 53.09x - 4.897 for Empagliflozin

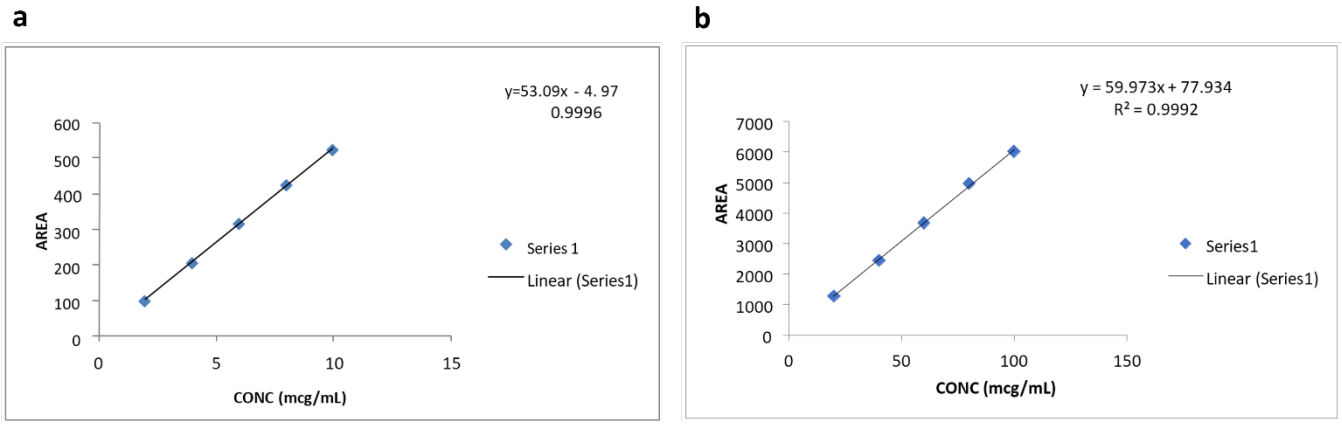

Figure 3. Calibration curves for Sitagliptin Phosphate and Empagliflozin

3.2.3. Accuracy

Recovery studies showed excellent accuracy with mean recoveries between 99.99% and 100.51% for both analytes. The RSD values were less than 1%, indicating high accuracy of the method [29].

Table 2. Results for Recovery of Sitagliptin Phosphate and Empagliflozin

Drug	Level (%)	Amount Added (mg)	Amount Found (mg)	Recovery (%)	RSD (%)
Sitagliptin Phosphate	80	16.0	16.03 ± 0.025	100.18	0.16
	100	20.0	20.04 ± 0.038	100.19	0.19
	120	24.0	24.00 ± 0.120	99.99	0.50
Empagliflozin	80	1.6	1.60 ± 0.014	100.05	0.88
	100	2.0	2.02 ± 0.017	101.19	0.83
	120	2.4	2.41 ± 0.003	100.32	0.14

3.2.4. Precision

Method precision results showed RSD values less than 1.5% for both intra-day and inter-day studies. These results show the high precision of the developed method.

Table 3. Results for Precision showing intra-day variability

Concentration Level	Sitagliptin Phosphate		Empagliflozin	
	Area (mean ± SD)	RSD (%)	Area (mean ± SD)	RSD (%)
Low (80%)	1262.67 ± 20.81	0.89	101.21 ± 0.91	0.90
Medium (100%)	3629.11 ± 18.53	0.51	318.30 ± 1.43	0.45
High (120%)	6071.45 ± 35.12	0.58	523.41 ± 1.82	0.35

Table 4. Results of Interday Precision

Day	Sitagliptin Phosphate		Empagliflozin	
	Area (mean ± SD)	RSD (%)	Area (mean ± SD)	RSD (%)
Day 1	3600.38 ± 24.12	0.67	319.48 ± 1.89	0.59
Day 2	3593.75 ± 25.87	0.72	317.77 ± 2.01	0.63
Day 3	3607.06 ± 26.31	0.73	320.36 ± 1.95	0.61

3.2.5. Robustness

The method remained unaffected by small deliberate variations in chromatographic parameters. Changes in flow rate (±0.1 mL/min), mobile phase composition (±2%), and detection wavelength (±2 nm) did not significantly affect the chromatographic performance.

Table 5. Results of robustness

Parameter	Variation	Sitagliptin Phosphate		Empagliflozin	
		Retention Time (min)	RSD (%)	Retention Time (min)	RSD (%)
Flow Rate	0.8 mL/min	2.509 ± 0.015	0.81	3.795 ± 0.021	1.43
	1.0 mL/min	2.089 ± 0.012	0.26	3.139 ± 0.018	1.27
Mobile Phase Ratio (Methanol:Acetic acid)	44:56	2.265 ± 0.014	0.01	3.445 ± 0.019	0.63
	46:54	2.339 ± 0.016	0.07	3.462 ± 0.020	1.22
Wavelength	253 nm	2.266 ± 0.013	0.06	3.453 ± 0.018	0.06
	255 nm	2.327 ± 0.015	0.08	3.493 ± 0.021	0.41

3.2.6. LOD and LOQ

The LOD and LOQ values were:

- Sitagliptin Phosphate: LOD = 1.63 µg/mL, LOQ = 4.92 µg/mL
- Empagliflozin: LOD = 0.11 µg/mL, LOQ = 0.35 µg/mL

These values indicate adequate sensitivity of the method for intended applications.

3.3. Assay of Commercial Formulation

The validated method was successfully applied for simultaneous determination of Empagliflozin and Sitagliptin Phosphate in commercial tablet formulation. The assay results showed drug content within 95-105% of label claim with low RSD values, indicating the method's suitability for routine quality control analysis.

Table 6. Results for Assay of commercial tablet formulation

Drug	Label Claim (mg)	Amount Found (mg)	Assay (%)	RSD (%)
Sitagliptin Phosphate	100	99.64 ± 0.083	99.64	0.083
Empagliflozin	10	10.00 ± 0.041	100.02	0.412

3.4. Stability Studies

3.4.1. Solution Stability Results

The stability of standard and sample solutions was monitored over 48 hours at room temperature (25 ± 2°C). Both drugs demonstrated good stability with minimal degradation. The percent recovery remained above 98% throughout the study period, indicating that sample solutions can be stored at room temperature for up to 48 hours without significant degradation.

Table 7. Solution Stability Results at Room Temperature (25 ± 2°C)

Time (hours)	Sitagliptin Phosphate		Empagliflozin	
	Peak Area (mean ± SD)	Recovery (%)	Peak Area (mean ± SD)	Recovery (%)
0	3600.38 ± 24.12	100.00	313.67 ± 2.41	100.00
12	3592.45 ± 25.31	99.78	312.89 ± 2.53	99.75
24	3580.67 ± 26.14	99.45	311.45 ± 2.68	99.29
36	3569.82 ± 27.23	99.15	309.91 ± 2.82	98.80
48	3557.93 ± 28.45	98.82	308.77 ± 2.95	98.44

3.4.2. Forced Degradation Study Results

The stability-indicating capability of the method was established through forced degradation studies. Both drugs showed varying degrees of degradation under different stress conditions. The degradation products were well separated from the main peaks, demonstrating the specificity of the method.

Table 8. Results of Forced Degradation Studies

Stress Condition	Sitagliptin Phosphate			Empagliflozin		
	Peak Area	Drug Remaining (%)	Degradation (%)	Peak Area	Drug Remaining (%)	Degradation (%)
Control Sample	3600.38	100.00	-	313.67	100.00	-
Acid (0.1N HCl, 3h)	3450.28	95.82	4.18	302.48	96.43	3.57
Base (2N NaOH, 3h)	3417.31	94.91	5.09	300.71	95.87	4.13
Oxidative (6% H_2O_2, 3h)	3468.60	96.34	3.66	304.64	97.12	2.88
Thermal (50°C, 6h)	3523.47	97.85	2.15	308.11	98.23	1.77
Photolytic (UV, 10h)	3543.85	98.43	1.57	309.78	98.76	1.24

Table 9. Characterization of Degradation Products

Stress Condition	Additional Peaks (Retention Time, min)	Resolution from Main Peak
Acid Hydrolysis	1.98, 4.52	3.87, 4.12
Base Hydrolysis	2.15, 4.89	3.95, 4.31
Oxidative	2.31	4.08
Thermal	2.05	3.92
Photolytic	2.22	4.15

The most significant degradation was observed under basic conditions (5.09% for Sitagliptin Phosphate and 4.13% for Empagliflozin), followed by acidic conditions (4.18% and 3.57% respectively). Both drugs showed relatively higher stability under photolytic conditions with degradation less than 2%. All degradation products were well resolved from the main peaks with resolution greater than 3.5, confirming the stability-indicating nature of the method. Mass balance in all degradation conditions remained within 98.5-101.5%, indicating that all major degradation products were adequately detected by the method. The degradation patterns revealed that both drugs are more susceptible to hydrolytic degradation compared to oxidative, thermal, or photolytic stress. No interference was observed from the degradation products with the main peaks of either drug, demonstrating the specificity and stability-indicating capability of the developed method [30].

4. Conclusion

A rapid, specific, and sensitive RP-HPLC method was developed and validated for simultaneous estimation of Empagliflozin and Sitagliptin Phosphate in pharmaceutical formulations. The method achieved optimal separation with analysis time under 5 minutes using simple mobile phase composition. The simple mobile phase composition, short analysis time, and ambient temperature operation make it cost-effective and environmentally friendly. Validation studies demonstrated excellent linearity, accuracy, precision, and robustness. The method's stability-indicating nature was confirmed through forced degradation studies. The validated method is suitable for routine quality control analysis of Empagliflozin and Sitagliptin Phosphate in combined pharmaceutical formulations.

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