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SIAM STUDIES BY PERFORMING FORCED DEGRADATION OF SITAGLIPTIN AND DAPAGLIFLOZIN USING RP-HPLC

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ABSTRACT
Background: A rapid, selective, and stability-indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method was established for the simultaneous quantification and forced degradation analysis of Sitagliptin phosphate monohydrate and Dapagliflozin propanediol monohydrate in active pharmaceutical ingredients and bulk formulations.
Methods: Chromatographic separation was achieved on a Cosmosil C18 column (250 mm × 4.6 mm, 5 µm particle size) using an isocratic mobile phase consisting of methanol and water (60:40 v/v) adjusted to pH 3.0 with orthophosphoric acid. The liquid chromatograph was operated at a flow rate of 0.8 mL/min, an injection volume of 20 µL, a system pressure of 14 to 15 MPa, and an isosbestic detection wavelength of 219 nm. Forced degradation profiling was conducted under basic hydrolytic (sodium hydroxide) and peroxide-mediated oxidative (3% H2O2) stress conditions.
Results: Sitagliptin and Dapagliflozin eluted at well-defined retention times of 2.789 minutes and 4.777 minutes, respectively, displaying an inter-analyte chromatographic resolution of 10.045. Column efficiency exceeded 4900 theoretical plates, and USP tailing factors were below 1.16 for both parent analytes. Base hydrolysis of Sitagliptin (2 N NaOH, 80 °C, 1 hour) produced three degradation products (retention times: 6.421, 7.922, and 12.220 minutes), whereas oxidative degradation (3% H2O2, 6 hours) formed a single degradation product (retention time: 5.357 minutes). Dapagliflozin exhibited greater chemical vulnerability, generating four distinct degradation products under alkaline hydrolysis (0.5 N NaOH, 60 °C, 1 hour; retention times: 2.803, 3.589, 6.283, 7.705, and 11.809 minutes) and four degradation products under oxidative stress (retention times: 2.583, 5.875, 7.292, and 10.546 minutes). Complete baseline resolution was maintained between all degradation product peaks and intact parent active drugs.
Conclusion: The proposed isocratic RP-HPLC procedure is specific, efficient, and stability-indicating, making it highly suitable for routine quality control, batch release, and degradation analysis of Sitagliptin and Dapagliflozin.

KEYWORDS: Sitagliptin phosphate; Dapagliflozin; RP-HPLC; Forced degradation; Stability-indicating; Isocratic elution.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) remains a major global public health challenge, driving the development of innovative multi-target pharmacological approaches and advanced analytical methodologies, such as validated reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with mass spectrometry and computational safety profiling [1]. In pharmaceutical manufacturing and quality control, monitoring process-related impurities and degradation profiles in active antidiabetic substances is essential to guarantee clinical efficacy and patient safety [2]. Among contemporary fixed-dose combination therapies, the co-formulation of sitagliptin phosphate—a competitive dipeptidyl peptidase-4 (DPP-4) inhibitor—and dapagliflozin propanediol monohydrate—a selective sodium-glucose cotransporter-2 (SGLT2) inhibitor—has gained widespread clinical acceptance, prompting the development of rapid ultraviolet (UV) spectrophotometric quantification methods [3]. To ensure product stability over its shelf-life, establishing stability-indicating RP-HPLC protocols capable of separating intact active pharmaceutical ingredients (APIs) from stress-induced degradation products under various environmental conditions is imperative [4]. Recent trends in chromatographic analysis emphasize the simultaneous determination of SGLT2 inhibitors alongside biguanides and other incretin-based antidiabetic agents in multi-component formulations [5]. Consequently, stability-indicating HPLC methods tailored for sitagliptin and dapagliflozin in commercial tablet formulations serve as vital tools for routine quality assurance [6].

Sitagliptin acts by preserving endogenous incretin hormones (GLP-1 and GIP) to enhance glucose-dependent insulin secretion, whereas dapagliflozin inhibits renal glucose reabsorption in the proximal convoluted tubule to induce glycosuria, representing a highly effective dual-action therapeutic strategy [7]. The simultaneous assay of these co-formulated active substances requires analytical techniques with superior chromatographic resolution,

baseline stability, and high peak capacity [8]. Analytical method validation for sitagliptin phosphate monohydrate and dapagliflozin propanediol monohydrate in finished tablet products must comply strictly with International Council for Harmonisation (ICH) Q2(R1) guidelines [9]. Similar stability-indicating liquid chromatographic methods have been successfully deployed for synthetic mixtures combining dapagliflozin with cardiovascular or metabolic agents [10]. The quantification of sitagliptin and dapagliflozin in bulk raw materials and finished commercial tablets requires demonstrated specificity to distinguish intact drug peaks from degradation products [11]. Furthermore, liquid chromatography-tandem mass spectrometry (LC-MS/MS) hyphenated techniques have played a key role in identifying stress degradants and elucidating chemical degradation pathways for gliflozin-gliptin combinations [12]. Spectrophotometric methods have also been reported for the simultaneous determination of dapagliflozin, sitagliptin, and metformin in triple-combination dosage forms [13]. Dual-wavelength UV-spectrophotometric protocols offer simple, cost-effective screening alternatives for routine assessment of sitagliptin and dapagliflozin [14].
In accordance with ICH Q1A(R2) stress testing guidelines, forced degradation profiling under hydrolytic, oxidative, thermal, and photolytic stress conditions provides critical insights into drug stability pathways [15]. Stability-indicating RP-HPLC studies on sitagliptin phosphate and dapagliflozin propanediol monohydrate demonstrate that stress-induced degradants must be completely resolved from main analyte peaks [16]. Comprehensive reviews of forced degradation methodologies highlight the importance of mobile phase optimization, pH control, and stationary phase selection in establishing stability-indicating power [17]. First-order derivative spectrophotometry has provided an alternative mathematical approach for resolved spectrum estimation in binary sitagliptin and dapagliflozin synthetic mixtures [18]. Multi-component stability testing of dapagliflozin with other gliptins and biguanides underscores the necessity of maintaining acidic mobile phase pH to suppress silanol ionization and prevent peak tailing [19]. Vierordt's simultaneous equation method has similarly been applied for preliminary binary ratio determination [20]. Analytical evaluations of Phase III combination compositions comprising sitagliptin and dapagliflozin revealed that existing liquid chromatographic methods often utilize complex gradient elutions or high buffer concentrations that generate elevated column backpressures [21].

Methodological research on dapagliflozin combined with second-generation gliptins has established key benchmark conditions for reversed-phase separations [22]. Multivariate optimization strategies, such as Quality by Design (QbD), have been increasingly applied to systematically refine liquid chromatographic parameters for antidiabetic fixed-dose combinations [23]. Ultra-performance liquid chromatography (UPLC) protocols have achieved rapid retention times for sitagliptin combined with newer SGLT2 inhibitors like ertugliflozin [24]. Stability studies on dapagliflozin co-formulations consistently demonstrate its inherent sensitivity to oxidative peroxide environments and alkaline hydrolysis [25]. Analytical method development for sitagliptin and dapagliflozin synthetic mixtures has underscored the significance of selecting an optimal isosbestic wavelength to balance molar absorptivity differences between the two drugs [26]. Chromatographic procedures designed for dapagliflozin raw materials have successfully isolated process-related organic impurities and degradation products [27]. Assay protocols for dapagliflozin co-formulated with metformin hydrochloride established linearity, precision, and accuracy parameters across therapeutic concentration ranges [28].

Application of QbD principles in RP-HPLC method development ensures robustness against minor deliberate variations in mobile phase organic content, pH, and flow rate [29]. High-performance thin-layer chromatography (HPTLC) has provided an alternative planar separation technique for dapagliflozin quality control [30]. Kinetic degradation investigations using UHPLC platforms confirmed first-order degradation kinetics for SGLT2 inhibitors under elevated temperature and humidity stress [31]. High-performance liquid chromatography methods optimized for gliptin-gliflozin combinations have also been adapted for bioanalytical and pharmacokinetic applications in biological matrices [32]. Early single-entity RP-HPLC assays for dapagliflozin established foundational retention parameters for C-aryl glucoside structures [33]. Subsequent stability-indicating RP-HPLC studies on fixed-dose combination tablets demonstrated that simple isocratic elution modes can achieve robust separation of drug substances from degradants [34]. Pioneering forced degradation profiling of dapagliflozin under basic, acidic, and peroxide stress established the susceptibility of its benzylic carbon and ethoxy phenyl ether linkages [35].

Building upon these foundational insights, the objective of the present study is to develop and validate a simple, rapid, and cost-effective isocratic RP-HPLC method utilizing a Cosmosil C18 column (250mm × 4.6mm × 5 µm) with a mobile phase of methanol:water (60:40 v/v, pH 3.0) at 219 nm for the simultaneous estimation and forced degradation profiling (alkali and oxidative stress) of Sitagliptin phosphate monohydrate and Dapagliflozin propanediol monohydrate in bulk API.

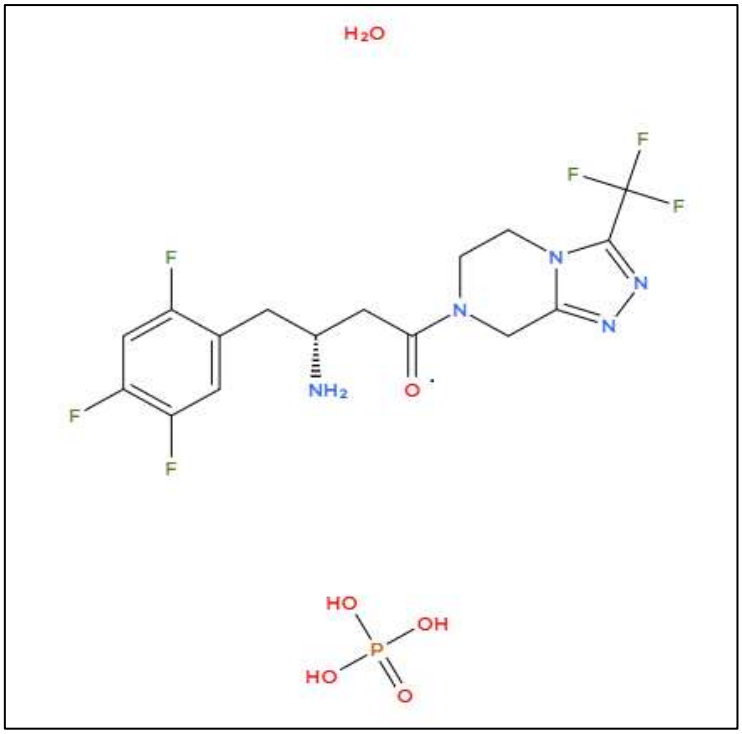

Figure 1: Sitagliptin Phosphate Monohydrate- Chemical Structure

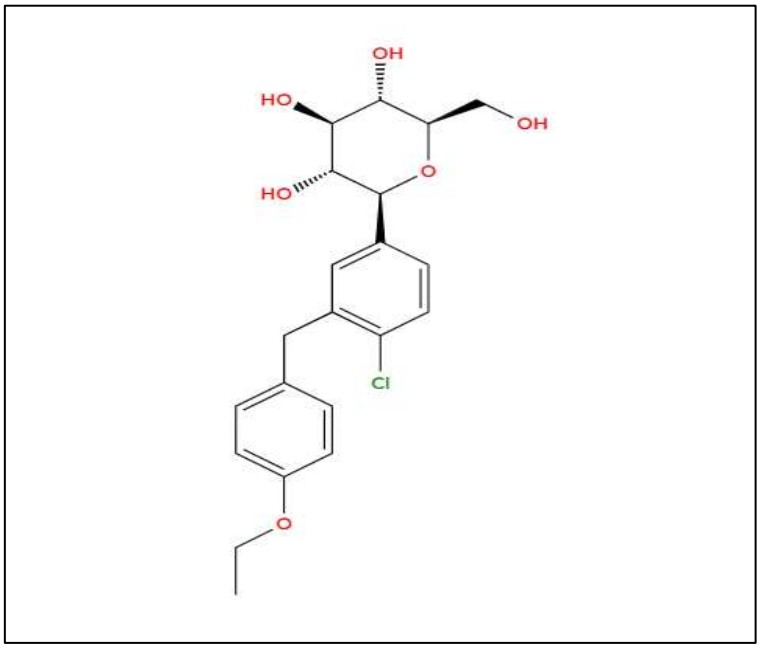

Figure 2: Dapagliflozin- Chemical Structure

MATERIALS AND METHODS

Chemicals and Reagents

Pharmaceutical-grade active pharmaceutical ingredient (API) working standards of Sitagliptin phosphate monohydrate and Dapagliflozin propanediol monohydrate, as well as commercial combined tablet formulations, were utilized. HPLC-grade methanol, orthophosphoric acid (o-phosphoric acid), sodium hydroxide (NaOH), and hydrogen peroxide (H2O2, 3% v/v) were procured from commercial chemical suppliers. High-purity double-distilled water was prepared in-house and used for mobile phase preparation, diluent preparation, and sample processing.

Instrumentation and Chromatographic Configuration

Chromatographic evaluations were carried out using a Shimadzu Prominence HPLC binary gradient system (Shimadzu Analytical (India) Pvt. Ltd.) equipped with an LC-20AD ultra-fast liquid chromatography (UFLC) dual-piston pump operating up to a maximum pressure limit of 40 MPa, an SPD-20A ultraviolet/visible (UV/Vis)

spectrophotometric detector, and LC Solution data acquisition software. Chromatographic separation was performed on a Cosmosil C18 analytical column (250 mm length × 4.6 mm internal diameter, 5 µm particle size). The optimized mobile phase consisted of an isocratic mixture of HPLC-grade methanol and purified water in a 60:40 v/v ratio. The mobile phase pH was adjusted to pH 3.0 ± 0.05 using o-phosphoric acid. The mobile phase was degassed using an ultrasonic bath and filtered through a 0.45 µm nylon membrane filtration unit prior to chromatographic operation. The liquid chromatograph was operated at an optimized flow rate of 0.8 mL/min with an injection volume of 20 µL under ambient column temperature conditions. System operating pressure stabilized in the range of 14 to 15 MPa. UV detection was executed at an isosbestic wavelength of 219 nm.

Preparation of Standard and Working Solutions

Primary stock solutions of Sitagliptin phosphate monohydrate (1000 µg/mL) and Dapagliflozin propanediol monohydrate (1000 µg/mL) were prepared individually by dissolving accurately weighed amounts of reference standards in the mobile phase diluent. Working standard solutions of Sitagliptin phosphate monohydrate (50 µg/mL) and Dapagliflozin propanediol monohydrate (5 µg/mL) were prepared by appropriate volumetric dilution with the mobile phase. A combined working standard solution containing 50 µg/mL Sitagliptin phosphate monohydrate and 5 µg/mL Dapagliflozin propanediol monohydrate was similarly prepared to evaluate inter-analyte chromatographic resolution and system suitability parameters.

Forced Degradation Protocols (Stress Testing)

Forced degradation studies were performed under basic hydrolytic and oxidative stress environments to evaluate the stability-indicating efficiency of the reversed-phase chromatographic procedure.

Alkali-Induced Hydrolysis (Base Degradation)

• **Sitagliptin Phosphate Monohydrate:** Accurately weighed 10 mg of Sitagliptin phosphate monohydrate was transferred into a reaction flask containing 10 mL of 2 N NaOH solution. The solution was condensed at 80 °C for 1 hour. After thermal exposure, the solution was allowed to cool to ambient temperature, adjusted to neutral pH (pH 7.0), and diluted with mobile phase to achieve a working concentration of 50 µg/mL prior to HPLC analysis.

• **Dapagliflozin Propanediol Monohydrate:** Accurately weighed 10 mg of Dapagliflozin propanediol monohydrate was dissolved in 5 mL of methanol and combined with 5 mL of 0.5 N NaOH solution. The reaction mixture was condensed at 60 °C for 1 hour. Upon cooling, the reaction mixture was neutralized to pH 7.0 and diluted with mobile phase to yield a working concentration of 5 µg/mL for chromatographic profiling.

Peroxide-Mediated Oxidation (Oxidative Degradation)

• **Sitagliptin Phosphate Monohydrate:** Accurately weighed 10 mg of Sitagliptin phosphate monohydrate was treated with 10 mL of 3% v/v H_2O_2 solution and maintained at room temperature for 6 hours. Following stress exposure, the sample was diluted with mobile phase to a working concentration of 50 µg/mL and injected into the chromatographic system.

• **Dapagliflozin Propanediol Monohydrate:** Accurately weighed 10 mg of Dapagliflozin propanediol monohydrate was treated with 10 mL of 3% v/v H_2O_2 solution and maintained at room temperature for 6 hours. The resulting solution was diluted with mobile phase to a final working concentration of 5 µg/mL and analyzed by RP-HPLC.

In parallel, reagent blank solutions (such as 3% H_2O_2 blank) were injected under identical chromatographic conditions to confirm that reagent matrix signals did not co-elute or interfere with parent drug peaks or degradation product peaks.

RESULTS AND DISCUSSION

Method Optimization and System Suitability

To establish an efficient, reproducible, and stability-indicating RP-HPLC procedure for the dual determination of Sitagliptin phosphate monohydrate and Dapagliflozin propanediol monohydrate, chromatographic variables including stationary phase selectivity, organic modifier concentration, aqueous phase pH, and detection wavelength were systematically optimized.

A Cosmosil C18 column (250 mm × 4.6 mm, 5 µm particle size) supplied the required lipophilic retention and silanol masking necessary to separate the highly polar triazolopiperazine derivative (Sitagliptin) and the glucoside-bearing C-aryl moiety (Dapagliflozin). The mobile phase composition of methanol and water in a 60:40 v/v ratio provided suitable analyte partitioning without generating excess column backpressure, maintaining operating pressures between 14 MPa and 15 MPa at a flow rate of 0.8 mL/min. Acidification of the aqueous component to pH 3.0 ± 0.05 using orthophosphoric acid suppressed silanol ionization and stabilized the protonated basic sites on Sitagliptin, yielding symmetrical chromatographic peaks with minimal tailing. An isosbestic wavelength of 219 nm was selected to ensure balanced absorbance responses for both target analytes.

System suitability testing was performed by analyzing individual working standard solutions of Sitagliptin phosphate monohydrate (50 ppm) and Dapagliflozin propanediol monohydrate (5 ppm). Sitagliptin eluted at a retention time of 2.788 minutes with a peak area response of 449,495, a theoretical plate count (N) of 4996, and a USP tailing factor (T) of 1.147. Dapagliflozin exhibited a retention time of 4.794 minutes, a peak area response of 377,332, a theoretical plate count of 7124, and a tailing factor of 1.157.

Parameter	Sitagliptin Standard (50 ppm)	Dapagliflozin Standard (5 ppm)	Acceptance Criteria
Retention Time (Rt, min)	2.788	4.794	Reproducible
Peak Area Response	449,495	377,332	Consistent
Theoretical Plates (N)	4996	7124	> 2000
USP Tailing Factor (T)	1.147	1.157	\le 1.5

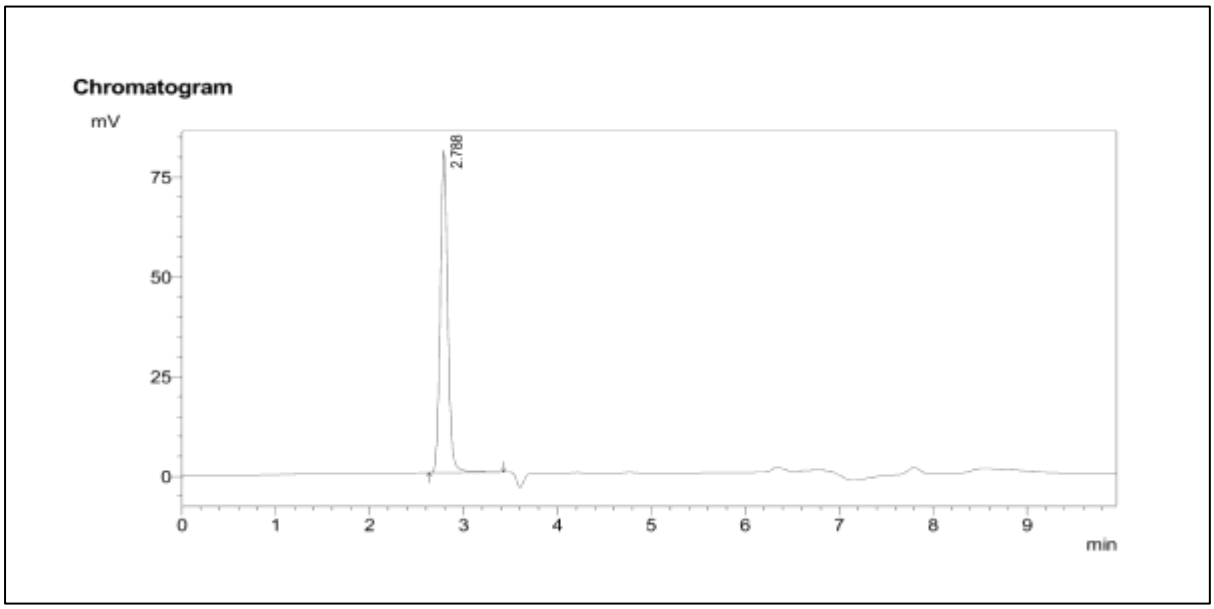

Figure 3: Sitagliptin phosphate monohydrate 50ppm

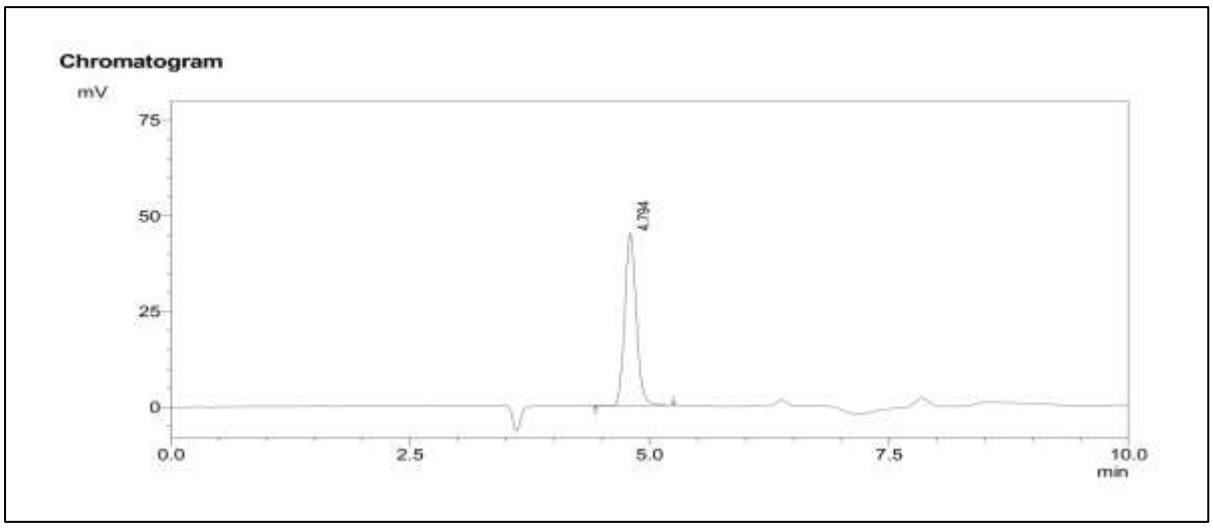

Figure 4: Dapagliflozin 5ppm

Simultaneous Quantitation in Combined Formulations

The capability of the optimized isocratic RP-HPLC system to resolve both drugs simultaneously was validated by injecting a combined standard mixture containing 50 ppm Sitagliptin phosphate monohydrate and 5 ppm Dapagliflozin propanediol monohydrate.

The resulting chromatogram demonstrated clean baseline resolution between the two active analytes. Sitagliptin eluted first at a retention time of 2.789 minutes with a peak area of 442,685, a theoretical plate count of 4921, and a tailing factor of 1.145. Dapagliflozin eluted second at a retention time of 4.777 minutes with a peak area of

384,611, a theoretical plate count of 6516, and a tailing factor of 1.158. The chromatographic resolution (Rs) calculated between the two analyte peaks was 10.045, exceeding the minimum threshold requirement (Rs > 2.0) and confirming complete separation without spectral or physical overlap.

Analyte Peak	Retention Time (Rt, min)	Peak Area	Resolution (Rs)	Theoretical Plates (N)	Tailing Factor (T)
Peak 1: Sitagliptin	2.789	442,685	—	4921	1.145
Peak 2: Dapagliflozin	4.777	384,611	10.045	6516	1.158

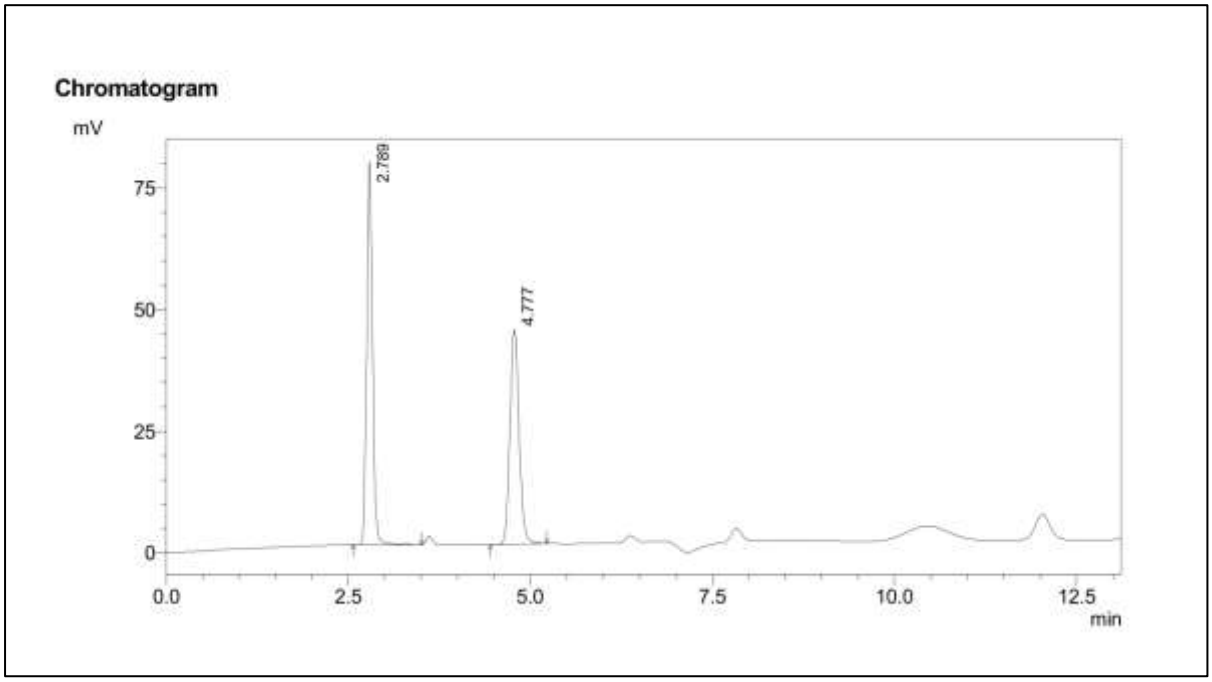

Figure 5: Combination Sitagliptin+Dapagliflozin (50+5ppm)

Forced Degradation Studies (Stress Testing)

To demonstrate the stability-indicating property of the method, forced degradation studies were carried out on Sitagliptin phosphate monohydrate and Dapagliflozin propanediol monohydrate under alkaline hydrolysis and hydrogen peroxide-mediated oxidative stress conditions.

Alkali-Induced Degradation

Exposure of Sitagliptin phosphate monohydrate (50 ppm) to 2 N sodium hydroxide at 80 °C for 1 hour produced significant chemical transformation. The chromatographic profile revealed three distinct degradation product peaks in addition to the parent drug. The intact Sitagliptin peak appeared at a retention time of 3.000 minutes (peak area: 544,431, N = 7257, T = 1.385). The three degradation products eluted at retention times of 6.421 minutes (peak area: 28,860, Rs = 18.705), 7.922 minutes (peak area: 90,407, Rs = 6.232), and 12.220 minutes (peak area: 204,199, Rs = 13.395). High resolution values (Rs \ge 6.232) across all peaks confirmed that base degradation products elute well clear of the parent drug signal.

For Dapagliflozin propanediol monohydrate (5 ppm), alkali hydrolysis with 0.5 N sodium hydroxide in methanol at 60 °C for 1 hour led to substantial drug cleavage. In primary testing, six chromatographic peaks were identified. The residual Dapagliflozin parent peak eluted at 4.743 minutes (peak area: 295,178, N = 7065, T = 1.216). Four distinct degradation product peaks were recorded at retention times of 2.803 minutes (peak area: 172,021), 3.589 minutes (peak area: 24,425), 6.283 minutes (peak area: 68,423), 7.705 minutes (peak area: 74,134), and 11.809 minutes (peak area: 53,075). In a confirmatory replicate run, the parent peak shifted slightly to 4.781 minutes (peak area: 295,724), accompanied by three major degradation peaks at 2.808 minutes (peak area: 167,410), 3.594 minutes (peak area: 23,588), 6.323 minutes (peak area: 20,672), and 7.769 minutes (peak area: 65,923).

Stress Condition	Target Analyte	Peak #	Retention Time (Rt, min)	Peak Area	Resolution (Rs)	Theoretical Plates (N)	Tailing Factor (T)

2 N NaOH, 80 °C, 1 h	Sitagliptin	1 (Parent)	3.000	544,431	—	7257	1.385
		2 (Degradant 1)	6.421	28,860	18.705	13033	1.164
		3 (Degradant 2)	7.922	90,407	6.232	15226	1.122
		4 (Degradant 3)	12.220	204,199	13.395	16131	1.075
0.5 N NaOH, 60 °C, 1 h	Dapagliflozin	1 (Degradant 1)	2.803	172,021	—	8846	1.493
		2 (Degradant 2)	3.589	24,425	5.652	8157	1.250
		3 (Parent)	4.743	295,178	6.002	7066	1.216
		4 (Degradant 3)	6.283	68,423	2.796	823	3.093
		5 (Degradant 4)	7.705	74,134	2.281	6913	0.855
		6 (Degradant 5)	11.809	53,075	10.989	15752	1.096

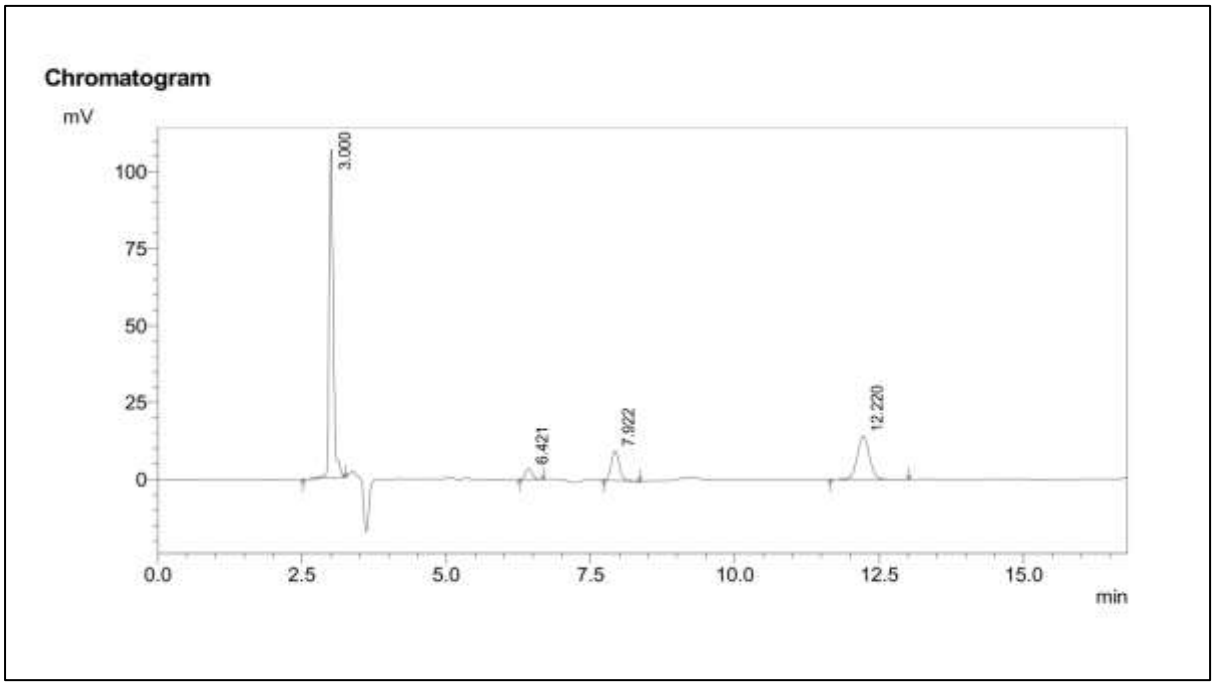

Figure 6: Sitagliptin phosphate monohydrate 50ppm Base Degradation (2N 80°C 1hr)

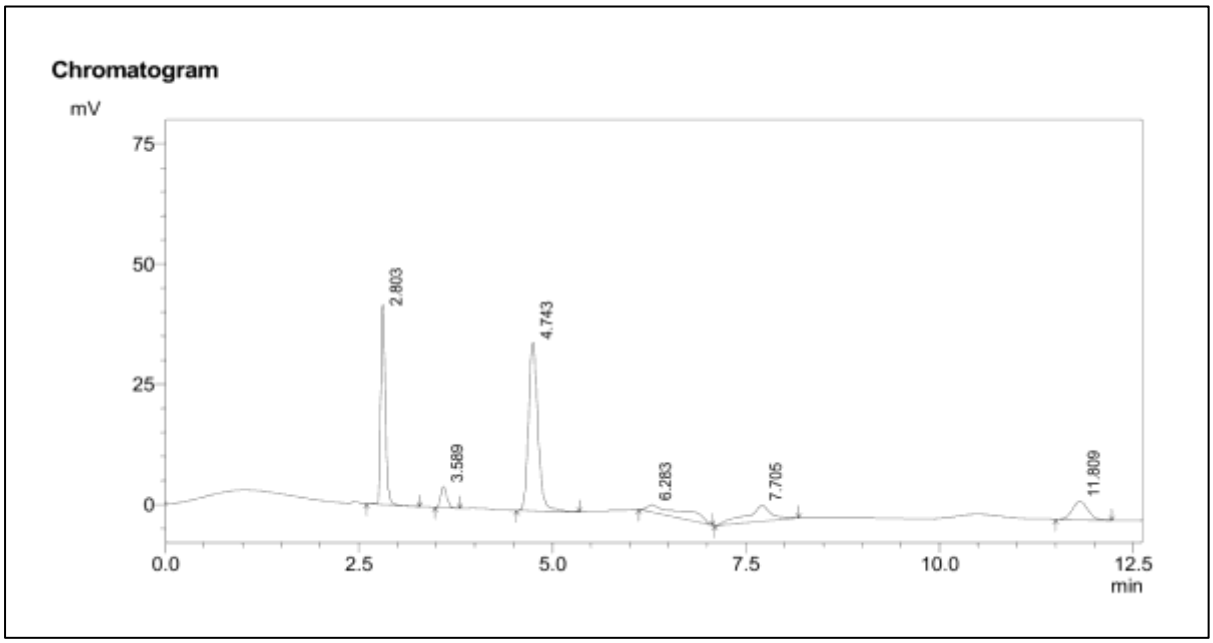

Figure 7: Dapagliflozin 5ppm Base (0.5N) Degradation 60°C 1hr

Oxidative Degradation

Prior to evaluating sample oxidation, a reagent blank containing 3% v/v hydrogen peroxide was injected. The hydrogen peroxide matrix signal eluted at 3.599 minutes with a peak area of 12,881,149, a theoretical plate count of 6872, and a tailing factor of 1.377.

Treatment of Sitagliptin phosphate monohydrate (50 ppm) with 3% v/v hydrogen peroxide for 6 hours at room temperature yielded a single degradation product peak. Intact Sitagliptin eluted at 2.724 minutes (peak area: 417,456, N = 4976, T = 1.144). The hydrogen peroxide reagent peak appeared at 3.604 minutes (peak area: 13,371,100, Rs = 5.339), while the single oxidative degradation product eluted cleanly at 5.357 minutes (peak area: 34,839, Rs = 9.429, N = 11872, T = 1.158).

Under identical oxidative stress conditions (3% v/v hydrogen peroxide for 6 hours at room temperature), Dapagliflozin propanediol monohydrate (5 ppm) displayed greater sensitivity, yielding four distinct degradation product peaks. The reagent peak eluted at 3.496 minutes (peak area: 1,735,858). The remaining Dapagliflozin core signal appeared at 4.303 minutes (peak area: 319,182, Rs = 3.896), flanked by four degradation product peaks eluting at retention times of 2.583 minutes (peak area: 60,585), 5.875 minutes (peak area: 25,886, Rs = 7.934), 7.292 minutes (peak area: 54,348, Rs = 5.158), and 10.546 minutes (peak area: 97,940, Rs = 11.172).

Stress Condition	Target Sample	Peak Assignment	Retention Time (Rt, min)	Peak Area	Resolution (Rs)	Theoretical Plates (N)	Tailing Factor (T)
Blank Reagent	3% H2O2	Matrix Blank	3.599	12,881,149	—	6872	1.377
3% H2O2, RT, 6 h	Sitagliptin	Peak 1: Sitagliptin	2.724	417,456	—	4976	1.144
		Peak 2: Reagent	3.604	13,371,100	5.339	6777	1.380
		Peak 3: Oxidative Degradant	5.357	34,839	9.429	11872	1.158
3% H2O2, RT, 6 h	Dapagliflozin	Peak 1: Degradant 1	2.583	60,585	—	4470	1.315
		Peak 2: Reagent Peak	3.496	1,735,858	5.760	7944	1.304
		Peak 3: Parent / Peak	4.303	319,182	3.896	6741	1.192
		Peak 4: Degradant 2	5.875	25,886	7.934	11686	1.182
		Peak 5: Degradant 3	7.292	54,348	5.158	12981	1.198
		Peak 6: Degradant 4	10.546	97,940	11.172	13605	2.186

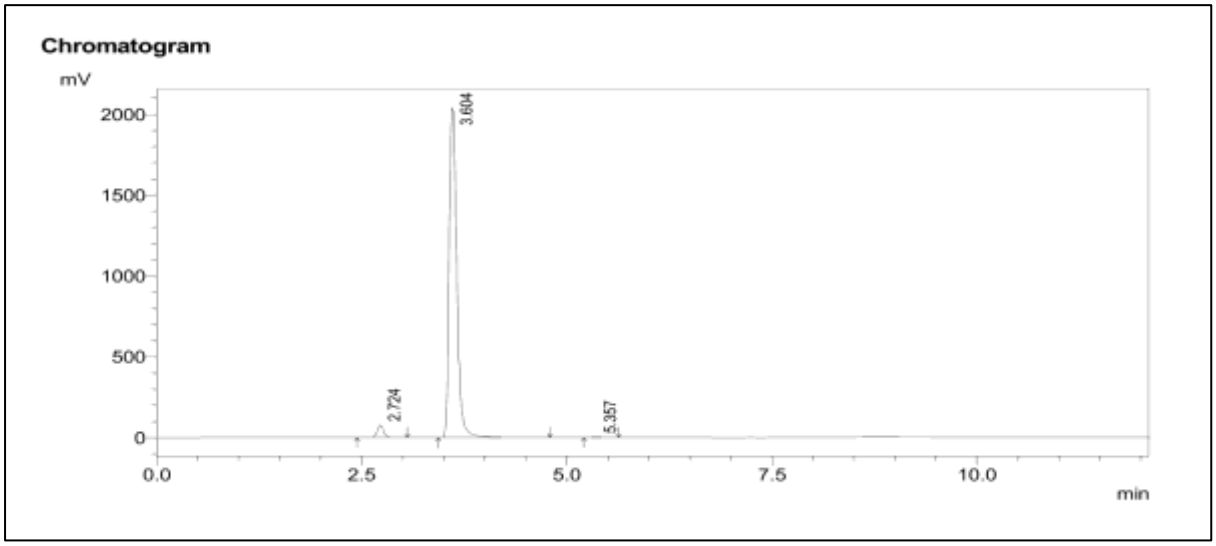

Figure 8: Sitagliptin phosphate monohydrate 50ppm H2O2 6hrs

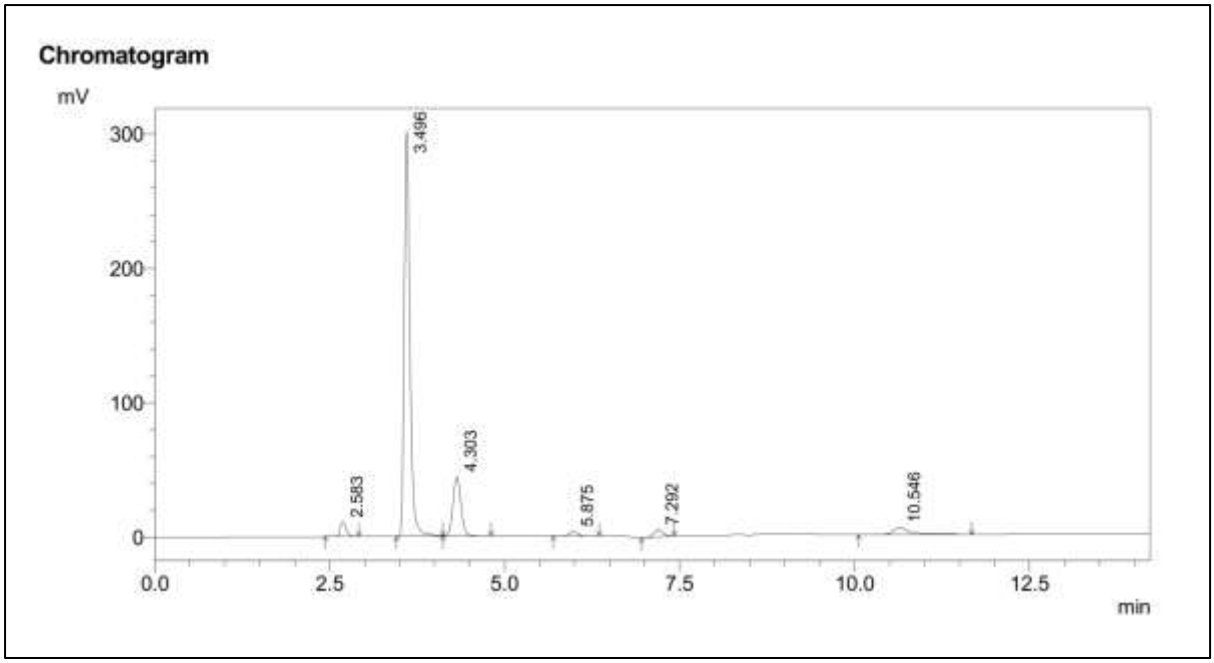

Figure 9: Dapagliflozin 5ppm H2O2 6hrs

DISCUSSION

The experimental findings demonstrate that the developed isocratic RP-HPLC method effectively separates Sitagliptin and Dapagliflozin while maintaining complete resolution from all stress-induced degradation products. The choice of an acidic mobile phase (pH 3.0) prevented secondary silanol interactions, ensuring sharp, symmetrical peaks (T \le 1.158) and high column efficiency (N > 4900) for both active pharmaceutical ingredients under standard run conditions.

The forced degradation profiles highlight distinct chemical susceptibilities between the two antidiabetic agents. Sitagliptin showed high stability under oxidative stress, generating only one degradation product (Rt = 5.357 min), but experienced notable cleavage under strong alkaline conditions, producing three degradants (Rt = 6.421, 7.922, and 12.220 min). Conversely, Dapagliflozin displayed greater vulnerability to both alkaline hydrolysis and peroxide oxidation, generating four distinct degradation product peaks under each stress state. The formation of multiple degradants in Dapagliflozin can be attributed to the oxidative vulnerability of its benzylic carbon and the base sensitivity of its C-glucoside structure.

Crucially, in every stress testing evaluation, all degradation product peaks maintained adequate resolution (Rs > 2.2) relative to the parent active drugs. This structural separation confirms the stability-indicating capability of the optimized HPLC method, establishing its suitability for routine quality assurance, shelf-life monitoring, and degradation analysis in pharmaceutical tablet formulations.

CONCLUSION

An isocratic stability-indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method was successfully developed and evaluated for the simultaneous determination and forced degradation profiling of Sitagliptin phosphate monohydrate and Dapagliflozin propanediol monohydrate. Utilizing a Cosmosil C18 analytical column (250 mm × 4.6 mm, 5 µm particle size) with a mobile phase consisting of methanol and water (60:40 v/v ratio, adjusted to pH 3.0 using orthophosphoric acid) at a flow rate of 0.8 mL/min and an isosbestic wavelength of 219 nm, the protocol achieved rapid and baseline-resolved separations within short run times. Under optimized operating conditions, intact Sitagliptin and Dapagliflozin eluted at retention times of 2.789 minutes and 4.777 minutes, respectively, displaying exceptional inter-analyte chromatographic resolution (Rs = 10.045), high column efficiency (N > 4900), and acceptable peak symmetry (T \le 1.158).

Forced degradation experiments verified the stability-indicating capacity of the chromatographic system under basic hydrolytic and oxidative stress conditions. Sitagliptin demonstrated relative stability against peroxide-mediated oxidation, generating a single degradation product (Rt = 5.357 minutes) when exposed to 3% H2O2 for 6 hours, whereas alkaline stress (2 N NaOH, 80 °C, 1 h) converted the drug into three distinct degradation products (Rt = 6.421, 7.922, and 12.220 minutes). Dapagliflozin exhibited higher chemical sensitivity, forming four fully resolved degradation product peaks under both basic hydrolysis (0.5 N NaOH, 60 °C, 1 h) and oxidative stress (3% H2O2, 6 h). Importantly, all stress-induced degradation peaks separated completely from intact active parent signals (Rs > 2.2) with zero baseline co-elution or spectral overlap.

In conclusion, the established RP-HPLC analytical method provides key operational benefits, including a simple isocratic mobile phase composition, low organic solvent usage, rapid sample throughput, and robust degradation profiling without requiring complex buffer salts or gradient programming. Consequently, this validated technique represents a reliable, cost-effective, and stability-indicating tool for routine quality control testing, batch release, and shelf-life stability monitoring of Sitagliptin and Dapagliflozin in both bulk active pharmaceutical ingredients and combined tablet dosage forms.

ABBREVIATIONS

API: Active Pharmaceutical Ingredient; DPP-4: Dipeptidyl Peptidase-4; GIP: Glucose-Dependent Insulinotropic Polypeptide; GLP-1: Glucagon-Like Peptide-1; H2O2: Hydrogen Peroxide; HPLC: High-Performance Liquid Chromatography; HPTLC: High-Performance Thin-Layer Chromatography; ICH: International Council for Harmonisation; ID: Internal Diameter; LC: Liquid Chromatography; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry; MeOH: Methanol; MPa: Megapascal; N: Number of Theoretical Plates; NaOH: Sodium Hydroxide; o-phosphoric acid: Orthophosphoric Acid; ppm: Parts Per Million; QbD: Quality by Design; RP-HPLC: Reversed-Phase High-Performance Liquid Chromatography; Rs: Chromatographic Resolution; Rt: Retention Time; SGLT2: Sodium-Glucose Cotransporter-2; SPD: Spectrophotometric Detector; T: USP Tailing Factor; T2DM: Type 2 Diabetes Mellitus; UFLC: Ultra-Fast Liquid Chromatography; UHPLC/UPLC: Ultra-High Performance Liquid Chromatography / Ultra-Performance Liquid Chromatography; USP: United States Pharmacopeia; UV/Vis: Ultraviolet / Visible Spectrophotometry; v/v: Volume per Volume Ratio.

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