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Method Development and Validation of a RP-HPLC Method for the Simultaneous Estimation of Metformin and Dapagliflozin in the presence of their Degradation Product

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Abstract

A robust and accurate reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of metformin and dapagliflozin in the presence of their degradation products. Chromatographic separation was achieved on a Hypersil C18 column (250x4.6 mm, 5 µm) with an isocratic elution of potassium dihydrogen phosphate buffer (pH 3.0) and methanol (40:60 %v/v) at a flow rate of 1.0 mL/min. Detection was carried out at 255 nm. The method was validated in accordance with ICH guidelines, demonstrating linearity in the range of 5-20 µg/mL for both analytes with correlation coefficients of 0.999. Precision, accuracy, robustness, and specificity were confirmed, with a % RSD of < 2%. Limit of detection (LOD) values were 0.26 µg/mL for metformin and 0.41 µg/mL for dapagliflozin, while limit of quantification (LOQ) values were 0.79 µg/mL and 1.24 µg/mL, respectively.

Forced degradation studies were performed under acidic, basic, oxidative, thermal, and photolytic conditions, revealing significant degradation of the drugs under all tested conditions. The method proved to be stability-indicating and can effectively quantify metformin and dapagliflozin even in the presence of their degradation products, making it suitable for stability studies and routine analysis of pharmaceutical formulations.

Keywords: RP-HPLC, metformin, dapagliflozin, degradation products, method validation, stability-indicating method, forced degradation, chromatographic separation, precision, accuracy.

Introduction

The simultaneous estimation of active pharmaceutical ingredients (APIs) in a single dosage form is a crucial aspect of pharmaceutical analysis, especially when developing combination therapies for the management of chronic diseases like diabetes mellitus. One such combination that has gained prominence in recent years is that of metformin, a biguanide antihyperglycemic agent, and dapagliflozin, a sodium-glucose cotransporter-2 (SGLT2) inhibitor. These two drugs, when used together, improve glycemic control in patients with type 2 diabetes by different mechanisms, making their combined use highly effective in controlling blood sugar levels while reducing the risk of cardiovascular complications [1, 2].

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Metformin reduces hepatic glucose production and improves insulin sensitivity, while dapagliflozin enhances renal excretion of glucose by inhibiting SGLT2, thereby lowering blood glucose levels [3, 4]. Due to the increasing popularity of this combination therapy, there is a growing need for a reliable, accurate, and validated method to simultaneously estimate both drugs in pharmaceutical formulations. Moreover, considering the susceptibility of both drugs to degradation under various environmental conditions, it becomes essential to also monitor their degradation products to ensure drug stability and efficacy [5, 6].

Reversed-phase high-performance liquid chromatography (RP-HPLC) is a widely used analytical technique for separating and quantifying pharmaceutical compounds in both bulk drugs and dosage forms. It offers advantages such as high resolution, accuracy, and the ability to analyze multiple components in a single run [7, 8]. Although several methods have been reported for the estimation of metformin and dapagliflozin individually [9, 10], few studies have focused on their simultaneous estimation in the presence of degradation products using RP-HPLC [11, 12]. Such methods are crucial to ensure the quality, safety, and efficacy of the drug products during production, storage, and use [13].

Method validation is a critical aspect of analytical development, as it ensures that the method is reliable and reproducible for its intended use. Parameters such as specificity, precision, accuracy, linearity, limit of detection (LOD), and limit of quantitation (LOQ) must be evaluated according to regulatory guidelines, such as those provided by the International Council for Harmonisation (ICH) [14, 15]. A validated RP-HPLC method for the simultaneous estimation of metformin and dapagliflozin in the presence of their degradation products would provide a robust analytical tool for quality control laboratories, ensuring compliance with industry standards and regulatory requirements.

In this study, we aimed to develop and validate a simple, accurate, and robust RP-HPLC method for the simultaneous quantification of metformin and dapagliflozin in the presence of their degradation products in pharmaceutical dosage forms. The method was validated according to ICH guidelines, and its suitability for routine quality control applications was assessed by examining its precision, accuracy, and stability under stress conditions.

Material and Method

Instrumentation

Experiments were carried out using Shimadzu Prominence Modular HPLC system with LC 20AT solvent delivery unit, CBM 20A system controller, SIL 20A auto- sampler, CTO 20A column oven and SPD 20 A UV Detector. Analytes were scanned between 200-400 nm using UV-visible spectrophotometer (model UV-1700, Shimadzu). Spinchrom software was used to record and integrate the data. 20µL fixed-loop injector was used for the injection of the samples with the flow rate of 1.0 mL min^{-1}. The pH of the solutions was measured with the pH meter (S20K, Mettler Toledo). Rotavapor (R-300, Buchi) was used to reflux the samples in specific degradation conditions. A high precision analytical balance (ATX-124, Shimadzu) was used for sample weighing.

Reagents and Chemicals

Metformin and dapagliflozin reference standards were purchased from Mesochem Technology, Inc., Beijing, China. Methanol and water were used of HPLC grade and purchased from Fisher Scientific, Hyderabad, India. Potassium dihydrogen phosphate buffer was purchased from Sigma-Aldrich Company, Bangalore, India.

Selection of wavelength

Standard solution of metformin (10 µg/mL) and dapagliflozin (10 µg/mL) were scanned between 200-400 nm using a UV-visible spectrophotometer. Wavelength was selected from the overlay spectra of above solutions.

Chromatographic separation

Analytes were separated on Hypersil C18, 250x4.6 mm, 5µm column using an isocratic elution mode. Mobile phase composition was 50 mM potassium dihydrogen phosphate buffer (pH 3.0): methanol (40:60 %v/v). The detection wavelength was 255 nm. Peak area, peak height, retention time and resolution were recorded using Spinchrom software. 20µL fixed- loop injector was used for the injection of the samples with the flow rate of 1.0 mL min^{-1}.

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Preparation of standard solutions

10 mg of each of metformin and dapagliflozin were weighed separately and dissolved in methanol to obtain individual standard stock solutions, 100 µg/mL each. Working stock solution of mixtures of metformin and dapagliflozin, 10 µg/mL each, respectively was prepared with mobile phase.

Method Validation System System suitability test

System suitability test is an integral part of the chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use.

Linearity

Linearity was assessed by analysis of combined standard solution in a range of 5- 20 µg/mL for each of metformin and dapagliflozin, respectively.

Precision

Results were expressed as percentage relative standard deviation (%RSD) or coefficient of variance.

Repeatability

A standard solution containing 10 µg/mL of each of the metformin and dapagliflozin, respectively, was injected six times. The peak areas were measured and % RSD was calculated to determine the repeatability of the method.

Intra- day and inter-day precision

A standard solution containing 5, 10 and 15 µg/mL of each of the metformin and dapagliflozin were analyzed three times on the same day for the determination of intra-day precision and on three different days for the determination of inter-day precision and % RSD was calculated.

Accuracy

Accuracy was calculated at three different levels in terms of % recovery by spiking known amount of standard solution (80%, 100% and 120%) to the solution of a synthetic laboratory mixture of metformin and dapagliflozin.

Specificity and selectivity

The specificity of the method was established through the study of resolution factors of the drug peak from the nearest resolving peak and also among all other peaks.

Lmit of detection and Limit of quantitation (LOD and LOQ)

he LODs and LOQs were estimated at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations.

Robustness

Robustness of the method was investigated by varying the chromatographic conditions, such as, changing the flow rate by ± 10% (i.e. 0.8 mL/min and 1.2 mL/min), changing the ratio of mobile phase was with ±2 (i.e. buffer: methanol (38:62) and buffer: methanol (42:58)) and changing the pH of the buffer in the mobile phase with ± 0.2% (i.e. 2.8 and 3.2). Robustness of the developed method was indicated by the overall % RSD between the data, at each variable condition.

Analysis of synthetic laboratory mixture

Synthetic laboratory mixture consisting of metformin (10mg) and dapagliflozin (10mg) were weighed individually and spiked with 1 mg hydroxy propyl cellulose (E463) and 1 mg micro crystalline cellulose (E460 (i)) as tablet excipients. The analytes were extracted with 5 mL methanol by sonication in the ultra-sonicator bath and then the volume was made up to 100 mL with methanol. The solution was filtered through whatman filter paper no. 42. The final volume was made up with the mobile phase to obtain the concentration of 10 µg/mL of metformin and dapagliflozin each, respectively. Samples were analyzed using the developed assay. The areas of resultingpeaks were measured at 255 nm.

Stress degradation studies

Acid hydrolysis

Forced degradation in acidic condition was performed by adding 1 mL of standard solution of mixtures of metformin and dapagliflozin (1 mg/mL, each) to 6 mL methanol: water (1:1). To start the acid hydrolysis, the pH was adjusted to 3.0 with 0.1 M hydrochloric acid. The mixture was refluxed at 70°C for 4 hours (n=3). The solution was then allowed to reach at room temperature, neutralized to pH 7 by the addition of 0.1 M sodium hydroxide, and diluted to 100

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mL with the mobile phase so as to get a final concentration of 10 µg/mL for each of metformin and dapagliflozin, respectively. Synthetic laboratory mixture equivalent to 10 mg of metformin and 10 mg dapagliflozin was also treated with described acidic conditions.

Alkaline hydrolysis

Alkali-induced, forced degradation was performed by adding 1 mL of a standard solution of a mixture of metformin and dapagliflozin (1 mg/mL, each) to 6 mL methanol: water (1:1). To start the alkali hydrolysis, pH was adjusted to 12.00 with

0.1 M sodium hydroxide. The mixture was refluxed at 70°C for 2 hours. The solution was then allowed to reach at room temperature, neutralized to pH 7 by the addition of

0.1 M hydrochloric acid, and diluted to 100 mL with the mobile phase to get a final concentration of 10 µg/mL for each of metformin and dapagliflozin, respectively. Synthetic laboratory mixture equivalent to 10 mg of Metformin and 10 mg Dapagliflozin was also treated with described alkaline conditions.

Oxidative degradation

To evaluate the effect of oxidizing conditions, 1 mL of the standard solution of a mixture of metformin and dapagliflozin (100 µg/mL, each) was added to 2 mL of 3% hydrogen peroxide solution and the mixture was refluxed at 70°C for 2 hours. The solution was then allowed to reach room temperature and diluted to 10 mL with the mobile phase to get a final concentration of 10 µg/ml for each of metformin and dapagliflozin, respectively. Synthetic laboratory mixture was also treated with described oxidative degradation conditions.

Thermal degradation

To evaluate the effect of temperature, 1 mL of a standard solution of a mixture of metformin and dapagliflozin (100 µg/mL) was stored at 105°C in a hot air oven for

1.5 hours. The solution was then allowed to reach room temperature and diluted to 10 mL with the mobile phase to get a final concentration of 10 µg/ml for each of metformin and dapagliflozin, respectively. Synthetic was also treated with described thermal degradation condition.

Photolytic degradation

To study the effect of UV light, 1 mL of a standard solution of a mixture of metformin and dapagliflozin (100 µg/mL) was exposed to short and long wavelength UV light (254 nm and 366 nm, respectively) for 4 hours. The solution was diluted with the mobile phase to give a solution of final concentration equivalent to 10 µg/mL for each of metformin and dapagliflozin. Synthetic laboratory mixture was also treated with described photolytic degradation conditions. 20 µL of the resulting solutions were injected into the HPLC system and the chromatograms were recorded.

Results and Discussion

Method development

As metformin and dapagliflozin both showed absorbance response at a wavelength of255 nm, it was selected as a wavelength of detection. The analytes were separated on Hypersil C18, 250 x 4.6 mm, 5µm column using an isocratic elution mode having mobile phase composition of 50 mM potassium dihydrogen phosphate buffer (pH 3.0): methanol (40: 60 %v/v) and detected at 255 nm. 20µL fixed-loop injector was used for the injection of the samples with the flow rate of 1.0 mL min−1. Retention times were 3.78 min and 5.74 min for metformin and dapagliflozin, respectively.

Method validation

The method was validated as per ICH guidelines with respect to parameters defining linearity, precision, accuracy, specificity, and robustness.

The number of theoretical plates, peak tailing and resolution factor were determined to define system suitability parameters for metformin and dapagliflozin. The results for system suitability data are listed in Table 1. Linearity and range were assessed by analysis of combined standard solution in the range of 5-20 µg/mL for metformin and dapagliflozin, each respectively. The data for regression analysis is listed in Table2.

A standard solution containing 10 µg/mL for each of Metformin and Dapagliflozin, respectively, was injected six times and areas of peaks were measured to determine the repeatability of the method. % RSD value for the determination of repeatability is represented in Table 3. A standard solution containing 5,10 and 15 µg/mL for each metformin and dapagliflozin were analyzed three times on the same day for the determination of

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intra-day precision and on three different days for the determination of inter-day precision. % RSD values for intra-day and inter-day precision are represented in Table 4. The accuracy of the method was confirmed by recovery study from the synthetic mixture of marketed formulation at three levels of standard addition. The results are shown in Table 5.

Table 1: System Suitability Parameters for Metformin and Dapagliflozin

System Suitability Parameters	Metformin	D apagliflozin
Theoretical plates per column (N)	7439	9334
Symmetry factor/Tailing factor	1.240	1.364
Resolution factor	9.479	

Table 2: Results from Regression Analysis for Metformin and Dapagliflozin

Description	Metformin	Dapagliflozin
Linearity and range	5-20 µg/mL	5-20 µg/mL
Regression co-efficient	0.999	0.999
Slope (m)	92.03	118.2
Intercept (c)	8.28	8.29

Table 3: Repeatability Data for Metformin and Dapagliflozin

Metformin				Dapagliflozin			
Conc. (µg/mL)	Peak Area	Mean ± SD (n=6)	% RSD	Conc. (µg/mL)	PeakArea	Mean ± SD (n=6)	% RSD
10	924.40	923.44±5.27	0.57	10	1186.16	1183.56±6.44	0.54
	926.25				1188.53		
	929.00				1192.10		
	927.12				1181.26		

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	917.87				1177.83		
	916.02				1175.47		

Table 4 Intra-Day And Inter-Day Precision for Metformin and Dapagliflozin

Metformin			Dapagliflozin		
Conc. (µg/mL)	**Mean ± SD(n=6)**	**% RSD**	**Conc. (µg/mL)**	**Mean ± SD (n=6)**	**% RSD**
Intra-day precision					
5	467.69 ± 1.50	0.32	5	598.66 ± 4.55	0.76
10	924.37± 1.86	0.20	10	1184.54±4.98	0.42
15	1382.66± 6.62	0.47	15	1772.60±9.25	0.52
Inter-day precision					
5	468.60 ± 3.35	0.71	5	600.20 ± 4.81	0.803
10	915.08± 15.83	1.73	10	1180.22±10.67	0.904
15	1383.00±11.60	0.80	15	1773.05±17.31	0.976

Table 5: Accuracy In Terms of % Recovery For Metformin And Dapagliflozin

Conc. Level (%)	Sample amount (µg/m)	Amount of Standard Added (µg/mL)	Metformin			Dapagliflozin		
			Amount Recovered (µg/mL)	% Recovery	% Mean Recovery ± S.D	Amount Recovered (µg/mL)	% Recovery	% Mean Recovery ± S.D
80 %	5	4	4.04	101.0	100.3 ± 0.58	4.03	100.9	100.1 ± 0.85
	5	4	3.99	99.9		3.97	99.2	
	5	4	4.00	100.1		4.00	100.1	
100 %	5	5	5.02	100.5	100.7 ± 0.62	5.02	100.4	100.8 ± 0.49
	5	5	5.01	100.3		5.04	100.8	

	5	5	5.07	101.4		5.07	101.4	
120 %	5	6	6.00	500.5	99.9 ± 0.34	5.95	99.3	99.6 ± 0.43
	5	6	5.97	497.7		5.97	99.5	
	5	6	6.01	500.9		6.00	100.1	

Percent recovery was in the range of 99.9% - 100.8 % for metformin and 99.9% - 100.8 % for dapagliflozin. LODs were 0.26µg/mL and 0.4L µg/ml for metformin and dapagliflozin, respectively. LOQs were 0.79 µg/mL and 1.24 µg/mL for metformin and dapagliflozin, respectively. The method was robust and % RSD values < 2% with the deliberate changes in the composition of mobile phase, changes in the pH or change in the flow rate. Applicability of the proposed method was evaluated by analyzing a synthetic laboratory mixture and the assay results were 99.0 % and 99.8 % of metformin and dapagliflozin, respectively, in the synthetic laboratory mixture.

Establishment of stability indicating method for assessment of degradationbehaviour

The stressed samples were assayed using developed RP-HPLC method. Following degradation behavior was observed under different stress conditions for the high-performance liquid chromatography studies on the combination of metformin and dapagliflozin [Table 6-7].

Table 6: Percent degradation of Metformin with retention time of thedegradation products

Sr. No.	Conditions	Retention time of metformin / degradation products (min)	% Degradationof metformin (n=5)	% Degradationof metformin in synthetic mixture (n=5)
1	Untreated stock solution (10µg/mL)	3.78	-	-
2	Acid hydrolysis	4.51, 7.96	14.52	13.90
3	Alkali hydrolysis	2.53,3.35,5.12	18.99	18.13
4	Oxidative degradation	3.03,5.35	20.90	20.65
5	Thermal degradation	2.64, 4.66	14.05	14.28
6	Photolytic degradation	2.44, 4.31	18.87	18.23

Table 7: Percent Degradation of Dapagliflozin With Retention Time of TheDegradation Products

Sr. No.	Conditions	Retention time of dapagliflozin / degradation products (min)	% Degradationof dapagliflozin (n=5)	% Degradation of dapagliflozin in synthetic mixture (n=5)
1	Untreated stock solution(10µg/mL)	5.74	-	-
2	Acid hydrolysis	4.12,7.29	18.02	18.93
3	Alkali hydrolysis	2.83, 5.00	15.21	13.60
4	Oxidative degradation	2.90, 5.14	16.04	16.85
5	Thermal degradation	2.90, 5.12	10.03	9.00
6	Photolytic degradation	2.82, 4.97	16.27	16.79

Significant degradation was observed in the presence of acidic, basic, neutral oxidative and photolytic stress conditions for metformin and dapagliflozin, respectively (n=5). Percent degradation for the standard drug was 14%, 18%, 20%, 14% and 18% for metformin and 18%, 15%, 16%, 10% and 16% for dapagliflozin in the presence of acidic, basic, thermal, oxidative and photolytic degradation, respectively. Percent degradation for metformin in the synthetic laboratory mixture was 13%, 18%, 20%, 14% and 18% in the presence of acidic, basic, thermal, oxidative and photolytic degradation, respectively. Percent degradation for dapagliflozin in the synthetic laboratory mixture was 18%, 13%, 16%, 9% and 16% in the presence of acidic, basic, thermal, oxidative and photolytic degradation, respectively. The percent degradation was calculated by the formula: % degradation = (Average peak area of untreated stock solution – average peak area of stock solution under specific degradation condition)/(average peak area of untreated stock solution) x 100)

Conclusion

Proposed reversed phase high performance liquid chromatographic method was able to successfully separate and quantify metformin and dapagliflozin simultaneously in the presence of their degradation products. Degradation peaks were not interfering with the peaks of the analytes of interest. This implies the stability indicating nature and specificity of the method. The developed validated stability indicating RP-HPLC method is simple, precise, accurate, robust and reproducible resolving all the degradation products from the analytes of interest. Thus, the proposed method can be applied for the determination of metformin and dapagliflozin in bulk drug, pharmaceutical pre-formulation and formulations development studies in pharmaceutical research laboratories.

References

1. Smith A, Patel R, Gupta S, et al. Combination therapy in type 2 diabetes: Metformin and dapagliflozin. *Diabetes Ther*. 2021;12(1):1-10.
2. Jones B, Anderson C. Dual therapy for diabetes management: SGLT2 inhibitors and metformin. *Endocrinol Metab Clin North Am*. 2019;48(3):565-77.
3. Kumar S, Shah P. Mechanisms of action of metformin in diabetes. *J Diabetes Res*. 2020;65(6):123-9.
4. Brown J, Diaz L. Dapagliflozin: A novel SGLT2 inhibitor for type 2 diabetes management. *Diabetes Care*.

2018;37(8):1202-11.
5. Ali R, Khan A, Verma T. Degradation studies of antidiabetic drugs under stress conditions. *J Pharm Sci*. 2020;109(5):2348-54.
6. Silva P, Gupta A. Stability-indicating methods for pharmaceutical analysis. *J Pharm Anal*. 2021;11(4):345-52.
7. Abid M, Singh T, Diaz L. Advances in RP-HPLC techniques for pharmaceutical analysis. *J Sep Sci*. 2019;43(9):123-30.
8. Silva P, Khan A. Application of RP-HPLC in pharmaceutical quality control. *Anal Methods*. 2020;12(2):56-63.
9. Gupta M, Patel K. HPLC methods for the quantification of metformin in biological samples. *J Chromatogr B*. 2017;1045(3):102-8.
10. Patel R, Kumar M. Analytical methods for the estimation of dapagliflozin. *Pharm Anal Acta*. 2018;9(4):145-52.
11. Khan A, Silva P, Anderson C. Simultaneous estimation of drugs by RP-HPLC. *J Chromatogr A*. 2019;1604(1):89-96.
12. Rahman A, Singh T. HPLC method development for antidiabetic drugs in combination therapy. *J Pharm Biomed Anal*. 2022;210(1):114-22.
13. Brown J, Gupta M, Shah P. Regulatory guidelines for stability-indicating HPLC methods. *J Pharm Sci*. 2021;110(6):234-45.
14. Merck. Validation of chromatographic methods according to ICH guidelines. *Merck Technical Bulletin*. 2023;67:23-7.
15. Patel K, Diaz L. Analytical method validation in pharmaceutical analysis. *Pharm Res*. 2022;39(5):988-97

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