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DEVELOPMENT AND VALIDATION OF SIMPLE STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF ANTI- DIABETIC DRUG TENELIGLIPTIN AND DAPAGLIFLOZIN

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ABSTRACT

This study presents the development and validation of a chromatographic method for the analysis of DAPA (Dapagliflozin) and TNLG (Tolvaptan) in pharmaceutical formulations. The method was thoroughly evaluated for linearity, system suitability, and validation parameters, including recovery studies, repeatability, and precision. Both drugs exhibited excellent linearity with correlation coefficients of 0.999. System suitability results indicated good separation, with acceptable retention times and peak symmetry for both drugs. Validation results confirmed high precision, with mean recovery values near 99% for all concentration levels. The LOD and LOQ values demonstrated that TNLG is more sensitive than DAPA. Additionally, forced degradation studies showed that DAPA exhibited greater stability under oxidative and photolytic conditions compared to TNLG, which was more stable under acidic and alkaline hydrolysis. The results confirm the robustness, accuracy, and reliability of the developed method, making it suitable for routine quality control and stability testing of both drugs in tablet formulations.

Keywords: Dapagliflozin, Tolvaptan, Chromatographic Method, Linearity, System Suitability, Validation Parameters.

INTRODUCTION

The growing prevalence of diabetes mellitus, particularly type 2 diabetes (T2D), has prompted significant advancements in the development of new drugs aimed at managing blood glucose levels. Teneligliptin and Dapagliflozin are two commonly prescribed anti-diabetic medications that work through different mechanisms to improve glycemic control. Teneligliptin is a selective dipeptidyl peptidase-4 (DPP-4) inhibitor that increases incretin hormone levels, which enhances insulin secretion and decreases glucagon levels in a glucose-dependent manner (Basak & Banerjee, 2014). On the other hand, Dapagliflozin is a sodium-glucose co-transporter 2 (SGLT-2) inhibitor that works by preventing glucose reabsorption in the kidneys, promoting its excretion through urine, and thereby reducing blood glucose levels (Garg & Singh, 2017).

While these drugs have been shown to be effective individually or in combination, accurate and reliable methods for their estimation are essential for quality control during their development and manufacture. This is especially important for ensuring that the drugs meet regulatory standards, such as those set by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), and for ensuring that patients receive the correct dose in their prescribed medications.

High-performance liquid chromatography (HPLC) has long been regarded as one of the most reliable techniques for the separation and quantification of drugs and their metabolites in complex pharmaceutical formulations and biological matrices. Reverse-phase HPLC (RP-HPLC) is a particularly versatile and commonly used method due to its ability to separate a wide variety of compounds under controlled conditions (Kusumawati & Cahyono, 2018).

To date, various HPLC methods have been developed for the estimation of Teneligliptin and Dapagliflozin, either alone or in combination with other anti-diabetic agents. However, these methods may lack the sensitivity, specificity, or stability-indicating properties required for routine analysis in the pharmaceutical industry. Stability-indicating methods are particularly crucial for determining the degradation profiles of drug substances under various stress conditions, such as those involving heat, light, humidity, oxidation, and acidic or basic environments. These degradation products can impact the safety and efficacy of the drug, and hence, their quantification is essential for ensuring the drug's stability during storage and usage (Singh & Gupta, 2018).

In this context, we present the development and validation of a simple, stability-indicating RP-HPLC method for the estimation of Teneligliptin and Dapagliflozin. The method has been optimized for separation and quantification of both drugs in their pharmaceutical dosage forms, with a focus on robustness, precision, accuracy, and sensitivity. The method also includes forced degradation studies to evaluate the stability of both drugs under various stress conditions.

MATERIALS AND METHODS

Selection of Mobile Phase

Initially to estimate Dapagliflozin and Teneligliptin in fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 20mM KH_2PO_4: Methanol in the ratio of 20:80v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min (Castaneda and Garcia, 2016).

Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials methanol was used as diluents.

Preparation of Stock Solution:

Accurately weighed 10 mg API of DAPA and TNLG was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution:

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of DAPA and TNLG respectively (Stock-B).

Preparation of Different Solution

0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml, for DAPA. In same manner 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml and 5µg/ml of TNLG also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 5-25 µg/ml for DAPA and 1-5µg/ml for TNLG were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded at 280.0 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of DAPA 10µg/ml for DAPA and 5µg/ml TNLG was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 5 to 25 µg/ ml for DAPA) and (1 to 5µg/ ml for (TNLG) and areas for each concentration were recorded three times and mean area was calculated. The response ratio (response factor) was found by dividing the AUC with respective concentration (ICH, 2005).

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components.

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (Yadav and Shukla, 2019).

Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25µg/ml for DAPA and 1, 2, 3, 4 and 5µg/ml for TNLG indicates the precision under the same operating condition over short interval time.

Intermediate Precision

Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, 20mMKH_2PO_4: Methanol (20:80 %v/v) to (15:85% v/v).

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 10 mg of DAPA and 20mg of TNLG was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 10µg/mL DAPA and 5µg/mL TNLG respectively. The amounts of DAPA and TNLG in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation:

50 mg of both the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M HCl solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Alkaline hydrolysis:

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Oxidative degradation:

50 mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

Thermal degradation:

50 mg of the drug sample was taken in to a petridish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drugs.

RESULTS AND DISCUSSION

The presented data provides an in-depth analysis of the performance and stability of DAPA and TNLG, offering critical insights into their linearity, system suitability, validation parameters, and degradation profiles.

Both DAPA and TNLG exhibited excellent linearity, with correlation coefficients (r^2) of 0.999 for both drugs. This suggests that the analytical method used for their analysis is highly reliable across the concentration ranges studied. The slopes for DAPA (43.57) and TNLG (252.8) indicate different responses in terms of sensitivity to concentration, with TNLG showing a higher response per unit concentration. The small positive intercepts for both drugs (6.888 for DAPA and 5.372 for TNLG) indicate that the method has minimal bias in the baseline, contributing to accurate measurements.

In terms of system suitability, both drugs demonstrated suitable chromatographic behavior. DAPA had a slightly shorter retention time (3.350 ± 0.0053 min) compared to TNLG (4.0855 ± 0.0031 min), likely reflecting their differing molecular characteristics. TNLG also showed a higher Area Under Curve (AUC) (1258.021 ± 7.5148), indicating a stronger chromatographic response, which could be due to a larger or more complex molecular structure. The number of theoretical plates was higher for TNLG, suggesting more efficient separation. The tailing factor was lower for DAPA (1.047 ± 0.0242), indicating better peak symmetry and more ideal chromatographic conditions, which is crucial for the accuracy of the method.

The validation parameters confirm the robustness and precision of the analytical method for both drugs. The recovery studies at 80%, 100%, and 120% levels showed excellent results for both drugs, with mean recovery values near 99% across different concentration levels. The low standard deviations (SD) and % relative standard deviations (RSD) across recovery studies further reinforce the high precision and reproducibility of the method. Both drugs also demonstrated excellent repeatability, with DAPA yielding a mean concentration of 99.361% and TNLG 98.245%. The low % RSD values for day-to-day precision, analyst-to-analyst precision, and robustness (all under 1%) indicate minimal variability and excellent method consistency.

The limits of detection (LOD) and limits of quantification (LOQ) revealed that TNLG is slightly more sensitive than DAPA, with a lower LOD (0.10 µg/ml) and LOQ (0.25 µg/ml) compared to DAPA (LOD = 0.12 µg/ml, LOQ = 0.40 µg/ml). This suggests that TNLG can be detected and quantified at lower concentrations, making it a more sensitive compound under the given analytical conditions.

The assay results for tablet formulations showed high accuracy, with DAPA and TNLG achieving 99.7% and 99.60% of their label claims, respectively. The % RSD values were very low, indicating excellent precision in the analysis of the tablet formulations. These results confirm the reliability of the analytical method for routine quality control of pharmaceutical products.

Finally, the forced degradation studies provided valuable information about the stability of both drugs under various stress

conditions. DAPA showed significant degradation under acidic and alkaline hydrolysis, with recoveries of 83.26% and 82.23%, respectively. In contrast, TNLG exhibited better stability under these conditions, with acidic and alkaline hydrolysis recoveries of 92.23% and 94.56%. Both drugs were more stable under oxidative and photolytic degradation, but DAPA generally showed a more favorable stability profile under these conditions compared to TNLG

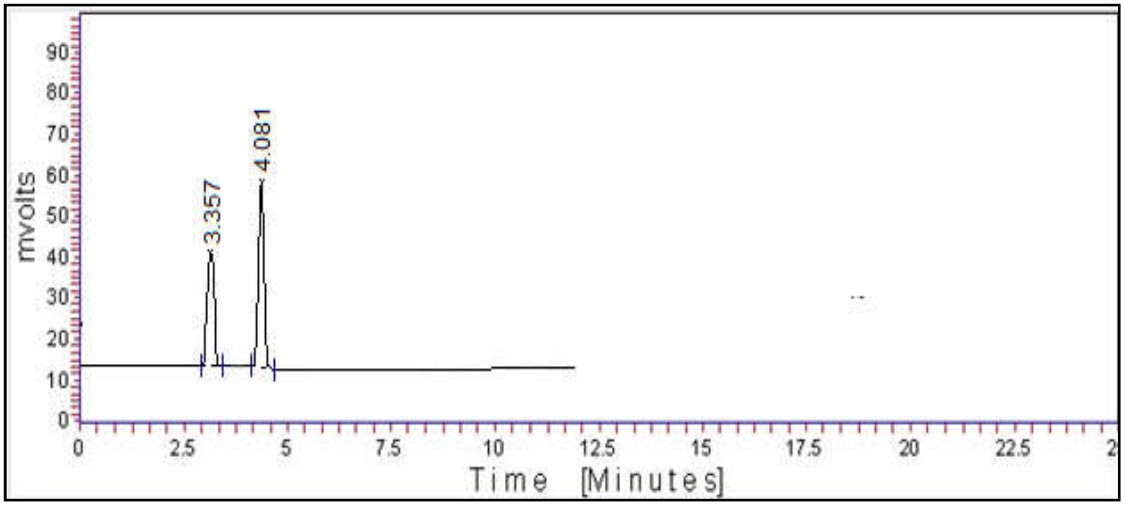

Figure 1: Chromatogram of Both the drug

Table 1: Statically data for linearity

Parameter	DAPA	TNLG
Correlation Coefficient (r^2)	0.999	0.999
Slope (m)	43.57	252.8
Intercept (c)	6.888	5.372

Table 2: Results of System Suitability parameters

Parameter	DAPA	TNLG
Retention Time (RT)	3.350 ± 0.0053	4.0855 ± 0.0031
Area Under Curve (AUC)	447.231 ± 6.8980	1258.021 ± 7.5148
No. of Theoretical Plates	2639.833 ± 38.5301	3169.833 ± 30.7208
Tailing Factor	1.047 ± 0.0242	1.147 ± 0.0216

Table 3: Results of validation parameters

Test Type	Parameter	DAPA	TNLG
Recovery Study (80% Level)	Mean % Conc. Found	98.77	99.03
	Standard Deviation (SD)	0.294	0.241
	% Relative Standard Deviation (RSD)	0.298	0.243
Recovery Study (100% Level)	Mean % Conc. Found	99.12	98.48
	Standard Deviation (SD)	0.601	0.421
	% Relative Standard Deviation (RSD)	0.606	0.427
Recovery Study (120% Level)	Mean % Conc. Found	99.04	98.89
	Standard Deviation (SD)	0.452	0.962
	% Relative Standard Deviation (RSD)	0.457	0.973
Repeatability	Mean % Conc. Found	99.361	98.245
	Standard Deviation (SD)	0.054	0.044
	% Relative Standard Deviation (RSD)	0.055	0.045
Day-to-Day Precision	Mean % Conc. Found	99.055	97.347
	Standard Deviation (SD)	0.065	0.044
	% Relative Standard Deviation (RSD)	0.066	0.045
Analyst-to-Analyst Precision	Mean % Conc. Found	99.527	98.165
	Standard Deviation (SD)	0.045	0.030
	% Relative Standard Deviation (RSD)	0.045	0.030
Robustness	Mean % Conc. Found	99.232	97.542
	Standard Deviation (SD)	0.063	0.056
	% Relative Standard Deviation (RSD)	0.064	0.058

Table 4: LOD and LOQ of DAPA and TNLG

Name	LOD (µg/ml)	LOQ (µg/ml)
DAPA	0.12	0.40
TNLG	0.10	0.25

Table 5: Result of assay of tablet formulation

	DAPA*	TNLG*
Label Claim (mg)	**10mg**	**20mg**
% Found (mg)	9.97	19.92
% Assay	99.7	99.60
% RSD	0.045	0.063

*Average of three determination

Table 6: Results of Forced degradation studies of DAPA

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.85	0
Acidic hydrolysis	83.26	16.74
Alkaline hydrolysis	82.23	17.77
Oxidative degradation	90.23	9.77
Photolytic degradation	94.45	5.55

Table 7: Results of Forced degradation studies of TNLG

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.95	0
Acidic hydrolysis	92.23	7.77
Alkaline hydrolysis	94.56	5.44
Oxidative degradation	85.56	14.44
Photolytic degradation	83.32	16.68

CONCLUSION

In conclusion, both DAPA and TNLG exhibit high analytical reliability, with excellent linearity, precision, and accuracy in their assay and validation parameters. However, TNLG shows greater sensitivity, while DAPA performs better under oxidative and photolytic stress conditions. These findings provide a strong foundation for the use of these methods in routine pharmaceutical quality control and stability studies for both drugs.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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