Ind. J. Pharm. Edu. Res., 2027; XX(XX):1-14.
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Original Article

A QbD Guided Development and Optimisation of RP-HPLC Method for Simultaneous Quantification of Linagliptin and Dapagliflozin Propanediol Monohydrate

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ABSTRACT

Background: Linagliptin (LIN) and Dapagliflozin Propanediol Monohydrate (DAP) are commonly used in combination for the management of type 2 diabetes mellitus, necessitating reliable simultaneous quantification in pharmaceutical formulations. An AQbD-guided RP-HPLC (Reversed-Phase High-Performance Liquid Chromatography) approach provides a systematic and robust strategy for developing and optimising the analytical method. **Objectives:** The objective of the present study was to develop, optimise, and validate a simple, specific, accurate, precise, robust, and sensitive RP-HPLC method for simultaneous quantification of LIN and DAP using an Analytical Quality by Design (AQbD) approach. **Materials and Methods:** The method was optimised using Cosmosil C18 column (250 mm x 4.6 mm, 5 µm), mobile phase made up of methanol: phosphate buffer in the proportion 70:30 %v/v, the flow rate was kept as 0.8 mL/min and 227 nm used as detection wavelength. Reversed phase chromatography was accomplished by utilising HPLC System (Analytical Technologies Pvt. Ltd.,) with UV detector. Box Behnken Design (BBD) was utilised for statistical analysis of data obtained after experimental runs. **Results:** The procedure was ascertained rectilinear in the concentration range of 10-50 µg/mL and 20-100 µg/mL of LIN and DAP respectively with R^2 value 0.9994 and 0.9996. Percentage recovery was determined to be 100.06 - 100.27% and 99.67-100.86 % for of LIN and DAP respectively. The detection limit for LIN and DAP was observed as 0.4624 µg/mL, 0.2807 µg/mL and quantitation limit for LIN and DAP was found to be 1.4014 µg/mL and 0.8506 µg/mL correspondingly. Greenness of method was assessed and greenness score was 0.61. **Conclusion:** The AQbD-based RP-HPLC method developed for simultaneous estimation of LIN and DAP was simple, accurate, precise, robust. The optimised technique was successfully validated as per framework outlined in ICH Q2(R^2) guidelines and found suitable for routine pharmaceutical analysis with moderate greenness.

Keywords: Analytical Quality by Design, Box Behnken Design, Dapagliflozin, Linagliptin.

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Received: 25-07-2026;
Revised: 13-08-2026;
Accepted: 06-10-2026.

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INTRODUCTION

Diabetes mellitus is a long-term metabolic condition characterised by sustained increases in blood glucose concentration. As reported by the World Health Organization, around 830 million individuals globally are affected by diabetes mellitus.[1] A recent study conducted by the Indian Council of Medical Research highlighted a high prevalence of both diabetes and prediabetes in India, estimating that nearly 101 million individuals are living with diabetes, while about 136 million are in the prediabetic stage. In addition, data from International Diabetes Federation indicate that India ranks second globally in terms of the number of people affected by type 2 diabetes mellitus, thereby contributing substantially to the global disease burden.[2] IUPAC name of LIN is 8-[(3*R*)-3-aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methyl quinazolin-2-yl) methyl] purine-2,6-dione[3] shown in Figure 1. LIN, a xanthine-based dipeptidyl peptidase-4 (DPP-4) inhibitor, is given orally once daily. It suppresses the enzymatic activity of DPP-4, leading to increased circulating levels of active incretin hormones, including glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide following food intake. This mechanism contributes to improved glycaemic control by enhancing insulin secretion from pancreatic β-cells while simultaneously reducing glucagon release from α-cells.[4] Chemical name of DAP is (2*S*,3*R*,4*R*,5*S*,6*R*) -2-[4-chloro-3-[(4 ethoxy phenyl) methyl] phenyl]-6- hydroxy methyl) oxane-3,4,5-triol; (2*S*)- propane-1,2-diol; hydrate[5] shown in Figure 1.

DOI: 10.5530/ijper.20270299

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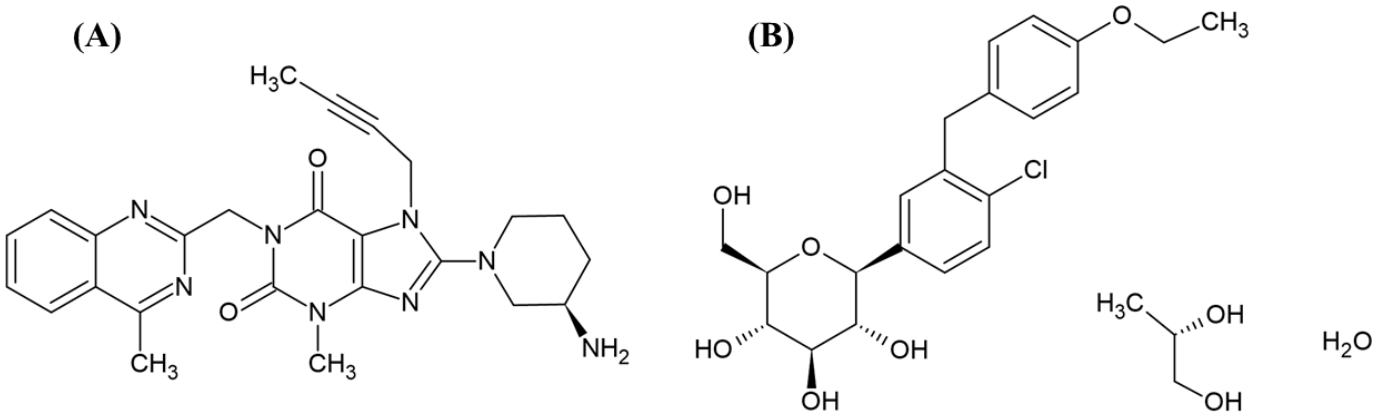


Figure 1: Chemical structure of (A) Linagliptin (B) Dapagliflozin Propanediol Monohydrate.

DAP, a sodium-glucose cotransporter-2 (SGLT2) inhibitor, is extensively utilised in the treatment of type 2 diabetes mellitus, providing effective glycaemic regulation along with additional cardiovascular and renal protective benefits.[4] Prashant Kumthekar *et al.*, in their study suggested that the fixed dose combination of and LIN 5 mg and DAP 10 mg tablet is superior to LIN 5 mg alone in terms of efficacy. The combination therapy represents a promising and effective approach for managing type 2 diabetes, resulting in notable improvements in glycaemic control. Additionally, both agents were generally well tolerated, with a low frequency of adverse events. This combination has gained significant therapeutic importance in the management of type 2 diabetes mellitus. Consequently, the development of reliable and sensitive analytical methods for their simultaneous quantification in bulk and pharmaceutical dosage forms has attracted considerable research interest.

A comprehensive literature review revealed that several UV spectrophotometric methods.[6–11] have been widely investigated for quantifying LIN and DAP, both as individual analytes and in combination with other pharmaceutical agents. In addition, several HPLC methods have been developed for the estimation of DAP and its combinations with other drugs.[12–14,16–23] , as well as for LIN and its combinations.[24–32] Furthermore, one HPTLC method.[15,33] has been reported. However, only a limited number of HPLC methods are available for the simultaneous determination of LIN and DAP.[34–36]

Existing methods predominantly rely on conventional trial-and-error approaches, lack QbD-based optimisation, do not include quantitative greenness assessment and solution stability studies. All reported methods for the estimation of LIN and DAP employ acetonitrile as the solvent, which is relatively expensive and consequently increases the overall cost of the method. Hence, there exists a clear need to develop a systematic, statistically optimised, cost-effective and efficient reversed phase high performance liquid chromatographic method with enhanced performance for the simultaneous quantification of LIN and DAP in pure drug substance and tablet dosage form.

MATERIALS AND METHODS

Chemicals and Reagents

Chromatography grade water, methanol and potassium dihydrogen phosphate were provided by Merck Life Sciences Pvt. Ltd., Mumbai, India. Nylon membrane filter (0.45 µm size) was made available from Merck Life Sciences Pvt. Ltd., Bangalore, India. Other chemicals utilised for this research work were analytical grade and solvents of HPLC grade.

Active pharmaceutical ingredient of LIN and DAP were obtained from Swapnroop Drugs and Pharmaceuticals, Chhatrapati Sambhajinagar, India. Tablet Juslina D -5/10 containing 5 mg of LIN and 10 mg of DAP, (Manufactured by Exemed Pharmaceuticals, GIDC, Vapi. Dist.- Valsad, Gujarat, India.) were bought from local pharmacy.

Instruments and software

Analysis of drug combination was performed by using HPLC System (HPLC 3000 series, Analytical Technologies Pvt. Ltd.,) with UV visible detector (UV - 3000M), HPLC workstation software was used for analytical data integration and utilised Cosmosil C18 column with dimensions 250 mm x 4.6 mm, 5 µm. UV visible Spectrophotometer (Analytical Technologies Ltd., 2012), sonicator (Labman Scientific Instruments, LMUC Series), analytical weighing balance (Wensar, DAB220), pH meter (Hanna Instruments, HI2211). Design expert was used for evaluation of experimental design study (Stat-Ease Inc., Minneapolis, USA, Version 13.0).

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Selection of mobile phase

LIN and DAP were soluble in methanol. Potassium dihydrogen phosphate buffer at pH 2.8 was used to control the ionisation of LIN and DAP, improve peak symmetry, minimise silanol interactions, and achieve adequate resolution with methanol as organic modifier in RP-HPLC. Mobile phase comprising of methanol: Phosphate buffer (10 mM KH_2PO_4) pH 2.8 in the ratio 70:30%v/v was selected for quantitation of LIN and DAP since it gave sharp, well resolved peaks. Then eluting solvents was vacuum degassed and filtered by using 0.45µ size membrane filter.

Preparation of phosphate buffer

Weighed accurately 0.137 g of Potassium dihydrogen phosphate (KH_2PO_4) and dissolved in 100 mL of water. The pH was adjusted to 2.8 using dilute orthophosphoric acid and measured using a calibrated pH meter. Before analysis, the pH meter was standardised with certified pH 4.0 and 7.0 buffer solutions in accordance with the manufacturer's instructions. The prepared buffer was subsequently passed through a 0.45 µm membrane filter and subjected to sonication before being used in the analysis. This solution was used in the mobile phase composition.

Preparation of standard stock solution

Weighed accurately 10 mg of LIN and DAP separately and dissolved in 10 mL of solvent (solvent was used as mobile phase) to produce 1000 ppm solution.

Preparation of sample solution

Accurately weighed twenty tablets and triturated utilising mortar and pestle. Weight of tablets was found to be 9.0 g and average weight of tablet was 204 mg. Powder analogous to 5 mg of LIN and 10 mg of DAP was accurately weighed and transferred in to 100 mL volumetric flask, dissolved in approximately 70 mL methanol, sonicated for 15 min, and filtered. An appropriate aliquot of the filtrate was further diluted with mobile phase (Methanol: Phosphate Buffer, 70:30 v/v) to obtain the required concentration for analysis.

Selection of wavelength for analysis

The overlain UV spectra of LIN and DAP were recorded in methanol over the wavelength range of 200-400 nm. Freshly prepared 10 µg/mL standard solutions of were analysed in U.V. visible spectrophotometer. LIN exhibited λ_{max} at 224 nm, whereas DAP showed a characteristic λ_{max} at 295 nm. The spectra intersected at 227 nm, indicating an isosbestic point where both analytes exhibited identical absorptivity. Therefore, 227 nm was selected as the analytical wavelength for simultaneous estimation.

HPLC method optimisation by AQbD approach

Analytical Target Profile (ATP)

Defining ATP is the most important step and first step while optimising analytical method by using experimental design technique. An ATP consists of performance characteristics for quality attributes including appropriate details on the product and process parameters to be determined with associated performance criteria.

Method Performance Characteristics (MPCs)

These are the method parameters that directly influence the ATP. Retention time, no. of theoretical plates used as method performance characteristics. MPCs should be controlled to obtain the desired output.

Analytical Procedure Parameter (APP)

These are factors or independent variables which having potential effect or they are critical for performance of method. Factors such as flow rate, mobile phase composition, pH are regarded as analytical procedure parameter.

Risk assessment

Risk assessment study was done by Ishikawa fishbone diagram and on the basis of literature available. Effect of critical procedure parameter on the dependent variable was evaluated. Effect of APP on MPC was studied in risk assessment.

Selection of Experimental Design

Box-Behnken Design (BBD) was utilised for method optimisation. It requires less experimental runs than Central Composite Designs (CCD) for the same number of independent variables, particularly when applying quadratic relationships. Hence BBD is less costly as compared to CCD. Therefore, BBD was implemented for optimisation HPLC technique. During making BBD three factors, two levels and five centre points were taken for study; 17 experimental runs were produced by design expert software which were performed (Table 1). Standard and sample preparations were made and subsequently injected into the chromatographic system. For both drugs Retention time, theoretical plates were measured as responses. The Design Expert software was utilised for evaluating the design of experiment study. (Stat-Ease Inc. Minneapolis, USA, Version 13.0). ANOVA with a quadratic approach was utilised to evaluate the coefficients and nature of the robustness. The significance of the independent variables was estimated by statistical technique ANOVA. Evaluation of the quadratic model's significance was carried out based on the *p*-value, F-value, and goodness-of-fit statistics.

The relationship between APP and MPCs was described using a second-order polynomial equation generated by the Box-Behnken Design:

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Table 1: DoE table showing the independent variables and corresponding responses.

	Factors			Responses			
Run 1	% Methanol (%)	Flowrate (mL/min)	pH	RT of LIN (min)	RT of DAP (min)	TP of LIN (plates/column)	TP of DAP (plates/column)
1	70	0.9	3	3.572	7.842	5546	11460
2	80	0.8	3	3.345	5.955	4116	11307
3	80	0.9	2.8	2.982	5.429	3544	10438
4	70	0.9	3	3.569	7.835	5551	11455
5	80	0.9	3.2	2.982	5.48	4635	10345
6	70	0.9	3	3.577	7.840	5539	11467
7	70	1	2.8	3.203	7.113	1883	11030
8	80	1	3	2.704	4.953	2029	9852
9	60	0.9	2.8	4.043	16.652	4345	12733
10	60	0.8	3	4.585	18.994	4658	13351
11	70	0.9	3	3.570	7.847	5545	11453
12	70	0.8	3.2	3.733	11.203	4733	12068
13	70	0.9	3	3.575	7.841	5549	11461
14	70	0.8	2.8	3.729	11.137	4309	12430
15	60	0.9	3.2	4.058	16.903	4514	13518
16	70	1	3.2	3.219	7.155	2160	10598
17	60	1	3	3.923	11.783	5488	12806

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2$$

Where Y represents the response (e.g., retention time, number of theoretical plates), β_0 is the intercept, β_1-β_3 are the linear coefficients, β_{12}-β_{23} are the interaction coefficients, β_{11}-β_{33} are the quadratic coefficients, and A, B, and C represent the selected APP.

The significance of the model and individual coefficients was evaluated using ANOVA, and the optimised chromatographic conditions were obtained using the desirability function approach.

Method Operable Design Region (MODR)

The Method Operable Design Region (MODR) represents a dynamic, multidimensional domain in which the combined influence of independent variables maintains minimal variability in performance. It is defined using numerical optimisation techniques, with clearly established boundaries for each individual response to ensure consistent and reliable outcomes.[37]

Analytical Method Control Strategy

A structured control strategy, developed from in-depth method knowledge and development studies, that ensures the analytical procedure consistently delivers accurate, reliable, and robust performance across its entire lifecycle.

Solution Stability Study

Standard solution stability was evaluated by conducting dedicated stability studies. Triplicate standard solutions were kept at ambient temperature and analysed after 24 h (day 1) and 48 h (day 2), followed by calculation of %RSD to assess consistency. Mobile phase stability was examined by monitoring any changes in appearance, along with system suitability and repeatability, after storage on the benchtop for the same time intervals.[38]

Validation of Analytical Procedure

The optimised high-performance liquid chromatographic method was validated in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Q2 (R^2) guidelines. Key performance characteristics evaluated included linearity, precision, accuracy, robustness, as well as the limits of detection and quantification.[39]

Assessment of Analytical Greenness

Two different ecological metrics were employed to assess the effect of developed technique on environment. Greenness AGREE,

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Complex GAPI and Analytical Eco-Scale was utilised to estimate the environmental compatibility. Evaluation is carried out by considering use of solvents, wastage produced and energy used during analytical procedure development.[40–41]

RESULTS

Analytical Target Profile (ATP)

The Analytical Target Profile of the proposed RP-HPLC method was to develop a simple, rapid, accurate, precise, robust analytical procedure for the simultaneous quantification of LIN and DAP in tablet dosage forms. The method was designed to achieve assay results within 98-102% of the label claim, precision with %RSD not exceeding 2%, adequate chromatographic resolution (resolution > 2), acceptable peak symmetry (tailing factor < 2), and a total chromatographic run time not exceeding 15 min.

Method Performance Characteristics (MPCs)

Retention time, theoretical plates were identified as method performance characteristics which were highly important parameters for method development.

Analytical Procedure Parameter (APP)

Factors or independent variables such as mobile phase composition (% organic phase i. e. methanol), flow rate and pH were recognised as analytical procedure parameter.

Risk assessment

Effect of analytical procedure parameter on the dependent variable was evaluated by using Ishikawa fishbone diagram and on the basis of literature available. Effect of APP on CQA was studied in risk assessment.

Experiments were performed as per Box Behnken Design using reverse phase HPLC. Retention time, theoretical plate, peak area, resolution etc. were assessed. Three independent variables selected found critical for analysis of LIN and DAP. The proposed design was statistically evaluated by ANOVA. The analysis provides contour plot, 3D response surface graph by plotting the response against other two factors while the third factor was kept maintained at a desired level, and representation of a response as two dimensional and three-dimensional response surface plots were shown in Figure 2. The p-value and F statistics values were noted for each dependent variable, confirming robustness of developed method at extended variations in conditions.

Retention time of optimised reversed phase liquid chromatographic method of LIN and DAP was 3.72 min, 11.13 min correspondingly. Resolution among two peaks was 23.27 confirming good separation. The corresponding peak areas were 309428 and 158064. Theoretical plates were measured to be 4309 and 12430, indicate column performance was efficient for the given conditions.

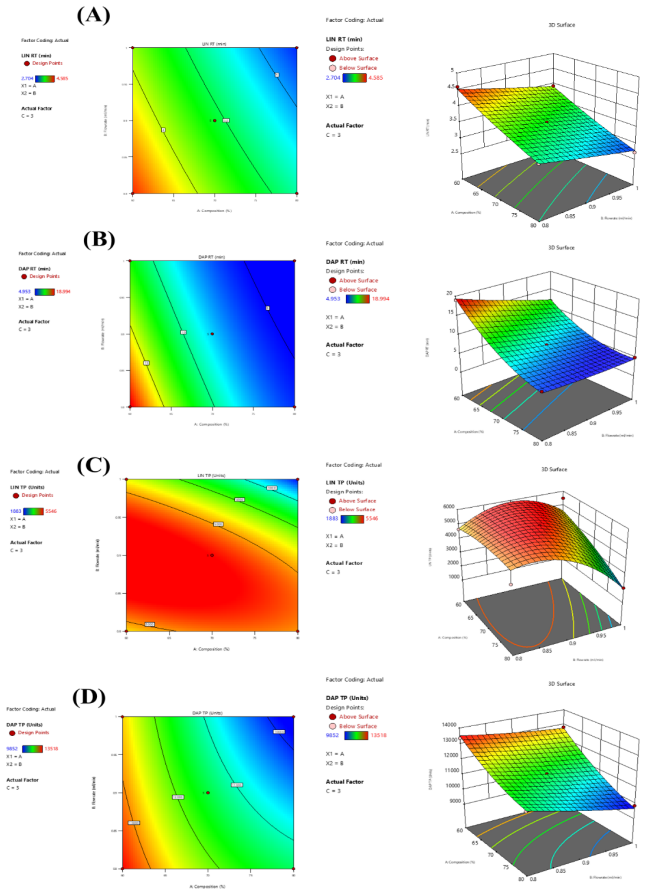


Figure 2: Response Surface Methodology (RSM) plots obtained from the Box-Behnken experimental design. (A) Retention time of Linagliptin; (B) Retention time of Dapagliflozin; (C) Theoretical plates of Linagliptin; and (D) Theoretical plates of Dapagliflozin.

Optimised Chromatographic Condition for analysis

Optimised mobile phase by Box Behnken design consisting of methanol and phosphate buffer pH 2.8 in ratio 70:30 %v/v and flow rate was 0.8 mL/min. Cosmosil C18 column (250 mm x 4.6 mm; 5 µm) was utilised for chromatographic estimation. Detection wavelength was 227 nm which is isosbestic point. Run time was 15 min. Sample volume 20 µL was used. Run time 15 min. The different interaction and quadratic effects of the composition of mobile phase and flow rate on retention time, number of theoretical plates were analysed using ANOVA tool (Figure 3).

Design Space

MODR was established in the mobile phase composition 62 to 71% methanol, buffer pH 2.8 to 3.2 and flow rate is 0.8 to 1 mL/min consistently met all predefined MPC acceptance criteria. The best conditions were determined through numerical optimisation, yielding the solution with the maximum desirability value (Table 2).

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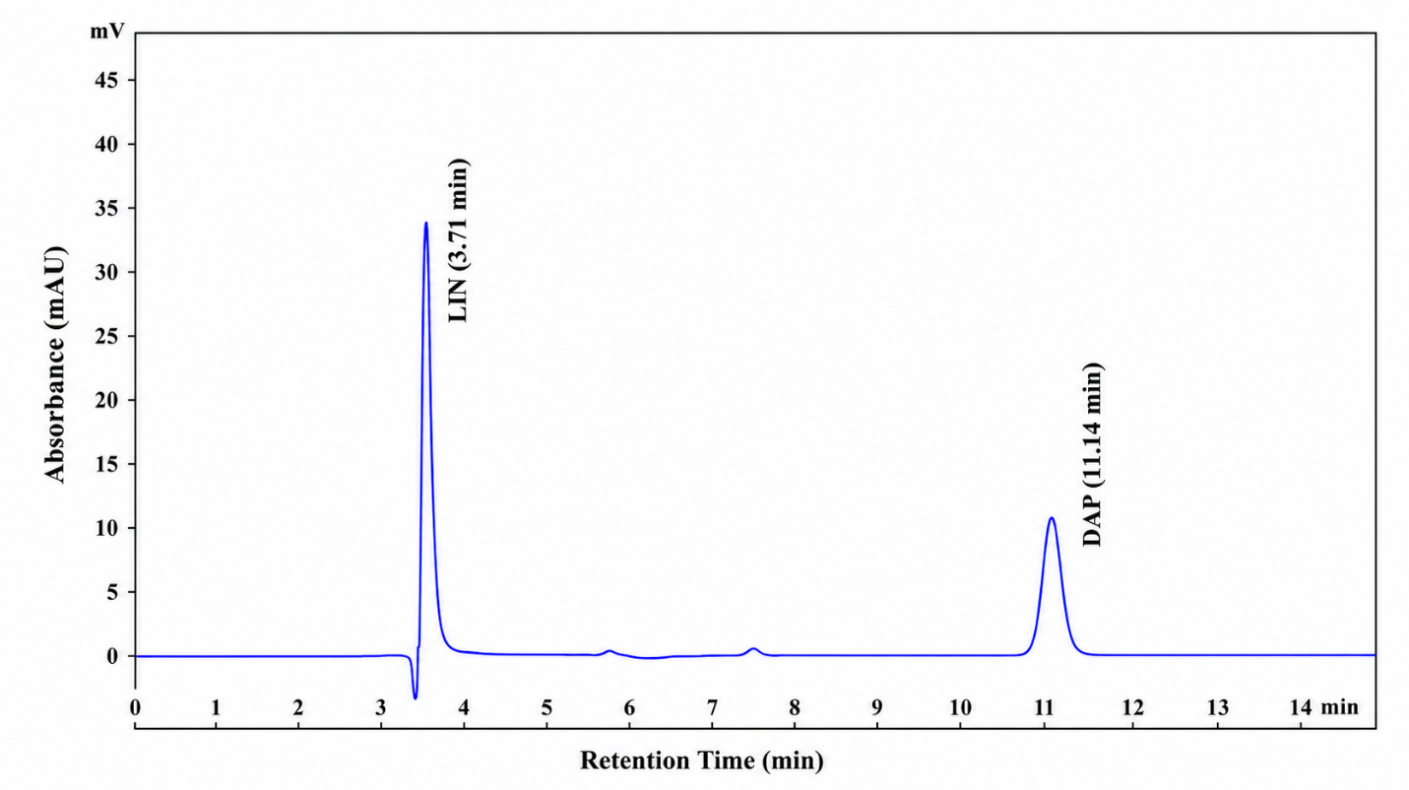


Figure 3: Optimised Chromatogram of LIN and DAP.

Table 2: Results of ANOVA, fit statistics of quadratic model for efficiency of BBD.

Dependent Variables	ANOVA		Fit statistics		
	p-value	F- value	Predicted R^2	Adjusted R^2	Adequate Precision
Retention time (LIN)	0.0001	122.04	0.8987	0.9855	40.70
Retention time (DAP)	0.0001	237.71	0.9478	0.9925	51.45
Theoretical plate (LIN)	0.0171	5.55	0.9670	0.7190	6.91
Theoretical plate (DAP)	0.0001	62.32	0.8840	0.9388	27.03

Analytical Control Strategy

Percentage composition of organic phase i.e. percentage of methanol and flow rate were identified as critical points which needs to be controlled to get within limit MPC results.

Validation of Analytical Procedure

System Suitability

Prior to sample analysis, the chromatographic system was evaluated through system suitability testing to ensure that all instrumental and separation parameters were operating satisfactorily. Six consecutive injections of the standard solution were carried out, and the %RSD of the peak areas remained within acceptable criteria, demonstrating consistent repeatability. Column performance was verified by satisfactory theoretical plate values, while peak symmetry was indicated by appropriate tailing factors. In addition, adequate resolution between closely eluting peaks confirmed effective separation. Overall, these findings establish that the system is fit for routine analytical use (Table 3). The system suitability results demonstrated acceptable chromatographic performance for both analytes. LIN exhibited a tailing factor of 1.36, while DAP showed a tailing factor within the acceptable range. Although LIN is a basic drug and may exhibit secondary interactions with residual silanol groups of the stationary phase, the optimised mobile phase composition and pH provided satisfactory peak symmetry. The observed tailing factor was well below the acceptance limit of 2.0, indicating that the method is suitable for routine quantitative analysis.

Specificity

The specificity of the method was evaluated by examining blank, standard, and sample preparations. No interfering signals were detected at the retention times corresponding to the analytes, demonstrating that excipients and other matrix components did

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Table 3: Results obtained from system suitability tests.

Parameters	Results		Acceptance criteria
	LIN	DAP	
Retention time (min)	3.71	11.14	NA
Tailing factor	1.36	0.95	NMT 2
Theoretical plate counts	6963	11602	NLT 2000
Resolution between LIN and DAP peak	-	24.70	NLT 2
Percentage RSD for peak area (six replicate injections)	0.1604	0.2510	NMT 2%

not influence the measurement. These findings confirm that the method is selective and suitable for the intended analysis, shown in Figure 4. The specificity of the proposed RP-HPLC method was demonstrated by comparing blank, standard, and sample chromatograms. The retention times of LIN and DAP in the sample solution were consistent with those observed in the standard solution. Minor additional peak observed in the sample chromatogram were attributed to formulation excipients and matrix constituents. These peaks were well resolved from the analyte peaks and did not interfere with the determination of either drug, confirming the specificity of the method.

Linearity

Method linearity was assessed using multiple standard solutions prepared at different concentration levels. For each analyte, a calibration plot was generated by correlating the measured chromatographic peak response with the corresponding concentration. The developed method exhibited linearity over the concentration varying from 10-50 µg/mL for LIN with R^2 value 0.9994 and 20-100 µg/mL for DAP with R^2 value 0.9996, indicating excellent proportionality between response and concentration (Figure 5).

Accuracy

The accuracy of the developed analytical procedure was determined using a standard-addition approach at three levels, namely 50%, 100%, and 150% of the nominal test concentration. Appropriate quantities of LIN and DAP reference standards were added to the previously analysed sample matrix, and each spiking level was examined in three independent determinations. The percentage recovery, standard deviation, and %RSD were calculated. The recoveries were found to be within acceptable limits, indicating that the method is accurate and free from interference of excipients. Low %RSD (>2%) values further confirmed the reliability of the method.

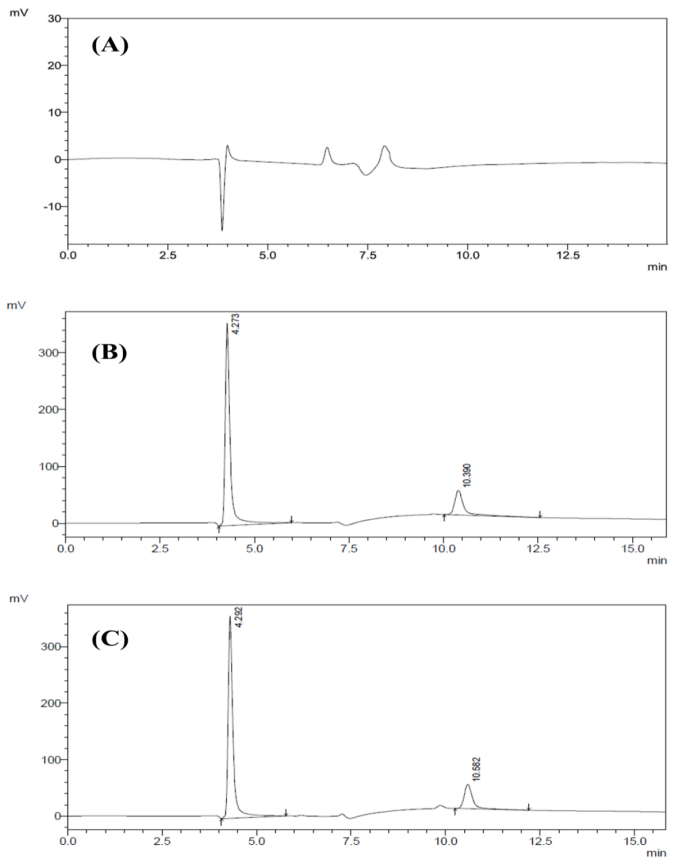


Figure 4: Representative chromatograms obtained under the optimised RP-HPLC conditions demonstrating method specificity. (A) Chromatogram Blank; (B) Chromatogram of the standard mixture; (C) Chromatogram of the tablet sample solution.

Precision

Precision of the method was assessed in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). For repeatability, multiple injections of the same concentration were analysed within a single day, while intermediate precision was evaluated on different days. Inter-day precision was assessed by analysing three independently prepared sample solutions at the working concentration on three consecutive days. The analyses were carried out under identical chromatographic conditions using the same instrument, column,

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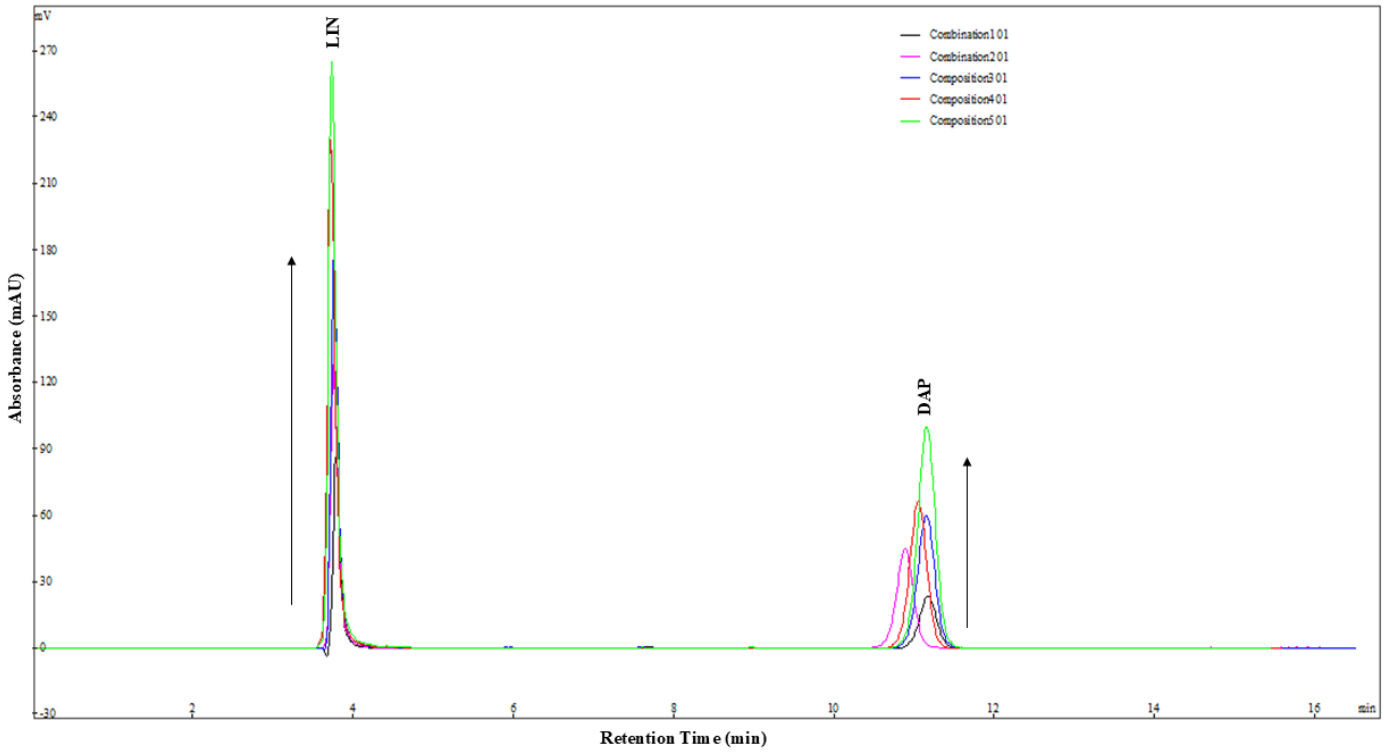


Figure 5: Overlay chromatograms used for linearity assessment of Linagliptin (LIN) and Dapagliflozin (DAP) under optimised RP-HPLC conditions. The increase in peak height and peak area with increasing concentration demonstrates the linear detector response over the investigated concentration ranges. Arrows indicate the direction of increasing concentration.

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mobile phase composition, flow rate, injection volume, and detection wavelength. The assay results obtained on different days were compared, and the percentage Relative Standard Deviation (%RSD) was calculated to evaluate method reproducibility. The %RSD values for peak area were found to be within acceptable limits, demonstrating that the method is precise and reproducible (Table 4).

Robustness

Robustness of the method was determined by making small deliberate variations in pH and detection wavelength. The effect of these changes on system suitability parameters and assay results was evaluated. The method remained unaffected by these minor variations, indicating its robustness and reliability during routine usage. The robustness of the optimised RP-HPLC procedure was assessed by intentionally modifying selected critical chromatographic conditions to determine the method's ability to maintain consistent analytical performance. The parameters studied included mobile phase pH (±0.2 units), flow rate (±0.1 mL/min), methanol composition (±2%), and detection wavelength (±2 nm). Sample solutions were analysed under each modified condition, and the assay results along with system suitability parameters were recorded. The method was considered robust when the %RSD of assay results was less than 2.0% and no significant changes in retention time, peak symmetry, or theoretical plate count were observed (Table 4).

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined according to ICH Q2(R^2) guidelines using the standard deviation of the response (σ) and the slope of the calibration curve (S). The following equations were employed:

$$\mathrm{LOD} = \frac{3.3\sigma}{S}$$

$$\mathrm{LOQ} = \frac{10\sigma}{S}$$

Where σ is the standard deviation of the intercepts of the regression lines and S is the slope of the corresponding calibration curve.

Assay

The assay of LIN and DAP in tablet dosage form was calculated using the external standard method by comparing the peak areas obtained from sample and standard solutions after applying the appropriate dilution and purity correction factors.

Table 4: Results of method validation.

Validation Parameters	LIN	DAP
Specificity	No interference	No interference
Linearity (correlation coefficient)	0.9994	0.9996
Range	10-50µg/mL	20-100µg/mL
Accuracy (%Recovery)	0.1623	0.0719
Level 1 (50%)	100.2741	100.8620
Level 2 (100%)	100.1485	99.6728
Level 3 (150%)	100.0672	99.6722
Precision - Intraday (%RSD)	0.2761	0.0571
Precision - Interday (%RSD)	0.2944	0.0405
LOD	0.4624	0.2807
LOQ	1.401	0.8506
Robustness - change in pH (%RSD)	0.4947	0.3951
Robustness - change in wavelength (%RSD) Robustness - Flow rate (%RSD) Robustness - Percentage of methanol (%RSD)	0.2173 0.5913 0.3980	0.7278 0.2648 0.2673

The percentage assay was calculated using the equation provided below.

$$\text{Assay}\ (\%) = \frac{A_T}{A_S} \times \frac{W_S}{W_T} \times \frac{D_T}{D_S} \times \frac{P}{100} \times \frac{\text{Average Tablet Weight}}{\text{Label Claim}} \times 100$$

Where:

A_T = Peak area of test solution,

A_S = Peak area of standard solutions,

W_S = Weight of working standard (mg),

W_T = Weight of sample taken (mg),

D_S = Standard dilution factor,

D_T = Sample dilution factor,

P = Purity of working standard (%),

Label Claim = Amount of drug claimed per tablet.

The proposed technique was adopted for the assay of tablet formulation. Analysis was done by injecting the blank, standard and sample in to the chromatographic system and peak areas of LIN and DAP were used for calculating the % assay by using the formula. The % purity of tablets of LIN and DAP was ascertained to be 99.63% and 100.13% respectively.

Result of solution stability

The mean peak areas obtained at Initial, 24 h, and 48 h were compared to evaluate solution stability. The overall %RSD was found to be 0.618%, and 0.0354 for LIN and DAP respectively, which is well below the acceptance criterion of 2.0%, indicating that the analytical solution remained stable for at least 48 h under the studied conditions (Table 5).

Assessment of Analytical Greenness

Greenness AGREE assessment

The developed HPLC procedure was scored 0.61 by using Greenness AGREE software indicates that the developed method is moderate green. AGREE scores range from 0 to 1. The GAPI pictogram revealed predominantly green and yellow zones, indicating acceptable environmental friendliness of the proposed HPLC method, with red zones mainly associated with the use of organic solvents. Greenness results were visually depicted in Figure 6. The environmental sustainability of the developed RP-HPLC procedure was evaluated using the AGREE (Analytical GREEnness Metric for analytical methods) assessment tool. The AGREE score ranges from 0 to 1, with higher values indicating better compliance with the twelve principles of Green Analytical Chemistry. In general, scores between 0.8 and 1.0 represent excellent greenness, scores between 0.6 and 0.8 indicate acceptable to good greenness, and scores below 0.6 suggest limited greenness. The obtained AGREE score of 0.61 demonstrates that the proposed method possesses an acceptable degree of environmental sustainability while maintaining suitable analytical performance for routine quality control applications.

Complex GAPI assessment

The Complex GAPI assessment revealed a predominance of green and yellow zones, indicating that most analytical steps exert low to moderate environmental impact. The red zones were mainly attributed to the use of methanol in the mobile phase and the associated solvent waste generated during chromatographic analysis. The proposed method offers environmental advantages compared with several previously reported methods that utilise acetonitrile as the principal organic component of the mobile phase. Acetonitrile is generally associated with higher toxicity, greater environmental burden, and increased disposal concerns compared with methanol. The replacement of acetonitrile with methanol, together with a relatively short analysis time and moderate solvent consumption, contributed to an improved environmental profile. Therefore, despite the presence of some red zones in the Complex GAPI pictogram, the overall assessment

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Table 5: Solution stability study data for LIN and DAP.

LIN	Time	Conc. (µg/mL)	Area (Units)	Mean	SD	% SD	% RSD
	Initial	50	1965343	1977281	10410	0.5265	0.618
		50	1982029				
		50	1984472				
	24 h	50	1954969	1955619	1182	0.0604	
		50	1954906				
		50	1956984				
	48 h	50	1959292	1956961	2860	0.1461	
		50	1953769				
		50	1957822				
DAP	Initial	100	1589486	1585358	3828	0.2414	0.0354
		100	1581924				
		100	1584664				
	24 h	100	1582857	1584432	2345	0.1480	
		100	1583312				
		100	1587128				
	48 h	100	1586132	1585451	1889	0.1192	
		100	1586906				
		100	1583315				

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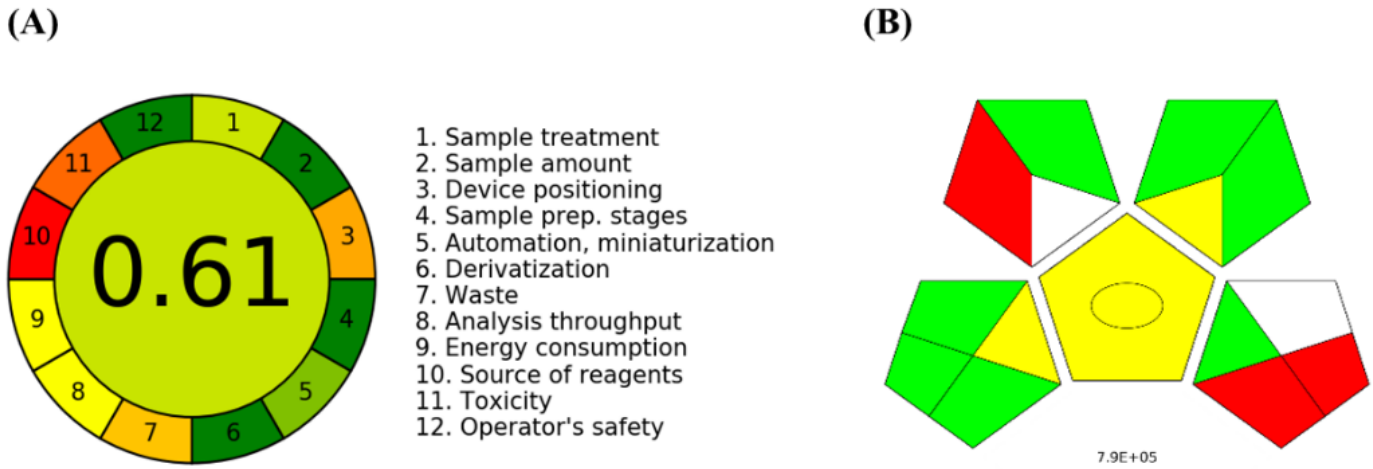


Figure 6: (A) AGREE (Analytical GREEnness Metric Approach) pictogram showing an overall greenness score of 0.61, indicating acceptable environmental sustainability. (B) Complex GAPI (Green Analytical Procedure Index) pictogram illustrating the environmental impact of individual analytical steps. The green, yellow, and red zones represent low, moderate, and high environmental impacts, respectively.

Table 6: Penalty Points Assessment for purposed method by Analytical Eco Scale.

Parameter	Observation	Penalty Points (Approx.)
Methanol (70%) in mobile phase	Hazardous organic solvent, but less problematic than acetonitrile	12
Phosphate buffer (30%)	Low environmental impact	1
Sample preparation using methanol	Additional solvent consumption	4
Energy consumption (HPLC instrument)	Conventional HPLC analysis	1
Occupational hazard	Standard laboratory handling	0
Waste generation (25 mL/analysis)	Between 10-100 mL	5
Total Penalty Points		23

supports the acceptable greenness and sustainability of the developed method.

Analytical Eco-Scale assessment

The developed RP-HPLC procedure was additionally assessed for its environmental sustainability using the Analytical Eco-Scale (AES) approach. The Eco-Scale score was calculated by assigning penalty points for reagent hazards, solvent consumption, energy requirements, occupational hazards, and waste generation. The proposed method employed methanol-phosphate buffer (70:30, v/v) as the mobile phase, generated approximately 25 mL of waste per analysis, and required conventional HPLC instrumentation. The total penalty points were estimated to be 23, resulting in an Analytical Eco-Scale score of 77. According to the Eco-Scale classification, methods with scores greater than 75 are considered excellent green analytical procedures. Therefore, the developed RP-HPLC method can be classified as an environmentally sustainable analytical method, supporting the conclusions obtained from the AGREE and Complex GAPI assessments (Table 6).

DISCUSSION

Box Behnken Design was employed for the optimisation of developed RP HPLC technique, which created 17 experimental runs with five repetitions of centre points, in order to evaluate effect, interactions of critical process parameters on dependent variables and to develop a model with sufficient statistical data. Composition of mobile phase solvents i. e. percentage of methanol, flow rate affects the retention time, no. of theoretical plates and peak area, separation of analyte etc. Optimising solvent ratio of mobile phase is very important for achieving good resolution, required retention time and optimum theoretical plates.[42]

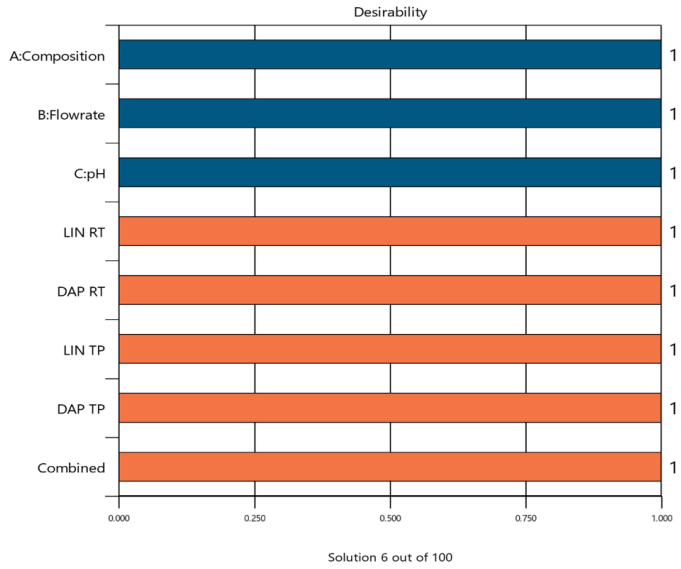


Figure 7: Derringer's desirability ramp plot generated using the Box-Behnken Design for numerical optimisation of the RP-HPLC method. Derringer's desirability function simultaneously optimises multiple chromatographic responses by transforming each response into an individual desirability value ranging from 0 (completely undesirable) to 1 (fully desirable), which are then combined into an overall desirability index.

The flow rate of diluent determines the time of analysis and peak shape of analytes. Maintaining appropriate flow rate can optimise the analysis time and resolution of peaks. pH is considered a key method attribute since it directly influences how analytes behave chemically, supports the robustness of the method, and helps maintain consistent and dependable analytical results. Therefore, careful regulation and monitoring of pH are crucial to ensure accuracy and proper method validation. Therefore, ratio of eluting solvent, flow rate, pH was identified as method performance characteristics. These method attributes were tested at three levels. BBD design was generated by Design Expert software. The optimised chromatographic conditions were selected by Derringer's desirability (Figure 7).

Optimised mobile phase by Box Behnken design consisting of methanol and phosphate buffer having pH 2.8 in ratio 70:30 %v/v and flow rate was 0.8 mL/min. Cosmosil C18 column (250 mm x 4.6 mm; 5 µm) was utilised for chromatographic estimation. Detection wavelength was 227 nm which is isosbestic point. Sample volume 20 µL was used. Run time was 15 min. The different interaction and quadratic effects of the composition of mobile phase and flow rate on retention time, number of theoretical plates were analysed using ANOVA tool. ANOVA results of LIN by Box Behnken Design, response surface methodology for retention time had *p*-value 0.0001 and

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F statistical value 122.04. For DAP *p*-value was 0.0001 and F statistical value 237.71. For LIN theoretical plates, the developed model was statistically significant (p=0.0171; F-value = 5.55). The Adequate Precision value of 6.91 exceeded the minimum desirable value of 4, indicating an acceptable signal-to-noise ratio for navigation of the design space. However, compared with other responses, the model demonstrated moderate predictive strength, suggesting that additional experimental factors not included in the present design may contribute to variability in theoretical plate count. Therefore, the model should be interpreted as suitable for optimisation within the studied experimental domain, while caution should be exercised when extrapolating predictions beyond the investigated factor ranges. For theoretical plate of DAP, the p-value was 0.0001 and F statistical value was 62.32. The refined quadratic model for DAP theoretical plates exhibited excellent predictive capability, with an Adjusted R^2 of 0.9388 and a Predicted R^2 of 0.8840. The close agreement between these values (difference = 0.0548) indicates good model predictability and absence of significant overfitting. The Adequate Precision value of 27.03 demonstrates an adequate signal-to-noise ratio, confirming the suitability of the model for navigating the experimental design space and optimisation studies as shown in Table 2. These responses had p-value below 0.05 and F statistical value above 2.5 shows that the optimised RP HPLC method was significant. Adequate precision for all models was more than 4 (which is desirable), suggest adequate signal. The optimised procedure was evaluated and found to be in full compliance with ICH guidelines; all validation parameters met the required acceptance criteria. Linearity was confirmed over a selected concentration range for LIN and DAP, with coefficients of correlation (R^2) were 0.9994 and 0.9996 for LIN and DAP correspondingly.

Compared with previously reported RP-HPLC methods for the simultaneous determination of LIN and DAP, the proposed AQbD-based method demonstrated several analytical advantages. Earlier methods generally relied on conventional trial-and-error optimisation approaches, whereas the present method employed a systematic AQbD strategy using Box-Behnken Design to establish a robust design space and identify method performance characteristics. The developed method exhibited excellent linearity within the selected concentration ranges, with correlation coefficients meets the acceptance criteria and sensitivity values (LOD and LOQ) comparable to those reported in previous studies. Precision studies showed %RSD values well below 2%, confirming excellent repeatability and intermediate precision. Accuracy was verified through recovery experiments, yielding recovery rates within acceptable limits. Precision studies were results in low RSD values, indicated that the method was reliable. Robustness was evaluated which reveals that there was no significant impact on the method performance. Furthermore, chromatographic performance parameters such as theoretical plate count, tailing factor, and resolution satisfied ICH acceptance criteria, indicating efficient separation and peak symmetry.

Greenness assessment was carried out by using AGREE, complex GAPI and analytical eco scale. Comparison with previously published RP-HPLC methods revealed that the proposed method provides analytical performance comparable to existing methods for simultaneous estimation of LIN and DAP. However, unlike the reported methods, the present work employed a Quality-by-Design (QbD) strategy using Box-Behnken Design for systematic method optimisation. Furthermore, the environmental sustainability of the method was comprehensively assessed using AGREE, Complex GAPI, and Analytical Eco-Scale tools. The Analytical Eco-Scale score of 77 and AGREE score of 0.61 indicate acceptable-to-excellent environmental performance. These features distinguish the present method from previously reported methods and support its suitability for routine quality control applications.

These findings suggest that the proposed AQbD-driven method provides a robust, reliable, and sustainable alternative to previously reported methods for routine quality control analysis of LIN and DAP in pharmaceutical dosage forms.

CONCLUSION

The reversed phase HPLC method was developed, optimised for concurrent quantification of LIN and DAP in their pure form and tablet dosage form by employing Box Behnken Design. The optimised technique was validated as per ICH guidelines. The analytical procedure validation results demonstrate that the developed RP-HPLC method is simple, precise, accurate, specific, and robust. Furthermore, the method exhibited acceptable environmental performance as demonstrated by AGREE, Complex GAPI, and Analytical Eco-Scale assessments. Therefore, the proposed method is suitable for routine quality control analysis of LIN and DAP in pharmaceutical formulations.

ACKNOWLEDGEMENT

We would like to express gratitude to the Department of Science and Technology, Government of India for providing the research facility at the School of Pharmacy under DST-FIST, which I utilised for my research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT AND SPONSORSHIP

The authors declare that no financial support or sponsorship was received for the research.

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AUTHORS CONTRIBUTION

Conceptualization and design: Ramdas Darade and Sanjay Pekamwar. Method development and validation: Ramdas Darade. Writing - original draft preparation: Ramdas Darade and Writing - reviewing and editing: Ramdas Darade and Sanjay Pekamwar. Interpretation of statistical data: Sanjay Pekamwar. Supervision: Sanjay Pekamwar. All authors reviewed and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data were analysed during study are available from corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ABBREVIATIONS

REFERENCES

1. Ngo VM, Bharot N, Donohue F, Kearney PM, Breslin JG, Roantree M. Analyzing and predicting diabetes with deep learning and visual insights. Intell Based Med. 2026;14:100368. doi:10.1016/j.ibmed.2026.100368
2. Kumthekar P, Upadhyay MR, Tamma RR, Kranti V, Bhattacharya R, Sharma PK, et al. Fixed-dose combination of dapagliflozin and linagliptin in individuals with type 2 diabetes mellitus inadequately controlled on metformin: A randomized double-blind multicenter trial. J Diabetol. 2025;16(1):50–56. doi: 10.4103/jod.jod_169_24.
3. Ito T, Naito Y, Shimmoto N, Ochiai K, Hayashi N, Okamura T. Long-term safety and effectiveness of linagliptin as add-on therapy in Japanese patients with type 2 diabetes: final results of a 3-year post-marketing surveillance. Expert Opin Drug Saf. 2021;20(3):363–372. doi: 10.1080/14740338.2021.1859477. PMID: 33269625.
4. Kalra S, Unnikrishnan AG, Chawla R, Mathur R, Junaidy KH, Shah M, *et al.* Rational use of dapagliflozin 5 mg: real-world prescribing trends and a practical ABCDEF framework for therapy personalization. J Diabetol. 2026;17(1):72-82. doi:10.4103/jod.jod_124_25 [✓ CrossRef Verified] [PMID: 42368878])
5. Dadhaniya K, Pandya V. A review on development and validation of analytical methods for simultaneous estimation of dapagliflozin, linagliptin and metformin in pharmaceutical dosage form. Int J Pharm Sci. 2025;3(5):4750-65. doi:10.5281/zenodo.15545251 [PMID: 42270437])
6. Mante GV, Gupta KR, Hemke AT. Estimation of dapagliflozin from its tablet formulation by UV-spectrophotometry. Pharm Methods. 2017;8(2):102–107. doi: 10.5530/phm.2017.8.16.
7. Zalte AG, Saudagar RB, Katkade PN. Validated UV-Spectroscopic estimation of Linagliptin Concentration in Bulk and Dosage form. Res J Pharm Technol. 2016;9(5):490–492. doi: 10.5958/0974-360X.2016.00090.1.
8. Joshi D, Singh B, Rautela A, Semwal N. Analytical method development and validation of UV-visible spectrophotometric method for the estimation of linagliptin. Glob J Nanomed. 2021;5(5):555671. doi:10.19080/GJN.2021.05.555671
9. Jani S, Shukla R, Patel P, Mehta B, Detholia K. Quantitative estimation of sitagliptin and dapagliflozin propanediol monohydrate in synthetic mixture using 1 order derivative spectroscopy simultaneous spectrophotometric analysis. International Journal of Pharmaceutical Chemistry and Analysis. 2022;9(1):28-34. doi:10.18231/j.ijpca.2022.005
10. Gajare S, Zalte A, Saudagar RB. Simultaneous spectrophotometric determination of linagliptin and metformin hydrochloride in combined tablet dosage form. Asian J Pharm Anal. 2017;7(2):79-83. doi:10.5958/2231-5675.2017.00013.8.
11. Mathew C, Varma S. Green analytical methods based on chemometrics and UV spectroscopy for the simultaneous estimation of empagliflozin and linagliptin. Asian J Pharm Anal. 2022:43–48. doi: 10.52711/2231-5675.2022.00008.
12. Verma MV, Patel CJ, Patel MM. Development and stability indicating HPLC method for dapagliflozin in API and pharmaceutical dosage form. Int J Appl Pharm. 2017;9(5):33-41. doi:10.22159/ijap.2017v9i5.19185.
13. Sarkar S, Patel VP. Method development and validation of dapagliflozin drug in bulk and tablet dosage form by RP-HPLC. Int J Pharma Res Health Sci. 2017;5(4):1755-9. doi:10.21276/ijprhs.2017.04.07.
14. Basha S, Sravanthi P. Development and validation of dapagliflozin by reversed-phase high-performance liquid chromatography method and it's forced degradation studies. Asian J Pharm Clin Res. 2017;10(11):101–105. doi: 10.22159/ajpcr.2017.v10i11.19705.
15. Suma BV, Deveswaran R, Shenoy P. A NEW HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN IN BULK AND TABLET DOSAGE FORM. Int J Pharm Pharm Sci. 2019:58–63. doi: 10.22159/ijpps.2019v11i8.34339.
16. More SS, Sonawane SS, Chhajed SS, Kshirsagar SJ. Development and validation of RP-HPLC method for simultaneous estimation of saxagliptin and dapagliflozin in tablets. Asian J Pharm Technol. 2018;8(3):145–148. doi: 10.5958/2231-5713.2018.00023.5.
17. Gundala A, Kvsrg P, Koganti B. Application of quality by design approach in RP-HPLC method development for simultaneous estimation of saxagliptin and dapagliflozin in tablet dosage form. Brazilian Journal of Pharmaceutical Sciences. 2019;55:e18129. doi: 10.1590/s2175-97902019000218129.
18. Kommineni V, Chowdary KPR, Prasad SVUM. Formulation of dapagliflozin and saxagliptin tablets and *in vitro* evaluation by RP-HPLC method. Asian J Pharm Anal. 2019;9(2):93–98. doi: 10.5958/2231-5675.2019.00018.8.
19. Bhavyasri K, Surekha T, Begum S, Sumakanth M. RP-HPLC method for dapagliflozin and metformin HCl in bulk and combined formulation. Arch Pharm Pract. 2021;12(4):106–110. doi: 10.51847/CzxI0wYrYr.
20. Patel YD, Patel PR, Bhatt J, Mehta B, Detholia K. Quantitative computation and stability evaluation of phase III composition comprising sitagliptin and dapagliflozin propanediol monohydrate by RP-HPLC. J Appl Pharm Sci. 2022;12(6):148–155. doi: 10.7324/JAPS.2022.120614.
21. Boggula N, Pandiyan PS. Development and validation of RP-HPLC method for the simultaneous estimation of dapagliflozin and saxagliptin in bulk and pharmaceutical dosage forms. Int J Pharm Sci Res. 2021;12(1):314-20. doi:10.13040/IJPSR.0975-8232.12(1).314-20.
22. Manasa M, Vijey Aanandhi M. Stability indicating simultaneous method development and validation of dapagliflozin and saxagliptin by RP-HPLC. Res J Pharm Technol. 2021;14(2):1045–1049. doi: 10.5958/0974-360X.2021.00187.6.
23. Vankalapati KR, Alegete P, Boodida S. Stability-indicating HPLC method development and validation for simultaneous estimation of metformin, dapagliflozin, and saxagliptin in bulk drug and pharmaceutical dosage form. Biomed Chromatogr. 2022;36(7):e5384. doi: 10.1002/bmc.5384. PMID: 35434817.
24. Vijaya Sri K, Anusha M, Reddy SR. A rapid RP-HPLC method development and validation for the analysis of linagliptin in bulk and pharmaceutical dosage form. Asian J Pharm Anal. 2015;5(1):16-20. doi:10.5958/2231-5675.2015.00003.4.
25. Mourad SS, El-Kimary EI, Hamdy DA, Barary MA. Stability-indicating HPLC-DAD method for the determination of linagliptin in tablet dosage form: Application to degradation kinetics. J Chromatogr Sci. 2016;54(9):1560–1566. doi: 10.1093/chromsci/bmw103. PMID: 27334290.
26. Rajbangshi JC, Alam MM, Hossain MS, Islam MS, Rouf ASS. Development and validation of a RP-HPLC method for quantitative analysis of linagliptin in bulk and dosage forms. Dhaka University Journal of Pharmaceutical Sciences. 2018;17(2):175–182. doi: 10.3329/dujps.v17i2.39173.
27. Veeresham C, Vemula P, Dodda D, Balekari U, Panga S. Simultaneous determination of linagliptin and metformin by reverse phase-high performance liquid chromatography method: An application in quantitative analysis of pharmaceutical dosage forms. J Adv Pharm Technol Res. 2015;6(1):25–28. doi: 10.4103/2231-4040.150368. PMID: 25709966.
28. Jadhav SB, Reddy PS, Narayanan KL, Bhosale PN. Development of RP-HPLC, stability indicating method for degradation products of linagliptin in presence of metformin HCL by applying 2 level factorial design; and identification of impurity-VII, VIII and IX and synthesis of impurity-VII. Sci Pharm. 2017;85(3):25. doi: 10.3390/scipharm85030025. PMID: 28653975.
29. Patel IM, Chhalotiya UK, Jani HD, Kansara D, Kachhiya HM, Shah DA. Simultaneous quantification of empagliflozin, linagliptin and metformin hydrochloride in bulk and synthetic mixture by RP–LC method. Future J Pharm Sci. 2021;7(1):182. doi: 10.1186/s43094-021-00332-1.
30. Kiran TNR, Parvathi P, Kumar JNS. Development and validation of RP-HPLC method for the simultaneous estimation of linagliptin, empagliflozin and metformin in solid dosage forms. Asian Journal of Pharmaceutical Analysis. 2020;10(3):117. doi:10.5958/2231-5675.2020.00021.6
31. Barbude P, Tawar M, Burange P. Method development using a UV visible spectrophotometer for the simultaneous estimation of metformin (MET), saxagliptin (SXG), and dapagliflozin (DGF) in marketed formulation. Asian J Pharm Anal. 2022:243–247. doi: 10.52711/2231-5675.2022.00039.
32. Unade TT, Pawar AKM. New validated stability-indicating RP-HPLC method for the simultaneous determination of metformin hydrochloride, linagliptin and empagliflozin in bulk and pharmaceutical dosage forms. Int J Appl Pharm. 2022:68–76. doi: 10.22159/ijap.2022v14i2.43626.
33. Shukla A, Chhalotiya U, Shah D, Tandel J, Kachhiya H, Parmar M. Development and validation of stability indicating HPTLC method for simultaneous estimation of Dapagliflozin and Linagliptin. Discover Chemistry. 2024;1(1):4. doi:10.1007/s44371-024-00002-0

www.ijper.org

34. Patel YN, Patel A. RP-HPLC method development and validation for simultaneous estimation of dapagliflozin propanediol monohydrate and linagliptin. Int J Pharm Sci Rev Res. 2023;81(2):97-103. doi:10.47583/ijpsrr.2023.v81i02.017.

35. Chhalotiya U, Shukla A, Shah D, Tandel J, Kachhiya H, Parmar M. Simultaneous estimation of dapagliflozin and linagliptin using reverse phase-HPLC with photo diode array (PDA). Journal of Chemical Metrology. 2024:19–29. doi: 10.25135/jcm.104.2401.3020.

36. Ghode PD, Kapse R, Sayare A, Pachauri A, Kandekar U, Deshpande T, *et al.* RP-HPLC method development and validation for the simultaneous estimation of dapagliflozin propanediol monohydrate and linagliptin in tablet dosage form. Afr J Biomed Res. 2024;27(3S):242-9. doi:10.53555/AJBR.v27i3S.1929.

37. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: ICH Q14: Analytical procedure development. 2022.

38. Gholve R, Pekamwar S, Wadher S, Kalyankar T. Stability-indicating RP-HPLC method development and validation for simultaneous estimation of telmisartan and rosuvastatin calcium in bulk and in tablet dosage form. Futur J Pharm Sci. 2021;7(1):224. doi:10.1186/s43094-021-00369-2

39. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: ICH Q2(R2): Validation of analytical procedures. https://database.ich.org/sites/default/files/ICH_Q2(R2)_Guideline_2023_1130.pdf Accessed on: 10 March 2026.

40. Płotka-Wasylka J, Wojnowski W. Complementary green analytical procedure index (ComplexGAPI) and software. Green Chemistry. 2021;23(21):8657–8665. doi: 10.1039/D1GC02318G.

41. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE—Analytical GREEnness Metric Approach and Software. Anal Chem. 2020;92(14):10076–10082. doi: 10.1021/acs.analchem.0c01887. PMID: 32538619.

42. Keerikkadu M, Bangera PD, Shetty A, Tippavajhala VK, Rathnanand M. QbD-optimised and validated RP-HPLC method development using Box-Behnken design for quantitative evaluation of dasatinib in lipid nanoparticles. Int J Appl Pharm. 2026:178–188. doi: 10.22159/ijap.2026v18i3.58317.

Cite this article: Darade RB, Pekamwar SS. A QbD Guided Development and Optimisation of RP-HPLC Method for Simultaneous Quantification of Linagliptin and Dapagliflozin Propanediol Monohydrate. Indian J of Pharmaceutical Education and Research. 2027;XX(XX):1-14.

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