

A Stability-Indicating RP-HPLC Approach for the Simultaneous Determination of Linagliptin and Dapagliflozin in Bulk and Marketed Formulation

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Abstract

A simple, rapid, accurate, precise, and stability-indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous determination of linagliptin (LNG) and dapagliflozin (DPM) in bulk drug and tablet dosage forms. Chromatographic separation was accomplished on a Shimadzu Shimpack C18 (250 mm × 4.6 mm, 5 µm) column with a mobile phase composed of 0.1% orthophosphoric acid and acetonitrile (40:60 v/v) at a flow rate of 1 mL/min, with UV detection at 230 nm. The retention periods for LNG were determined to be 3.651 minutes and for DPM to be 5.989 minutes. The method was validated in accordance with the ICH Q2(R2) guidelines. The concentration ranges of 5–25 µg/mL for LNG ($R^2 = 0.9991$) and 10–50 µg/mL for DPM ($R^2 = 0.9990$) were used to establish linearity. The limits of quantitation (LOQ) for LNG and DPM were 1.548 µg/mL and 0.669 µg/mL, respectively, while the limits of detection (LOD) were 0.523 µg/mL and 0.220 µg/mL. The mean percentage recoveries for both medications were 99.71 to 100.69, indicating exceptional accuracy. Precision investigations (repeatability, intraday, and interday) produced % RSD values that were significantly lower than 2.0. The method's stability-indicating nature was confirmed by forced degradation experiments conducted under acidic, alkaline, oxidative, thermal, and photolytic stress conditions, which confirmed the efficient resolution of both analytes from their degradation products. The assay that was developed was effectively implemented in the analysis of commercial formulations, demonstrating a high degree of recovery and minimal variability. This technique is appropriate for the routine quality control analysis of combined pharmaceutical formulations.

Keywords- Linagliptin, Dapagliflozin, RP-HPLC, Method Validation, Stability-Indicating Method, Forced Degradation Studies.

Introduction

Diabetes mellitus is a chronic metabolic disorder that is distinguished by ongoing hyperglycemia that is the result of defects in insulin secretion, insulin action, or both. Progressive microvascular and macrovascular complications, such as diabetic nephropathy, retinopathy, neuropathy, cardiovascular disease, and heart failure, are associated with type 2 diabetes mellitus (T2DM), which accounts for the majority of diabetes cases. As a result, modern pharmacotherapy is designed to mitigate the risk of cardiovascular and renal complications in addition to achieving adequate glycaemic control. Newer classes of glucose-lowering agents, particularly sodium–glucose cotransporter-2 (SGLT2) inhibitors and dipeptidyl peptidase-4 (DPP-4) inhibitors, have become increasingly popular due to the growing recognition of cardiorenal protection as a critical therapeutic objective. SGLT2, a transporter that is predominantly expressed in the proximal renal tubules, is potently, selectively, and reversibly inhibited by dapagliflozin.[1–4]

The preponderance of renal glucose reabsorption from the glomerular filtrate is attributed to SGLT2. Independent of insulin secretion, the inhibition of this transporter results in a reduction in renal glucose reabsorption and an increase in urinary glucose excretion, thereby reducing blood glucose concentrations. Intravascular volume and intraglomerular pressure are both reduced by this mechanism, which is also linked to natriuresis and osmotic diuresis[3, 4].

Dapagliflozin has exhibited clinically significant cardiovascular and renal benefits in addition to its glucose-lowering action. Dapagliflozin significantly reduced the risk of cardiovascular mortality or hospitalisation for heart failure in the DECLARE–TIMI 58 trial compared to placebo (hazard ratio [HR] 0.83, 95% CI 0.73–0.95) and renal events (HR 0.76, 95% CI 0.67–0.87). Subsequent evidence from DAPA-CKD demonstrated that dapagliflozin reduced the risk of a composite renal or cardiovascular outcome in patients with chronic kidney disease, including individuals without diabetes, supporting the broader cardiorenal role of SGLT2 inhibition.[3, 5]

Dapagliflozin is a C-glucoside derivative that contains a chlorophenyl and ethoxybenzyl moiety. Its IUPAC name is

(2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-6-(hydroxymethyl)oxane-3,4,5-triol.[6]

The polarity, solubility, chromatographic retention, and spectroscopic characteristics of the molecule are influenced by its multiple hydroxyl groups and aromatic structural features.[2, 3, 5, 7]

Linagliptin is a potent and highly selective dipeptidyl peptidase-4 (DPP-4) inhibitor. Its IUPAC name is (R)-8-(3-(R)-aminopiperidin-1-yl)-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1H-purine-2,6-dione.[6]

Linagliptin is a potent, highly selective, orally active DPP-4 inhibitor and is used for the treatment of T2DM. The enzyme dipeptidyl peptidase-4 (DPP-4) rapidly degrades the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP). Inhibition of DPP-4 leads to an increase in amount and activity of these incretin hormones which results in stimulation of insulin secretion and inhibition of glucagon secretion in a glucose dependent manner. The risk of hypoglycemia with linagliptin is low because of the glucose-dependent insulintropic action in the absence of medications that independently increase insulin secretion. [8–10]

Use of linagliptin in patients with renal impairment is one of the important pharmacological features. Unlike most other DPP-4 inhibitors, linagliptin is eliminated predominantly via nonrenal routes and dose adjustment is generally not necessary based on renal function alone. It has also been widely studied for cardiovascular safety. In the CARMELINA randomised clinical trial of participants with T2DM at high cardiovascular and renal risk, linagliptin was non-inferior to placebo for the primary cardiovascular outcome. No significant increase in major cardiovascular events or composite renal endpoint was noted. Twelve These properties make linagliptin an attractive therapeutic option for patients in whom renal function is a major consideration.[9, 11]

The combined use of dapagliflozin and linagliptin is pharmacologically justified by their complementary mechanisms. Dapagliflozin primarily reduces plasma glucose by increasing urinary glucose excretion through SGLT2 inhibition, whereas linagliptin enhances glucose-dependent insulin secretion and reduces glucagon secretion through DPP-4 inhibition. Consequently, their amalgamation offers complementary mechanisms of glycaemic regulation that do not directly depend on the same pharmacological pathway. Fixed-dose combinations that contain dapagliflozin and linagliptin have been the subject of significant interest due to their ability to simplify treatment regimens and enhance therapeutic convenience and adherence in patients who require combination therapy. [9–11]

The simultaneous presence of two drugs that are structurally and physicochemically distinct in a pharmaceutical formulation presents an analytical challenge. Dapagliflozin and linagliptin exhibit significantly distinct chromatographic and spectroscopic characteristics. Dapagliflozin comprises numerous hydroxyl groups and a relatively polar glucoside structure, while linagliptin contains numerous nitrogen-containing heterocyclic moieties. Therefore, in order to ensure the accurate separation and quantification of both analytes in the presence of excipients and potential degradation products, simultaneous determination necessitates the optimisation of analytical conditions and enough selectivity.

Numerous analytical methods have been reported for the determination of dapagliflozin and linagliptin, such as UV–visible spectrophotometry, HPLC, HPTLC, and, more recently, quality-by-design (QbD)-assisted chromatographic applications.[10–15,16] The appeal of UV spectrophotometric procedures is derived from their low operating costs, rapid analysis, simplicity, and relatively minimal instrumentation requirements. In recent studies, simultaneous UV methods have been reported for dapagliflozin and linagliptin in combined pharmaceutical dosage forms. These methods are based on simultaneous-equation and area-under-the-curve approaches. Nevertheless, spectrophotometric methods may be influenced by overlapping absorption spectra and generally offer less information about individual components and degradation products than chromatographic techniques.

HPLC-based methods are frequently employed for pharmaceutical quality-control applications due to their enhanced selectivity. RP-HPLC methods for the simultaneous estimation of dapagliflozin and linagliptin in bulk drug and tablet formulations, as well as stability-indicating methods capable of resolving degradation products, are included in recent literature. HPTLC has also been examined as a cost-effective and environmentally sustainable alternative for the simultaneous determination of these medications. A recent QbD-assisted HPTLC method which successfully separated linagliptin and dapagliflozin showed the importance of systematic optimisation of chromatographic variables. Twenty-one In recent years, experimental design has been introduced in QbD-based RP-HPLC procedures to improve the robustness and reproducibility of chromatographic conditions .

The analytical landscape for this drug combination is still developing. Recently, an RP-HPLC study emphasised the increasing importance of stability-indicating analytical procedures for dapagliflozin–linagliptin formulations combining chromatographic quantification with LC–MS/MS characterisation of degradation products. Nineteen An additional recent study reported a green and QbD-optimized RP-HPLC procedure, which emphasised the reduction

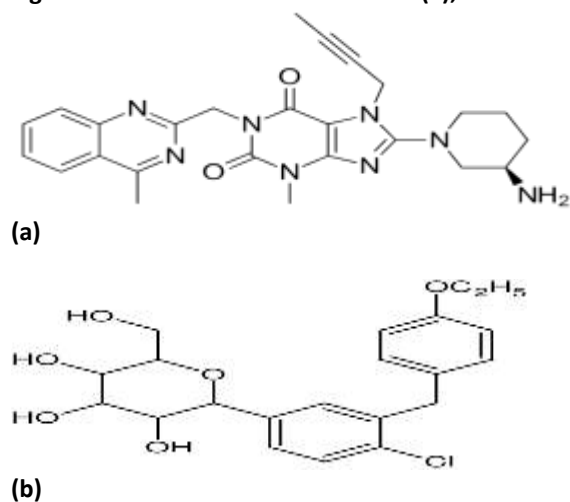
of solvent burden and the systematic optimisation of the method. Eighteen These advancements underscore the significance of creating analytical procedures that are not only precise and accurate, but also sufficiently straightforward, rapid, robust, and appropriate for routine pharmaceutical quality control.

In accordance with ICH Q2(R2), analytical procedures that are intended for quantitative determination must exhibit their fitness for purpose through suitable validation characteristics, such as specificity/selectivity, accuracy, precision, linearity, and range, and, where applicable, detection and quantitation limits and robustness. The present Q2(R2) guideline is applicable to analytical procedures that are employed for the release and stability testing of pharmaceutical products and drug substances. It offers a unified framework for the demonstration of the performance of analytical procedures. Twenty-two Consequently, it is imperative to conduct the systematic validation of a proposed method prior to its implementation in the routine analysis of pharmaceutical formulations.[18–23]

Although numerous analytical procedures are available, there is still a requirement for simple, economical, accurate, precise, sensitive, and reproducible analytical methods that can simultaneously determine dapagliflozin and linagliptin in bulk drug substances and combined tablet formulations using commonly available laboratory instrumentation. In particular, an analytical strategy that integrates both UV spectrophotometry and RP-HPLC can offer complementary approaches. UV spectrophotometer is simple and economical whereas RP-HPLC gives better selectivity and separation capacity.

Hence, the present study was undertaken to develop and validate simple, accurate, precise, sensitive and reproducible UV spectrophotometric and RP-HPLC methods for simultaneous estimation of dapagliflozin and linagliptin in pharmaceutical tablet dosage forms and bulk drugs. The developed methodologies were evaluated systematically for their analytical performance as per ICH guidelines for the quantitative analysis of the drugs in their combined dosage form. The developed procedures are expected to provide a practical and reliable analytical tool for routine quality-control analysis of pharmaceutical formulations containing dapagliflozin and linagliptin.

Fig. 1. The chemical structure of LGN (a), and DPM (b).



2. Materials and methods

2.1. Chemicals and reagents

LNG, DPM were supplied samples by Glenmark Pharmaceuticals, Sinnar, Nashik, India. HPLC grade acetonitrile and methanol were obtained from Merck Life Science Private Limited, India and combination medication (brand name- Linapil-D, Alkem Laboratories Ltd) was purchased from local pharmacy store. Water for HPLC was acquired from a Milli-Q system (Merck).

2.2. Instruments and software

The UV-Visible double-beam spectrophotometer (Model UV-1900i, Shimadzu, Japan) and FT-IR spectrophotometer (ALPHA-T, Bruker, Germany) were employed. The analytical data were collected and processed by the LabSolutions

LC software package of the chromatographic system Shimadzu LC-2030C Plus, Prominence-I (Shimadzu, Japan). The column temperature was maintained at 35°C and detection was performed at 230 nm. The samples were weighed on a Shimadzu AP 225 WD digital balance. The sonication was performed with the help of ultrasonic cleaner, model D-120/LH.

2.3. Chromatographic condition

Chromatographic examination was performed on a Shimadzu Shimpack C18 (250 mm x 4.6 mm, 5µm particle size) column. Mobile phase comprised of ACN: 0.1% OPA in the ratio of 40:60 v/v. The samples were prepared in mobile phase. The flow rate was 1 ml/min and the injection volume was 15µL. UV detector and column temperature were 230 nm and 35 °C, respectively. The overall analytical duration (run time) was 10 min with an injection volume of 15 µL for each sample.

2.4. Preparation of standard solution

Separately, LGN (5 mg) and DPM (10 mg) were accurately weighed and dissolved in methanol in a 10 mL volumetric vial. The stock that was obtained contains 1000 µg/mL of DPM and 500 µg/mL of LGN. Additionally, the mobile phase was employed as a diluent to combine the two stock solutions, resulting in an appropriate concentration. The concentration ratio of LGN to DPM was 1:2 to obtain the mixed standard solutions. The targeted concentration was prepared for analysis after the resulting solution was filtered through a 0.22 µm membrane filter.

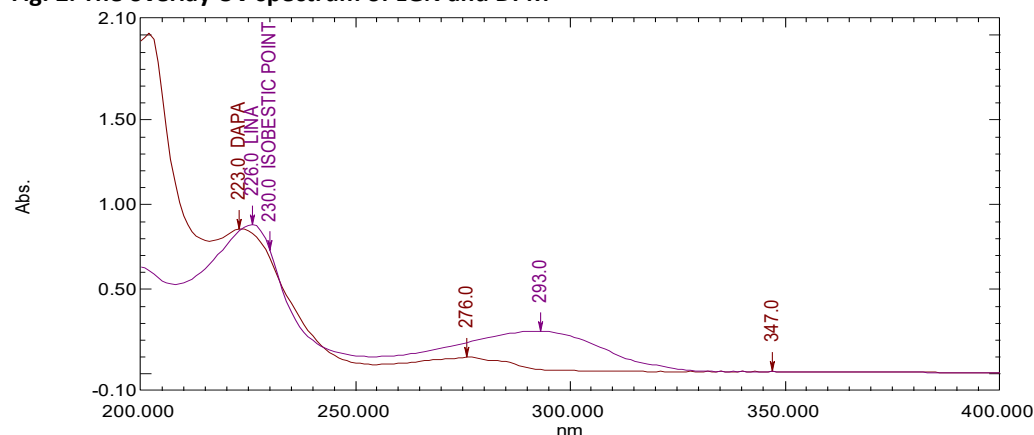
2.5. Preparation of sample solution

The powdered tablet formulation, which is equivalent to a single dose unit and contains 5 mg of LGN and 10 mg of DPM, was precisely weighed and transferred to a 100 mL volumetric flask. To facilitate the extraction of the combined drugs in methanol, the drug mixture was sonicated for 20 minutes with approximately 50 mL of methanol. The volume was subsequently adjusted to 100 mL using methanol. The solution that was extracted was filtered through a 0.22 µm membrane filter, and the mobile phase was prepared to contain the desired concentrations (50-250%).

2.6. Determination of working wavelength

Working standard solutions of LGN and DPM were prepared and scanned in the UV region (200–400 nm) separately, and the resultant overlay spectrum obtained was used to determine the suitable working wavelength. The overlay UV spectrum is presented in **Fig. 2** The isobestic point for analysis was selected at 230 nm as the suitable wavelength for the simultaneous determination of LGN and DPM.

Fig. 2. The overlay UV spectrum of LGN and DPM



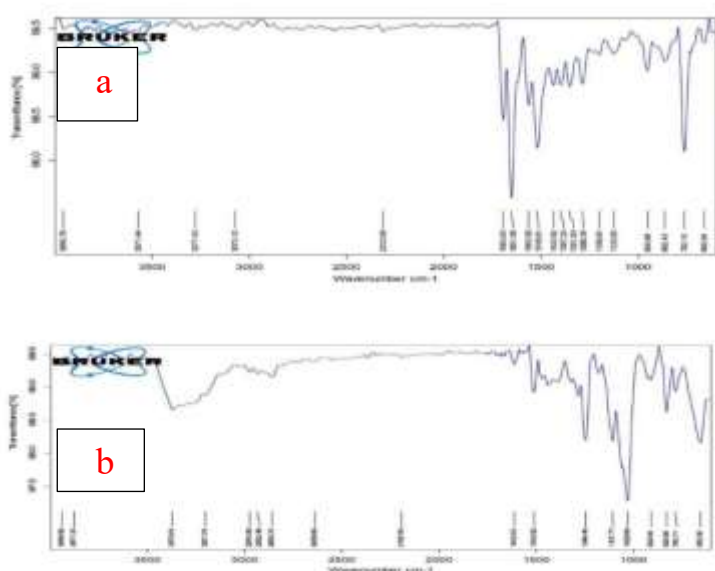
2.7. IR Spectra for primary identification of LGN and DPM

FT-IR characterisation of linagliptin: In the FT-IR spectrum of linagliptin, the absorption bands at approximately 3330–3285 cm⁻¹ were characterised as N–H stretching vibrations, while the band at 2944 cm⁻¹ was attributed to aliphatic C–H stretching. Around 1697 and 1654 cm⁻¹, characteristic carbonyl stretching vibrations were observed.

The absorption band at 1564 cm^{-1} was attributed to N–H bending, while the bands in the $1506\text{--}1450\text{ cm}^{-1}$ region were associated with aromatic C=C/C=N and C–H bending vibrations. The C–N stretching vibrations were responsible for the bands observed at $1291\text{--}1287\text{ cm}^{-1}$. The drug's identity was verified by the observed characteristic bands, which were consistent with the reported FT-IR characteristics of linagliptin. IR spectra given in Fig. 3a.

FT-IR characterisation of dapagliflozin: The FT-IR spectrum of dapagliflozin exhibited characteristic absorption bands that corresponded to its main functional groups. A broad absorption band in the $3350\text{--}3400\text{ cm}^{-1}$ range was attributed to the O–H stretching vibration. The aliphatic C–H stretching vibrations were ascribed to the bands observed between $2920\text{--}2940$ and $2850\text{--}2900\text{ cm}^{-1}$. Aromatic skeletal vibrations were associated with absorption bands in the $1600\text{--}1650\text{ cm}^{-1}$ region, whereas bands in the lower wavenumber region were primarily attributed to C–H bending and C–O stretching vibrations. The identity of dapagliflozin was further substantiated by the characteristic fingerprint-region absorptions. IR spectra given in Fig. 3b.

Fig. 3. The FTIR spectrum of LNG (a), and DPM (b).



3. Method Development & Validation

The RP-HPLC method was systematically developed and optimized using a step-by-step approach to achieve high resolution, sharp peak symmetry, and a stable baseline within a 10-minute runtime. Trial 1: The analytes were rapidly eluted at the injection void volume as a result of the high solvent strength in a preliminary investigation that utilised an organic-rich mobile phase (ACN: 0.1% OPA in water, 60:40 v/v) at room temperature. Trial 2: The organic modifier's intensity was reduced to 50:50 v/v to initiate retention; however, peak symmetry remained poor, with significant tailing. Trial 3: The analyte retention was optimised away from the solvent front by utilising a 40:60 v/v mobile phase ratio. A small shift in retention duration and baseline drift were the outcomes of the operations conducted at ambient temperature. Trial 4 (Optimised): By increasing the column temperature to $35\text{ }^{\circ}\text{C}$ and maintaining a mobile phase ratio of 40:60 v/v, the column backpressure was reduced, peak tailing was eliminated, and a very reproducible and precise peak form was achieved.

The developed HPLC method was validated based on ICH Q2 (R2). Validation parameters included specificity, accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), system appropriateness and robustness.

3.1. System suitability test

Prior to analysis, system suitability testing was implemented to evaluate the HPLC system's functionality. A mixed standard solution containing LGN at a concentration of $10\text{ }\mu\text{g/mL}$ and DPM at a concentration of $20\text{ }\mu\text{g/mL}$ was injected in six replicates, with a volume of $10\text{ }\mu\text{L}$ for each injection. The study examined parameters such as R_t , peak

area, tailing factor, number of theoretical plates, and resolution (Rs).

3.2. Linearity

Mixed standard solutions were prepared at five distinct concentration levels in accordance with the target concentration to evaluate linearity. The concentration ranges for LGN and DPM are 5-15 and 10-50 µg/mL, respectively, in relation to the respective target concentrations of 50%, 100%, 150%, 200%, and 25%. By conducting regression analysis and plotting peak area against concentration, calibration curves were generated. The following equations were used to evaluate the sensitivity of the method in terms of LOD and LOQ values from the slope of the calibration curve (S) and the standard deviation of the y-intercepts (σ):

$$\text{LOD} = 3.3 \times (\sigma/S)$$

$$\text{LOQ} = 10 \times (\sigma/S)$$

3.3. Accuracy

Accuracy was examined to check the effect of excipients on the results of the analytical data. The experiment was conducted by standard addition method at target levels of 80, 100 and 120% according to ICH guidelines. The already known concentration of LGN and DPM was added to the tablet matrix. The concentration after addition was determined in triplicate at specified levels of additional concentration and eventually recovery was estimated.

3.4. Precision

The method precision was calculated by percentage relative standard deviation (% RSD) for repeatability, intraday and interday analysis. The intra-day precision was assessed by analysing each level of concentration in triplicate on a day. The interday precision was determined by performing the analysis on three consecutive days. The repeatability, intraday and interday were studied by analysing the produced solution at 80, 100 and 120% of the target concentration. The %RSD from these measurements was used to confirm the reliability and reproducibility of the method.

3.5. Robustness

The robustness of the approach was assessed by making small and purposeful variations in the chromatographic parameters including flow rate, column oven temperature and wavelength of detection. % RSD for each modification was determined to study the robustness of method.

3.6. Forced degradation studies

In accordance with the ICH Q1A (R2) guidelines, forced degradation conducted to evaluate the specificity and stability-indicating potential of the developed HPLC method. The formulation was treated to a number of stress conditions such as acidic, basic, oxidative, photolytic, and thermal conditions.[24–27]

3.7. Assay of tablet formulation

The developed RP-HPLC method was successfully applied to the quantitative analysis of the marketed formulation Linapil-D tablets that claim to contain 5 mg of LGN and 10 mg of DPM. Twenty tablets were weighed and powdered for analysis. 5 mg of LGN and 10 mg of DPM (equal amounts of LGN and DPM) were taken in a 100 ml volumetric flask. To this 50 ml methanol was added and sonicated for 20 min diluted with methanol up to the mark and passed through a 0.22 µm membrane filter. The final concentration of LGN and DPM (10 and 20 µg/mL) was created by taking 1 mL of above prepared stock and diluted with diluent. The area was compared to the standard area and the concentration of assay was determined.

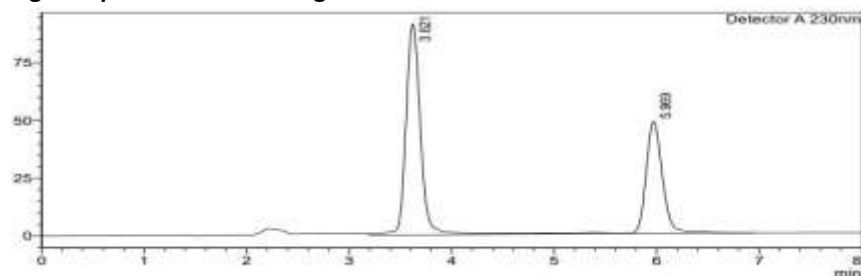
4. Results

4.1. Method development and optimization

The chromatographic method was created systematically for simultaneous determination of LNG and DPM utilizing RP- HPLC system on Shimadzu Shimpack C18 column (250mm x 4.6 mm, 5 µm particle size). All the trials were done using UV detection at 230 nm, selected isobestic point from UV spectral overlay obtained from UV spectroscopy. The optimized mobile phase consisted of a binary mixture of Acetonitrile and 0.1% Orthophosphoric Acid (OPA) in

water in a ratio of 40:60 v/v. The system was operated under isocratic elution mode at a constant flow rate of 1.0 mL/min.

Fig. 5. Optimized Chromatogram of LNG and DPM.



4.2. Method validation

The RP-HPLC technique for simultaneous quantification of LNG and DPM in pharmaceutical formulations was validated according to ICH Q2 (R2). The characteristics parameters for validation such as system suitability, linearity, sensitivity, precision, accuracy, specificity, forced degradation studies, robustness and assay of marketed formulations were investigated to establish the reliability and applicability of the method for routine analysis.

4.2.1. System suitability

The performance of the chromatographic system was checked by performing the system suitability test prior to validation. The obtained data indicated satisfactory chromatographic performance for both analytes under optimised circumstances as summarised in Table 2. The RT was found to be 3.651 ± 0.82 min for LNG and 5.989 ± 0.65 min for DPM, which shows the good separation. The theoretical plates were calculated to be 5322 ± 97.66 for LNG and 8955.67 ± 122.86 for DPM indicating high column efficiency. The USP tailing factors were 1.123 ± 0.124 for LNG and 1.386 ± 0.097 for DPM ≤ 2 . The resolution was determined to be 15.82 ± 0.827 which is substantially above the frequently accepted threshold of $R_s > 1.5$ exhibiting good separation of both analytes. The repeatability (%RSD) was 0.5903 for LNG and 0.8237 for DPM.

Table 2. System suitability testing parameters of suggested RP-HPLC technique.

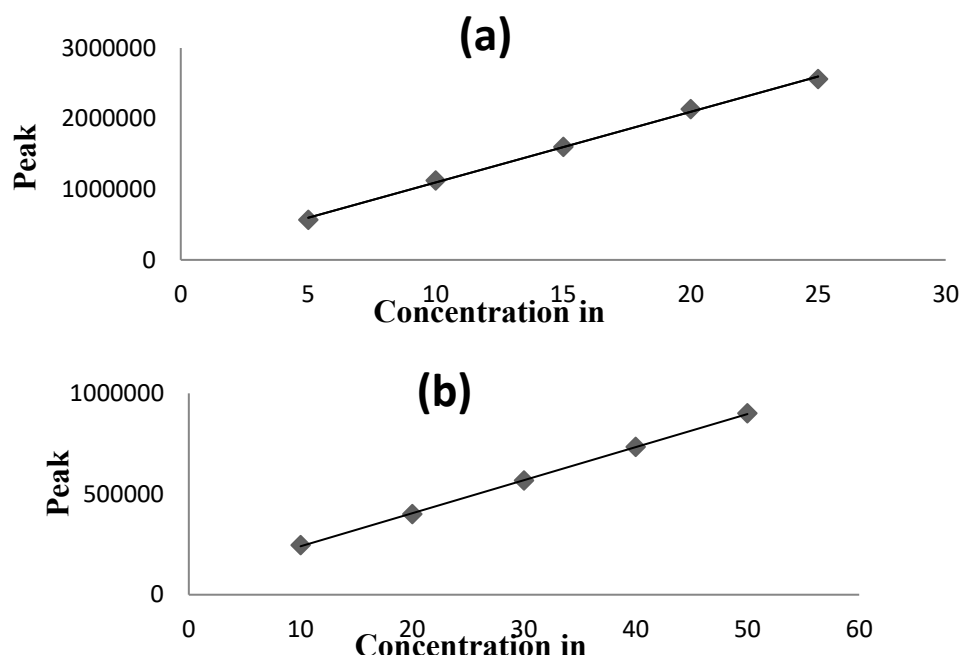
Parameters	LNG (Mean ^a $\pm$ % RSD ^b)	DPM (Mean ^a $\pm$ % RSD ^b)	Acceptable limit
Rt (min)	3.651 ± 0.82	5.989 ± 0.65	-
Peak Area	1122787 ± 5831	399705 ± 2622	-
Number of theoretical plates	5322 ± 97.66	8955.67 ± 122.86	> 2000
USP tailing factor	1.123 ± 0.124	1.386 ± 0.097	T of ≤ 2
Resolution	-	15.82 ± 0.827	R of > 2
Repeatability (% RSD)	0.5903	0.8237	Less than 2

^a Mean of six replicates; ^b % RSD, Relative standard deviation.

4.2.2. Linearity and sensitivity

The linearity of the developed method was calculated from area against five different concentration levels of LNG from 5–25 $\mu\text{g/mL}$ and DPM from 10–50 $\mu\text{g/mL}$ as shown in (Fig. 5). The regression equation of LNG was $Y = 99983.14x + 96904.43$ and the correlation coefficient (R^2) was 0.9991. Likewise, the regression equation for DPM was $Y = 16416.74x + 76390.66$ with correlation coefficient (R^2) of 0.999. As shown in Table 3.

Fig. 5. Linearity plot of LNG (a), and DPM (b).



The slope of the linearity curve and the standard deviation was used to determine the LOD and LOQ, and the respective values for LNG and DPM was 0.523 $\mu\text{g/mL}$; 1.548 $\mu\text{g/mL}$ and 0.220 $\mu\text{g/mL}$; 0.669 $\mu\text{g/mL}$ respectively were found.

Table 3. Sensitivity parameters for LNG and DPM

Parameter	LNG	DPM
Regression Equation	$Y = 99983.14x + 96904.43$	$Y = 16416.74x + 76390.66$
Slope	99,983.14	16,416.74
Intercept	96,904.43	76,390.66
R^2	0.9991	0.9990
Standard Error of Estimation	37,013.75	4,674.49
LOD	0.523 $\mu\text{g/mL}$	0.220 $\mu\text{g/mL}$
LOQ	1.548 $\mu\text{g/mL}$	0.669 $\mu\text{g/mL}$

4.2.3. Accuracy

The approach of accuracy was done using standard addition method at three concentration levels equal to 80, 100 and 120% of the target concentration. Accuracy is the degree to which the measured value is in close proximity to the genuine or accepted reference value. Recovery experiments were conducted at various concentration levels to assess the precision of the developed method. The percentage recovery for LNG and DPM varied from 99.64% to 101.25%, shown in Table.4.

Table.4. Recovery study parameters for LNG and DPM

Drug	Level (%)	Std Conc. $\mu\text{g/mL}$	Test $\mu\text{g/mL}$	Conc.	Peak Area	% Recovery	%RSD
LNG	80	10	8		1893904	99.85	0.24
	100	10	10		2141530	100.38	0.57
	120	10	12		2290098	99.71	0.46
DPM	80	20	16		666669	99.89	0.17

100	20	20	738033	100.69	0.90
120	20	24	800969	100.31	0.27

4.2.4. Precision

Precision studies were carried out to evaluate repeatability, intraday precision, and interday precision, with the findings expressed as percent relative standard deviation (% RSD). The comprehensive findings are represented in **Table 5**. The percentage relative standard deviation (RSD) values for repeatability assessments ranged from 0.514% to 0.650% for LNG and from 0.727% to 0.877% for DPM. Intraday accuracy assessments produced % RSD values of 0.496–0.606% for LNG and 0.788–0.944% for DPM. Interday accuracy assessments demonstrated % RSD values spanning from 0.512 to 0.710% for LNG and from 0.583 to 1.022% for DPM over the two days tested. The recorded % RSD values for all parameters remained comfortably inside the permissible limit of 2.0%, indicating that the established procedure exhibits exceptional precision and reproducibility.

Table 5. Precision study for LNG and DPM

Repeatability				
Concentration Level	LNG		DPM	
	Mean $\pm$ SD	% RSD	Mean $\pm$ SD	% RSD
80%	1896601 $\pm$ 9743	0.514	665661 $\pm$ 5768	0.867
100%	2133374 $\pm$ 12958	0.607	732547 $\pm$ 5327	0.727
120%	2296535 $\pm$ 14927	0.650	797653 $\pm$ 6999	0.877
Intraday precision (Day 1)				
80%	1896601 $\pm$ 9410	0.496	668220 $\pm$ 5909	0.884
100%	2133374 $\pm$ 11794	0.553	732305 $\pm$ 5774	0.788
120%	2296535 $\pm$ 13910	0.606	797370 $\pm$ 7530	0.944
Interday precision (Day 2)				
80%	1896601 $\pm$ 9940	0.524	667939 $\pm$ 3896	0.583
100%	2133374 $\pm$ 14250	0.668	732200 $\pm$ 6035	0.824
120%	2296535 $\pm$ 16302	0.710	797094 $\pm$ 8143	1.022
Interday precision (Day 3)				
80%	1896990 $\pm$ 9714	0.512	667790 $\pm$ 4314	0.646
100%	2133374 $\pm$ 12958	0.607	732658 $\pm$ 5077	0.693
120%	2296835 $\pm$ 14927	0.650	797751 $\pm$ 6704	0.840

SD: Standard Deviation; % RSD: % Relative standard deviation (n = 3 repeatability, intraday, interday)

4.2.5. Robustness

The results of robustness studies are shown in **Table 6**. The reliability and stability of the method were highlighted, since it was still reliable and consistent after intentional changes in analytical conditions.

Table 6. Robustness study data.

Parameters	Changed conditions	LNG 50 ppm			DPM 15 ppm		
		Peak area	% Assay	% RSD	Peak area	% Assay	% RSD
Flow rate (mL/min)	0.9	1113243	99.15	0.65	398106	99.60	0.72
	1	1122787	100.00	0.52	399705	100.00	0.55
	1.1	1116612	99.45	0.78	396188	99.12	0.89
Column oven temperature (°C)	30	1121103	99.85	0.59	397107	99.35	0.66
	35	1124134	100.12	0.71	398826	99.78	0.58
	40	1108752	98.75	0.85	401384	100.42	0.81
Detection wavelength	228	1126717	100.35	0.62	395308	98.90	0.94
	230	1118520	99.62	0.74	400305	100.15	0.63

(nm)	232	1113805	99.20	0.69	397906	99.55	0.77
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4.2.6. Specificity

Specificity was established by the analysis of chromatograms of blank, placebo, standard, and sample solutions. The retention times of LNG and DPM did not show overlapping peak

4.2.7. Forced degradation studies

The stability-indicating RP-HPLC method was developed by exposing LNG and DPM to different stress conditions as per ICH Q1A(R2) guidelines. The degradation investigations encompassed acidic, alkaline, oxidative, thermal, and photolytic stress to assess the efficacy of the devised chromatographic technique in separating the analytes from their degradation byproducts.

Acidic Hydrolysis: Under acidic hydrolysis (0.1 N HCl, 60°C for 1 hr), LNG and DPM underwent 12.55 and 14.56 degradation, respectively. As illustrated in (Fig. 6(a)), an additional degradation peak appeared at approximately 5.677 min, while the peaks corresponding to both analytes remained well resolved without interference.

Alkaline Hydrolysis: Exposure to alkaline hydrolysis (0.1 N NaOH, 60°C for 1hr) resulted in 13.85 degradation of LNG and 13.52 degradation of DPM. As shown in (Fig. 6(b)), two degradation products were detected at approximately 3.362 min and 5.47 min.

Oxidative Degradation: Oxidative degradation was carried out using 3% hydrogen peroxide at room temperature for 3 h. LNG exhibited relatively low degradation (8.24), whereas DPM showed significantly lower degradation (4.22). The chromatogram (Fig. 6(c)) revealed multiple degradation peaks at approximately 4.773 min and 5.408 min, all of which were baseline separated from the parent drug peaks.

Thermal Degradation: During thermal degradation (60°C for 24hrs), LNG and DPM showed 3.88 and 3.52 degradation, respectively. The chromatogram (Fig. 6(d)) did not exhibit any major interfering degradation peaks near the analyte retention times, suggesting limited formation of chromatographically detectable degradation products under the selected thermal stress conditions. Both drug peaks retained acceptable peak symmetry and resolution.

Photolytic Stress: Under photolytic stress (sunlight exposure for 24 h), LNG and DPM underwent no degradation. (Fig. 6(e))

Fig. 6. Forced degradation study data

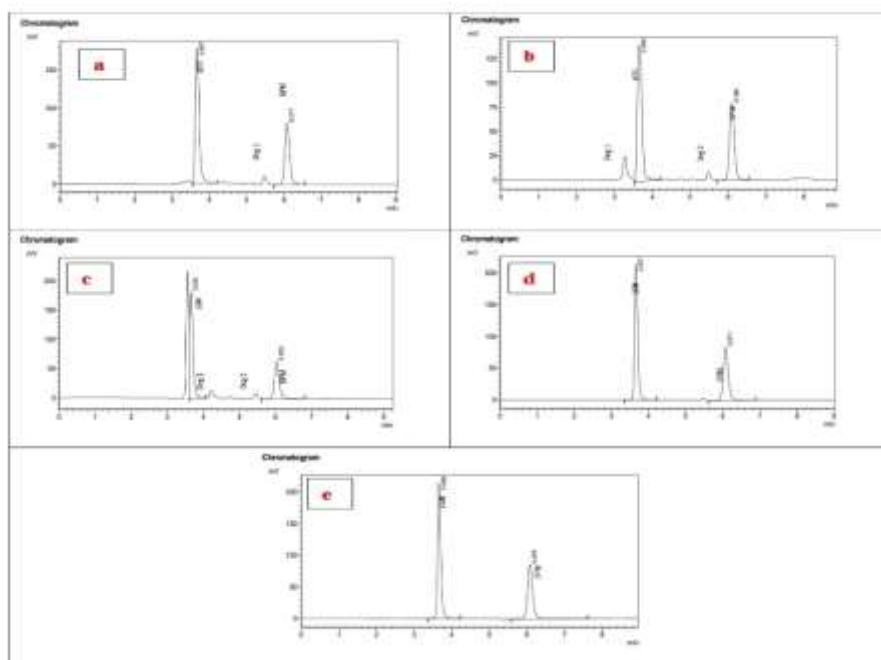


Table.7. Forced degradation study data

Stress environment	Experimental condition	% Degradation	
		LNG	DPM
Acidic	0.1 N HCl, 60°C for 1hr	12.55	14.56
Alkaline	0.1 N NaOH, 60°C for 1hr	13.85	13.52
Oxidative	3% H ₂ O ₂ at RT, 3 h	8.24	4.22
Thermal	Hot air oven at 60°C for 24hrs	3.88	3.52
Photolytic	Exposure to natural sunlight for 24 h	5.52	4.88

*RT- Room temperature

4.2.8. Assay of marketed formulation

The developed RP-HPLC method was effectively employed for the assessment of commercialised tablet formulation containing LNG and DPM. The average assay values were determined to be 99.97 % for LNG and 99.88 % for DPM of the labelled claim. The comparable % RSD values were 1.29 % for LNG and 1.06 % for DPM.

5. Discussion

The simultaneous determination of two structurally and physiochemical different antidiabetic drugs, linagliptin (LNG) and dapagliflozin (DPM) in a single tablet formulation poses an analytical challenge because of the large differences between the polar glucoside core of DPM and nitrogen-rich heterocyclic structure of LNG. This problem was successfully overcome by a revised RP-HPLC approach using a Shim pack C18 column and a mobile phase of acetonitrile and 0.1% OPA (40:60 v/v) which achieved baseline resolution between the two drugs in 10 min. The chosen isobestic point at 230 nm was sufficiently sensitive for both components.

The column performance was confirmed by system suitability tests, where high theoretical plate numbers (5322 for LNG and 8956 for DPM) and acceptable USP tailing factors (1.123 and 1.386, respectively) were obtained. The high resolution value of 15.82 between the two peaks demonstrates the separation effectiveness of the technique. The method validation performed according to ICH Q2(R2) standards has proved that the analytical system is quite robust and meets the requirements. The linearity graphs showed good linear relationships throughout the concentration ranges tested with excellent correlation coefficients ($R^2 > 0.9990$). The lower values of LOD and LOQ indicate that the technique is very sensitive for the determination of traces of both active pharmaceutical ingredients (APIs).

The accuracy was checked using the conventional addition method and the mean recovery values were found to be within 99.64-101.25. The lowest variance shows that common tablet excipients do not interact with the matrix peak signals. Precision results of repeatability, intraday and interday studies were all below 2.0 % RSD (maximum interday % RSD of 0.710 for LNG and 1.022 for DPM), which satisfies the stringent reproducibility levels needed for quality control processes. Deliberate changes in flow rate, temperature and wavelength were done to demonstrate the robustness of the system and the % RSD values obtained were less than 1.0 indicating that slight changes in the typical laboratory parameters do not affect the analytical performance.

Stability showing efficacy of this chromatographic technology was investigated in forced degradation studies. In acidic stress (0.1 N HCl), the degradation rates for LNG and DPM were 12.55 and 14.56, respectively, whereas the degradation rates were 13.85 and 13.52 in alkaline hydrolysis (0.1 N NaOH), respectively. Oxidative stress caused a worsening of 8.24 for LNG and 4.22 for DPM. Both medicines were stable against thermal stress (degradations <4.0) and photolytic stress (degradations <6.0). Importantly, all the newly created degradation products were successfully resolved from the primary parent drug peaks at baseline without any peak interferences. The practical efficiency of the technique was demonstrated when applied to commercial tablets of Linapil-D, leading to accurate testing (99.97 for LNG and 99.88 for DPM) with low sample variance.

6. Conclusion

A simple, sensitive, accurate, specific, precise, stability indicating RP-HPLC approach was successfully developed and thoroughly validated for simultaneous detection of linagliptin (LNG) and dapagliflozin (DPM) in bulk drug forms and combined commercial tablet formulations. The chromatographic system showed good performance under the optimum conditions with sharp and symmetrical peaks and retention times of 3.651 min and 5.989 min for LNG and DPM, respectively, with good resolution. The method fulfilled all the routine performance parameters described in the ICH Q2(R2) guidelines, showing good linearity in the ranges studied ($R^2 \geq 0.9990$), good sensitivity with low limits

of detection and quantification, and high precision with mean recovery percentages maintained between 99.64 and 101.25.

The accuracy analysis consisting of repeatability, intraday and interday also demonstrated % RSD values far below the strict industry limit of 2.0 confirming the exceptional reproducibility of the system. The method was confirmed to be highly specific and truly stability indicating as all the secondary degradation products were baseline resolved from the primary parent drug peaks without any chemical or structural interference by performing forced degradation studies under different environmental stress conditions (acidic, alkaline, oxidative, thermal and photolytic).

The utility of the analytical technique was demonstrated by its effective application to the analysis of commercial Linapil-D tablet assays, giving exact recoveries of 99.97 for LNG and 99.88 for DPM with minor variation between samples. The simple composition of the isocratic mobile phase, the short analysis run times, the minimal solvent consumption and the extremely good tolerance to very small changes in the operating parameters, make this approach a very efficient and low cost analytical instrument. This makes it well suited for routine high-throughput quality control, industrial batch release testing and long-term stability monitoring of mixed pharmaceutical dosage forms in quality control laboratories.

Acknowledgements

The authors gratefully acknowledge Dr. D. P. Hase, Head of the Department, and Dr. D. N. Raut, Dean of Research and Development, Amrutvahini College of Pharmacy, Sangamner, Maharashtra, India, for their valuable support, guidance, and encouragement during the course of this research. The authors also sincerely thank Glenmark Pharmaceuticals Ltd., Sinnar, Nashik, Maharashtra, India, for providing the gift samples of Dapagliflozin (DPM) and Linagliptin (LN) used in the present study.

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