International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 2, 2022

Original Article

NEW VALIDATED STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF METFORMIN HYDROCHLORIDE, LINAGLIPTIN AND EMPAGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

TAREKEGN TADESSE UNADE[1], A. KRISHNAMANJARI PAWAR[2*]

[1,2]A. U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam 530003, Andhra Pradesh, India
*Email: akmpawar@andhrauniversity.edu.in

Received: 16 Nov 2021, Revised and Accepted: 28 Dec 2021

ABSTRACT

Objective: The purpose of the present study is to develop simple, fast, accurate, precise, and robust stability-indicating reverse phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of metformin HCl, empagliflozin, and linagliptin in their combinations.

Methods: Separation was performed on Agilent Eclipse XDB-C18 (250 mm x 4.6 mm, 5 µm) column with a mobile phase consisting of 0.1 % triethylamine (pH =3) buffer and acetonitrile in the ratio 40: 60 (v/v) at a flow rate of 1 ml/min. Detection of the analytes was carried out at a wavelength of 240 nm with a photodiode array detector. The developed method was validated as per the International Conference on Harmonization (ICH) guidelines.

Results: The retention time values under the optimized condition were 2.660 min, 3.586 min, and 5.412 min for metformin HCl, linagliptin, and empagliflozin, respectively. The method was linear over a concentration range of 100 µg/ml-1500 µg/ml, 0.5 µg/ml-7.5 µg/ml, and 2.5 µg/ml-37.5 µg/ml for metformin HCl,linagliptin and empagliflozin respectively. The limit of detection (LOD) of the method was found to be 4.00 µg/ml, 0.02 µg/ml, and 1.00 µg/ml for metformin HCl, linagliptin, and empagliflozin, respectively. The degradation peaks were clearly resolved from the parent drug peaks in the chromatograms of forced degradation studies.

Conclusion: The validated method was successfully applied for the determination of metformin HCl, linagliptin, and empagliflozin in their combined tablet dosage forms and hence can be used for the routine quality control of the drugs in pharmaceutical bulk, and dosage forms.

Keywords: Stability indicating, RP-HPLC, Metformin HCl, Linagliptin, Empagliflozin

DOI: https://dx.doi.org/10.22159/ijap.2022v14i2.43626. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by high blood glucose levels (hyperglycemia). It is a major health problem worldwide, which is associated with morbidity, mortality, reduced quality of life, and increased healthcare costs [1, 2]. DM is classified into type 1 and type 2, and type 2 DM is the predominant type that accounts for about 90-95% of cases of diabetes [3]. Insulin alone or in combination with oral hypoglycemic agents for type 1 and oral hypoglycemic agents for type 2 is the recommended medication for the treatment of diabetes along with lifestyle management [4].

Metformin hydrochloride is among the biguanide class of oral hypoglycemics. Chemically it is, 3-(diaminomethylidene)-1,1-dimethylguanidine; hydrochloride (fig. 1). The liver is presumably the primary site of metformin function and its main mechanism of action is inhibition of hepatic gluconeogenesis. It is the drug of choice for the treatment of type II diabetes, particularly in overweight and obese people and individuals with normal kidney function [5, 6].

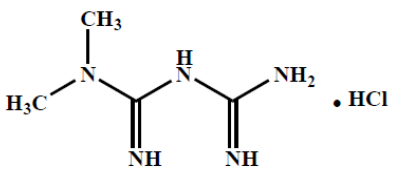

Fig. 1: Chemical structure of metformin HCl

Linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitors family of oral hypoglycemic agents. Chemically it is, 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-ethylquinazolin-2-yl) methyl]-3,7-dihydro-1H-purine-2,6-dione] (fig. 2). Inhibition of DPP-4 prevents the rapid cleavage of incretins and as a consequence, higher endogenous incretin levels enhance glucose-induced insulin secretion. The net effect results in lowering fasting and post-prandial blood glucose level. Linagliptin is indicated for the treatment of type 2 DM alone or in combination with other agents in addition to diet and exercise [7, 8].

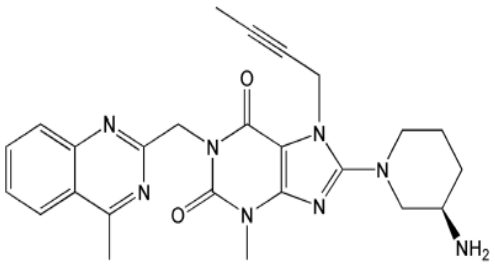

Fig. 2: Chemical structure of linagliptin

Empagliflozin is among the new sodium-glucose co-transporter-2 (SGLT2) inhibitors. Chemically it is, 1-chloro-4-[b-Dglucopyranos-1-yl]-2-[4-([S]-tetrahydrofuran-3-yl-oxy) benzyl (fig. 3). Inhibition of SGLT2, the transporters primarily responsible for the reabsorption of glucose in the kidney, results in increased urinary excretion of glucose and consequently results in lowering of blood glucose level. Empagliflozin is used along with diet and exercise and sometimes with other medications to lower blood sugar levels in people with type 2 DM [9].

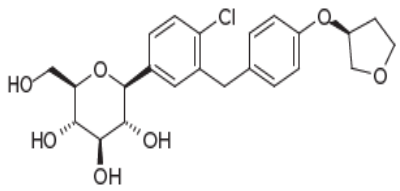

Fig. 3: Chemical structure of empagliflozin

Fixed-dose combinations (FDCs) of metformin HCl, empagliflozin and linagliptin are a new option in adults with type 2 DM to improve glycemic control [10]. FDCs are effective choices for patients needing glucose lowering with multiple agents, with advantages of reducing pill burden, low risk of weight gain, and hypoglycemia as compared to monotherapy [11, 12].

An extensive literature search revealed that several analytical methods were reported for the analysis of metformin HCl, linagliptin, empagliflozin, a combination of metformin and linagliptin, a combination of metformin and empagliflozin, and a combination of linagliptin and empagliflozin using Ultraviolet spectroscopy [13-16], reverse phase high-performance liquid chromatography (RP-HPLC) [17-25], and ultra-performance liquid chromatography (UPLC) [26,27]. However, only a few RP_HPLC methods were reported for the simultaneous determination of metformin HCl, linagliptin, and empagliflozin in their recently approved fixed dosage combinations [28-30] and thus, there is a need to develop rapid, sensitive, and cost-effective stability indicating RP-HPLC method for the simultaneous estimation of metformin HCl, linagliptin and empagliflozin in fixed dosage combinations. Hence, the present study was aimed to develop a simple, sensitive, rapid, accurate, and precise stability-indicating RP-HPLC method for the simultaneous determination of metformin HCl, linagliptin, and empagliflozin in their bulk and combined dosage forms.

MATERIALS AND METHODS

Chemicals and reagents

The reference standards of empagliflozin, linagliptin, and metformin HCl (more than 99 % purity) were procured from Biocon, Bangalore, and the tablet dosage forms were purchased from the market. HPLC grade acetonitrile and analytical grade chemicals such as triethylamine (TEA), orthophosphoric acid (OPA), sodium hydroxide, hydrochloric acid, and hydrogen peroxide were purchased from E. Merck Limited, Mumbai. Purified water was prepared by using 0.45 Millipore Milli-Q water purification systems.

Apparatus and instrumentation

An Agilent technologies model 1260 infinity series HPLC equipped with quaternary pumps and photodiode array (PDA) detector was employed in this study. The output signal was monitored and integrated by Openlab CDS EZ Chrom A.04.05 software. Metler Toledo ME204 analytical balance, Hover Labs LMPH-9 pH meter, Remi ultrasonicator, Millipore vacuum filtration unit, Borosil double distillation apparatus, and Kemi hot air oven were used.

Chromatographic conditions

Separation was performed using Agilent Eclipse XDB-C18 (250 mm x 4.6 mm, 5 µm) as a column with mobile phase 0.1 % TEA adjusted to pH 3 with orthophosphoric acid and acetonitrile in a ratio of 40: 60 (v/v). The samples were analyzed using 10 µl injection volume, maintaining the flow rate at 1.0 ml/min with a runtime of 6 min, and the temperature was maintained at ambient conditions. Detection and purity establishment of the drugs was achieved using a PDA detector at 240 nm wavelength.

Preparation of buffer solution and mobile phase

TEA (0.1 %) solution of pH = 3 was prepared by adding 1 ml of TEA in 1 L purified water, filtering through 0.45 µ membrane filter paper, sonicating, and adjusting the pH with previously filtered OPA solution. The mobile phase was prepared by mixing 0.1 % TEA buffer and acetonitrile in a ratio of 40: 60 (v/v). The mobile phase was used as a diluent.

Preparation of standard solution

Accurately weighed and transferred 1000 mg of metformin HCl, 5 mg of linagliptin, and 25 mg of empagliflozin pure powders into a 100 ml volumetric flask. Approximately 70 ml of diluent was added, sonicated for 15 min to dissolve, and then diluted to the volume. Five mill liters of the above solution was further transferred to 50 ml volumetric flask, made up to the volume with diluents and filtered through 0.45 µ Nylon syringe filter to give a standard working solution of 1000 µg/ml, 5 µg/ml, and 25 µg/ml of metformin HCl, linagliptin and empagliflozin respectively.

Preparation of sample solution

Ten tablets, labeled to contain 1000 mg of Metformin HCl, 5 mg of Linagliptin, and 25 mg of Empagliflozin per tablet, were weighed to determine the average weight. Then the tablets were finely powdered and a quantity of the powder equivalent to the weight of 1 tablet was accurately transferred to a 100 ml volumetric flask. Approximately 70 ml of diluent was added; the mixture was sonicated for 15 min and diluted to final volume with the diluent. Five mill liters of the above solution was further transferred to 50 ml volumetric flask, made up to the volume with diluents and filtered through 0.45 µ Nylon syringe filter to give a final concentration of 1000 µg/ml, 5 µg/ml, and 25 µg/ml of metformin HCl, linagliptin and empagliflozin respectively.

Method validation

The developed method was evaluated for validation parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, and LOQ, according to ICH Q2 (R1) guidelines [31].

System suitability

The system suitability test was carried out by performing six replicate injections of a working standard solution containing 1000 µg/ml metformin HCl, 5 µg/ml linagliptin, and 25 µg/ml empagliflozin.

Specificity

The specificity of the method was evaluated by performing the analysis of working standard solution, sample solution, blank solution, and placebo solution to examine the blank and placebo chromatograms for any interfering peaks within the retention time of the analyte peaks.

Linearity

An appropriate volume of aliquots from standard metformin HCl, linagliptin and empagliflozin stock solutions were transferred to different volumetric flasks. The volumes were adjusted to the mark with diluent to give a solution containing concentration of 100 µg/ml,250 µg/ml,500 µg/ml,750 µg/ml,1000 µg/ml,1250 µg/ml and 1500 µg/ml of metformin HCl; 0.5 µg/ml,1.25 µg/ml,2.5 µg/ml,3.75 µg/ml,6.25 µg/ml and 7.5 µg/ml of linagliptin; and 2.5 µg/ml,6.25 µg/ml,12.5 µg/ml,18.75 µg/ml,25 µg/ml,31.25 µg/ml and 37.5 µg/mof empagliflozin.

Precision

The method precision (repeatability) was performed by carrying out six independent assays of the test sample at 1000 µg/ml of metformin HCl, 25 µg/ml of empagliflozin, and 5 µg/ml of linagliptin against the reference standard. The intermediate precision was evaluated by carrying out six independent assays of test samples on different days at 1000 µg/ml of metformin HCl, 25 µg/ml of empagliflozin, and 5 µg/ml of linagliptin against the reference standard.

Accuracy

Concentrations of drugs at 50 %, 100%, and 150 % levels were spiked to the pre-analyzed sample solution and were injected into the HPLC system each in triplicate. The % mean recovery at each of the concentration levels was calculated to determine the accuracy.

Robustness

Working standard solution containing 1000 µg/ml of metformin HCl, 25 µg/ml of empagliflozin, and 5 µg/ml of linagliptin was prepared as per the test method and injected into the HPLC system at variable conditions such as flow rate of±0.1 ml/min and organic phase composition of mobile phase by±5% to study the robustness of the method.

Forced degradation study

Forced degradation studies were carried out as per ICH guidelines Q1A (R2) [32]. Samples of metformin HCl, linagliptin, and empagliflozin were exposed to different stress conditions, such as acidic, alkaline, oxidative, thermal, and photostability conditions, for the forced degradation studies. In the case of acidic and alkali degradation, samples were treated with 1 M HCl and 1 M NaOH at 60 °C for 30 min. Oxidative degradation was done using 30% v/v H_2O_2 at 60 °C for 30 min. Thermal degradation was conducted by exposing the powder sample to 60 °C for 24 h in an oven. Photostability was checked by exposing the sample to UV light by placing it in a UV chamber for 24 h. For degradation under hydrolysis conditions, the samples were treated with water for 2 h at 60 °C. After the stipulated time, all of the samples were cooled to room temperature, the acid and base treated samples were neutralized and analyzed using the optimized chromatographic conditions to assess the degradation of drugs and stability, indicating nature of the method.

RESULTS

Method development and optimization

This work was majorly emphasized to establish new stability-indicating RP-HPLC method for the simultaneous quantification of metformin HCl, linagliptin, and empagliflozin in their recently approved fixed dosage combinations. After multiple systematic trials, the optimum chromatographic condition having well-resolved peaks with better peak shape was achieved by using an isocratic mobile phase composed of 0.1 % TEA (pH =3) buffer and acetonitrile (40: 60 v/v) at a flow rate of 1.0 ml/min, injection volume of 10 µl, column temperature 25 °C, and detection wavelength 240 nm. Separation on Agilent Eclipse XDB-C18 (250 mm x 4.6 mm, 5 µm) column gave the desired chromatographic parameters for the optimized condition. The retention time values under the optimized condition were 2.660 min, 3.586 min, and 5.412 min for metformin HCl, linagliptin, and empagliflozin, respectively. The final optimized chromatogram is presented in fig. 4.

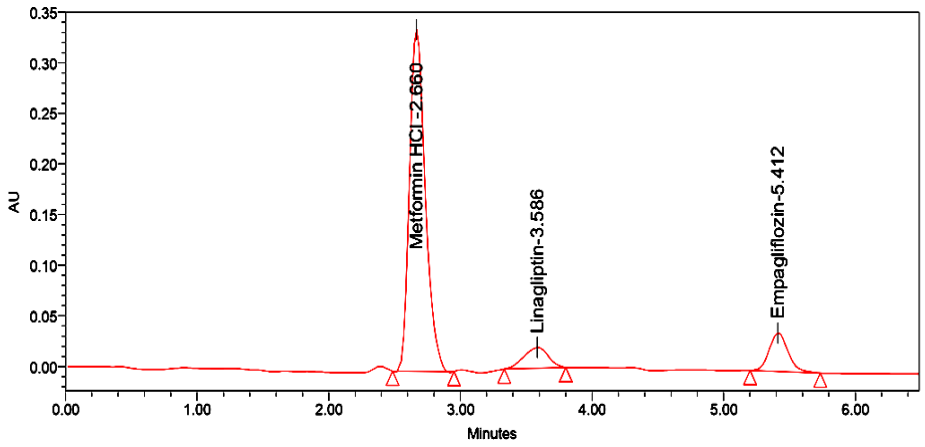

Fig. 4: Optimized chromatogram of the proposed method

Method validation

System suitability test

System suitability was enumerated by performing six independent injections of working standard solutions of the drugs. Parameters including % relative standard deviation (RSD), the number of theoretical plates, resolution, and tailing factors were calculated. The %RSD values for peak response were found to be 0.11%, 0.12% and 0.08% for metformin HCl, linagliptin and empagliflozin respectively (table 1). The tailing factors were less than 1.5, and all the parameters met the requirements for the system suitability.

Specificity

The specificity of the method was evaluated by performing an analysis of the standard solution, sample solution, placebo, and blank solution for the presence of possible interferences. The HPLC chromatogram for the placebo and blank showed no interfering peaks. The placebo chromatogram is presented in fig. 5.

Linearity

Seven point calibration curves were obtained in a concentration range of 100 µg/ml-1500 µg/ml for metformin HCl, 0.5 µg/ml-7.5 µg/ml for linagliptin, and 2.5 µg/ml-37.5 µg/ml for empagliflozin. Peak area and concentration data were subjected to least square regression analysis and the response of the drugs was found to be linear in the investigated concentration ranges. The linear regression equations were y = 2795x+67884 for metformin HCl, y = 49054x+229 for linagliptin and y = 15278x+2772 for empagliflozin. The coefficient of determination (R^2) values was 0.999, 0.9995, and 0.9996 for metformin HCl, linagliptin, and empagliflozin, respectively. The linearity curves are presented in fig. 6-8.

Table 1: System suitability data of proposed method

Parameters	Peak name		
	Metformin HCl	Linagliptin	Empagliflozin
Retention time	2.667±0.006	3.588±0.004	5.413±0.01
Peak Area	2793177±3102	263307±313	378483±314
Number of theoretical plates (N)	3012±46.7	4513±98.4	6902±69.0
Tailing factor (T)	1.09±0.06	0.91±0.05	1.07±0.08
Resolution (R)	-	3.56±0.43	5.78±0.10
% RSD (peak area)	0.11	0.12	0.08

mean±Standard Deviation (SD) (n= 6)

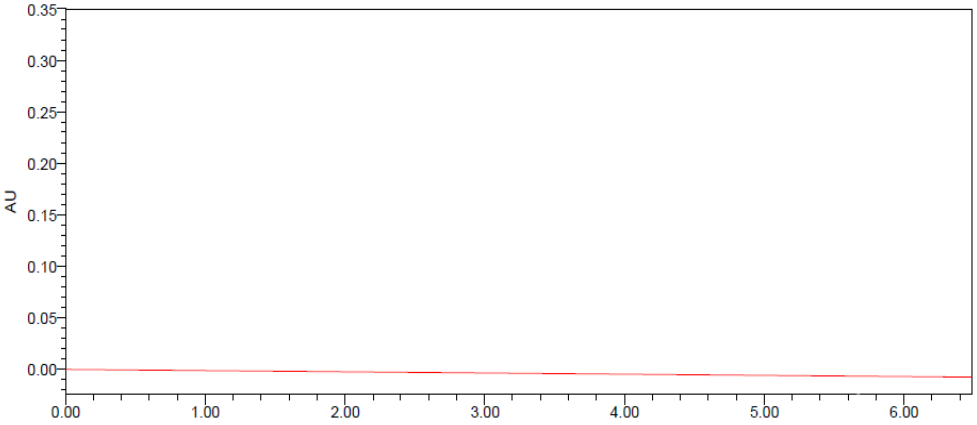

Fig. 5: Specificity chromatogram of placebo

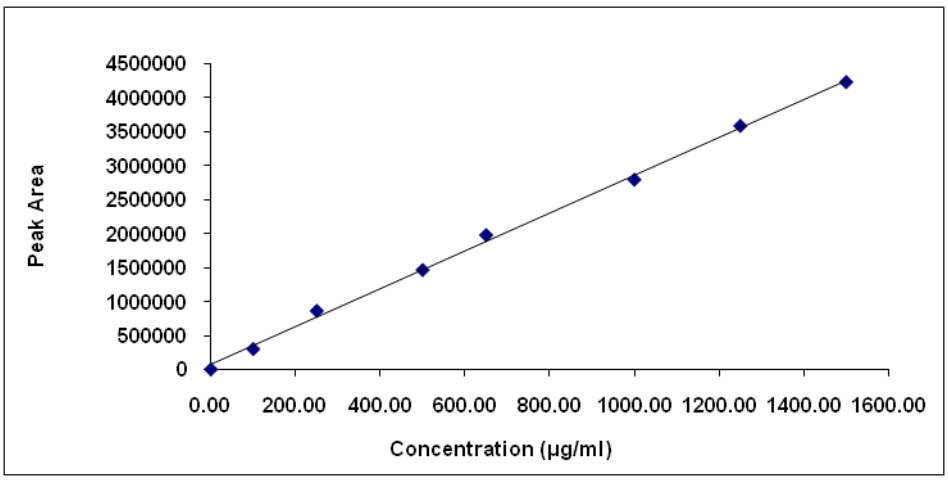

Fig. 6: Standard calibration graph of metformin HCl

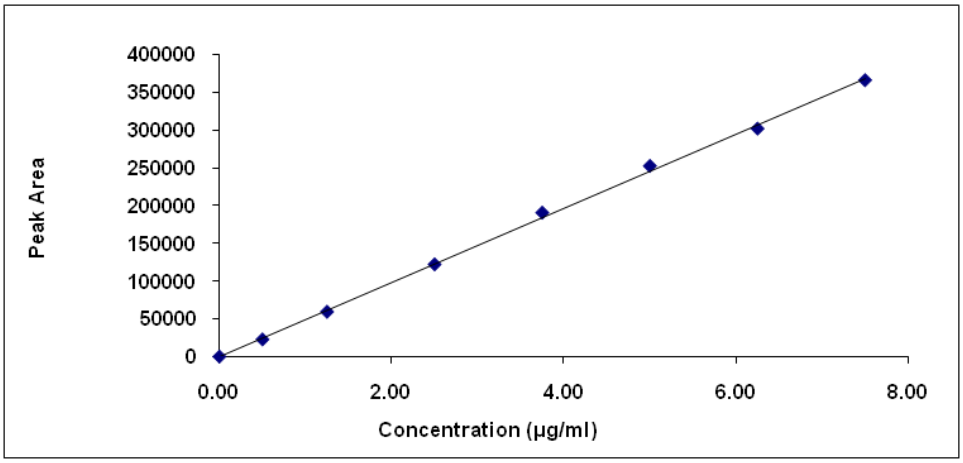

Fig. 7: Standard calibration graph of linagliptin

Limit of detection (LOD) and limit of quantification (LOQ)

Method's sensitivity was checked by evaluating the limits of detection (LOD) and limit of quantification (LOQ). For the LOD and LOQ studies, three replicates of the analytes at the lowest concentration were prepared as per the test method and injected into the HPLC system. LOD was established by identifying the concentration which gave a signal-to-noise (S/N) ratio of 3, whereas LOQ was established by identifying the concentration, which gave an S/N ratio of 10 [30]. The LOD measures were 4.0 µg/ml, 0.02 µg/ml, and 1.0 µg/ml for metformin HCl, linagliptin and empagliflozin respectively, while the LOQ values were 13.4 µg/ml, 0.07 µg/ml and 3.3 µg/ml for metformin HCl, linagliptin and empagliflozin respectively. The result of LOD and LOQ is presented in table 2.

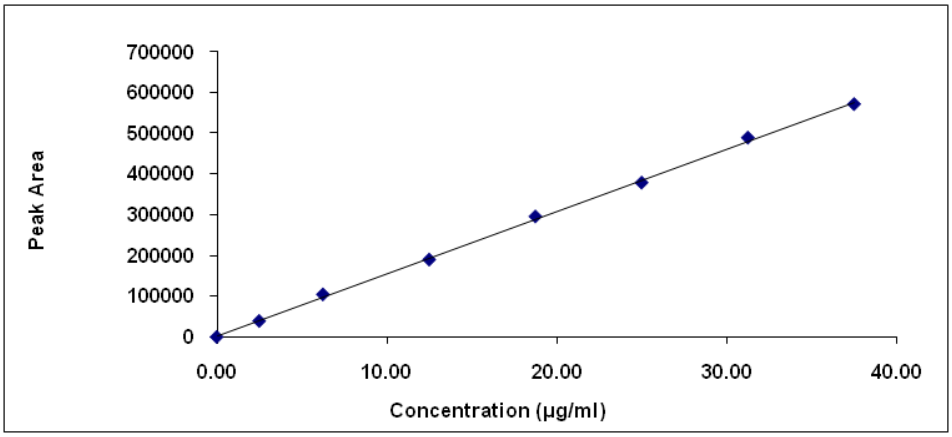

Fig. 8: Standard calibration graph of empagliflozin

Table 2: LOD and LOQ data of proposed method

Parameter	Measured values (µg/ml)		
	Metformin HCl	Linagliptin	Empagliflozin
LOD	4.0±0.1	0.02±0.0	1.00±0.06
LOQ	13.4±0.05	0.07±0.01	3.3±0.08

mean±SD (n= 3)

Precision

The chromatograms of six injections for method precision studies and six injections for intermediate precision studies were recorded and % RSD values were calculated (table 3). The % RSD of peak responses for method precision were 0.495%,0.557% and 0.795% for metformin HCl, linagliptin and empagliflozin respectively, while the % RSD of peak responses for intermediate precision were 0.79%, 0.82% and 0.0.8% for metformin HCl, linagliptin and empagliflozin respectively. The results demonstrated the appropriate precision of the developed method.

Accuracy

Accuracy of the proposed method was ascertained by performing recovery studies using the standard addition method by spiking the known quantities of standards at 50, 100, and 150 % each in triplicate to the pre-analyzed samples of metformin HCl, linagliptin, and empagliflozin. The recoveries were found to be 99.49-100.87%, 99.23-100.40%, and 99.67-100.67% for metformin HCl, linagliptin, and empagliflozin with % RSD values less than 1.64. The accuracy result is presented in table 4.

Table 3: Precision data of proposed method

Parameter	Metformin HCl		Empagliflozin		Linagliptin	
	Peak area	% Assay	Peak area	% Assay	Peak area	% Assay
	Method precision					
Mean	2782618	99.6	377760	99.8	263529	100.1
SD	13781.66	0.504	2180.90	0.565	2093.88	0.781
%RSD	0.495	0.51	0.577	0.57	0.795	0.78
Intermediate precision						
Mean	2781702	99.6	376814	99.6	263368	100
SD	21842.3	0.797	3090.66	0.829	2147.683	0.819
% RSD	0.785	0.8	0.82	0.83	0.815	0.82

(n= 6)

Table 4: Accuracy data of the proposed method

Recovery level	Amount added (mg)	Peak area	Amount recovered (mg)	% Mean recovery±SD	% RSD
Metformin HCl					
50%	500.00	1390076	497.43	99.49±1.11	1.12
100%	1000.00	2790502	998.57	99.86±0.23	0.23
150%	1500.00	4227571	1512.81	100.87±0.51	0.51
Linagliptin					
50%	2.5	132086	2.51	100.4±0.40	0.39
100%	5.0	262538	4.99	99.73±0.50	0.50
150%	7.5	391953	7.44	99.23±1.63	1.64
Empagliflozin					
50%	12.5	188600	12.46	99.67±0.67	0.67
100%	25.0	377751	24.95	99.83±0.38	0.38
150%	37.5	571332	37.74	100.67±0.21	0.21

(n= 3)

Table 5: Robustness data of proposed method

Robustness conditions	% RSD*		
	Metformin HCl	Linagliptin	Empagliflozin
Flow rate minus (0.9 ml/min)	0.77	0.46	0.70
Flow rate plus (1.1 ml/min)	0.91	1.05	0.53
Organic phase compositionminus (55%)	0.87	0.55	0.78
Organic phase compositionplus (65%)	0.67	1.04	0.65

*Each value is % RSD of triplicate measurements (n= 3)

Robustness

The robustness study was performed by slight modification in the flow rate of the mobile phase and composition of the mobile phase. The % RSD of peak areas of the robust conditions was calculated to cheek the reproducibility of the method. The % RSDs of peak response were ranged between 0.46%-1.05% for the deliberately altered method conditions. Robustness data is presented in table 5.

Forced degradation studies

The forced degradation studies showed that degradation peaks were observed when the drug samples were stressed with acid, base, and peroxide, while no apparent degradation peaks were seen in photolytic and hydrolysis degradation. The % of degradation was shown within the range of 12.9-2.9% at various stresses conditions. The maximum degradation (12.9%) was enumerated for linagliptin in acid stress conditions, while the minimum (2.9 %) was recorded for metformin HCl in hydrolysis stress conditions. For all the forced degradation samples the purity angles were less than the purity threshold. Results of forced degradation studies are presented in table 6.

Assay of tablet dosage forms

The proposed method was used for the assay of commercially available tablets containing combinations of Metformin HCl, Linagliphtin, and Empagliflozin. The assay was performed in triplicates. The assay percentage of Metformin HCl, Linagliphtin, and Empagliflozin was found to be within the limits of 99.8-100.1%. Results of the formulation analysis are presented in table 7.

Table 6: Result of forced degradation studies at various stress conditions

	Stress condition				
	Control	Acid	Alkali	Photo	Hydrolysis
	Metformin HCl				
% degradation	-	12.4	11.6	3.3	2.9
Purity angle	0.134	0.107	0.117	0.141	0.138
Purity threshold	1.228	1.229	1.235	1.272	1.256
Linagliptin					
% degradation	-	10.1	10.7	3.4	3.0
Purity angle	0.134	0.11	0.119	0.143	0.135
Purity threshold	1.228	1.226	1.233	1.27	1.259
Empagliflozin					
% degradation	-	12.9	12.1	4.5	3.2
Purity angle	0.134	0.133	0.12	0.14	0.135
Purity threshold	1.228	1.228	1.232	1.273	1.259

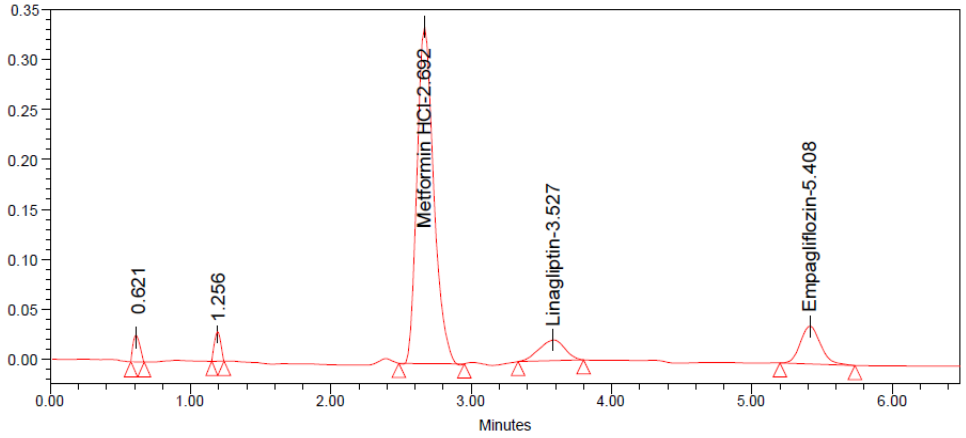

Fig. 9: Chromatogram of acid degradation study

Table 7: Assay result of tablet dosage form

Component	Label claim (mg per tablet)	*Amount found (mg)	**%
Metformin HCl	1000	998	99.8±0.4
Linagliptin	5	5.05	100.1±1.0
Empagliflozin	25	24.95	99.8±0.6

**mean±SD (n= 3)

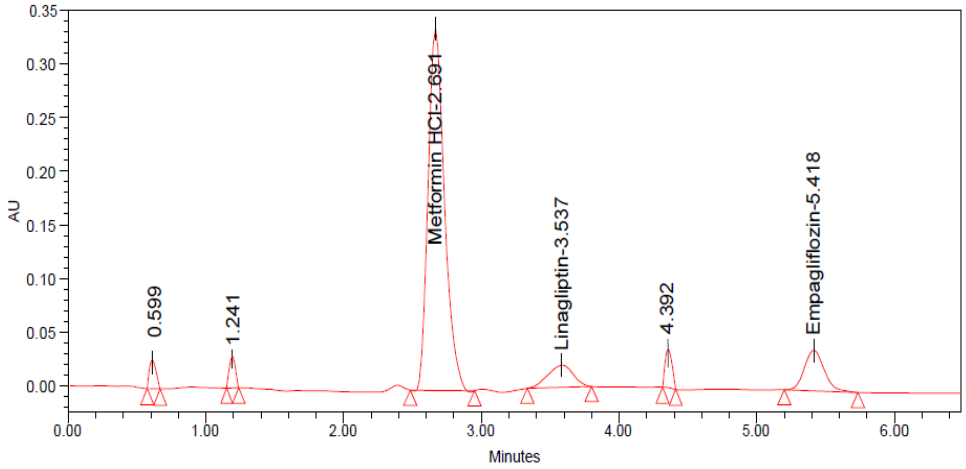

Fig. 10: Chromatogram of alkali degradation study

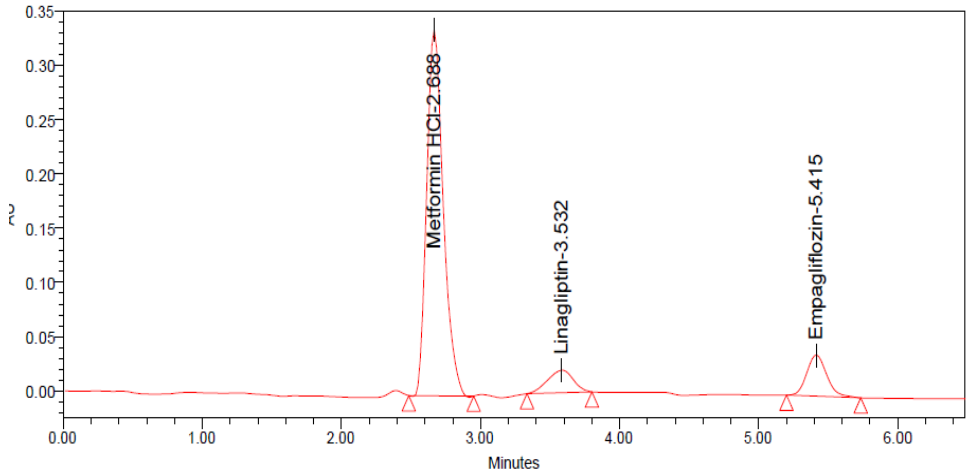

Fig. 11: Chromatogram of photodegradation study

DISCUSSION

As compared to previous literature reports; fast, sensitive, and cost-effective stability-indicating analytical method was developed for the simultaneous estimation of metformin HCl, linagliptin, and empagliflozin [28, 29]. The analytical method was developed and optimized to determine suitable chromatographic conditions for obtaining sharp and well-resolved peaks of metformin HCl, linagliptin, and empagliflozin with minimal tailing. After several trials on different RP columns such as Zodiac C18 (150 mm x 4.6 mm, 5 µm), X-bridge Phenyl (150 mm x 4.6 mm, 5 µm), and Eclipse XDP-C18 (250 mm x 4.6 mm, 5 µm), the optimum separation between the analytes was achieved on Agilent Eclipse XDB-C18 (250 mm x 4.6 mm, 5 µm) column. Along with columns, different mobile phase compositions at different pH and flow rates were also evaluated and optimum separation was achieved by using 0.1 % TEA (pH =3) buffer and acetonitrile (40: 60 v/v) as a mobile phase at a flow rate of 1.0 ml/min. In a previously reported method, it required a long run time (18 min) due to longer retention of empagliflozin peak [30], whereas in this method, the overall run time was 6 min because of elution of analyte peaks at a shorter possible time without compromising resolution and this signifies that the proposed method is rapid and cost-effective.

All the system suitability parameters were within the acceptable limit of ICH guidelines [31], the resolutions were greater than 2, the theoretical plate count was greater than 2,000, tailing factors were less than 2, and % RSDs for peak response were less than 2 (table 1). The developed method was found to be selective as the HPLC chromatogram for the placebo proves that there were no co-eluting peaks at the retention time of metformin HCl, linagliptin, and empagliflozin; this clarifies that the excipients used in formulations didn't interfere with analyte determination. High values of coefficient of determination (R^2) for metformin HCl, linagliptin, and empagliflozin indicate the worthy linearity of the proposed method for the simultaneous analysis of the drugs in their combined dosage forms [31, 32]. As compared to previously reported methods, this method covers a wider linearity range i.e. about 10%-150% of the working concentration ranges of each drug, whereas in previously reported methods, the linearity of the calibration curve was ranged in 10%-100% [28-30]

The very low measures of LOD and LOQ indicate better sensitivity of the proposed method for the analysis of metformin HCl, linagliptin, and empagliflozin [30-31]. The proposed method showed a remarkable outcome compared to a previous report with respect to low LOD and LOQ, particularly for metformin HCl [29]. The %RSD values of both method and intermediate precision were less than 2, which implies that the proposed method is precise. The proposed method was found to be accurate because the % recoveries were between the acceptable range of 98%-102% and %RSDs were less than 2 as per ICH guidelines [31, 32].

Small but deliberate changes in method parameters such as flow rate minus (0.9 ml/min), flow rate plus (1.1 ml/min), mobile organic phase composition minus (55 %), and mobile organic phase composition plus (65%) didn't result in significant alliterations of peak responses for the analyzed drugs (% RSD less than 2). These

low %RSD responses indicate that the robustness variations have no massive influence on the analytical output of the proposed RP-HPLC method. The % of degradation was shown within the range of 12.9-2. 9 % at various stresses conditions and the % of degradation from a previous study was found within 7.76-0.13 % [29]. The proposed method was stability indicating as it is evidenced by clear separation of degradation peaks from analyte peaks in the chromatograms of forced degradation studies (fig. 9 and 10). Moreover, for all the forced degradation samples, the purity angles were less than the purity threshold; this indicates that there is no interference from the degradants in quantifying the analytes in combined dosage forms and thus, the developed method is considered to be stability-indicating [32].

CONCLUSION

New stability indicating RP-HPLC method was successfully developed and validated for the simultaneous determination of metformin HCl, linagliptin, and empagliflozin. The developed method was found to be simple, specific, accurate, precise, and robust. The method was successfully applied for the determination of metformin HCl, linagliptin, and empagliflozin in their combined tablet dose formulations and hence can be used for the routine analysis of these drugs in pharmaceutical bulk, combined, and individual dosage forms.

ACKNOWLEDGMENT

The authors would like to thank the Department of Pharmaceutical Analysis and Quality assurance, A. U. College of Pharmaceutical Sciences, Andhra University for their support.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author TTU is mainly involved in this research work for the compilation of his Ph. D. thesis. Author AKMP has guided all through the work.

CONFLICT OF INTERESTS

Authors have declared that no conflict of interest exists.

REFERENCES

1. Ohiagu FO, Chikezie PC, Chikezie CM. Pathophysiology of diabetes mellitus complications: metabolic events and control. Biomed Res Ther. 2021 Mar 31;8(3):4243-57. doi: 10.15419/bmrat.v8i3.663.
2. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018 Feb 28;138:271-81. doi: 10.1016/j.diabres.2018.02.023, PMID 29496507.
3. International Diabetic Federation [internet]. Type 2 diabetes. Vol. 54. p. B-1160 Brussels, Belgium. Avenue Hermann-Debroux. Available from: https://www.idf.org/aboutdiabetes/type-2-diabetes.html. [Last accessed on 20 Jul 2021]
4. Simo R, Hernandez C. Treatment of diabetes mellitus: general goals, and clinical practice management. Rev Esp Cardiol. 2002 Apr 1;55(8):845-60. doi: 10.1016/s0300-8932(02)76714-6, PMID 12199981.
5. Song R. Mechanism of metformin: A tale of two sites. Diabetes Care. 2016 Feb 01;39(2):187-9. doi: 10.2337/dci15-0013, PMID 26798149.
6. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. Diabetologia. 2017 Aug 03;60(9):1577-85. doi: 10.1007/s00125-017-4342-z, PMID 28776086.
7. Tella SH, Akturk HK, Rendell M. Linagliptin for the treatment of type 2 diabetes. Diabetes Manag. 2014;4(1):85-101. doi: 10.2217/dmt.13.55.
8. Chen XW, He ZX, Zhou ZW, Yang T, Zhang X, Yang YX, Duan W, Zhou SF. Clinical pharmacology of dipeptidyl peptidase 4 inhibitors indicated for the treatment of type 2 diabetes mellitus. Clin Exp Pharmacol Physiol. 2015 Oct 01;42(10):999-1024. doi: 10.1111/1440-1681.12455, PMID 26173919.
9. Chawla G, Chaudhary KK. A complete review of empagliflozin: most specific and potent SGLT2 inhibitor used for the treatment of type 2 diabetes mellitus. Diabetes Metab Syndr. 2019 May-Jun;13(3):2001-8. doi: 10.1016/j.dsx.2019.04.035, PMID 31235127.
10. Center for Drug Evaluation and Research [internet]. Application number: 212614Orig1s000-Clinical Pharmacology and pharmaceutics review. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/212614Orig1s000ClinPharmR.pdf. [Last accessed on 20 Aug 2020]
11. Lingvay I, Beetz N, Sennewald R, Schuler Metz A, Bertulis J, Loley C, Lang B, Lippert C, Lee J, Manning LS, Terada D. Triple fixed-dose combination empagliflozin, linagliptin, and metformin for patients with type 2 diabetes. Postgrad Med. 2020 May 04;132(4):337-45. doi: 10.1080/00325481.2020.1750228, PMID 32366156.
12. Li J, Lian H. Recent development of single preparations and fixed-dose combination tablets for the treatment of non-insulin-dependent diabetes mellitus: A comprehensive summary for antidiabetic drugs. Arch Pharm Res. 2016 May 26;39(6):731-46. doi: 10.1007/s12272-016-0762-4, PMID 27230777.
13. Padmaja N, Veerabhadram G. Development and validation of an analytical method for simultaneous estimation of empagliflozin and linagliptin in bulk drugs and combined dosage forms using UV-visible spectroscopy. Pharm Lett. 2015;7(12):306-12.
14. Patil SD, Chaure SK, Kshirsagar S. Development and validation of UV spectrophotometric method for Simultaneous estimation of Empagliflozin and Metformin hydrochloride in bulk drugs. Asian J Pharm Anal 2017;7(2). doi: 10.5958/2231-5675.2017.00019.9.
15. Rane SS, Chaudhari RY, Patil VR, Barde LG. Development and validation of UV spectrophotometric method for simultaneous estimation of empagliflozin and linagliptin in bulk drugs and pharmaceutical dosage form. Doctrines Integr Med Pharm Sci. 2021;01(1):44-53.
16. Mishra K, Soni H, Nayak G, Patel SS, Singhai AK. Method development and validation of metformin hydrochloride in tablet dosage form. J Chem. 2011;8(3):1309-13. doi: 10.1155/2011/768014.
17. Kar M, Choudhury PK. HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. Indian J Pharm Sci. 2009;71(3):318-20. doi: 10.4103/0250-474X.56031, PMID 20490303.
18. Chhetri HP, Thapa P, Van Schepdael A. HPLC method for the quantification of metformin hydrochloride in bulk and dosage forms. Int J Pharm Sci Res. 2013 Jul 01;4(7):2600-4.
19. Sekhar Reddy BRC, Bhaskar Rao NV, Saraswathi K. A validated stability-indicating HPLC assay method for linagliptin. Pharm Sin. 2014;5(5):131-7.
20. Vemula P, Dodda D, Balekari U, Panga S, Veeresham C. 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 Jan 30;6(1):25-8. doi: 10.4103/2231-4040.150368, PMID 25709966.
21. Kavitha KY, Geetha G, Hariprasad R, Kaviarasu M, Venkatnarayanan R. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of linagliptin and metformin in pure and pharmaceutical dosage form. J Chem Pharm Res. 2013;5(1):230-5.
22. Mallikarjuna RN, Gowri SD. RP-HPLC method for simultaneous estimation and stability-indicating study of metformin and linagliptin in pure and pharmaceutical dosage forms. Int J Pharm Pharm Sci. 2015 Mar 01;7(3):191-7.
23. Sirigiri N, Subramanian NS, Kumar Reddy GN. Stability indicating method development and validation for simultaneous estimation of linagliptin and empagliflozin in tablets by HPLC. Saudi J Pharm Sci. 2018 Aug 30;4(8):884-96.
24. Raut AN, Jawarkar SG, Khodke VS, Khole VA. Method development, validation by simultaneous estimation of empagliflozin and linagliptin by RP-HPLC method. J Pharm Sci Innov. 2020;9(1):1-4.

25. El-sheik R, Hassan WS, Youssef E, Hamdi AY, Badahdah NA, Alzuhiri ME. Development and validation of rapid stability-indicating high-performance liquid chromatography method for the determination of linagliptin and empagliflozin in pure and dosage forms. Asian J Pharm Clin Res. 2020 Apr 07;13(4):172-7.
26. Gopal NM, Sridhar C. A validated stability-indicating ultra-performance liquid chromatographic method for simultaneous determination of metformin hydrochloride and empagliflozin in bulk drug and tablet dosage form. Int J App Pharm. 2017 May 01;9(3):45-50. doi: 10.22159/ijap.2017v9i3.17441.
27. Vankalapati KR, Alegete P, Boodida S. Stability-indicating ultra-performance liquid chromatography method development and validation for simultaneous estimation of metformin, linagliptin, and empagliflozin in bulk and pharmaceutical dosage form. Biomed Chromatogr. 2021;35(4):e5019. doi: 10.1002/bmc.5019. PMID 33146442.
28. Kiram TN, Parvathi P, Suresh Kumar TN. Development and validation of RP-HPLC method for the simultaneous estimation of linagliptin, Empagliflozin, and metformin in solid dosage forms. Asian J Pharm Anal. 2020 Aug 04;10(3):1-5.
29. Gurrala S, Raj S, Cvs S, Anumolu PD. Quality-by-design approach for chromatographic analysis of metformin, empagliflozin and linagliptin. J Chromatogr Sci. 2021 Apr 05;59(4):1-27. doi: 10.1093/chromsci/bmab030, PMID 33822920.
30. 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. Futur J Pharm Sci. 2021 Aug 30;7(1):182. doi: 10.1186/s43094-021-00332-1.
31. International Conference on Harmonization. ICH Harmonized tripartite guideline. Validation of Analytical Procedures: Text and Methodology. Vol. Q2. Geneva, Switzerland; 2005. p. R1.
32. International Conference on Harmonization. ICH Harmonized tripartite guideline. Stability Testing of New Drug Substances and Products. Vol. Q1A. Geneva, Switzerland; 2003. p. R2.