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

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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

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].

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-ethyquinazolin-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].

Empagliflozin is among the new sodium-glucose co-transporter-2 (SGLT2) inhibitors. Chemically it is, 1-chloro-h-1-[\(\text{D-glucopyranos-1-yl}\)]-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].
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 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 milliliters 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 milliliters 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, 5.0 µg/ml, 6.25 µg/ml, 7.5 µg/ml, 10.0 µg/ml, 12.5 µg/ml, 18.75 µg/ml, 25 µg/ml, 31.25 µg/ml and 37.5 µg/ml of linagliptin and empagliflozin respectively.

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 alkaline 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 H2O2 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 a 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 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 alkaline 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 H2O2 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 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 (R²) 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                     | Metformin HCl | Linagliptin | Empagliflozin |
|--------------------------------|---------------|------------|---------------|
| Retention time                 | 2.66±0.006    | 3.58±0.004 | 5.41±0.01     |
| Peak Area                      | 279±3177±3102 | 263307±313 | 378432±314    |
| Number of theoretical plates (N)| 3012±46.7     | 451±98.4  | 6902±69.0     |
| Tailing factor (T)             | 1.09±006      | 0.91±0.05 | 1.07±0.08     |
| Resolution (R)                 | 3.65±0.43     | 5.76±0.10 |               |
| % RSD (peak area)              | 0.11          | 0.12       | 0.08          |

mean±Standard Deviation (SD) [n= 6]
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.
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 0.88% 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 2: LOD and LOQ data of proposed method

| Parameter | Measured values (µg/ml) |
|-----------|-------------------------|
| LOD       | 4.0±0.1                 |
| LOQ       | 13.4±0.05               |

Table 3: Precision data of proposed method

| Parameter | Metformin HCl | Linagliptin | Empagliflozin |
|-----------|---------------|-------------|---------------|
| Mean      | 2782618       | 377760      | 263529        |
| SD        | 13781.66      | 2180.90     | 2093.38       |
| RSD       | 0.495         | 0.577       | 0.795         |
| Intermediate precision | 2781702 | 376814 | 263368 |
| SD        | 21842.3       | 3090.66     | 2147.683      |
| RSD       | 0.785         | 0.82        | 0.815         |

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   | 998.37                | 99.86±0.23         | 0.23  |
| 150%           | 1500.00           | 4227571   | 1512.01               | 100.07±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 composition minus (55%) | 0.87   | 0.55          | 0.78        |
| Organic phase composition plus (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 check 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, Linagliptin, and Empagliflozin. The assay was performed in triplicates. The assay percentage of Metformin HCl, Linagliptin, and Empagliflozin was found to be within the limits of 99.8-100.1%. Results of the formulation analysis are presented in table 7.
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 (R²) 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 alterations of peak responses for the analyzed drugs (% RSD less than 2). These
low %RSD responses indicate that the robustness variations have a 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.

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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.

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