



Original Article

RP-LC Method in Gradient mode for Combined Quantification of Metformin, Linagliptin, and Empagliflozin in Combination Tablets

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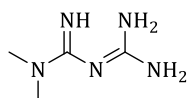
Fixed dosage forms

ABSTRACT

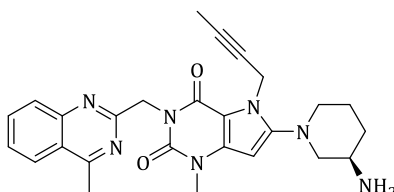
It has been developed and validated an accurate, sensitive, precise, quick, and gradient reverse phase HPLC (RP-HPLC) technique for combined quantification of Metformin, Linagliptin, and Empagliflozin in raw materials and pharmaceutical combined tablets. With acetonitrile as the organic solvent, the best separation was achieved on a 250 mm x 4.6 mm i.d., 5- μ -particle size Inertsil®-Octadecyl-silyl-3V-Reverse-Phase-C₁₈-column with acetonitrile as the non-polar-modifier and Dipotassium *mono*-hydrogen phosphate buffers system [0.03M] in water with pH 3.0 adjusted with *ortho*-phosphoric acid (0.1 %v/v) in the gradient mode of elution as an elution solvent at a speed of 1.0 mL.min⁻¹. UV detection was at 230-nm. Metformin had a retention time of 2.421 minutes, Linagliptin's was 8.187 minutes, and Empagliflozin's was 11.71 minutes. With a correlation coefficient of about 0.9999, the peak-response was obtained as a function of concentration over the range of 80-480 mcg/mL for metformin, 0.4-2.4 mcg/mL for linagliptin, and 0.8-4.8 mcg/mL for empagliflozin. Metformin, Linagliptin, and Empagliflozin were shown to have a percentage assay of 99.92, 99.72, and 99.74, respectively. Metformin, Linagliptin, and Empagliflozin, each have a limit of detection of 0.04 g/mL, 0.004 g/mL, and 0.004 g/mL, respectively. Metformin, Linagliptin, and Empagliflozin, each have a limit of quantification (LOQ) of 0.12 ng/mL, 0.012 g/mL, and 0.012 g/mL, respectively. The presence of excipients in the formulation had no effect on the assay method. The procedure is appropriate for usage in QC- laboratories since it is quick and precise.

GRAPHICAL ABSTRACT

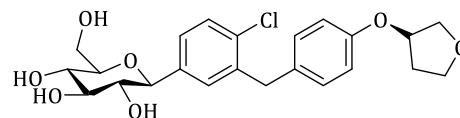
Metformin



Linagliptin



Empagliflozin



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Introduction

Empagliflozin inhibits the Na-glucose-co-transporter system that can be taken orally (SGLT2). The molecular weight is 450.91D and the chemical formula is $C_{23}H_{27}ClO_7$. Empagliflozin is a non-hygroscopic white to yellowish powder [1-3]. It's soluble in water, C_2H_5OH , and acetonitrile; it's soluble in 1:1 proportion of CH_3CN-H_2O ; and essentially not soluble in toluene⁴. Linagliptin is given by mouth, which inhibits the di-peptidyl-peptidase-4 (DPP-4) [4]. It is soluble in water, methanol, ethanol, and acetonitrile, as well as soluble in 50 percent acetonitrile/water, and essentially insoluble in toluene⁴. Linagliptin is an orally administered dipeptidyl peptidase-4 (DPP-4) inhibitor. The chemical formula is $C_{25}H_{28}N_8O_2$, and the molecular weight is 472.54. Linagliptin is soluble in methanol, ethanol very sparingly, isopropanol only very faintly, and acetone only very weakly. Metformin-HCl is a crystalline white-to-off-white powder with a molecular weight of 165.63D and a molecular formula of $C_4H_{11}N_5 \cdot HCl$. Metformin-HCl is soluble in water but almost insoluble in acetone, pet-ether, and chloroform. The pKa-of metformin is 12.4 and metformin hydrochloride in a 1% aqueous solution has a pH of 6.68. Trijardy® is a novel FDC for diabetes therapy which combines extended-release metformin (metformin-XR) with empagliflozin, an SGLT2 inhibitor, and linagliptin, a DPP-4 inhibitor. Each TRIJARDY XR film coated tablet contains an extended-release metformin-HCl central tablet covered with the empagliflozin as fast-released pharmacological ingredient. In the literature, RP-HPLC approach has been published for measuring the potency of metformin and other oral anti-diabetic drugs [5-8]. Nevertheless, no techniques for combined determination of metformin-HCl-with-linagliptin & empagliflozin have been published in oral fixed dosage form. Furthermore, no official or preliminary monograph on this combination of analytes has been published in any of the compendial pharmacopoeias [9]. The goal of this study was to develop an accurate and efficient RP-HPLC method to estimate a new combination of antidiabetic drugs in fixed dosage forms for oral administration. The validation of the devised

approach is also addressed in this study, as per ICH standards [12].

Martials and Methods

- Metformin, 99%, Linagliptin of 99% and Empagliflozin of 99% pure are acquired from Sigma-Aldrich Chemicals, Mumbai, India.
- Rankem-Fine-Chemicals of HPLC- Grade-Acetonitrile.
- Rankem-Fine-Chemicals AR_grade: DiPotassium-monohydrogen-phosphate (dibasic, K_2HPO_4) [0.03M].
- *Ortho*- H_3PO_3 , 85% (v/v) obtained from Quligen-Fine chemicals.
- Chromatographic-Grade water.

Chromatographic-Instrument

Quantitative HPLC was carried out on a Shimadzu Prominence high-performance liquid chromatograph with a PDA detector module, which included an automated injector with a 20-l injection volume and a quadra-pump. The utilized column was a Reverse-Phase-Inertsil-Octa-Decyl-S-3V- C_{18} column (250 mm x 4.6 mm internal diameter with particle size 5 μm). Spin-chrome Software was installed on the HPLC equipment. The column temperature 40 °C was adjusted and eluted over 20.0 minutes at a mobile solvent speed of 1.0 mL.min⁻¹ under linear gradient conditions. The organic modifier is acetonitrile, while the mobile phase is water with Dipotassium hydrogen phosphate [0.03M] and a pH of 3.0 adjusted with *ortho*-phosphoric acid (0.1 percent v/v) (0.1% v/v). It was degassed and filtered *via* 0.45 m Nylon membrane filters before use. For three analytes, UV detection at 230 nm was used as isobestic wavelength detection with a PDA detector.

Preparation of Primary Standard Drug Solutions

To make the primary standard stock solution, 400mg of Metformin, 2 mg of Linagliptin, and 4 mg of Empagliflozin were dissolved in a volumetric flask (100 mL) with 20 mL of diluent (1:1 v/v $CH_3CN:H_2O$), sonicated for 15 minutes, and then brought up to 100mL with diluents to get the primary standard stock solution containing 4000 $\mu g \cdot mL^{-1}$ of Metformin, 20 $\mu g \cdot mL^{-1}$ of Linagliptin and 40 $\mu g \cdot mL^{-1}$ of Empagliflozin.

Preparation of Working Standard Drug Solution

In a 50 mL volumetric flask containing 400 $\mu\text{g}\cdot\text{mL}^{-1}$ Metformin, 2 $\mu\text{g}\cdot\text{mL}^{-1}$ Linagliptin, and 4 $\mu\text{g}\cdot\text{mL}^{-1}$ Empagliflozin, the working standard solution was produced up to 50 mL with diluent (1:1 v/v $\text{CH}_3\text{CN}:\text{H}_2\text{O}$) from 5 mL of the above-mentioned primary-stock solution.

Sample Preparation

Twenty Trijardy-XR® combination tablets, which contain an extended-release metformin-HCl central tablet covered by the empagliflozin and linagliptin as fast-released pharmacological ingredient components are available in 10 mg empagliflozin/5 mg linagliptin/1000 mg metformin-HCl extended-release combined tablet dosage forms, were accurately weighed, and the mean weight was determined, as well. An equivalent-quantity of the homogeneous powder equivalent to 400 mg metformin, 4 mg empagliflozin, and 2 mg linagliptin was transferred, and 50 mL 0.02 M KH_2PO_4 and 0.02 M K_2HPO_4 added. $\text{CH}_3\text{OH}:\text{H}_2\text{O}$ at ratio of 90:10 (v/v) was added as diluent, sonicated for 30 minutes, diluted to 100 mL and pH adjusted to 7.0 with ortho- H_3PO_3 in water. The resulting solution was miscible completely by sonication for 30 min, diluted up to 100 mL with ortho-phosphoric acid. Metformin 400 $\mu\text{g}\cdot\text{mL}^{-1}$, Linagliptin 2 $\mu\text{g}\cdot\text{mL}^{-1}$, and Empagliflozin 4 $\mu\text{g}\cdot\text{mL}^{-1}$ are all present in the sample solution. The individual linear regression equations were used to determine the amounts of metformin, empagliflozin, and linagliptin in tertiary mixture of sample solution form fixed-dosage-forms.

Linearity

Aliquots of Metformin, Linagliptin, and Empagliflozin working stock solutions were placed in various 10mL volumetric flasks and made the volume up to the 10mL with the mobile phase, yielding in final strengths of 80-480 $\mu\text{g}\cdot\text{mL}^{-1}$, 0.4-2.4 $\mu\text{g}\cdot\text{mL}^{-1}$, and 0.8-4.8 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively (Table 1). The peak areas and retention times of each of these drug solutions (loaded at 10 μL) were measured three times in the column. Using a PDA-detector set at 230 nm, a linearity-graph was generated by plotting peak areas versus Metformin, Linagliptin, and Empagliflozin concentrations in $\mu\text{g}\cdot\text{mL}^{-1}$.

Accuracy

The approach's accuracy was found by evaluating the drugs' recovery by using the standard-spiking method. To assess if the analytes contained in the formulation caused positive or negative interventions, known amounts of each drug equivalent to 10 percent standard drug solution were added to 80 percent, 100 percent, and 120 percent of the target test concentrations a formulation mixture. Each set-of-addition was replicated three times at each dilution level. The results are compared to a competent reference standard after extraction of sample preparation. The percentage of analytes re-covered by the assay was used to assess the accuracy. Table 4 shows the results of accuracy investigations on standard solution and process-related impurity; the recovery measurements suggest that the procedure was accurate.

Precision

Quality-control samples in 100 % (w/v) dilution were used to assess intraday and interday precision. On the same day, six replicates of the target concentrations were examined for intraday variation, and six replicates were examined for inter-day variation on three different days. The method's repeatability is indicated by the low RSD value (1%) (Table 4).

Limits of Detection and Quantification

The method's LOD was set at the lowest concentrations of active pharmaceutical components with a signal-to-noise (S/N) ratio of around 3 (LOD). The lowest active therapeutic medication concentrations which can be assessed with acceptable precision and accuracy while maintaining a signal-to-noise (S/N) ratio of roughly 10 (LOQ).

Method Applicability

Our study group evaluated the newly created method by applying it to pharmaceutical tablets for estimating Metformin, Linagliptin, and Empagliflozin.

Method Validation Tests

Method precision (RSD, percent), method accuracy (recovery percent & %RSD), linear range (r^2), and LOD & LOQ were explored as recommended method validation characteristics.

Result and Discussion

Linearity

With a correlation coefficient of 1.0, the graph of chromatographic-peak areas of all analytes versus respective concentrations was indicated to be linear in the band of 80-480 µg. mL⁻¹ for Metformin, 0.4-2.4 µg.mL⁻¹ for Linagliptin, and 0.8-4.8 µg.mL⁻¹ for Empagliflozin (Table 1). The least

square fit data of linear regression analysis was derived from the measurements given in Table 2. Metformin's regression formula is $y = 1E+06x$, Linagliptin's is $y=122817x$, and Empagliflozin's is $y = 187710x$. Table 2 presents the regression parameters for this technique that include slope, intercept, and % RSD. These findings suggest that there was a significant correlation.

Table 1: Evaluation of precision with-in-day and day-to-day analysis

With-in-day Precision study of 100% dilution containing Metformin (400 µg/MI), Linagliptin (2 µg/mL) and Empaglifozin (4 µg/mL)							Day-to-Day Precision study of 100% dilution containing – Metformin (400 µg/mL), Linagliptin (2 µg/mL) and Empaglifozin (4 µg/mL)					
Metformin			Linagliptin		Empagliflozin		Metformin		Linagliptin		Empagliflozin	
S. No	R.T	Peak-Area	R.T	Peak-Area	R.T	Peak-Area	R.T	Peak-Area	R.T	Peak-Area	R.T	926267
1	2.421	5735858	8.187	622809	11.716	935541	2.425	5771972	8.194	607055	11.693	927709
2	2.422	5738972	8.189	606552	11.713	922964	2.426	5773228	8.195	608105	11.696	928509
3	2.422	5753564	8.189	605189	11.702	921717	2.426	5778001	8.196	608723	11.692	929952
4	2.424	5743597	8.191	605106	11.700	922997	2.426	5779250	8.200	609552	11.699	930743
5	2.425	5747941	8.194	605885	11.704	923517	2.426	5784303	8.198	610453	11.693	931079
6	2.425	5751030	8.194	606724	11.698	923612	2.426	5792709	8.193	610354	11.685	929043.2
Average	2.423	5745160.3	8.191	608710.8	11.705	925058	2.426	5779910.5	8.196	609040.3	11.693	1877.9
Std. Dev	0.0017	6929.4	0.0028	6939.0	0.0073	5179.8	0.0042	7679.2	0.0024	1334.2	0.0046	0.2
% RSD	0.060	0.1	0.033	1.1	0.061	0.6	0.019	0.1	0.031	0.2	0.040	926267

Table 2: Regression analysis & Operating-System Suitability Results

Study-Parameter	Metformin	Linagliptin	Empagliflozin
Retention Time (min)	2.421	8.187	11.716
Tailing Factor	1.454	1.399	1.090
Peak areas	5735858	622809	935541
Percentage of peak areas	78.65	8.53	12.82
Theoretical Plates	3033.879	17953.186	31545.673
Resolution	0.000	27.439	13.884
Linear range in (µg/mL)	80-480	0.4-2.4	0.8-4.8
Limit-of-Detection (µg.mL ⁻¹)	0.04	0.004	0.004
Limit-of-Quantification (µg.mL ⁻¹)	0.12	0.012	0.012
Correlation-Coefficient (r ²)	0.998	0.998	0.998
Assay-in-Percentage (%)	99.92	99.32	99.74

Accuracy

Individual recovery of analyte at 80 %-dilution level on w/v basis, 100 %-dilution level on w/v basis and 120 %-dilution level on w/v basis of prescribed concentrations was 98.1 percent to

102.45 percent, demonstrating the method's accuracy. The RSD was usually less than 1% in these data, demonstrating that the technique seems to be very accurate and generates consistent results (Table 3).

Table 3: Summary of the standard calibration Curve for Linearity experiment

Amount-of Metformin (µg/mL)	Chromatogram	Amount-of Linagliptin (µg/mL)	Chromatogram	Amount-of Empagliflozin in µg/mL	Chromatogram
80	Peak- Area	0.4	Peak- Area	0.8	Peak- Area
160	1196011	0.80	124271	1.6	189169
240	2351071	1.20	244392	2.4	370631
320	3525071	1.60	373497	3.2	562549
400	4690599	2	492631	4.0	750593
480	5756301	2.4	609699	4.8	939968

Precision

Table 4 summarizes the intra-day and inter-day fluctuation in precision analysis. The method's repeatability is indicated by the low RSD value

(less than 1%). These results indicate that the approach has a high level of precision and repeatability, both within a single analytical run and across multiple runs (Table 4).

Table 4: Accuracy evaluation by Spike-analysis method

Accuracy parameter	Standard-addition with 80%w/v of the Working Standard			Standard-addition with 100%w/v of the Working Standard			Standard-addition with 120% (w/v) of the Working Standard		
	Empagliflozin (3.2 µg/mL)	Linagliptin (1.60 µg/mL)	Metformin (320 µg/mL)	Empagliflozin (4.0 µg/mL)	Linagliptin (2.0 µg/mL)	Metformin (400 µg/mL)	Empagliflozin (4.8 µg/mL)	Linagliptin (2.4 µg/mL)	Metformin (480 µg/mL)
Amount added	0.40	0.20	40	0.4	0.2	80	0.4	0.2	80
Amount Found	3.57	1.78	358	4.36	2.19	479	5.22	2.41	558
% Recovery	99.16	98.88	99.44	99.09	99.54	99.79	100.2	99.58	99.64
% RSD.	0.02	0.04	0.15	0.28	0.54	0.22	0.62	0.74	0.82

Robustness

At three different levels, -ve2, 0, and +ve2, the influence of minute but intentional alterations in the separation parameters was explored. The experimental settings were purposely changed at three distinct levels to determine the robustness of this approach, and the RT and chromatographic responses were evaluated. The effect was

observed by modifying one factor at a time. The RT and the method response were not affected by changing the stationary phase, pH of elution solvents by 0.2 units (pH=3.2 and pH=2.8), or mobile phase flow rate by 1.0 mL.min⁻¹ (0.8 and 1.2 mL.min⁻¹), demonstrating that the procedure was robust. Table 5 summarises the findings.

Table 5: The method's Robustness can be tested by changing the chromatographic settings (n=3, 100%w/v Working dilution contains Metformin-400 µg/mL, Linagliptin- 2 µg/mL and Empagliflozin-4 µg/mL)

S.No.	Study of Peak areas with Different HPLC Column			Study of Peak areas with Mobile Phase pH increase			Study of Peak areas with Mobile Phase pH decrease		
	Metformin	Linagliptin	Empagliflozin	Metformin	Linagliptin	Empagliflozin	Metformin	Linagliptin	Empagliflozin
1	6035770	633834	971248	5658854	545876	837626	5289148	639916	979780
2	6045001	634525	971486	5661336	542994	828083	5298146	641478	980233
3	6047370	635116	972876	5660917	548447	826794	5302371	642333	981138
Mean	6042713	634492	971870	5660369	545772	830834	5296555	641242	980384
% RSD	0.101	0.101	0.090	0.023	0.500	0.712	0.128	0.191	0.070
S.No.	Study of Retention times with Different HPLC Column			Study of Retention times with Mobile Phase pH increase			Study of Retention times with Mobile Phase pH decrease		
	Metformin	Linagliptin	Empagliflozin	Metformin	Linagliptin	Empagliflozin	Metformin	Linagliptin	Empagliflozin
1	2.420	8.166	11.732	2.257	7.943	10.973	2.345	8.128	11.718
2	2.417	8.162	11.722	2.259	7.937	10.972	2.347	8.126	11.713
3	2.418	8.163	11.728	2.257	7.930	10.970	2.347	8.124	11.718
Mean	2.418	8.164	11.728	2.257	7.937	10.972	2.346	8.126	11.716
% RSD	0.044	0.029	0.044	0.041	0.080	0.016	0.061	0.022	0.027

Limit-of-Detection & Limit-of-Quantifications

The limit-of-detection (LOD) for Metformin is 0.04µg/mL, and for Linagliptin and Empagliflozin was found to be 0.004 µg.mL⁻¹. The limit-of-quantification (LOQ) for Metformin is 0.12 µg.mL⁻¹

, and for Linagliptin and Empagliflozin was found to be 0.012 µg.mL⁻¹. These numbers illustrate the method's high sensitivity, which is essential in most investigations, as well as the fact that it can

be used to detect and quantify analytes over a wide concentration range.

Specificity

The RTs for Metformin, Linagliptin, and empagliflozin were determined to be 2.42 minutes for Metformin, 8.18 minutes for Linagliptin, and 11.72 minutes for empagliflozin, according to the

representative chromatogram given in Figure 1. When the pharmaceutical tablet matrices were evaluated, no indication of excipient interference signals was observed in the respective RTs of the chromatogram. It indicates that the analytes were not disturbing the probable merging peaks. As a result, this technique can be employed with certainty.

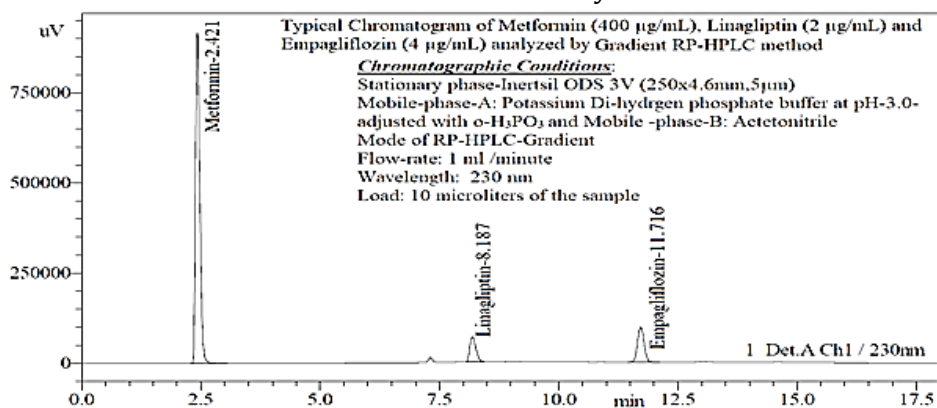


Figure 1: Chromatogram of Metformin-400µg/mL, Linagliptin-2µg/mL and Empagliflozin- 4µg/mL analyzed by the optimized Gradient RP-HPLC method

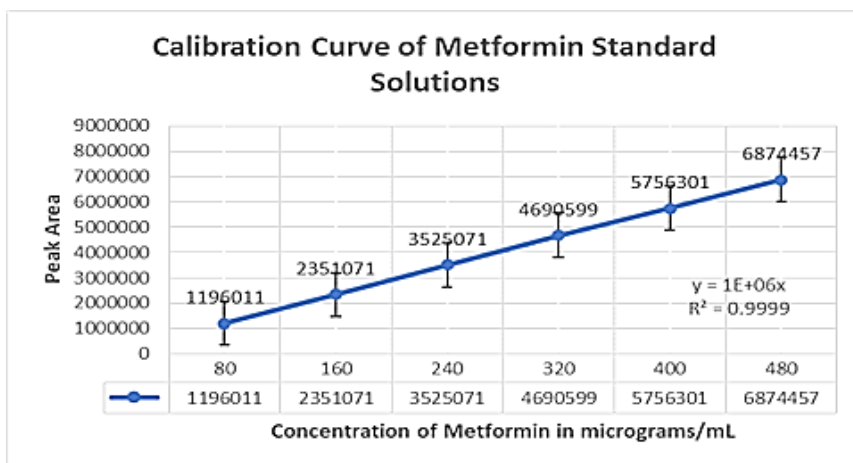


Figure 2: Linearity graphs of metformin for dilution of standard solutions

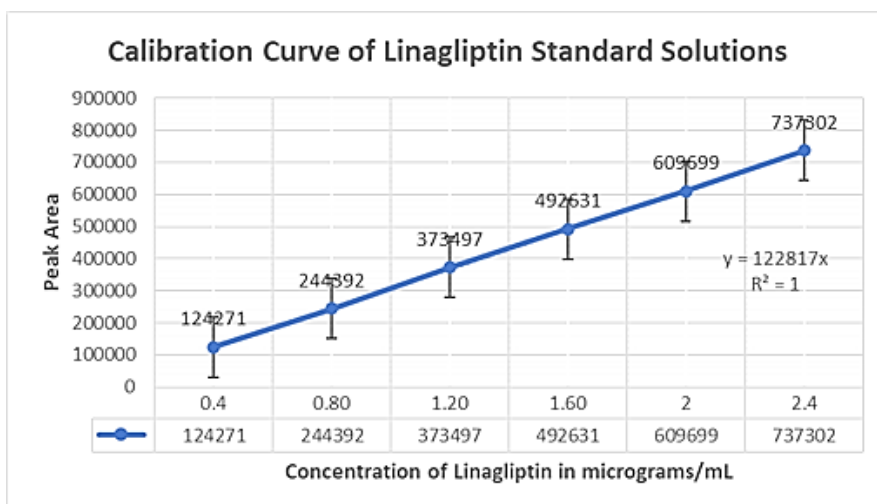


Figure 3: Linearity graphs of Linagliptin for dilution of standard solutions

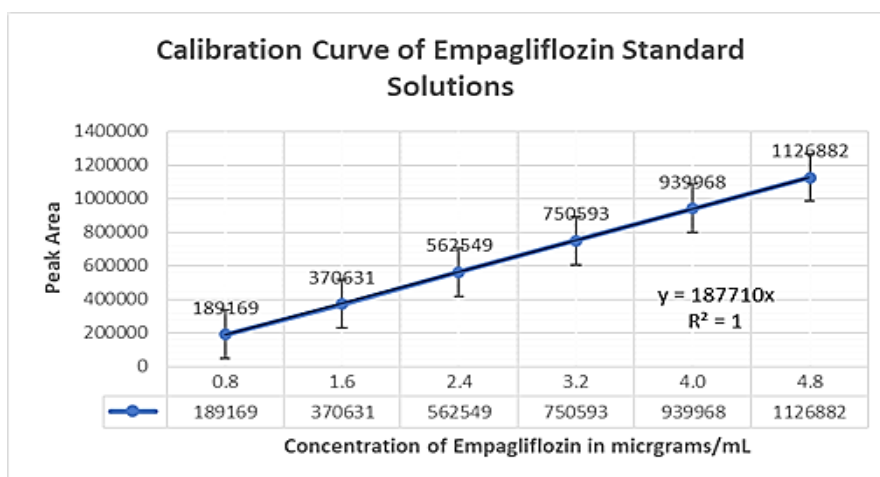


Figure 4: Linearity graphs of empagliflozin for dilution of standard solutions

A gradient RP- HPLC procedure for assaying the active ingredients was developed due to lack of an easy, reproducible, and quick-to-use method for the determination of Metformin, Linagliptin, and Empagliflozin concentrations in formulary matrices. We examined the effect of various HPLC technique variables on the final result of the study to optimize the chromatographic parameters, various proportions of CH₃OH-H₂O, CH₃CN-H₂O, and CH₃CN-KH₂PO₄ buffer were tested. After several early investigatory tests, CH₃CN-KH₂PO₄ buffer system [pH 3.0], it was chosen over other mobile phases because it resulted in improved resolution of pharmacologically active components. This procedure gives the good separation of analytes after multiple exploratory & investigatory trail runs. All three active pharmaceutical analytes had an excellent UV sensitivity and were interference-free at 230 nm. The analyte peaks were highly defined and without any incidence of tailing under these conditions. The aforementioned set of conditions were chosen for additional validation after considering the entire body of data acquired from this extensive study.

Conclusion

In this study, an efficient and commonly available HPLC method was devised for analyzing Metformin, Linagliptin, and Empagliflozin in pharmaceutical matrices. This method's key advantages are its significantly reduced run times, ease of use, and of operation. All of these features are critical in operation, especially when analyzing a large number of samples. The validation

experiments demonstrated that the procedural approach has a large calibration concentration range, adequate precision, accuracy, and practically reliable sensitivity. The method can be used for regular analysis in formulation QC-studies and allows for a straightforward, selective, sensitive, and specific assessment of Metformin, Linagliptin, and Empagliflozin.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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References

- [1]. Yadav C.H., Babu A.M., Development and Validation of Head-space Gas Chromatographic Method in Tandem with Flame ionized detection for the determination of Residual solvents in Simeprevir API Synthesis. *Research Journal of Pharmacy and Technology*, 2021, **14**:5175 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [2]. Mallibabu A., Yadav C.H., Pathi P.J., Development and validation of a stability indicating method by ultra performance liquid chromatography for the determination of vancomycin drug in vancomycin injection, *World Journal of Pharmaceutical Research*, 2022 [[Publisher](#)]
- [3]. Hazarathaiah, C., The Estimation of Mepivacain Hydrochloride Injection Liquid. *International Journal of Engineering and Techniques*, 2018, **4**:628 [[PDF](#)], [[Google Scholar](#)]
- [4]. Bailey C.J., Day C., Fixed-dose single tablet antidiabetic combinations. *Diabetes, Obesity and Metabolism*, 2009 **11**:527 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [5]. Ayoub B.M., UPLC simultaneous determination of empagliflozin, linagliptin and metformin. *RSC advances*, 2015 **5**:95703 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [6]. Shafi M.S.S., Begum A., Saradhi N., Bioanalytical method development and validation of linagliptin in plasma through LC-MS/MS. *International Journal of Bioassays*, 2014, **3**:3146 [[Google Scholar](#)]
- [7]. Shah P.A., Shrivastav P.S., George A., Mixed-mode solid phase extraction combined with LC-MS/MS for determination of empagliflozin and linagliptin in human plasma. *Microchemical Journal*, 2019, **145**:523 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [8]. Donepudi S., Achanta S., Validated HPLC-UV method for simultaneous estimation of linagliptin and empagliflozin in human plasma. *International Journal of Applied Pharmaceutics*, 2018, **10**:56 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [9]. Abdel-Ghany M.F., Abdel-Aziz O., Ayad M.F., Tadros M.M., New LC-UV methods for pharmaceutical analysis of novel anti-diabetic combinations. *Acta Chromatographica*, 2017, **29**:448 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [10]. Mabrouk M.M., Soliman S.M., El-Agizy H.M., Mansour F.R., A UPLC-DAD method for simultaneous determination of empagliflozin and three related substances in Standard-addition human plasma. *BMC Chem.*, 2019, **13**:83 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [11]. Bhole R.P., Wankhede S.B., Pandey M., Stability indicating HPTLC method for simultaneous estimation of empagliflozin and linagliptin in pharmaceutical formulation. *Analytical Chemistry Letters*, 2017, **7**:76 [[Crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

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