RESEARCH

Simultaneous quantification of empagliflozin, linagliptin and metformin hydrochloride in bulk and synthetic mixture by RP–LC method

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Abstract

Background: Extensive literature review revealed that no RP–LC method has been developed for simultaneous estimation of EMPA, LINA and MET in combined dosage form. This is a newer combination approved by USFDA on 4th June 2019 and it is launch in the United State Market on 27th January 2020.

Result: A simple, sensitive, specific, precise and accurate reverse phase—high performance liquid chromatography (RP- HPLC) method has been developed for simultaneous estimation of Empagliflozin, Linagliptin and Metformin HCl in bulk and synthetic mixture. Phenomenex C_{18} column (250 mm × 4.6 mm, 5 μm) was used as stationary phase for chromatographic separation through isocratic elution using Acetonitrile: Methanol: Water in a ratio (27: 20: 53, v/v/v) pH 4 adjusted with 1% Ortho-phosphoric acid as mobile phase at flow rate 1 ml/min. PDA detector was used for simultaneous analysis of all three drugs at common wavelength 223 nm and the each injection volume was 20 μl. The linearity range for Empagliflozin, Linagliptin and Metformin HCl was found to be 0.5–5 μg/ml, 0.25–2.5 μg/ml, and 50–500 μg/ml, respectively. The retention time for Empagliflozin, Linagliptin and Metformin HCl was found to be 14.5 min, 3.4 min and 2.01 min, respectively. The percentage (%) recovery was found to be 99.98–100.81% for Empagliflozin, 99.33–100.57% for Linagliptin and 100.65–101.35% for Metformin HCl respectively.

Conclusion: As per the international Conference on Harmonisation (ICH) Q2 (R1) guideline, proposed RP–LC method validation has been carried out. The proposed RP–LC method was repeatable and selective as per statistical analysis and it can be use for simultaneous estimation of Empagliflozin, Linagliptin and Metformin HCl in bulk and synthetic mixture. The proposed method might be applied for simultaneous estimation of all three drugs in pharmaceutical formulation.

Keywords: Empagliflozin (EMPA), Linagliptin (LINA), Metformin HCl (MET), Validation, RP–HPLC

Background

Empagliflozin (EMPA) is used as a sodium glucose co-transporter-2 (SGLT-2) inhibitor to improve glycemic control in adult patients with type 2 diabetes. SGLT-2 co-transporters reabsorb glucose from the glomerular filtrate in kidney and the glucuretic action resulting from inhibition of SGLT-2 which reduces renal absorption and lowers down the renal threshold for glucose, therefore

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increases glucose excretion which reduces hyperglycae-mia and also helps in blood pressure reduction [1, 2]. Chemically EMPA is 1-chloro-4-(glucopyranos-1-yl)-2-(4-(tetrahydrofuran-3-yloxy)benzyl)benzene and having empirical formula is $C_{23}H_{27}ClO_7$ with molecular weight 450.91 g/mole (Fig. 1A).

Linagliptin (LINA) is having competitive, reversible DPP-4 inhibitory action which is responsible for DPP-4 breakdown reduction of GLP-1 and glucose-depend-ant insulinotropic polypeptide (GIP). From beta cells of the pancreas, GLP-1 and GIP stimulate the release of insulin during inhibiting release of glucagon from pancreatic beta cells. These effects together reduce the breakdown of glycogen in the liver and increase insulin release in response to glucose [2–4]. Chemically LINA is (R)-8-(3-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methylquinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione and having empirical formula is $C_{25}H_{28}N_8O_2$ with molecular weight 472.5422 g/mole (Fig. 1B).

N-dimethylmethanimidamide hydrochloride is Met-formin Hydrochloride (MET). Metformin is belongs to antihyperglycemic agent of the biguanide class which is used for the management of type II diabetes. MET is having empirical formula $C_4H_{11}N_5.HCl$ with molecular weight 165.625 g/mole (Fig. 1C).

Metformin HCl is drug of choice for type II diabetes patient which lowers blood glucose concentrations in type II diabetes without causing hypoglycemia. It is also known as an insulin sensitizer which leads to decrease in insulin resistance which leads to significant reduction of plasma fasting insulin level [5].

On 4th June 2019, USFDA is approved a newer com-bination and is launch in the United State Market on 27th January 2020. Combination works by three complementary mechanisms which help in managing blood glucose in adult with type 2 diabetes [6].

A far-reaching literature survey carried out for quan-titative analysis of EMPA, LINA and MET revealed that the attempts have been made to develop liquid chroma-tographic methods for the estimation of EMPA, LINA and MET alone and combination with other drugs [7–11, 11–14] which represent that no RP–LC method has been reported in literature review for simultaneous estimation of EMPA, LINA and MET in combined dosage form. LC methods are preferred method of analysis due to their accuracy, precision and sensitivity of instrument. RP–LC method is more advantageous over HTPLC which is require a small sample size which can be modified on depending on level of quantitation needed and gives reli-able results. Therefore, attempt has been made for the simultaneous estimation of EMPA, LINA and MET in synthetic mixture by RP–LC method.

Methods

Instruments

HPLC system consisted of binary pump (Model Waters 515 HPLC pump), rheodyne loop injector and PDA detector (Waters 2998). Empower- version 2 software is used for data collection and analysis. Phenomenex C_{18} column (250 mm × 4.6 mm, 5 μm) is used for separa-tion through isocratic elution using Acetonitrile: Meth-anol: Water in a ratio (27: 20: 53, v/v/v) pH 4 adjusted with 1% Ortho-phosphoric acid as mobile phase at flow rate 1 ml/min. The photo diode array (PDA) detector is used for detection monitoring at common wavelength 223 nm and the each injection volume was 20 μl. The cal-ibrated Sartorius CP124S (Sartorius Corporation, United State) instrument is used for weighing of all the active
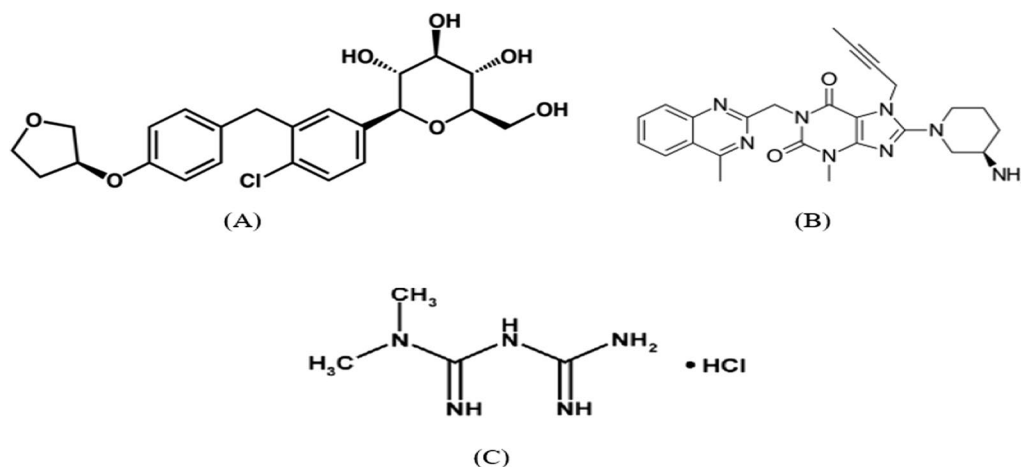

Fig. 1 Chemical structure of (**A**) Empagliflozin. (**B**) Linagliptin. (**C**) Metformin HCl

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pharmaceutical ingredients, excipients and chemicals which is having weighing sensitivity 0.01 mg.

Chemicals and reagents

Analytically pure active pharmaceutical ingredients EMPA (99.23%), LINA (98.92%), and MET (99.12%) was procured as a gratis samples from Torrent Pharmaceutical Pvt. Ltd., Ahmedabad, Gujarat, India, Zydus Cadila Healthcare Ltd., Ahmedabad, Gujarat, India and Sun Pharmaceutical Pvt. Ltd., Vadodara, Gujarat, India respectively. Methanol (HPLC grade) and acetonitrile (HPLC grade) were obtained from SRL Pvt. Ltd. Mumbai, India, HPLC grade water—Milli Q integral Water purification system, Merck KGaA, Darmstadt, Germany, and Ortho-phosphoric acid (AR grade) obtained from SRL Pvt. Ltd. Mumbai, India.

Chromatographic system
Preparation of mobile phase

By taking premix Acetonitrile: Methanol: Water (27:20:53, v/v/v) pH 4.0 adjusted with 0.6 ml of 1% O—phosphoric acid in 200 ml reservoir was used as a mobile phase. For degassing the mobile phase prior to use, the reservoir was sonicated for 20 min and mixtures of solvent were used as mobile phase.

Preparation of standard stock solution

Precisely weigh 100 mg of MET, 2.5 mg of LINA and 5 mg of EMPA and transfer into the three different 10 ml volumetric flask containing 2.0 ml of methanol and the volume was made upto the mark with methanol which gives the stock solution having concentrations 10000 μg/ml of MET, 250 μg/ml of LINA and 500 μg/ml of EMPA, respectively. Pipette out the 2.5 ml of MET, 0.5 ml of LINA and 0.5 ml of EMPA from the above stock solution, transfer into volumetric flask of 50 ml and make up the volume with methanol to acquire the concentration of working standard stock solution 500 μg/ml of MET, 2.5 μg/ml of LINA and 5 μg/ml of EMPA.

Calibration curve

From above working standard stock solution, pipette out appropriate volume of aliquots of MET (500 μg/ml), LINA (2.5 μg/ml) and EMPA (5 μg/ml) are transferred to different 10 ml volumetric flask and volume was adjusted up to the mark with the mobile phase to give a final concentration range of 50, 100, 200, 300, 400 and 500 μg/ml for MET, 0.25, 0.5, 1, 1.5, 2 and 2.5 μg/ml for LINA and 0.5, 1, 2, 3, 4 and 5 μg/ml for EMPA. By using the proposed chromatographic conditions, each solution was analyzed and the chromatogram was recorded. Calibration curves were constructed by plotting peak area v/s concentration and regression equations were computed.

Validation

According to the International Conference on Harmonization (ICH) guidelines Q2 (R1) guidelines [15], validation of the proposed developed RP–LC method was carried out.

Linearity

Linearity was studied by preparing standard solution of 7 different concentrations of 50, 100, 200, 300, 400 and 500 μg/ml for MET, 0.25, 0.5, 1, 1.5, 2 and 2.5 μg/ml for LINA and 0.5, 1, 2, 3, 4 and 5 μg/ml for EMPA. The chromatogram of each concentration was recorded 5 times from freshly prepared seven different concentrations for MET, LINA and EMPA. The terms of slope, intercept and correlation coefficient of MET, LINA and EMPA was used for the assessment of linearity. The calibration curves were developed by plotting concentration v/s peak area (n=5).

Precision

Precision study was designed in expressions of intra-day and inter-day precisions. Intraday and Interday precision was carried out by analyzing sample solution at three levels covering low, medium and high concentration of the calibration curve three times on the same day for intraday precision study and over a period of three different days (n=3) for interday precision study, 50, 200 and 500 μg/ml for MET, 0.25, 1 and 2.5 μg/ml for LINA and, 0.5, 2 and 5 μg/ml for EMPA. The chromatogram was recorded and peak areas obtained were used to calculate mean and % RSD values. The repeatability studies were carried out by estimating the response at 200 μg/ml for MET, 1.0 μg/ml for LINA and 2.0 μg/ml for EMPA 6 times and % RSD of area are reported.

Accuracy

The accuracy study was performed by calculating recovery of MET, LINA and EMPA with the help of standard addition method. Known amount of MET, LINA and EMPA at 80, 100, and 120% levels were spiked to pre quantified sample solution and the amount of MET, LINA and EMPA were estimated by putting the value of peak area to the regression equation of calibration curve.

Limit of detection and limit of quantification

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can detect. The limit of quantification (LOQ) is the lowest amount of analyte that can be quantitatively determined with appropriate precision and accuracy. As per ICH guideline, LOD and LOQ were calculated using the following equation:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Fig. 2 Absorbance spectra of calibration range of MET, LINA and EMPA (200–400 nm)
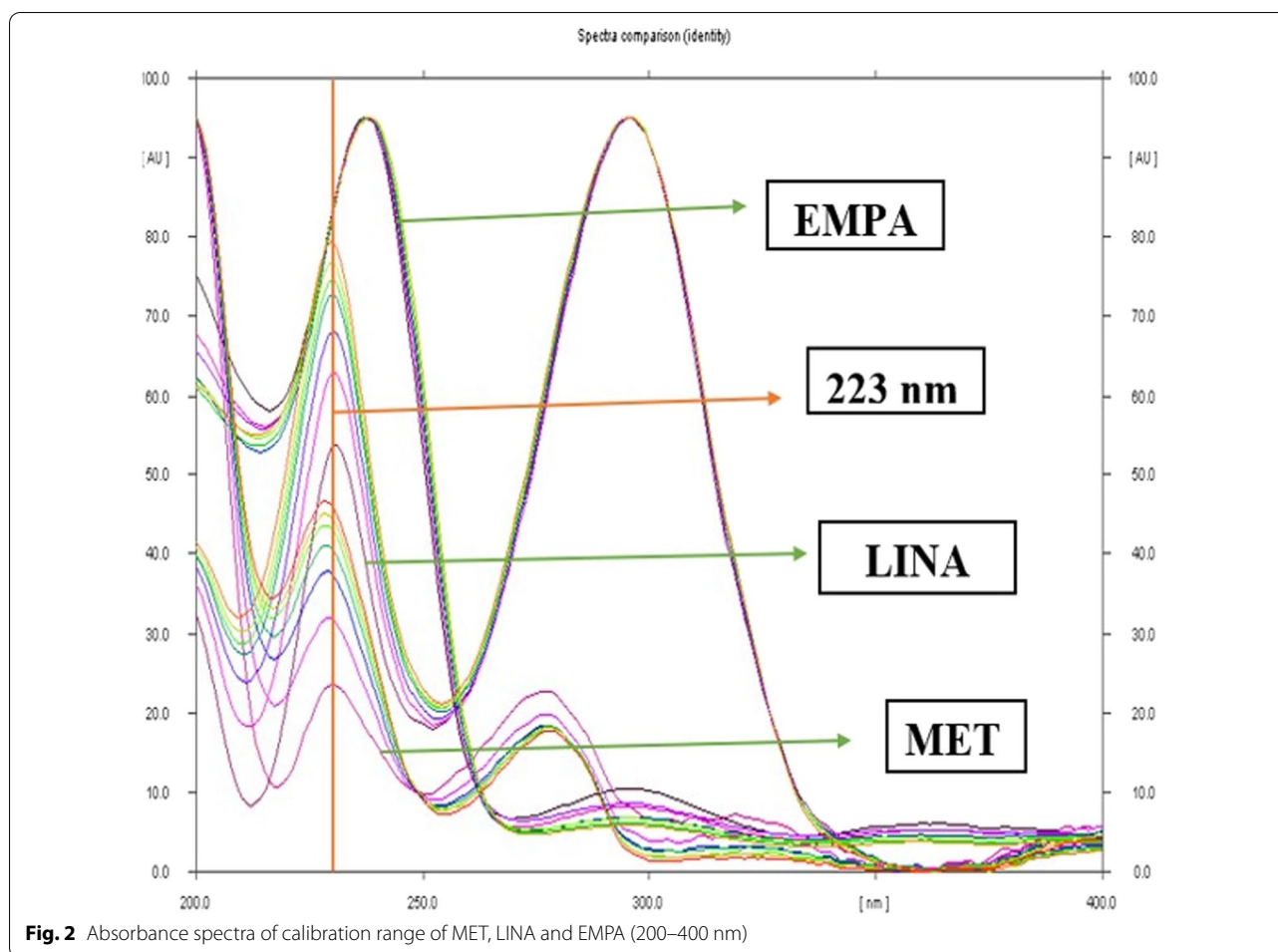
where, σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness
The robustness study has been carried out by a deliberate change in the proposed RP–LC chromatographic condition parameters like detection wavelength, flow-rate of mobile phase and the mobile phase solvent make on the results were examined for the concentration of 200 μg/ml for MET, 1 μg/ml for LINA and 2 μg/ml for EMPA. The average and %RSD of peak retention time were calculated.

Specificity
Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might be including impurities, degradants, preservatives, and excipients for checking the interference which are used in synthetic mixture. The developed method was found to be specific.

System suitability
The adequate performance of liquid chromatographic system can be assessed by System suitability parameters. A system suitability test requires ensuring that a given operating system may be normally applicable because of normal variation in equipment, supplies and techniques. System suitability also require for the verification of the resolution and reproducibility of the chromatographic system which are adequate for the analysis to be done.

Analysis of synthetic mixture [16]
The synthetic mixture of MET, LINA and EMPA was prepared in ratio of 500 mg: 2.5 mg: 5 mg respectively. Common excipients like Hydroxypropyl methylcellulose 0.34 g [HPMC], Polyethylene glycol 0.24 g [PEG], Magnesium stearate 0.15 g, Talc 0.18 g were weighed accurately and transfer into motor pestle along with 0.1 g of EMPA, 0.05 g of LINA, and 10 g of MET which is equivalent to 20 tablets. Synthetic mixture powder (0.554 g) was accurately weighed and transferred into 100 ml volumetric flask containing 20 ml of methanol. It was sonicated for
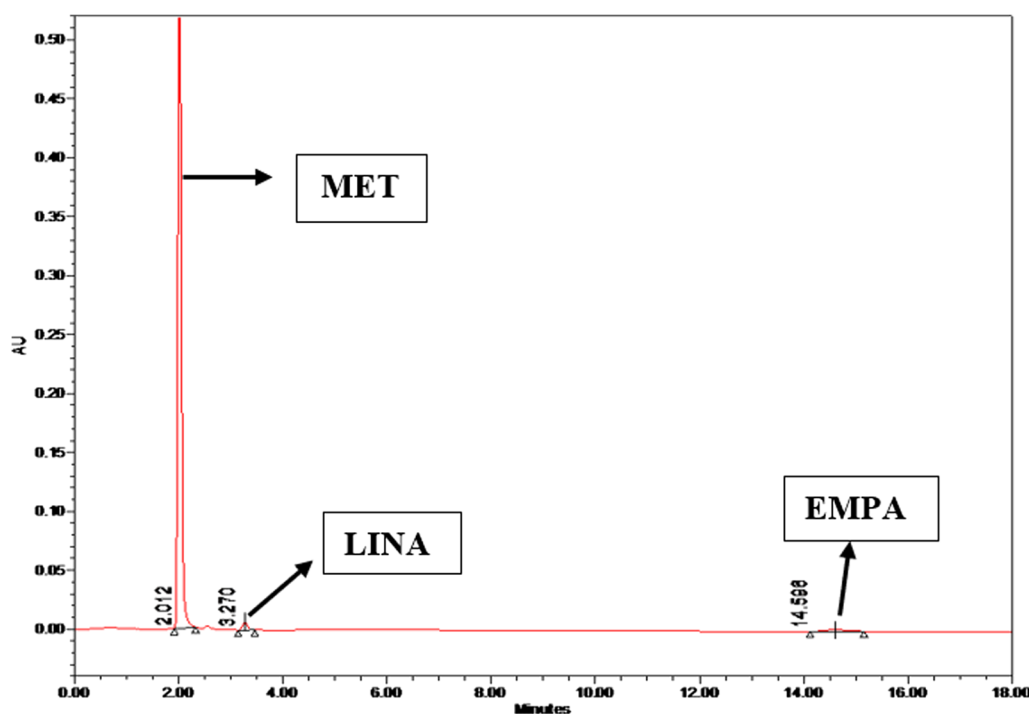
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Fig. 3 Chromatogram of MET (50 μg/ml), LINA (0.25 μg/ml) and EMPA (0.5 μg/ml) using Acetonitrile: Methanol: Water (27: 20: 53, v/v/v) pH 4.0 adjusted wit 1% OPA

Table 1 Regression Analysis of calibration curve

Parameter	MET	LINA	EMPA
Range (μg/ml)	50–500	0.25–2.5	0.5–5
Regression coefficient (R^2)	0.9981	0.9967	0.9968
Slope of regression equation	50,738.2	91,942	43,503
Standard deviation of slope	686.03	1928.75	244.10
Intercept of regression equation	755,315.8	50,312	3715.22
Standard deviation of intercept	86,870.96	1146.97	475.10

15 min and solution was filtered using Whatman filter paper No.42. Filtrate was collected in another 100 ml volumetric flask and the residue was washed with few amount of methanol, the filtrate and residue was combined and methanol was added upto the mark. From the above solution, 0.4 ml of aliquot was pipette out into the 10 ml volumetric flask and methanol was added up to the mark to obtain absolute concentration of 200 μg/ml for MET, 1.0 μg/ml for LINA and 2.0 μg/ml for EMPA respectively. The chromatogram was recorded at 223 nm and the quantification was carried out by keeping these values in the regression equation of calibration curve.

Results

Selection of analytical wavelength

The solution of EMPA, LINA and MET having the concentration of 10 μg/ml was scanned in the range of 200-400 nm, all three drug shows considerable absorbance at common wavelength 223 nm. Therefore 223 nm was a choice of an analytical wavelength for the analysis of all three drugs. The overlay spectra for selection of analytical wavelength are shown in Fig. 2.

Optimization of mobile phase

Several combinations of solvents were tried for optimization of mobile phase like Methanol: Water (80:20, v/v), Acetonitrile: Water (60:40, v/v), Acetonitrile: Methanol (50:50, v/v) and Acetonitrile: Methanol: Water (20:25:50, v/v/v) pH 4.0 adjusted with 1% Ortho-phosphoric acid (OPA). The mobile phase containing Acetonitrile (ACN): Methanol: Water (27:20:53, v/v/v) pH 4 adjusted with 0.6 ml of 1% Ortho-phosphoric acid (OPA) and showed satisfactory results at a flow rate of 1 ml/min. The retention time was found to be 2.01 min for MET, 3.2 min LINA and 14.5 min for EMPA. Where, total run time of analysis was 18 min (Fig. 3).
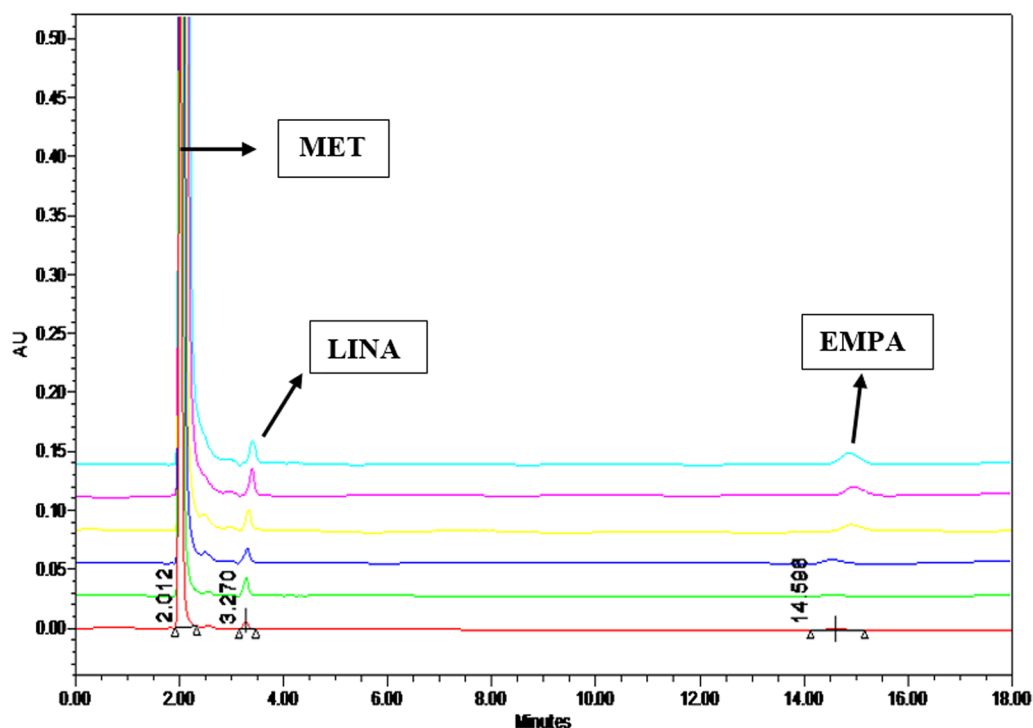
Fig. 4 Overlay Chromatogram of MET (50–500 μg/ml), LINA (0.25–2.5 μg/ml) and EMPA (0.5–5 μg/ml) using Acetonitrile: Methanol: Water (27: 20: 53, v/v/v) pH 4.0 adjusted with 1% OPA

Table 2 Summary of Validation Parameters

Parameter	MET	LINA	EMPA
Range (μg/ml)	50–500	0.25–2.5	0.5–1
Limit of detection (LOD)	5.65	0.04	0.03
Limit of quantification (LOQ)	17.12	0.12	0.10
Retention time	2.01	3.2	14.5
Accuracy (%)	100.65–101.35	99.33–100.57	99.98–100.81
Precision (%RSD)			
Intraday (n = 3)	0.21–1.12	0.20–1.27	0.29–1.69
Interday (n = 3)	1.10–1.91	0.76–1.53	0.70–1.78
Instrumental precision (%RSD)			
Repeatability (n = 6)	1.12	0.97	0.62

Linearity

The linearity study was performed by considering the clinical dose ratio of all the three drugs. The method was found to be linear in the range 50–500 μg/ml for MET, 0.25–2.5 μg/ml for LINA and 0.5–5 μg/ml for EMPA with the correlation coefficient 0.9981, 0.9967 and 0.9968 respectively. The data of regression analysis of calibration curve was shown in Table 1 and overlay spectra of MET (50–500 μg/ml), LINA (0.25–2.5 μg/ml) and EMPA (0.5–5 μg/ml) at 223 nm shown in (Fig. 4). The correlation coefficient values were found to be more than 0.9950 which indicate that the method is linear in relationship with respect to recorded area and concentrations.

Table 3 Accuracy studies of proposed method

Amount of drug taken from samples (μg/ml)			Amount of standard drug spiked (μg/ml)			Average Amount of recovered Standard (μg/ml) (n = 3)			% Recovery ± SD (n = 3)		
MET	LINA	EMPA	MET	LINA	EMPA	MET	LINA	EMPA	MET	LINA	EMPA
200	1	2	160	0.8	1.6	161	0.81	1.58	100.62 ± 0.95	101.25 ± 0.62	98.75 ± 0.80
200	1	2	200	1	2	203	0.98	2.02	101.50 ± 0.72	98.00 ± 1.1	101.00 ± 0.56
200	1	2	240	1.2	2.4	237	1.18	2.38	98.75 ± 0.84	98.33 ± 0.92	99.17 ± 1.12

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Table 4 Robustness studies of proposed method

Normal Condition	Changed condition	MET (200 µg/ml) (n = 3)		LINA (1 µg/ml) (n = 3)		EMPA (2 µg/ml) (n = 3)	
		Rt mean ± SD	%RSD	Rt mean ± SD	%RSD	Rt mean ± SD	%RSD
Wavelength 223 nm	221	2.14 ± 0.03	1.63	3.37 ± 0.06	1.80	14.3 ± 0.15	1.06
	225	2.08 ± 0.03	1.66	3.36 ± 0.05	1.71	14.3 ± 0.15	1.06
Flow rate 1 ml/min	0.9 ml/min	2.15 ± 0.03	1.67	3.43 ± 0.05	1.51	14.23 ± 0.12	0.8
	1.1 ml/mi	1.80 ± 0.01	0.84	2.87 ± 0.04	1.60	13.04 ± 0.25	1.95
Acetonitrile and Methanol (HPLC Grade) SRL Pvt.Ltd	Acetonitrile and Methanol (HPLC Grade) E-Merck Pvt. Ltd	2.06 ± 0.03	1.55	3.44 ± 0.06	1.74	14.35 ± 0.21	1.47
	Acetonitrile and Methanol (HPLC Grade) Rankem Pvt. Ltd	2.06 ± 0.04	1.94	3.44 ± 0.05	1.70	14.36 ± 0.15	1.06

Table 5 System suitability of proposed method

Parameter	MET	LINA	EMPA
Retention time (min)	2.01	3.4	14.5
Capacity factor (k′)	0.82	2.09	12.18
Theoretical plate (N)	2238.21	32,021	2426.62
Tailing factor (T$_f$)	1.25	1	1
Resolution (Rs)	2.52	22.38	–

Precision

The intraday and interday variation in the method responses were studied using precision study. The intraday and inter-day precision was performed and the %RSD of MET, LINA and EMPA was found to be 0.21–1.12, 0.20–1.27 and 0.29–1.69 for intraday and % RSD of MET, LINA and EMPA was found to be 1.10–1.91, 0.76–1.53, and 0.71–1.78 respectively. Instrumental precision was carried out by performing injection repeatability test and the %RSD of MET, LINA and EMPA was found to be 1.12%, 0.97% and 0.62%. The data of intraday, interday and repeatability was shown in Table 2. The variability in the responses was less than 2% for intra-day, inter-day and repeatability study, which indicate that the method is precise.

Accuracy

The percentage (%) recoveries of MET, LINA and EMPA was determined by a known amount of standard was spiked into pre-analyzed sample solutions. The recoveries were found to be 100.65–101.35% for MET, 99.33–100.57% for LINA and 99.89–100.81% for EMPA respectively. The accuracy data was shown in Table 3. The percentage (%) recoveries for all three drugs were found to be in the range of 95 to 102%, which indicate the method is accurate.

Limit of detection and Limit of quantification

The LOD and LOQ were carried out by signal to noise ratio. The lowest amount of drug (LOD) for MET, LINA and EMPA was found to be 5.65 µg/ml, 0.04 µg/ml, 0.03 µg/ml and the lowest amount of quantification (LOQ) was 17.12 µg/ml, 0.12 µg/ml and 0.10 µg/ml respectively.

Specificity

The specificity study was performed to check the interference of excipients used in the preparation of synthetic mixture and there is no any interference observed by excipients at the time of elution of all three different drugs at the respective retention time. The developed method was found to be specific.
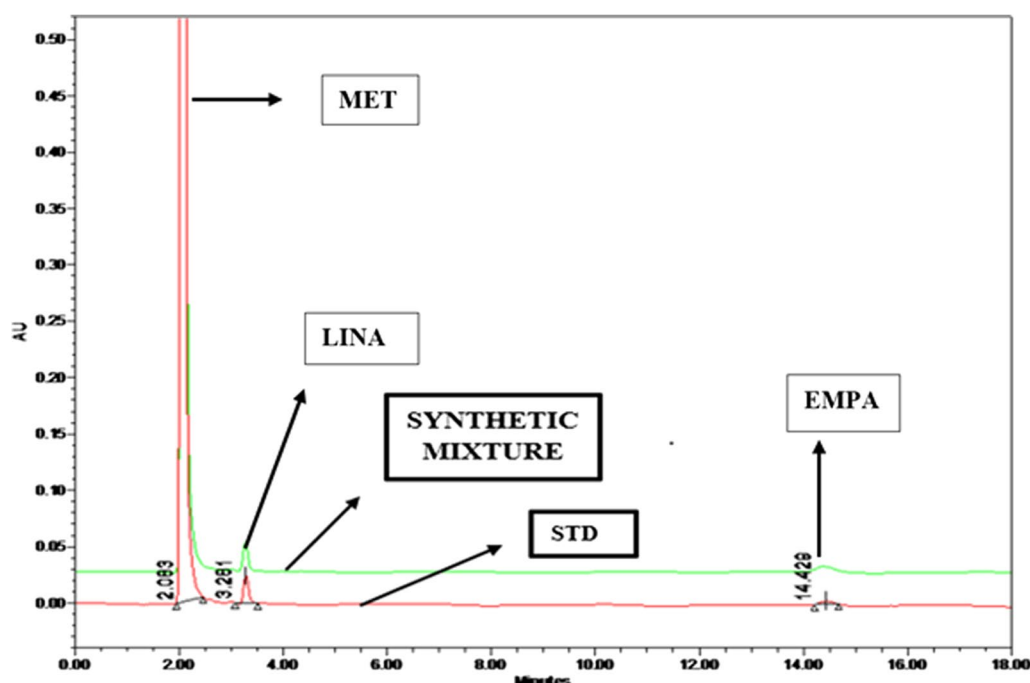
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Fig. 5 Overlay Chromatogram of standard and synthetic mixture of MET (200 µg/ml), LINA (1 µg/ml) and EMPA (2 µg/ml) using Acetonitrile: Methanol: Water (27: 20: 53, v/v/v) pH 4.0 adjusted wit 1% OPA

Robustness

After an introducing small and deliberate changes in chromatographic condition parameters like change in detection wavelength, flow rate, and make of mobile phase solvent, the % RSD of retention time of all three drugs was found to be less than 2% which confirming that the proposed method is robust. The data of robustness studies is shown in Table 4.

System suitability

The results of system suitability test are reported in Table 5.

Analysis of synthetic mixture

The proposed RP–LC method is applied for the simultaneous estimation of MET, LINA and EMPA from the prepared synthetic mixture. The % amount of drug for MET, LINA and EMPA was found to be 100.92–101.39%, 98.55–100.76% and 99.33–101.22%, respectively. The overlay Chromatogram of standard and prepared synthetic mixture of MET (200 µg/ml) LINA (1 µg/ml) and EMPA (2 µg/ml) are shown in (Fig. 5).

Discussion

The proposed RP–LC method is used for simultaneous estimation of EMPA, LINA and MET in bulk and synthetic mixture. Optimization of chromatographic conditions was achieved and with the help of Phenomenex C_{18} column (250 mm × 4.6 mm, 5 µm) through isocratic elution by Acetonitrile: Methanol: Water in the ratio of (27: 20: 53, v/v/v) pH 4.0 adjusted with 1% Ortho—phosphoric acid as mobile phase at flow rate 1 ml/min, the separation of all three drugs were achieved. The linearity for MET, LINA and EMPA was found to be 50–500 µg/ml, 0.25–2.5 µg/ml and 0.5–5 µg/ml, respectively. Table 4 is represent accuracy data and the % recoveries for MET, LINA and EMPA were found to be 100.65–101.35%, 99.33–100.57% and 99.89–100.81%, respectively. The specificity study was performed to check the interference of excipients used in the preparation of synthetic mixture and there is no any interference observed by excipients at the time of elution of all three different drugs which represent the method is specific. Table 5 represent the data of Robustness of the proposed developed RP–LC method is a measure of its ability to remain unaffected by small but deliberate change of the chromatographic method parameters. It was estimated by small changes in the chromatographic conditions like change in detection wavelength (± 2 nm), change in flow rate (± 0.1 ml) and change in mobile phase solvent make which represents

the minor changes does not affect on the symmetry of peak and retention time (R_t) of MET, LINA and EMPA which confirming the reliability and robustness of the method. The percentage (%) amount of all three drugs were found to be more than 98% in the assay of synthetic mixture which specify that the method provides accurate and precise results. As compared to reported TLC method, the proposed RP–LC method is more sensitive, accurate, robust and precise [17].

Conclusions

The proposed study describes that RP–LC method was developed and validated for the simultaneous estimation of EMPA, LINA and MET in bulk and synthetic mixture as per ICH Q2(R1) guidelines and found to be sensitive, accurate and precise. The proposed RP–LC method was repeatable and selective as per statistical analysis and the proposed method might be applied for simultaneous estimation of all three drugs in pharmaceutical formulation. As compared to reported TLC method, the proposed RP–LC method is more sensitive, accurate, robust and precise therefore R–LC method provides more accurate results and requires less quantity of sample. Due to complete automation, HPLC is preferred over TLC method as a pharmacopoeia method of analysis. The results published HPTLC manuscript was found to be effective for qualitative analysis, however proposed RP–LC method was found to be more accurate for quantitative analysis.

Abbreviations
HPLC: High Performance liquid chromatography; RP–HPLC: Reverse Phase High Performance liquid Chromatography; EMPA: Empagliflozin; LINA: Linagliptin; MET: Metformin HCl; OPA: Ortho phosphoric acid; LOQ: Limit of Quantitation; LOD: Limit of Detection; %RSD: Relative standard deviation; Rt: Retention time.

Authors' contributions
All authors associated with this research work declared that there is no conflict of interest for publication of work. All authors have read and approved the manuscript. The contribution of each authors are mentioned below: IMP: He is post graduate student and above work has been carried out by him as dissertation work. UKC: He is a mentor of IMP and under his noble guidance proposed method has been developed and validated as per ICH guideline. He is also giving training for ease of operation sophisticated reverse phase liquid chromatography instrument and involved in interpretation of data. HDJ: She is a mentor of IMP and under her noble guidance student can understand the concept of authentication of Active Pharmaceutical Ingredients by different analytical techniques She is also involved in interpretation of data. HMK: She is a co-mentor of KP and under her noble guidance student can understand the concept of mobile phase selection and how to optimize chromatographic conditions. She is also involved in interpretation of data. DK: He is a helping hand throughout cited research work. DAS: Through his good relationship with pharmaceutical industry we have received all active pharmaceutical ingredients and he is having sound technical knowledge Waters HPLC software system. All authors read and approved the final manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
No competing interests to declare.

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References
1. Drug bank, Empagliflozin, https://www.drugbank.ca/drugs/DB09038. Accessed 9 July 2019
2. Pubchem, Empagliflozin, https://pubchem.ncbi.nlm.nih.gov/compound/Empagliflozin. Accessed 15 September 2019
3. Drug bank, Linagliptin, https://www.drugbank.ca/drugs/DB08882. Accessed 16 July 2019
4. Pubchem, Linagliptin, https://pubchem.ncbi.nlm.nih.gov/compound/Linagliptin. Accessed 16 July 2019
5. Drug bank Metformin HCl, https://www.drugbank.ca/drugs/DB00331. Accessed 8 July 2019
6. Approval date for the combination, https://www.biospace.com/article/releases/u-s-fda-accepts-new-drug-application-for-triple-combination-tablet-for-adults-with-type-2-diabetes/ . Accessed 16 July 2019
7. Padmaja N, Veerabhadram G (2016) Method development and validation of RP-HPLC method for the estimation of empagliflozin in API. Int J Pharm Sci Res 7(2):724–727. https://doi.org/10.13040/IJPSR.0975-8232.7(2).724-27
8. Madhusudhan P, Reddy MR, Devanna N (2015) RPHPLC Method Development and Validation for Simultaneous Determination of Linagliptin and Empagliflozin in Tablet Dosage Form. Int Adv Res J Sci Eng Tech 2(2):95–99. https://doi.org/10.17148/IARJSET
9. Godasu SK, Sreenivas SA (2017) A new validated RP-HPLC method for the determination of Metformin HCl and Empagliflozin in its bulk and Pharmaceutical dosage forms. Int J Pharm Sci Res 8(5):2223–2232. https://doi.org/10.13040/IJPSR.0975-8232.8(5).2223-32
10. Rajbangshi JC, Alam MM, Hossain MS, Islam MS, Rouf A (2018) Development and Validation of a RP-HPLC Method for Quantitative Analysis of Linagliptin in Bulk and Dosage Forms. Dhaka Uni J Pharm Sci 17(2):175–182
11. doi:https://doi.org/10.3329/dujps.v17i2.39173
12. Pandya RH, Rathod R, Maheswari DG (2014) Bioanalytical method development and validation for simultaneous determination of linagliptin and metformin drugs in human plasma by RP-HPLC method. Pharmacophore 5(2):202–218
13. Shirisha S, Haque MA, Sireesha D, Bakshi V, Harshini S (2014) Development and validation of RP-HPLC method for simultaneous estimation of metformin and linagliptin in combined pharmaceutical dosage form. Int J Pharm Res Sci 2:491–495

14. Varaprasad C, Asif M, Ramakrishna K (2015) RP-HPLC method for simultaneous estimation of metformin and linagliptin in tablet dosage form. Rasayan J Chem 8(4):426–432.

15. Chhetri HP, Thapa P, Van Schepdael A (2014) Simple HPLC-UV method for the quantification of metformin in human plasma with one step protein precipitation. Saudi Pharm J 22(5):483–487. https://doi.org/10.1016/j.jsps.2013.12.011

16. Kar M, Choudhury PK (2009) HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. Ind J Pharm Sci 71(3):318–320. https://doi.org/10.4103/0250-474X.56031

17. ICH, (Q2R1), Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization, Geneva, Switzerland, (2005).

18. Boheringer Ingelheium International GMBH. Pharmaceutical composition, Methods for treating and uses thereof. WO 2017/093419 A1, 2017.

19. Patel IM, Chhalotiya UK, Jani HD, Kansara D, Shah DA (2020) Densitometric simultaneous estimation of combination of empagliflozin, linagliptin and metformin hydrochloride used in the treatment of type 2 diabetes mellitus. JPC- J Planar Chromat 33(2):109–118. https://doi.org/10.1007/s00764-020-00026-z

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