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Research Article

DEVELOPMENT AND VALIDATION OF RAPID STABILITY-INDICATING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF LINAGLIPTIN AND EMPAGLIFLOZIN IN PURE AND DOSAGE FORMS

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ABSTRACT

Objective: A new, simple, rapid, sensitive, and accurate stability-indicating high-performance liquid chromatography (HPLC) method was developed and validated for the quantitative determination of linagliptin (LNG) and empagliflozin (EMP) in pure and tablet dosage forms.

Methods: An isocratic HPLC method, using a C_{18} reversed-phase column (150 mm×4.6 mm i.d., particle size 5 µm) with an isocratic binary mobile phase consisting of phosphate buffer and acetonitrile (65:35, v/v), was investigated to separate the drug from its stress degradation products. The flow rate was 1.0 mL/min at ambient temperature and photodiode array detector is used at 226 nm for detection. The developed method was validated for system suitability, linearity, accuracy, precision, limits of detection and quantitation, specificity, stability, and robustness.

Results: The retention time of LNG and EMP was found to be 3.276±0.002 and 6.966±0.0006 min, respectively. The calibration curve was found to be linear with the equation y=158926.39X+11.139, with a correlation coefficient of R^2=0.9991 for LNG and y=22688.45X+4.259, with a correlation coefficient of R^2=0.9994 for EMP over a concentration range of 2.5–7.5 µg/mL and 5.0–15 µg/mL for LNG and EMP, respectively. The limits of detection were 0.29 and 0.48 µg/mL for LNG and EMP, respectively, and the limits of quantification were 0.89 and 1.5 µg/mL for LNG and EMP, respectively. The recovery values of this method are 101.11% and 101.48% for LNG and EMP, respectively, and the reproducibility is within 0.070 and 0.277 for LNG and EMP, respectively.

Conclusion: The proposed method is a rapid stability-indicating HPLC method that can be applied for the determination of LNG and EMP in pure and tablet dosage forms.

Keywords: Linagliptin, Empagliflozin, Rapid stability-indicating high-performance liquid chromatography method, Method validation, Dosage forms.

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INTRODUCTION

Linagliptin (LNG) is a more potent dipeptidyl peptidase (DPP)-4 inhibitor than other drugs that belong to the same class for the treatment of Type II diabetes. LNG is a competitive and reversible DPP-4 enzyme inhibitor that slows the breakdown of insulinotropic hormone glucagon-like peptide-1 for better glycemic control in diabetes patients. Empagliflozin (EMP) is a sodium glucose cotransporter-2 inhibitor indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with Type 2 diabetes. The combination of LNG and EMP is served as an adjuvant to diet and exercise to improve glycemia control in adults with Type-2 diabetes who know to have the cardiovascular disease [1-3]. LNG chemically, 8-[(3R)-3-aminopiperidin-yl]-7-(but-2yn-1-yl)-3-methyl-1-[(4-methyl quinazolin-2-yl)methyl]-3,7-dihydro-1H-purine-2,6-dione. EMP, chemically designated as (2S,3R,4R,5S,6R)-2-[4-chloro-3-({4-[(3S)-oxolan-3-yloxy]phenyl} methyl)phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol (Fig. 1).

Literature review revealed that few methods were described for the determination of LNG and EMP alone or in combination with other drugs from pharmaceutical dosage forms and in human plasma including spectrophotometry [4-7], ultra-performance liquid chromatography (LC) [8], LC–mass spectroscopy [9], and high-performance LC (HPLC) [10-25] techniques.

The aim of the present work is to develop and validate simple, fast, and reliable stability-indicating reverse-phase HPLC method with ultraviolet (UV) detection for the simultaneous determination of LNG and EMP in pure and pharmaceutical dosage forms. The proposed method can overcome the problems in all previously reported HPLC methods such as long time of analysis and expensive detectors, as shown in Table 1.

METHODS

Instrumentation

HPLC apparatus (Agilent 1200, Agilent, USA) equipped with UV detector DAD system. The pH measurements were made on a Hanna pH meter equipped with a combined glass-calomel electrode (Portugal) (HI: 9321).

Chemicals and reagents

Analytical HPLC grade solvents were used in all experiments, including acetonitrile and methanol (LAB-SCAN, Analytical Sciences, Gliwice, UL, Sowinskiego, Poland). Potassium dihydrogen orthophosphate, sodium hydroxide (NaOH), hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2 30%, v/v) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Double distilled water was used.

LNG (99.40%) and EMP (99.70%) were kindly supplied by Zeta Pharma, Egypt. The pharmaceutical dosage forms used were Glyxambi tablets (Eli Lilly and Company; Boehringer Ingelheim Pharmaceuticals, Inc.,

Table 1: Chromatographic methods reported for the determination of LNG and EMP in dosage forms

Chromatographic conditions			LOD (µg/mL)		Concentration range (µg/mL)		References
Mobile phase	**Flow rate (mL/min)**	**Detection (UV at) (nm)**	**LNG**	**EMP**	**LNG**	**EMP**	
Phosphoric acid:acetonitrile (45:50, v/v)	1.0	245	-	-	12.5–75	25–150	[19]
0.1% perchloric acid:acetonitrile (60:40, v/v)	1.0	230	0.43	0.03	12.5–75	12.5–75	[20]
0.1% phosphoric acid (pH 4.5):acetonitrile (68:32, v/v)	1.0	218			0.01–10	0.01–10	[12]
Methanol:phosphate buffer (KH_2PO_4, K_2HPO_4) (pH 3.0) (70:30, v/v)	1.0	254	0.0372	2.17	20–1000	10–50	[21]
Methanol:acetonitrile:0.1% ortho phosphoric acid (30:60:10, v/v)	1.0	246	0.03	0.06	2.5–15	5.0–30	[22]
Potassium dihydrogen phosphate buffer pH (3.4):methanol (70:30, v/v)	1.0	240	0.39	0.76	50–150	50–150	[23]
Phosphate buffer 0.01 M:acetonitrile (65:35, v/v)	1.0	226	0.29	0.48	2.5–7.5	5.0–15	Proposed work

LNG: Linagliptin, EMP: Empagliflozin, LOD: Limit of detection

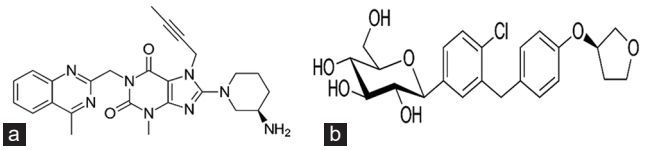

Fig. 1: The chemical structure of (a) linagliptin and (b) empagliflozin

5.0 mg LNG and 10 mg EMP per tablet) and Empacoza plus tablets (Zeta Pharma, Egypt, 5.0 mg LNG and 10 mg EMP per tablet).

Chromatographic conditions

The chromatographic separation was performed using ODS-3 InertsilC18 (150 mm×4.6 mm), 5.0 µm particle size column; the column temperature was maintained at 25±2°C. The autosampler utilized methanol as a rinse solution, the total run time was 6.0 min. The elution quaternary pump ran an isocratic flow using mobile phase consisting of a mixture of phosphate buffer and acetonitrile (65:35, v/v) at a flow rate of 1.0 mL/min. The eluate was monitored at 226 nm using UV diode array detector. The retention time of the drug was found to be LNG and EMP which were found to be3.276±0.002 and 6.966±0.0006 min, respectively. The injection volume was 20 µL. Mobile phase was used as diluent during the standard and test samples preparation.

Preparation of standard solutions

A stock solution of LNG or EMP (10 µg/mL) was prepared by dissolving 10 mg of LNG and EMP in mobile phase in 100 mL volumetric flask, then shaken and sonicated for 10 min till completely dissolved and then complete the volume to 100 mL with mobile phase. The working standard solutions were prepared by diluting aliquots of stock solution with mobile phase to obtain final concentrations ranging from 2.5–7.5 µg/mL to 5.0–15 µg/mL for LNG and EMP, respectively. Working solutions of the drugs were stable for 1 week.

Construction of calibration curves

Aliquots of standard solution, ranging from 2.5–7.5 µg/mL to 5.0–15 µg/mL for LNG and EMP, respectively, were prepared in a series of 10 mL volumetric flasks, 20 µL were injected into the instrument. Detection was performed at the wavelength of 226 nm. The calibration graph was constructed by plotting the peak areas obtained at the wavelength of 226 nm versus the corresponding injected concentrations.

Assay for tablets dosage forms

Twenty tablets of Glyxambi and Empacoza plus were weighed, finely powdered, and an accurately weighed amount of the powdered tablets equivalent to 5.0 mg LNG and 10 mg EMP which were transferred to 100 mL measuring flask and dissolved in 50 mL of mobile phase, sonicated for 10 min, and the solution was filtered through a 0.45 µm membrane filter and then the final solution was completed to volume with mobile phase. The proposed procedure was then completed as mentioned above.

Table 2: System suitability and statistical analysis

Parameters	LNG	EMP
System suitability		
t_R±SD (min)	3.276±0.002	6.966±0.0006
Number of theoretical plates (n)	6687	11895
k'	0.219	1.590
Linearity and regression data		
Linearity range (µg/mL)	2.5-7.5	5.0-15
Limit of detection (µg/mL)	0.29	0.48
Limit of quantitation (µg/mL)	0.89	1.50
Slope (b)±RSD	158926.39±0.362	22688.45±0.451
Intercept (a)±RSD	11.139±0.708	4.259±0.824
Correlation coefficient (R^2)	0.9991	0.9994
Comparison with a reported method [20]		
t-test[a]	0.116	0.196
F-ratio[a]	1.536	1.818

[a]Theoretical value for t and f at confidence limit at 95% confidence level and five degrees of freedom (p=0.05) are 2.571 and 5.05, respectively, SD: Standard deviation, RSD: Relative standard deviation

RESULTS

DISCUSSION

Method optimization

The conditions affecting the chromatographic performance of LNG and EMP were carefully studied to recognize the most suitable chromatographic system. Hence, the optimum chromatographic performances were achieved when using isocratic mobile phase composed of phosphate buffer and acetonitrile (65:35,v/v) with a flow rate of 1.0 mL/min, injection volume 20 µL, column temperature 25°C, and detection wavelength 226 nm. The results of three runs indicate high system suitability (Table 2). The retention time (t_R) values LNG and EMP were found to be 3.276±0.002 and 6.966±0.0006 min, respectively (Fig. 2).

Method validation

The developed method was validated for system suitability, linearity, sensitivity, precision, accuracy, robustness selectivity, and

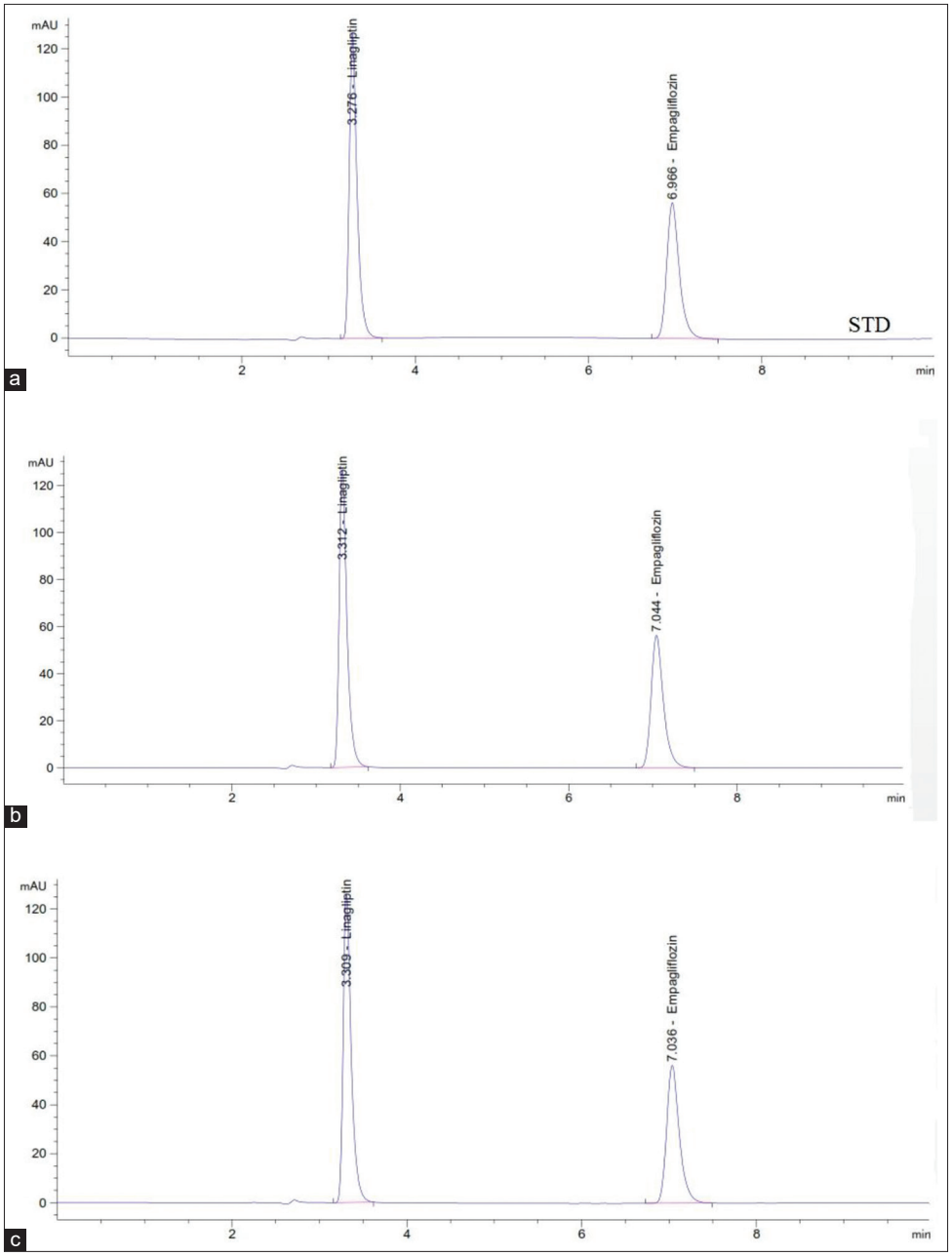

Fig. 2: Chromatogram of linagliptin and empagliflozin from (a) raw material, (b) Empacoza plus tablet, and (c) Glyxambi tablet

specificity and is applied for forced degradation studies as per the ICH guidelines [26].

Linearity

The linearity of LNG and EMP was established by eight-point calibration curve, concentration ranging from 2.5 to 7.5 µg/mL and 5.0 to 15 µg/mL for LNG and EMP, respectively. The graph of the peak area against concentration proved linear graph with regression equations; y=158,926.39x+11.139 and y=22,688.45x+4.259, with a correlation coefficients (R^2=0.9991 and 0.9994) for LNG and EMP, respectively.

Sensitivity

The limit of detection is defined as the injected quantity giving S=N of 3 (in terms of peak area) and were found to be 0.29 and 0.48 µg/mL for LNG and EMP, respectively. The limit of quantification is defined as the injected quantity giving S=N of 10 (in terms of peak area) and was found to be 0.89 and 1.50 µg/mL for LNG and EMP, respectively (Table 2).

Precision

The intraday repeatability (precision) of the developed method was assessed by analyzing six replicate injections of the standard solution at three different concentrations on the same day. The same was done for interday precision test except that the injection of the samples was every day for 5 days. The precision of the method was determined by calculating relative standard deviation (RSD %). The results in Table 3 show that the method is reproducible and there were high intra- and inter-day precisions (RSD ≤0.435%).

Accuracy

Accuracy of the method was established by performing recovery studies according to the ICH guidelines. Spiked samples were prepared by spiking pre-analyzed sample solutions with standard drug at three different concentration levels (50%, 100%, and 150% level). Mean percentage recovery values at three different concentrations of the two drugs were calculated. The % mean recovery was ranged from 100.13 to 101.20% and 100.40 to 101.70% for LNG and EMP, respectively (Table 3).

Robustness

The robustness of the present method was evaluated in terms of temperature, flow rate, column to column, wavelength of detection, and injection volume (Table 4). The slight variations in the examined factors had no significant effect on the shape of the peak. The results of coefficient of variation % indicate that the method is more sensitive to changes in the wavelength and the flow rate greater than to changes in the other factors. Compared with retention times (t_R-values), peak areas were more affected with the slight changes in the chromatographic conditions.

Selectivity and specificity of the method

The resulted peak after tablet analysis is found to be homogeneous and there are no coeluting peaks indicating specificity of the method. Comparison between the chromatogram of the raw LNG and EMP and that of extracted LNG and EMP from tablets indicate that the excipients in the formulation did not interfere with the determination of LNG and EMP.

Accuracy and application

Analysis of LNG and EMP in Empacoza plus and Glyxambi tablets by the proposed method showed high accuracy with a mean recovery range of 100.71±0.541% and 100.81±0.589% and 101.48±0.254% and 101.64±0.289% for LNG and EMP, respectively (Table 5). The results were compared with a reported method [20]. The values of t and f indicate that there is no significant difference between both methods.

Stability tests

The results (Fig. 3) of stress degradation indicate that LNG and EMP are strongly affected with reflux with HCl or NaOH. Reflux with H_2O_2 and

Table 3: Intra- and interday precision and accuracy of LNG and EMP (n=5)

Injected amount (µg/mL)	Intraday			Interday		
	Observed amount±SD	RSD %*	Accuracy (recovery %)**	Observed amount±SD	RSD %*	Accuracy (recovery %)**
LNG						
2.5	2.53±0.003	0.119	101.20	2.52±0.004	0.159	100.80
5	5.05±0.003	0.059	101.0	5.01±0.002	0.040	100.20
7.5	7.58±0.033	0.435	101.07	7.51±0.013	0.173	100.13
EMP						
5	5.06±0.007	0.138	101.20	5.02±0.011	0.219	100.40
10	10.17±0.003	0.029	101.70	10.08±0.015	0.149	100.80
15	15.23±0.016	0.105	101.56	15.11±0.01	0.066	100.73

*RSD (%)=SD×100/mean. **Accuracy (recovery %)=(Observed amount/Injected amount)×100. SD: Standard deviation, RSD: Relative standard deviation, LNG: Linagliptin, EMP: Empagliflozin

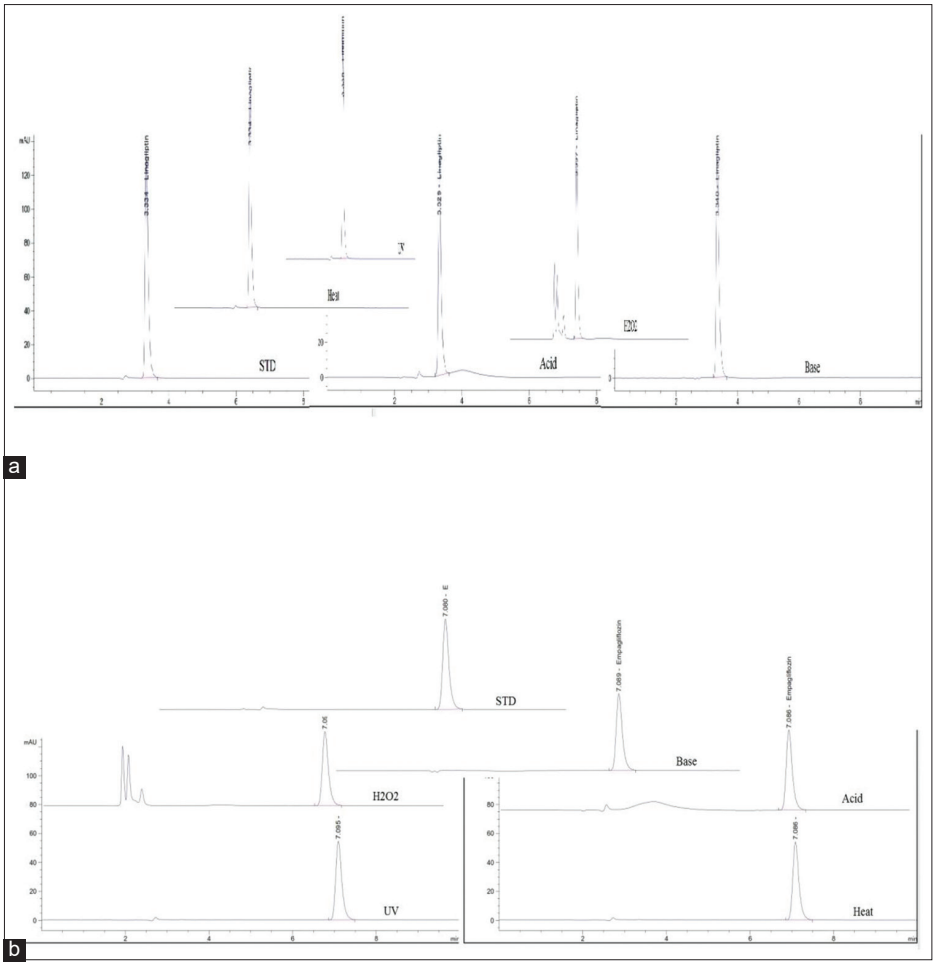

Fig. 3: Separation of (a) linagliptin and (b) empagliflozin from degradants after stress conditions

Table 4: Robustness of the proposed method

Changes factors	Temp. (°C)		Flow rate (mL/min)		Column to column		Wavelength of detection (nm)		Injected volume (µL)	
LNG										
Changes	23, 25, and 27		0.95, 1.0, and 1.05		ODS-3V, ODS		224, 226, and 228		19.9, 20, and 20.1	
Tested parameter	Peak area	t_R	Peak area	t_R	Peak area	t_R	Peak area	t_R	Peak area	t_R
CV (%)	0.125	0.51	0.131	0.98	0.039	0.33	0.864	0.82	0.453	0.73
EMP										
Changes	23, 25, and 27		0.95, 1.0, and 1.05		ODS-3V, ODS		224, 226, and 228		19.9, 20, and 20.1	
Tested parameter	Peak area	t_R	Peak area	t_R	Peak area	t_R	Peak area	t_R	Peak area	t_R
CV (%)	0.502	0.47	0.228	0.67	0.095	0.29	0.917	0.87	0.508	0.65

LNG: Linagliptin, EMP: Empagliflozin, CV: Coefficient of variation

Table 5: Statistical analysis of results obtained by the proposed method applied on tablets compared with a reported method

	LNG			EMP		
	Proposed method	Reported method [20]		Proposed method		Reported method [20]
	Empacoza plus tablets	Glyxambi tablets		Empacoza plus tablets	Glyxambi tablets	
Mean recovery[a]	100.71	100.81	100.52	101.48	101.64	101.29
±SD	0.541	0.589	±0.545	0.254	0.214	±0.289
±RSD%	0.537	0.584	±0.542	0.251	0.210	±0.286
Variance	0.292	0.346	0.297	0.065	0.046	0.084
SE	0.242	0.263	0.242	0.114	0.096	0.129
t-value[b]	0.150	0.212		0.547	1.146	
F-value[b]	1.014	0.856		1.296	1.845	

[a]Average of five determinations (n=5). [b]Theoretical values for t and f at confidence limit at 95% confidence level and five degrees of freedom (p=0.05) are 2.776 and 6.39, respectively

exposure to UV radiation leads to degradation of EMP and LIN, but the effect here is weaker than that in the case of HCl and NaOH. There is no interference with the peak of the intact drug, indicating that the method is stability indicating (Fig. 3). Hence, the proposed analytical method is also useful for the determination of LNG and EMP stability in sample of pharmaceutical dosage form.

CONCLUSION

Precise, simple, accurate, robust, and cost-effective stability-indicating HPLC method was developed for the routine and quantification analysis of LNG and EMP in pure forms and tablets. The method was successfully validated in terms of linearity, precision, and accuracy as per the ICH guidelines. Compared with the published chromatographic methods, this method represents a strong reduction of the analysis time and it is considered as a stability-indicating method. A high recovery of LNG and EMP in tablets was achieved. The proposed method ensured a precise and accurate determination of LNG and EMP in tablet formulations and is a stability-indicating method. No interference from the excipients was noticed. Hence, it can be concluded that the proposed method was a good approach for obtaining reliable results and found to be suitable for the routine analysis and quality control and percentage degradation of pharmaceutical preparations containing both drugs either individually or in combination.

AUTHORS' CONTRIBUTIONS

Prof. Dr. Ragaa El Sheikh has generated the research idea and interpreted the data and helped to draft the manuscript. Prof. Dr. Wafaa El Sayed Hassan has suggested the research idea and participated in the design of the study. Miss. Eman Helmy Youssef was prepared the solutions, carried out the experiments, interpreted the data, and helped to draft the manuscript. Dr. Abdulrahman Y. Hamdi has suggested the research idea and participated in the design of the study. Mr. Naif Ahmed Badahdah has participated in the design of the study and carried out the experiments. Mr. Muneer Esa Alzuhiri has participated in the design of the study and carried out the experiments. Prof. Dr. Ayman A. Gouda helped in check spelling, reducing the plagiarism, interpreting the data, reviewed the manuscript, and submitted the manuscript for publication.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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None.

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