Research Article

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Green quantitative methods for linagliptin and empagliflozin in dosage forms

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Abstract: The frequent drugs prescribed for type 2 diabetes patients are linagliptin (LNG) and empagliflozin (EMG) in different drug formulations. The objective of this research is to create and validate selective and simple methods to evaluate both medications in their dosage forms. Method A for the assay of LNG is based on the drug oxidation using an iodate/iodide mixture, while Method B involves the determination of EMG using permanganate oxidation. The regression graphs had good linearity in the ranges of 0.25–20 and 0.20–1.5 $\mu g \cdot ml^{-1}$ for the two drugs, respectively. The limits of detection were 0.082 and 0.065 $\mu g \cdot ml^{-1}$ for LNG and EMG, respectively. The two methods were validated and applied for the assay of the drugs in dosage forms successfully.

Keywords: green, spectrophotometry, linagliptin, empagliflozin, pharmaceutical

1 Introduction

Linagliptin (LNG) and empagliflozin (EMG) are the latest prescribed hypoglymatic agents. The molecular name for LNG is (8-[(3*R*)-3-aminopiperidin-1-yl]7-(but-2-yn-1-yl)-3-methyl-1[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1*H*-purine-2,6-dione]) (Figure 1a). It is a dipeptidyl-peptidase-4 inhibitor class-related effective hypoglycemic medication [1]. This class of DPP-4 inhibitors is considered to provide a new therapeutic approach to the management of type 2 diabetes, which attempts to decrease glucagon levels and increase glucose-dependent insulin release.

Chemically, EMG is known as (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-[(3*S*)-oxolan-3-yl]oxyphenyl]methyl] phenyl-6-(hydroxymethyl) oxane-3, 4, 5-triol (Figure 1b). It inhibits the sodium-glucose co-transporter-2 (SGLT-2) and was approved to treat type 2 diabetes [2]. SGLT-2 controls a significant amount of glucose absorption into the kidneys [3].

Only a few analytical techniques were employed, according to a review of the literature. For the analysis of LNG and EMG, spectrophotometric [4–9], spectrofluorimetric [10], electrochemical [11], and various types of liquid chromatography methods [12–19] were reported.

Many chromatographic procedures developed for the determination of LNG and EMG in several drug combinations are usually adopted using very expensive chromatographic techniques and utilizing toxic organic reagents and solvents or using derivatization steps and complicated procedures.

In pharmaceutical analysis, colorimetric methods became widely used due to their simplicity compared to the tedious experimental steps involved in many methods, such as heating and separation. In addition, the enhanced selectivity of such methods is due to the specific absorbance change during chemical reactions. As a result, kinetic spectrophotometric methods in particular owe the advantage of monitoring the change in color as a function of time, which results in enhancing the selectivity of the method by reducing the interference effects from other active components and physical properties. The researched medications (LNG and EMG) have no established analytical methods based on kinetic assays of the pharmaceuticals in dose forms.

The objective of the current work was to create new, straightforward, environmentally friendly, and selective methods for the assessment of both LGB and EMG in pharmaceutical formulations and as bulk materials using kinetic spectrophotometry.

The developed methods were based on the implementation of simple oxidation reactions of both LNG and EMG without interaction with other ingredients in their drug formulations [20,21]. The proposed methods are selective, eco-friendly, and cost-effective since they use non-toxic inorganic reagents, and no organic solvents and reagents were used.

The first approach was related to a novel colorimetric method for measuring LNG in pharmaceutical formulations or pure form. The process depends on the selective

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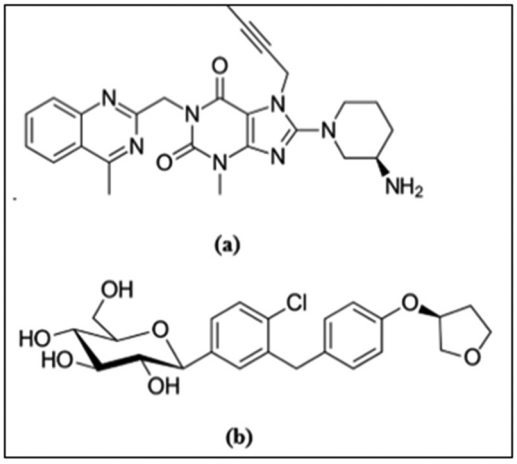

Figure 1: Structure of LNG (a) and EMG (b).

reduction of potassium permanganate in basic media by the LNG medication at room temperature, which produces the green manganate ion, whose maximal absorption wavelength is 610 nm.

The second method involved potassium iodate (KIO_3) as a selective oxidizing agent for the oxidation of EMG in the presence of potassium iodide (KI) to produce a stable iodine that was monitored at 350 nm spectrophotometrically. The two methods were developed according to the recommended green chemistry principles and sustainability [22]; as a result, both chemical processes eliminate the use of hazardous reagents and organic solvents, limit the amount of waste, and improve the safety conditions [23].

2 Materials and methods

2.1 Instrument

A double-beam UV–Vis spectrophotometer (Shimadzu 1900, Japan) has a scanning speed of 200 nm·min^{-1}, a bandwidth of 2.0 nm, and a wavelength range of 200–900 nm. It has a wavelength accuracy of 0.2 nm. Quartz cells with a diameter of 10.0 mm were used to test the absorbance values under temperature control.

2.2 Reagents

During this study, analytical-reagent chemicals and highly purified water were used.

Pure LNG and EMG drugs and pharmaceutical formulations and pure samples of LNG and EMG were kindly received from Jordanian Pharmaceutical Company, Amman-Jordan. The commercial drug formulations for LNG (Trajenta®) tablets are labeled to contain 5.0 mg LNG in each tablet. Each tablet of EMG (Jardiance®) contains 10 mg and was purchased from a local pharmacy.

2.3 Stock standard solutions

Standard solutions of LNG and EMG were prepared by serial dilutions of 100 μg·ml^{-1} or by dissolving appropriate amounts of both pure drugs in 100 ml of distilled water.

2.3.1 Reagents

A stock solution of 1.0×10^{-3} M was made by dissolving an appropriate mass of potassium permanganate ($KMnO_4$) in a 250 ml volumetric flask, and 1.0 M sodium hydroxide (NaOH) solution was used. A stock solution of 200 μg·ml^{-1} of KIO_3 and 2.0% KI solutions were used and stored in a refrigerator at 5°C for a week of use.

2.4 Methods

2.4.1 Procedure for LNG

Different volumes of LNG standard solution were transferred into 10.0 ml volumetric flasks. Then 1.0 ml of 0.1 M NaOH and 1.0 ml of 1.0×10^{-3} M of $KMnO_4$ solution were added to each volumetric flask and diluted to the mark. After 15.0 min at 25°C, the absorbance at 610 nm was measured for comparison against the reagent blank. The absorbance after a set period of time was plotted against the concentration of the test substance to create the standard calibration plot.

2.4.2 Procedure for EMG

Different volumes of 20.0 μg·ml^{-1} EMG solution were transferred into a 10.0 ml volumetric flask, then 2.0 ml of 0.12 M KI and 1.0 ml of 0.01 M of KIO_3 were added, and the volume of each flask was complete to 10 ml. After 15.0 min, the absorbance values were measured at 350 nm for each flask using glass cuvettes.

The drug solutions were equilibrated at 25°C in a water bath. Then different amounts of the EMG stock solution were added to a series of 10.0 ml volumetric. After that, 1.0 ml of KIO_3 and 1.0 ml of KI were added to each flask, and then distilled water was used to adjust the volume. The absorbance at 350 nm was measured after 15.0 min. Plotting EMG absorbance versus concentration allowed the construction of the calibration curve.

2.4.3 Applications to pharmaceutical forms

Ten tablets containing both LNG and EMG were pealed and then ground using an agate mortar and weighed properly. After that, aliquot of each was taken, weighed, and transferred to 50 ml flasks, where they were solubilized with water and filtered through a 0.2 µm pore size membrane. The solutions of both drugs were suitably diluted with water to get the final concentration, as shown in Table 3. After that, the spectrophotometric methods were used to determine the concentration of both drugs, as discussed in the procedure of calibration of these drugs.

3 Results and discussion

3.1 Absorption spectra

It is well known that $KMnO_4$ solution exhibits a significant absorption band at 525 nm in its absorption spectrum. In the presence of the basic permanganate solution, the reduction of permanganate by LNG in an aqueous solution produces a green color manganate ion with an absorption peak

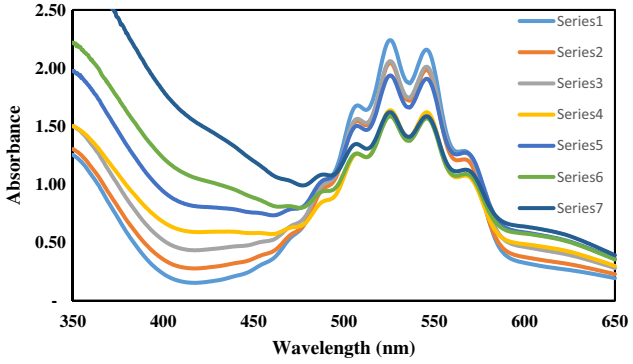

Figure 2: Effect of increasing the concentration of LNG on the spectrum of $KMnO_4$.

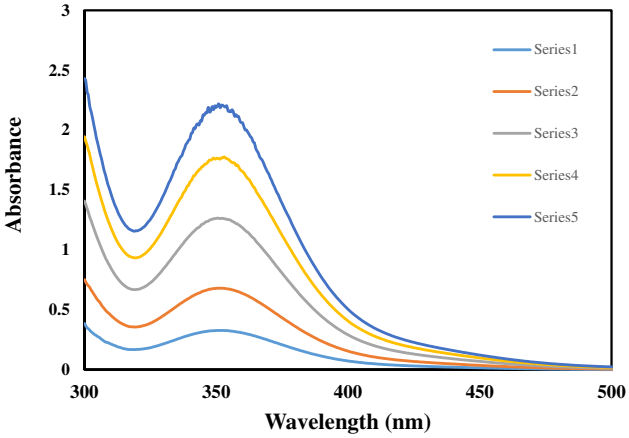

Figure 3: Effect of increasing the concentration of EMG after the reaction with KIO_3/I^-.

at 610 nm, as shown in Figure 2. Although there are no reported mechanisms for oxidation of LNG using alkaline permanganate, there are many reported works that investigate the mechanism of such oxidation of organic compounds bearing primary and secondary amine groups to undergo one-electron reduction of permanganate (MnO_4^-) to manganate ion (MnO_4^{2-}) in alkaline medium, according to the following equation [24]:

$$MnO^-_{4(aq)} + R\text{-}NH_2 + OH^-_{(aq)} \rightarrow MnO^{2-}_{4\,(aq)}.$$

For the assay of LNG in dosage forms, it is advised to utilize the fixed-time kinetic approach because the absorbance at 610 nm increases slowly over time to enhance the precision of the method. The second method for EMG is based on the reduction of EMG by potassium iodate in the presence of iodide ions. Then, an iodine/iodide complex ($3I_3^-$) is formed, and the absorbance of this complex is measured at 350 nm at constant time and temperature, as shown in Figure 3. The reaction equation of the formation of the triiodide complex is as follows:

$$IO^-_{3(aq)} + 8I^-_{(aq)} + 6H_{(aq)} + \rightarrow\ 3I^-_{3(aq)} + 3H_2O.$$

The mechanism of oxidation of EMG by using iodine as an oxidizing agent is similar to many documented methods of oxidizing organic compounds compared with an alcohol group [25]. This process involves producing iodine as a result of the oxidation of iodide ions by iodate in an acidic medium, which then oxidizes the alcohol group of the selected drug using iodine. It was found that under the same conditions as the oxidation of LNG, EMG does not react with alkaline permanganate, allowing for the determination of both drugs in combined formulations.

3.2 Effect of time and temperature

It was found that the oxidation reactions occurred at 25°C or room temperature. The reaction that occurs when LNG is heated results in the formation of manganese dioxide (MnO_2), which lowers sensitivity and precision. In order to simplify the analytical process and eliminate byproducts, the operation was carried out at room temperature. The results shown in Figure 4 reveal that the reaction reaches a constant absorbance at about 15 min.

The oxidation of EMG is due to the oxidation of alcohol groups present in its structure. Because the absorbance at 350 nm rises slowly with time, the effect of time on the progress of time at fixed temperature was studied, and the results are shown in Figure 5, which reveals that the reaction reaches a constant absorbance after about 15.0 min. It is suggested to use a fixed-time kinetic method for the estimation of EMG in pharmaceutical forms. It was also found that LNG does not react with iodate at the same conditions applied for the oxidation of EMG. The experimental factors that influence the formation and stability of the oxidation product were identified and optimized as follows.

3.3 Effect of amount of oxidizing agents

The effect of the amount of $KMnO_4$ was studied by varying its concentration while keeping the concentration of NaOH constant at 25°C. As shown in Figure 6, the effect of the amount of $KMnO_4$ was studied by continuous variation of the volume of $KMnO_4$ solution while keeping all other quantities constant; the optimum volume shown in Figure 6 was 1.0 ml, which resulted in obtaining the maximum value of absorbance at 610 nm.

The effect of the amount of KIO_3 was studied by varying its concentration while keeping the concentration of KI constant at 25°C. As shown in Figure 7, the optimum volume of KIO_3 was considered to be 1.0 ml resulting from finding the maximum absorbance at this value.

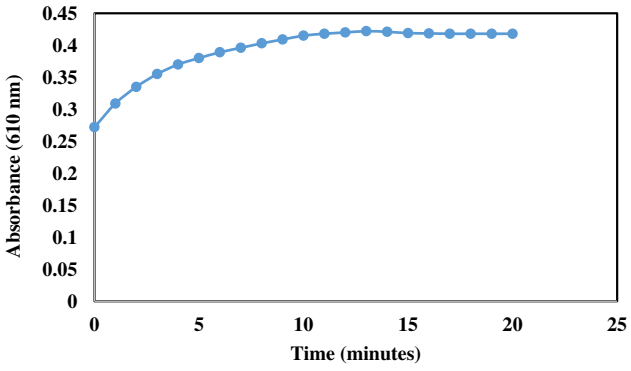

Figure 4: Effect of time on the absorbance at 610 nm in the presence of LNG.

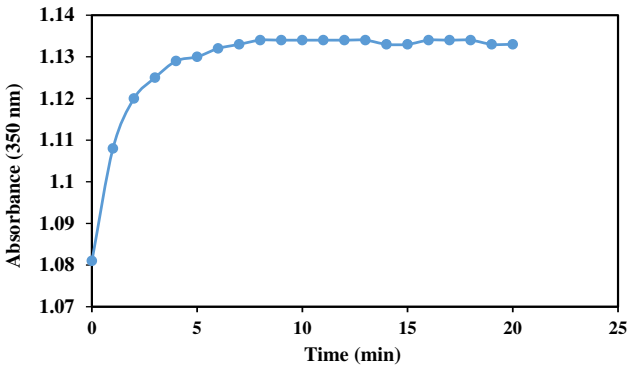

Figure 5: Effect of time on the absorbance at 350 nm in the presence of EMG.

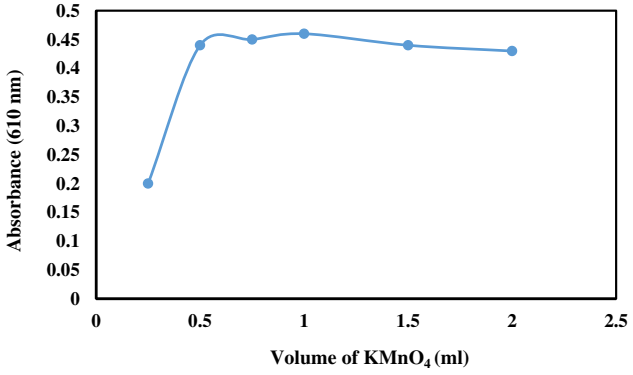

Figure 6: Effect of the volume of $KMnO_4$ on the absorbance at 610 nm in the presence of LNG.

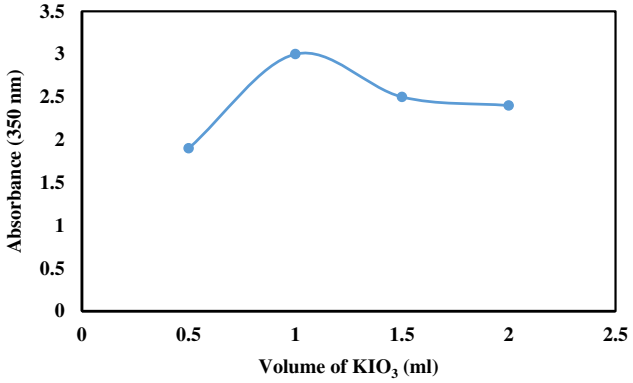

Figure 7: Effect of the volume of KIO_3 on the absorbance at 350 nm in the presence of EMG.

3.4 Validation of the methods

3.4.1 Linearity, detection, and quantification limits

The test of the examined medicines in pure form over concentration ranges of 0.2–20 and 4 0.2–1.5 g·ml^{-1} for LNG and EMG, respectively, was performed using optimal conditions and a fixed-time approach. Beer's law was used to plot the calibration curves with n = 5. The correlation coefficients for the two methods, which were evaluated for extremely excellent linearity, are displayed in Table 1.

Table 1: Statistical analysis for the determination of LNG and EMG

Parameter	LNG	EMG
Wavelength (nm)	610	350
Beers law limits (μg·ml^{-1})	0.25–20	0.20–1.5
Slope (b)	0.012	1.776
Intercept (a)	0.334	−0.502
Correlation coefficient (r)	0.998	0.993
LOD (μg·ml^{-1})	0.082	0.065
LOQ (μg·ml^{-1})	0.25	0.18

The sensitivity of the procedures was assessed using limits of detection (LOD) and limits of quantitation (LOQ), and the results are according to the advice of ICH [26]. The LOD was calculated using the formula LOD = 3.3 × σ/s, where σ is the standard deviation of five replicate measurements of the blank and s is the slope of the calibration plot. A further description of LOQ is 10 × σ/s.

Five determinations at three distinct concentration levels were made in order to evaluate the two techniques' accuracy and precision. The established techniques' relative standard deviation (RSD%) for precision and percent recovery for accuracy were calculated. Table 2 displays the relative standard deviation values for various medication concentrations based on calibration curves. These results of precision and accuracy demonstrated that the suggested methods are repeatable and reproducible.

3.5 Pharmaceutical preparations

The LNG and EMG in pure and dose forms were effectively determined using the indicated spectrophotometric procedures. Table 3 presents the findings. The proposed

Table 2: Evaluation of intra-day and inter-day precision and accuracy for LNG and EMG and precision

Methods	Added (μg·ml^{-1})	Intra-day			Inter-day		
		Found ± SD[a]	Precision RSD %[b]	Recovery (%)	Found ± SD[a]	Precision RSD %[b]	Recovery (%)
(LNG)							
Level 1	5.0	4.92 ± 0.09	1.83	98.4	4.84 ± 0.09	1.83	96.7
Level 2	10.0	9.89 ± 0.14	1.42	98.9	9.80 ± 0.14	1.42	98.0
Level 3	15.0	14.88 ± 0.26	17.5	99.2	14.80 ± 0.26	17.5	98.7
(EMG)							
Level 1	5.0	4.94 ± 0.29	5.9	98.0	4.89 ± 0.39	5.9	97.8
Level 2	10.0	9.94 ± 0.18	1.81	99.4	9.86 ± 0.26	1.81	98.0
Level 3	15.0	14.92 ± 0.34	2.28	99.5	14.80 ± 0.81	2.28	98.7

a: Average of five determinations, b: Standard deviation.

Table 3: Application to drug formulations

Drug	Claimed (μg ml^{-1})	Found ± SD[b]	Recovery (%)[c]	Found using reference method[d]	Recovery (%)
(LNG)					
Level 1	5.0	4.95 ± 0.08	99.0	4.98 ± 0.15	99.6
Level 2	10.0	10.1 ± 0.12	101.0	9.88 ± 0.28	98.8
Level 3	15.0	14.95 ± 0.34	99.6	14.94 ± 1.28	99.6
(EMG)					
Level 1	5.0	4.94 ± 0.25	98.8	4.92 ± 0.41	98.8
Level 2	10.0	9.90 ± 0.18	99.0	9.90 ± 1.20	99.0

a: Average of five determinations, b: Standard deviation, c: percent recovery, d: using reference methods.

processes underwent validation using reference methodologies. These techniques were compared to those for LNG [20] and EMG [21] in dose formulations that had been published. The statistical findings demonstrate good precision and accuracy in the assay of the pharmaceuticals under study using the suggested and cited techniques (Table 3).

4 Conclusions

LNG and EMG were estimated using newly developed techniques based on the oxidation of the two drugs using permanganate and iodate oxidizing agents, respectively. The developed methods were cost-effective and selective compared to other commonly used methods; moreover, the techniques were tested for precision and accuracy and applied successfully for the assay of both drugs in real dosage forms.

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Kirby M, Yu D, Conor S, Gorrell MD. Inhibitor selectivity in clinical application of DPP-4 inhibition. Clin Sci. 2010;118(1):31–41. doi: 10.1042/CS20090047.

[2] Bays H. From victim to ally: the kidney as an emerging target for the treatment of diabetes mellitus. Curr Med Res Opin. 2009;25(3):671–81. doi: 10.1185/03007990802710422.

[3] Levine MJ. Empagliflozin for type 2 diabetes mellitus: An overview of phase 3 clinical trials. Curr Diabetes Rev. 2016;13(4):405–23. doi: 10.2174/1573399812666160613113556.

[4] Sangeetha RK, Subashri T. Analysis of linagliptin in tablet dosage form by UV spectroscopy method, its derivatives and difference spectra. Eur J Pharm Med Res. 2016;3(11):536–40.

[5] Vijaya K, Anusha A, Sudhakar M. UV-spectrophotometric method for the estimation of linagliptin in bulk and pharmaceutical formulations. Asian J Res Chem. 2016;9(1):47–50. doi: 10.5958/0974-4150.2016.00009.2.

[6] Sujan B, Mohammad SH. Development and validation of a simple and rapid UV spectrophotometer method for the assay of linagliptin in bulk and marketed dosage form. Ind J Nov Drug Del. 2013;5(4):221–4.

[7] Ayoub BM, EI-Bagary RI, Elkadly EF. Spectrophotometric methods for the determination of linagliptin in binary mixture with metformin hydrochloride and simultaneous determination of linagliptin and metformin hydrochloride using RP-HPLC. Int J Biomed Sci. 2013;9(1):41–7.

[8] Lilian S. Development of economic UV spectrophotometric method for determination of linagliptin in its ternary mixture with empagliflozin and metformin: Comparison to economic pharmaceutical analysis in literature. Pharm Lett. 2016;8(13):267–9.

[9] Wael AD, Mohammad H, Tayel AA, Mousa M, Zainab Z, Lina AT, et al. Spectrophotometric analysis of Empagliflozin tablets as SGLT2 inhibitors in pharmaceutical samples. J Appl Pharm Sci. 2022;12(10):140–6. doi: 10.7324/JAPS.2022.121015.

[10] Mahmoud AO, Ahmed MH, Gamal AS, Naggar AH, Sayed MD. Diarylpyrrolone based fluorophore for the selective spectrofluorometric method for determination of Linagliptin antidiabetic drug in pharmaceutical tablets. Microchem J. 2019;148:555–60. doi: 10.1016/j.microc.2019.05.046.

[11] Ates AK, Celikkan HN. Voltammetric determination of linagliptin in bulk and plasma sample using an electrochemical sensor based on L-cysteine modified 1T-MoS2 nanosheets. Microchem J. 2021;167:1–6. doi: 10.1016/j.microc.2021.106308.

[12] Prathyusha V, Dilip D, Umamahesh B, Shyam P, Veeresham C. Simultaneous determination of linagliptin and metformin by reverse phase-high performance liquid chromatography method: An application in quantitative analysis of pharmaceutical dosage forms. J Adv Pharm Technol Res. 2015;6(1):25–8. doi: 10.4103/2231-4040.150368.

[13] Sharmila D, Achanta S. Validated HPLC-UV method for simultaneous estimation of linagliptin and empagliflozin in human plasma. Int J Appl Pharm. 2018;10(3):56–61.

[14] Panikumar AD, Sowndarya NS, Rajeshwari G, Radhagayathri A, Sunitha G. Quantification of linagliptin by chemical derivatization with appliance of chromogenic reagents. J Appl Chem Res. 2016;11(2):39–50.

[15] El-Desouky EA, Abdel EA, Ashraf AF, Ahmed AZ, Ahmed HA, Morshedy S. Determination of linagliptin and empagliflozin by UPLC and HPTLC techniques aided by lean six sigma approach. Biomed Chromatogr. 2021;35(7):1–12. doi: 10.1002/bmc.5102.

[16] Ahmad R, Hailat M, Jaber M, Alkhawaja B, Rasras A, AlShdefat R, et al. RP-HPLC method development for simultaneous estimation of empagliflozin, pioglitazone, and metformin in bulk and tablet dosage forms. Acta Pol Pharm Res. 2021;78(3):305–15. doi: 10.32383/appdr/139635.

[17] Shah PA, Shrivastav PS, George A. Mixed-mode solid phase extraction combined with LC-MS/MS for determination of empagliflozin and linagliptin in human plasma. Microchem J. 2019;145:523–31. doi: 10.1177/1469066719879297.

[18] Wattamwar T, Mungantiwar A, Halde S, Pandita N. Development of simultaneous determination of empagliflozin and metformin in human plasma using liquid chromatography–mass spectrometry and application to pharmacokinetics. Eur J Mass Spectrom. 2020;26(2):117–30. doi: 10.1177/1469066719879297.

[19] Ayoub BM, Mowaka S. LC-MS/MS determination of empagliflozin and metformin. J Chromatogr Sci. 2017;55(7):742–7. doi: 10.1093/CHROMSCI/BMX030.

[20] Sunitha G, Panikumar DA, Sahitya M, Nikitha G, Keerthi T. Spectrophotometric estimation of linagliptin using ion-pair complexation and oxidative coupling reactions – A green approach. Thai J Pharm Sci. 2020;44(4):245–50.

[21] Patil SD, SKMAH, RahmanPrajkta, Sanjay UV, K. Development and validation of simple UV- spectrophotometric method for the determination of empagliflozin. Asian J Pharm Anal. 2017;7(1):43–8. doi: 10.5958/2231-5675.2017.00004.7.
[22] Ivanković A, Dronjić A, Bevanda AM, Talić S. Review of 12 principles of green chemistry in practice. Int J Sustain Green Energy. 2017;6:39–48.
[23] Lawrence H, Keith Liz U, Gron, Jennifer L. Young green analytical methodologies. Chem Rev. 2007;2107:2695–708. doi: 10.1021/cr068359e.
[24] Nafisur R, Sumaiya KH. Kinetic modelling for the assay of nortriptyline hydrochloride using potassium permanganate as oxidant. AAPS PharmSciTech. 2015;16(3):569–78. doi: 10.1208/s12249-014-0230-8.
[25] Shaza A, Amir A. Validated green spectrophotometric kinetic method for determination of Clindamycin Hydrochloride in capsules. BMC Chem. 2021;15:29. doi: 10.1186/s13065-021-00755.
[26] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2(R1), Complementary Guideline on Methodology dated 06 November 1996, ICH, London; 2005.