Egypt. J. Chem. **Vol. 67**, No. 7 pp. 443 - 449 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Stability Indicating Assay Method of Metformin, Linagliptin and Empagliflozinin Pharmaceutical Dosage Form by HPLC Method

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Abstract

A new reversed phase high performance liquid chromatographic method for simultaneous determination of Metformin HCl, Linagliptin and Empagliflozin has been developed and validated to be a simple, sensitive, rapid, specific, precise, and accurate method. Chromatographic separation achieved on C18 column (4.6-mm x 25-cm; 5-µm) Thermohypersil. through isocratic elution using Water: Acetonitrile in ratio (65:35 v/v) adjusted to pH 3.5 with Orthophosphoric acid as a mobile phase at flow rate of 1.0 ml/min. UV detection was operated at 269 nm and injection volume was 10 µl. Linearity range for Metformin HCl, Linagliptin and Empagliflozin was 10000-30000, 50-150 and 250-750 µg mL-1, respectively. The proposed method showed good linearity, accuracy, precision and successfully applied for determination of the three drugs in laboratory prepared mixtures forms and so it is suitable for quality control of them. The method described is quite suitable for routine analysis in pharmaceutical preparations.

Keywords: HPLC, Metformin; Linagliptin; Empagliflozin; Method validation.

1. Introduction

Metformin hydrochloride (MET): chemically Ndimethylmethanimidamide hydrochloride. Metformin is aantihyperglycemic drug in the biguanide group of medications that is used to treat type 2 diabetes (Fig. 1). Metformin reduces blood glucose levels in people with type 2 diabetes without producing hypoglycemia, it is considered as an antihyperglycemic medication. As an insulin sensitizer, metformin is well known for lowering insulin resistance and lowering plasma fasting insulin levels to a clinically meaningful degree. Metformin is the treatment of choice for patients with obese type 2 diabetes [1].

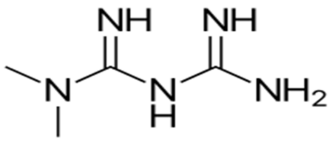

Figure 1: Chemical structures of Metformin

Linagliptin (LINA): chemically (R)-8-(3-aminopiperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methylquinazolin-2- ylmethyl)-3,7-dihydro-purine-2,6-dione (Fig. 2). LINA is a reversible, competitive DPP-4 inhibitor. Inhibiting this enzyme reduces GLP-1 and glucose-dependent insulin tropic polypeptide (GIP) degradation. GLP-1 and GIP enhance the release of insulin from pancreatic beta cells while suppressing glucagon release of from pancreatic beta cells. When combined, these effects lower the liver's breakdown of glycogen and enhance insulin release in response to glucose [2, 3].

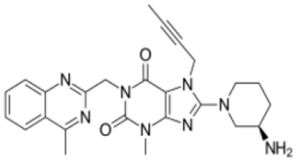

Figure 2: Chemical structures of Linagliptin

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EJCHEM use only: Received date 09 September 2022; revised date 30 December 2023; accepted date 07 January 2024

DOI: 10.21608/EJCHEM.2024.161798.6953

Empagliflozin (EMPA): chemically 1-chloro-4-(glucopyranos-1-yl)-2-(4-(tetrahydrofuran-3-yloxy) benzyl) benzene. EMPA is a sodium glucose cotransporter-2 (SGLT-2) inhibitor that has been proven to enhance glycemic control in adults with type 2 diabetes when combined with diet and exercise (Fig. 3). SGLT2 co-transporters are in charge of reabsorbing glucose from the glomerular filtrate in the kidney. The glucuretic effect caused by SGLT2 inhibition decreases renal absorption and lowers the renal glucose threshold, resulting in increased glucose excretion. Furthermore, it contributes to lower hyperglycemia, as well as weight loss and blood pressure reduction [2, 4].

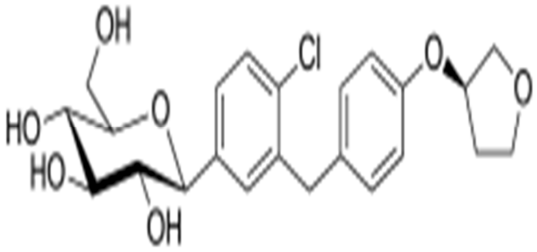

Figure 3: Chemical structures of Empagliflozin

On June 4, 2019, this newer combination was approved for a phase II clinical trial. according to USFDA report, the combination works using three complementary processes to help regulate blood glucose in adult with type 2 diabetes who could profit from having empagliflozin and linagliptin as part of their treatment regimen, in addition to metformin [5].

A survey of the literature on the quantitative determination of MET, LINA, and EMPA several attempts have been performed to establish analytical methods for the measurement of MET, LINA, and EMPA by high-performance thin-layer chromatography (HPTLC) for individual and combination with other medications [5-11].

Literature review found that no approach has been created for simultaneously determining of MET, LINA, and EMPA together in one dosage form. As a result, the current study's goal is to use the HPLC method to simultaneously estimate MET, LINA, and EMPA in bulk and synthesized mixtures.

Low chromatographic method published for the combination dosage form. Therefore, the present work was to establish a simple, rapid, sensitive, and cost-effective HPLC method for routine analysis.

2. Experimental

2.1. Chemicalsand reagents

Reference standards of metformin hydrochloride from Wanbury (India), Linagliptin, from Lee pharma (India) and Empagliflozin from Optrix (India). HPLC grade Methanol from Merck (Germany). HPLC grade Acetonitrile from Romil (England). Orthophosphoric acid from Scharlau (Spain). Water for chromatography from Merck (Germany).

2.2. Equipment and chromatographic conditions

Agilent 1200 with photodiode (PDA) detector used. HPLC separation and quantification were conducted on a ThermoHypersil C18 (4.6-mm x 25-cm; 5-µm). Water: Acetonitrile in ratio (65:35 v/v) Adjusted to pH 3.5 with Orthophosphoric acid used as mobile phase. Which was run isocratically. The mobile phase injected to the system at an injection rate of 1.0 ml/min. All measurements performed at room temperature. The volume injected was 10 µl. The detector was adjusted at 269 nm. The run time was timed at 7.5 min. Table 1 shows the optimum chromatographic condition.

Table1: Optimized chromatographic conditions

Parameters	Conditions
Stationary phase	Thermo Hypersil C18 , 250 x 4.6 mm, 5 µm
Mobile phase	Water: Acetonitrile (65:35 v/v) pH 3.5
Flow rate (mL min^{-1})	1.0
Run time (min)	7.5
Column temperature	Ambient (25 ^{0}C)
Injection volume (µL)	10
Detection wavelength	269 nm

2.3. Preparation of standard solution

Precisely weigh 1000 mg of metformin hydrochloride standard, 5mg Linagliptin and 25 mg Empagliflozin into 50 ml volumetric flask, add 25 ml of methanol, sonicate for 5 min then cool and complete the volume with methanol.

2.4. Preparation of Test solution

Weight ten tablets of Trijardy XR (1000 mg metformin, 5 mg Linagliptin and 25 mg Empagliflozin), finely powdered, and an accurately weighed amount of the powdered tablets equivalent to one tablet into 50 ml volumetric flask, with 25 ml of methanol, sonicate for 5 min then cool and finish the volume with methanol.

2.5. Method Validation

Linearity

The proposed method's linear calibration plots were obtained across concentration ranges of 10000 - 30000 µg mL^{-1} (10000, 16000, 20000, 24000 and 30000 µg mL^{-1}) for metformin hydrochloride, 50 – 150 µg mL^{-1} Linagliptin (50, 80, 100, 120 and 150 µg mL^{-1}) and 250 - 750 µg mL^{-1} (250, 400, 500, 600 and 750 µg mL^{-1}) for Empagliflozin. Triplicate injections were made for each standard solution.

Accuracy

Accuracy was evaluated by standard addition method of metformin hydrochloride, Linagliptin and Empagliflozin sulfate. This method known amounts of metformin hydrochloride, Linagliptin and Empagliflozin sulfate were added to the previously analysed sample solution and then experimental and true values were compared. Three levels were made corresponding to 80%, 100% and 120% of the nominal analytical concentration.

Acid hydrolysis

In round-bottom flasks, 1.0 mL of the solution was forced degraded in acidic medium. The mixtures were then refluxed for 2 hours with 10 mL of 1 N HCl. Before analysis, this solution was neutralized using 1 N NaOH. (Fig. 6)

Base hydrolysis

In round-bottom flasks, 1.0 mL of the solution was forced degraded in basic medium. The mixtures were then refluxed for 2.0 hours with 10 mL of 1 N NaOH. Before analysis, this solution was neutralized using 1 N HCl. (Fig. 7)

Oxidative hydrolysis

In round-bottom flasks, 1.0 mL of the solution was forced degraded with hydrogen peroxide. These mixtures kept for 2.0 hours with 10 mL of 3% (w/v) hydrogen peroxide. (Fig. 8)

Precision

The determination of intra-day and inter-day precision was used to investigate repeatability. The intra-day precision assessed by injecting five successive injections of three different concentrations on the same day, and the inter-day precision was determined by injecting the same solutions three days in a row. The peak area's relative standard deviation (RSD%) was then calculated to indicate precision.

Robustness

To assess the approach's robustness, premeditate adjustments were done in the experimental circumstances of the proposed method. Minor adjustments were made to the mobile phase composition, flow rate, and pH of the buffer solution to achieve this goal. The impact of these adjustments on chromatographic parameters such as retention time, tailing factor, and theoretical plate count was subsequently determined.

***Limit* of detection (LOD) and limit of quantitation (LOQ)**

Limits of detection (LOD) were calculated according to the expression 3.3σ/S, where S is the slope of the calibration curve and σ is the standard deviation of the response. Limits of quantification (LOQ) were established by using the formula 10σ/S. LOD and LOQ were scientifically verified by injections of pure standard at the LOD and LOQ concentrations.

3. Results and Discussion

The composition of the mobile phase and distinct stationary phases influenced peak shape, tailing factor, retention factor, theoretical plates, and resolution significantly. The goal of this work is to create a method that can be used to successfully separate and quantify the studied. For the simultaneous measurement of metformin hydrochloride, linagliptin, and empagliflozin, either separately or in pharmaceutical formulations, a simple, selective, sensitive, and accurate HPLC approach was used. Metformin hydrochloride, linagliptin, and empagliflozin had distinct resolutions with retention times of 2.19, 3.72, and 6.78 minutes, respectively. There were no interferences at the retention times for metformin hydrochloride, linagliptin, or empagliflozin due to the placebo (Figs. 4 and 5). Method validation was performed according to ICH guidelines (12).

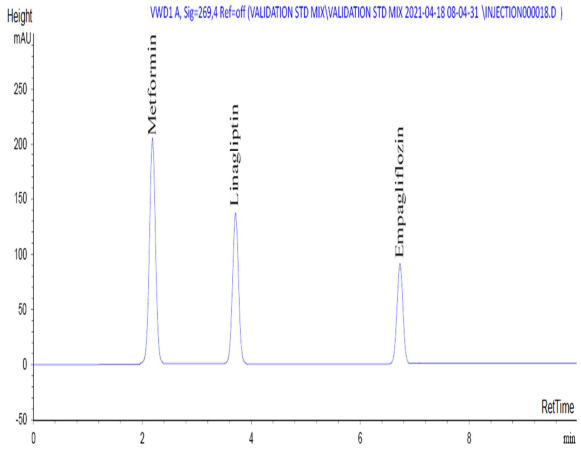

Figure 4: A typical chromatogram for MET, LIN and EMP standard drug.

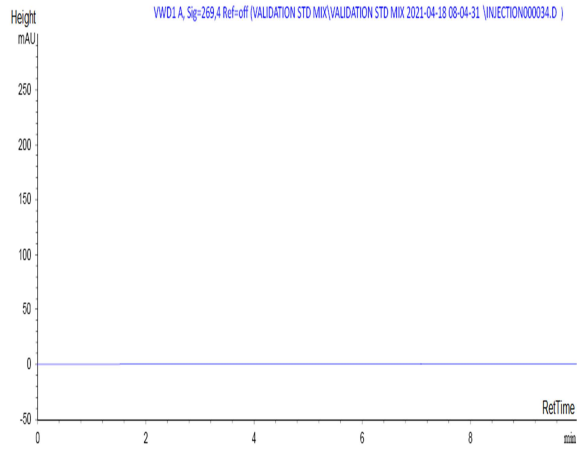

Figure 5: HPLC chromatogram of placebo

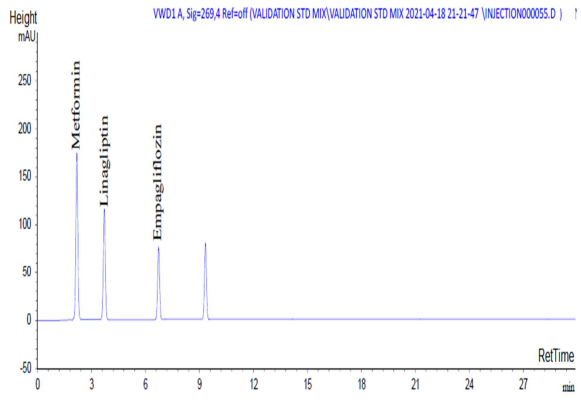

Figure 6: HPLC chromatogram of acid degradation

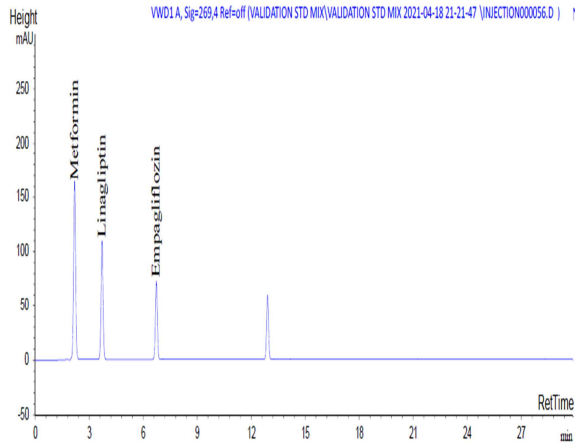

Figure 7: HPLC chromatogram of base degradation

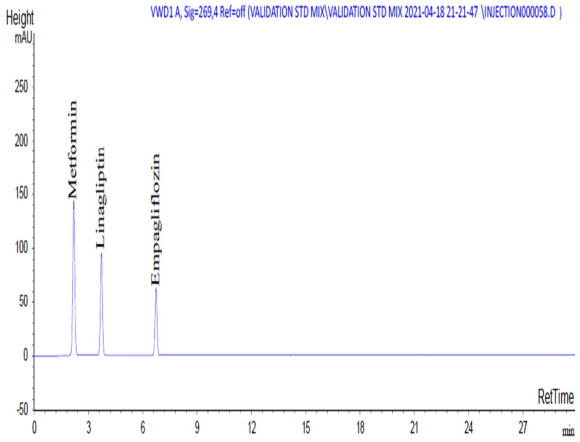

Figure 8: HPLC chromatogram of oxidation degradation

Linearity of the proposed method was evaluated and it was evident in the concentration range of 10000-30000µg mL^{-1} for MET, 50-150 µg $mL^{-1}$for LINand 250-750 µg $mL^{-1}$for EMP. Good linearity was evident by the high value of the correlation coefficient are shown in (Figs. 9, 10 and 11). The correlation between the analyte concentration and peak area is described by linear regression equations with high value of correlation coefficient (r) all results were listed in Table 2. The regression equations were calculated and found to be;

Y1 = 0.0975x - 3.5907, r1 = 0.9999

Y2 = 11.301x - 0.7041, r2 = 0.9999

Y3 = 1.8181x - 1.4978, r3 = 0. 9998

Where Y1, Y2 and Y3 are the peak area/10^3 for MET, LINand EMPconcentrations respectively and r1, r2 and r3 are the correlation coefficients.

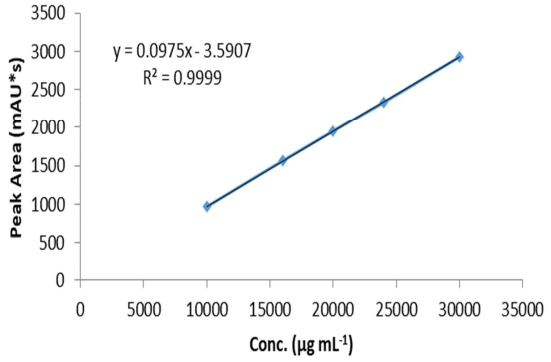

Figure 9: Calibration curve of Metformin using Agilent 1200 with DAD 269 nm.

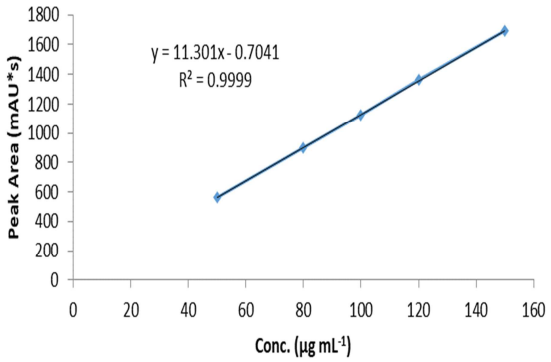

Figure 10: Calibration curve of Linagliptin using Agilent 1200 with DAD 269 nm.

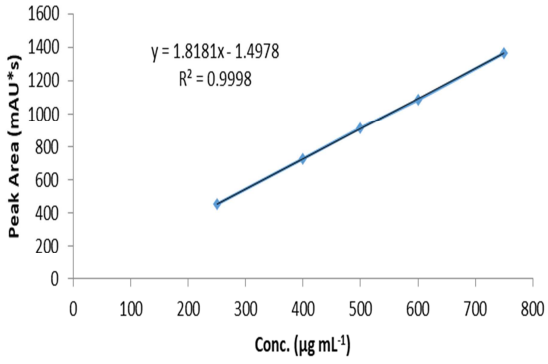

Figure 11: Calibration curve of Empagliflozin using Agilent 1200 with DAD 269 nm.

Table 2: Characteristics of the proposed methods used in assay of drugs under study

Parameters	MET	LIN	EMP
Linearity range/µg mL^{-1}	10000-30000	50-150	250-750
Slope	0.0975x	11.301x	1.8181x
Intercept (a)	-3.5907	-0.7041	-1.4978
Correlation coefficient	0.9999	0.9999	0.9998
Detection limit/µg mL^{-1}	1666.67	8.34	41.67
Quantification limit/µg mL^{-1}	5000	25	125
Tailing factor	1.10	1.12	1.25
Theoretical plate no.	4773	4428	4992

Regression equation: Y= a + bC, where Y is the area under peak, a is the intercept, b is the slope and C is the concentration.

Serial dilutions used to assess the limit of detection (LOD) and limit of quantitation (LOQ). LOD was measured to be 1666.67 µg mL^{-1}, 8.34 µg mL^{-1} and 41.67 µg mL^{-1} for metformin hydrochloride, linagliptin and empagliflozin, correspondingly (signal to noise ratio of 3:1). LOQ was measured to be 5000 µg mL^{-1}, 25 µg mL^{-1} and 125 µg mL^{-1} for metformin hydrochloride, linagliptin and empagliflozin; correspondingly (signal to noise ratio of 10:1).

Accuracy and recovery of the method was evaluated by standard addition method on different pharmaceutical formulation where good recoveries show no interference from excipients and the proposed approach is accurate. The described method generated and evaluated three levels of solutions (80, 100, and 120% of the nominal analytical concentrations).

Intra-day precision assessed by injecting five successive injections of three different concentrations on the same day and inter-day precision was determined by injecting the same solutions three days in a row. Precision was represented by the relative standard deviation (RSD%) of the peak area. Table 3 shows the intra-day and inter-day precision results. In this study, the mobile phases, standard solutions, and sample solution were all subjected to long-term (3-day) stability tests. The stability of these solutions was investigated by conducting the experiment and examining for changes in separation, retention, and asymmetry of the peaks, which were then compared to the pattern of the chromatogram of freshly made solutions.

Table 3: Intra-day and inter-day precision of the proposed HPLC method.

Drug	Actual concentration (µg mL-1)	Intra-day precision measured concentrations (µg mL^{-1}) ± SD; RSD (%)	Inter-day precision measured concentrations (µg mL^{-1}) ± SD; RSD (%)
MET	16000	16007 ± 2.08; 0.01	16004 ± 4.36; 0.03
	20000	20007 ± 4.93; 0.02	20007 ± 5.86; 0.03
	24000	24014± 6.08; 0.03	24014 ± 6.43; 0.03
LIN	80	80.27± 0.38; 0.47	80.37± 0.21; 0.26
	100	100.10± 0.10; 0.10	100.01± 0.01; 0.01
	120	120.20 ± 0.10; 0.08	120.18 ± 0.20; 0.16
EMP	400	400.22± 0.10; 0.03	399.30± 0.72; 0.18
	500	499.87 ± 0.21; 0.04	499.83 ± 1.22; 0.24
	600	599.10 ± 0.92; 0.15	598.03 ± 1.97; 0.33

Robustness of the method was performed by slightly varying chromatographic conditions. The results showed that slight variations in chromatographic conditions had a negligible effect on the chromatographic parameters (Tables 4, 5 and 6).

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Table 4: Robustness study formetformin

Chromatographic conditions	Assay (%)	t_R (min)	Theoretical plates	Tailing
Column temperature (20^0C)	100.31	2.18	3818	1.02
Column temperature (25^0C)	100.15	2.17	3817	1.10
Column temperature (30^0C)	100.22	2.19	3824	1.04
Flow rate (0.8 mL min^{-1})	99.97	2.44	3820	1.11
Flow rate (1.0 mL min^{-1})	100.11	2.20	3822	1.03
Flow rate (1.2 mL min^{-1})	100.01	2.01	3801	1.10
Buffer (pH 3.3)	99.91	2.22	3822	1.05
Buffer (pH 3.5)	99.88	2.20	3830	1.04
Buffer (pH 3.7)	99.17	2.21	3833	1.07

Table 5: Robustness study forlinagliptin

Chromatographic conditions	Assay (%)	t_R (min)	Theoretical plates	Tailing
Column temperature (20^0C)	100.11	3.74	3213	1.15
Column temperature (25^0C)	99.08	3.71	3211	1.11
Column temperature (30^0C)	100.01	3.76	3222	1.10
Flow rate (0.8 mL min^{-1})	99.92	3.91	3211	1.14
Flow rate (1.0 mL min^{-1})	99.78	3.70	3215	1.10
Flow rate (1.2 mL min^{-1})	99.16	3.51	3206	1.13
Buffer (pH 3.3)	100.13	3.74	3215	1.17
Buffer (pH 3.5)	99.86	3.73	3225	1.10
Buffer (pH 3.7)	99.61	3.77	3229	1.17

Table 6: Robustness study forempagliflozin

Chromatographic conditions	Assay (%)	t_R (min)	Theoretical plates	Tailing
Column temperature (20^0C)	99.22	6.79	2968	1.11
Column temperature (25^0C)	99.87	6.78	2967	1.12
Column temperature (30^0C)	99.18	6.81	2967	1.15
Flow rate (0.8 mL min^{-1})	99.77	6.85	2965	1.10
Flow rate (1.0 mL min^{-1})	99.09	6.79	2968	1.11
Flow rate (1.2 mL min^{-1})	99.87	6.53	2965	1.11
Buffer (pH 3.3)	99.27	6.78	2971	1.10
Buffer (pH 3.5)	99.88	6.79	2970	1.11
Buffer (pH 3.7)	100.02	6.81	2975	1.12

System suitability tests founded on the idea that the equipment, electronics, analytical procedures and samples constitute an integral system that can be evaluated as whole. System suitability used to ensure system performance before or during the analysis of the drugs. System suitability checked by calculating the asymmetry factor (AS), tailing factor (T), theoretical plates (N) and resolution (R), where the system was found to be suitable as shown in Table 7.

Table 7: Summary of system suitability tests

Parameters	metformin	linagliptin	empagliflozin
T	1.03	1.11	1.09
R[b]	—	5.33	12.54
N	3817	3213	2967
AS	1.01	1.12	1.11
RSD[a] (peak areas)	0.70	0.66	0.61
RSD[a] (retention time)	0.11	0.14	0.17

T, Tailing factor; N, no. of theoretical plates; R, resolution factor; As, asymmetry factor.
[a] RSD for five determinations.
[b] The resolution factor (R) calculated to the nearest peak in order.

Table (8): Peak purity summery for the three active materials after degradation

Material Name	Acid degrdn	Base degrdn	Oxi. degrdn	Heat degrdn
Metformin	0.991	0.994	0.992	0.995
Linagliptin	0.997	0.996	0.999	0.998
Empagliflozin	0.996	0.995	0.997	0.998

4. Conclusion

The validated HPLC method developed for the quantitative determination of metformin hydrochloride, linagliptin and empagliflozin in the Egyptian marketing in the same run by using simple, low cost, short time high performance HPLC method. The developed method was validated by testing its linearity, accuracy, precision, specificity, limits of detection and quantitation. This method enables simultaneous determination of metformin hydrochloride, linagliptin and empagliflozin using mobile phase contain (Water: Acetonitrile in ratio (65:35 v/v) Adjusted to pH 3.5) and DAD 269 nm at flow 1.0 ml/min with injection volume 10 µl. Because of the good separation and resolution of the chromatographic peaks, the proposed HPLC method could be adopted for the quantitative routine analysis.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

There are no conflicts to declare.

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