Asian Journal of Chemistry; Vol. 33, No. 2 (2021), 484-488

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23019

Development and Validation of a Stability-Indicating HPLC Method for Empagliflozin and Linagliptin in Tablet Dosage Form

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Received: 10 October 2020; *Accepted*: 6 January 2021; *Published online*: 15 January 2021; AJC-20238

A simple, stability indicating high performance liquid chromatographical (HPLC) method was developed and validated for the estimation of empagliflozin and linagliptin in combined dosage forms. Chromatographical separation was optimized by isocratic HPLC using C-18 column [BDS 250 mm × 4.6 mm, 5 µm] utilizing a mobile phase consisting a mixuture of 0.1% orthophosphoric acid and acetonitrile (60:40 v/v) running at a rate of 1 mL/min and monitoring effluents at 230 nm. The retention time of empagliflozin and linagliptin was 2.05 min and 4.10 min, respectively. Correlation coefficient (r^2) was 0.999 for both empagliflozin and linagliptin. The precision of method for the analysis of empagliflozin and linagliptin were 0.33 and 0.22, respectively. The accuracy of method (as recovery) was 100.96 to 101.48% for empagliflozin and 100.09 to101.13% for linagliptin. The results indicate the present method is accurate, precision and rugged as these results are within the specified limits. Therefore, the validated economical methodology can be applied for forced degradation study of empagliflozin and linagliptin in solid dosage forms.

Keywords: Empagliflozin, Linagliptin, Optimization, Validation, Stress study, Stability. HPLC method.

INTRODUCTION

The combination of linagliptin and empagliflozin is on the market as tablets formulation for oral use for the management of type 2 diabetes and cardiovascular risk. Empagliflozin is a sodium-glucose co-transporter (SGLT2). The chemical name of empagliflozin is (1*S*)-1,5-anhydro-1-C-{4-chloro-3-[(4-{[(3*S*)-oxolan-3-yl]oxy}phenyl)- methyl]phenyl}-D-glucitol. Linagliptin is a orally-active dipeptidyl peptidase-4 (DPP-4) inhibitor. The chemical name of linagliptin is 8-[(3*R*)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methyl quinazolin-2-yl)methyl]-3,7-dihydro-1*H*-purine-2,6-dione [1-5]. Many HPLC strategies were reported for estimation of linagliptin and empagliflozin separately or together with other medication in pharmaceutical dosage forms and in human plasma [6-10]. The stability indicating HPLC and HPTLC for drugs and strategies are reported for estimation of linagliptin separately or together with alternative agents [11-18]. Only one UPLC methodology was reported for simultaneous determination of empagliflozin, linagliptin and metformin [19]. However, to the best of our knowledge, no stability indicating methodology is reported for simultaneous determination of linagliptin and empagliflozin in pharmaceutical formulation by RP-HPLC. A stability indicating RP-HPLC methodology for the simultaneous determination of linagliptin and empagliflozin is reported here.

EXPERIMENTAL

HPLC grade, acetonitrile and analytical grade orthophosphoric acid were purchased from Merck, Germany. Linagliptin was obtained as a gift sample from Hikma Pharmaceuticals LLC, Industrial Area, Amman, Jordan, while empagliflozin working standard was purchased from JOSWE-Company, Amman, Jordan.

The HPLC Finnigan Surveyor (Thermo-Electron Corporation, San Jose, USA) system consisted of Alliance waters

2695 with UV-Vis plus detector with dual absorbance monitoring mode with running on Windows based acquiring software. HPLC column BDS [BDS 250 mm × 4.6 mm, 5 µm] was used for the analysis of analytes. Sonicator, pH meter (Sartorius), digital balance (Sartorius) were used for conducting the experiments. Mobile phase filtration unit Pall-Q Life Sciences, Switzerland was used for the filtration of mobile phase prior to routine use.

Standard solutions preparation

Standard preparation: Drug equivalent to 12.5 mg and 25.0 mg of linagliptin and empagliflozin, respectively were weighed in 25 mL dry volumetrically flask separately. Methanol (20 mL) was added and sonicated for 0.5 h and then the volume was made up to the mark with diluents to receive the stock solution of linagliptin (500 µg/mL) and empagliflozin (1000 µg/mL), respectively. Different samples of linagliptin and empagliflozin were prepared using the aliquots of stock solutions.

Chromatographic conditions: The wavelength of the detector was selected to 230 nm. Separation was achieved in isocratic mode on column of BDS C18 (4.6 × 250 mm, 5 µm particle size). The optimized mobile phase consisting a mixture of 0.1% orthophosphoric acid and acetonitrile (60:40 v/v) running at a rate of 1 mL/min. The separation of analytes was monitored at 230 nm using UV-Vis detector. The mobile phase filtered through nylon Millipore (0.2 µm) membrane filter before use. The separation was carried out at 30 ºC. The developed HPLC method was validated as per the ICH guidelines.

RESULTS AND DISCUSSION

Method validation: The developed method was validated as per the ICH guideline for the linearity, precision, accuracy, ruggedness and applied for routine use [20,21]. The retention time (t_r) of empagliflozin and linagliptin was found to be 2.05 min and 4.10 min, respectively (Fig. 1).

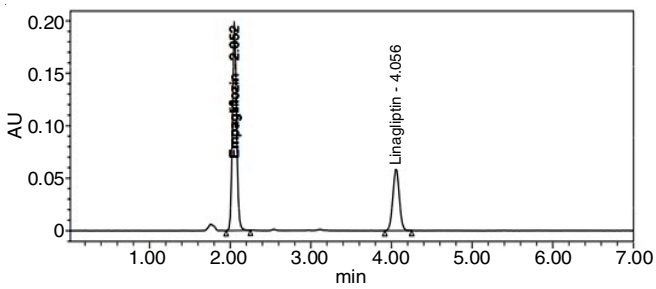


Fig. 1. Representative chromatograph of empagliflozin and linagliptin using optimized mobile phase

Linearity: Linearity range for empagliflozin and linagliptin were ranged from 12.5-75 and 25-150 µg/mL, respectively (Table-1). The correlation coefficient was found to be 0.999 and 0.999 for both empagliflozin and linagliptin (Fig. 2).

TABLE-1
LINEARITY DATA FOR EMPAGLIFLOZIN AND LINAGLIPTIN

Empagliflozin		Linagliptin	
Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area
25	456801	12.5	235592
50	950080	25.0	445338
75	1490729	37.5	677762
100	1966013	50.0	890059
125	2397570	62.5	1092101
150	2863491	75.0	1353505
Slope	19259	Slope	17696
Intercept	2326	Intercept	8178
r^2	0.9987	r^2	0.9989

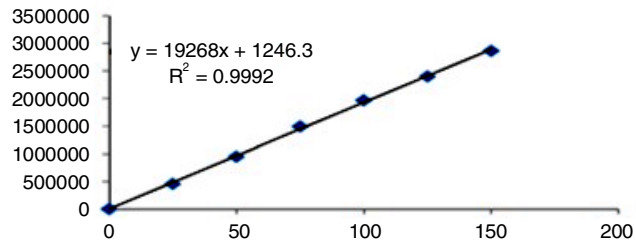
Limit of detection (LOD) and limit of quantification (LOQ): The LOD is calculated using formula 3.3σ/s, wherever "σ" is variance of the intercept obtained from the calibration curve and "s" is that the slope of the calibration curve, while the LOQ is calculated using the formula 10σ/s. The calculated LOD and LOQ are shown in Tables 2 and 3.

TABLE-2
LOD AND LOQ RESULTS FOR ANALYSIS OF EMPAGLIFLOZIN

Conc. (µg/mL)	Area 1	Area 2	Area 3	Avg. area
25	459227	452382	458793	456801
50	952951	947693	949595	950080
75	1493832	1487160	1491194	1490729
100	1993278	1968338	1936422	1966013
125	2408715	2391128	2392867	2397570
150	2876214	2850396	2863864	2863491
Intercept	1179	1443	1115	1246
Slope	19383	19213	19209	19268
Standard deviation of intercept				173.9
LOD (µg/mL)				0.03
LOQ (µg/mL)				0.09

Precision: The intraday precision was calculated from the area count of the repetitive analysis (n = 6) of empagliflozin (100 µg/mL) and linagliptin (50 µg/mL) solution, respectively (Table-4). The RSD of empagliflozin and linagliptin were 0.24 and 0.1, respectively.

Intermediate precision: Intermediate precision of the analytical methodology was calculated by performing the



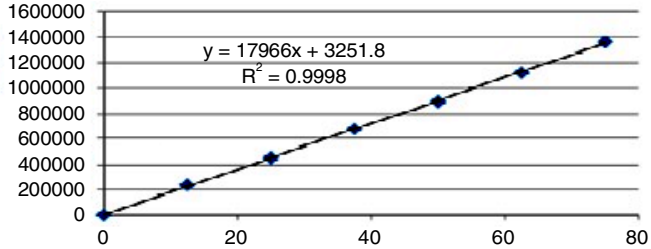


Fig. 2. Representative calibration curve for the analysis of empagliflozin and linagliptin using developed HPLC method

TABLE-3
LOD AND LOQ RESULTS FOR ANALYSIS OF LINAGLIPTIN

Conc. (µg/mL)	Area 1	Area 2	Area 3	Avg. area
12.5	235592	237271	235362	236075
25.0	445338	457617	457576	453510
37.5	677762	679083	670439	675761
50.0	890059	894655	899345	894686
62.5	1092101	1132198	1127290	1117196
75.0	1353505	1357424	1373638	1361522
Intercept	4380	4839	535.1	3251
Slope	17766	17998	18133	17966
Standard deviation of intercept				233.5
LOD (µg/mL)				0.43
LOQ (µg/mL)				1.32

TABLE-4
METHOD PRECISION DATA OF
EMPAGLIFLOZIN AND LINAGLIPTIN

S. No.	Area of empagliflozin (100 µg/mL)	Area of linagliptin (50 µg/mL)
1	1919332	876871
2	1927659	876313
3	1923605	877356
4	1923801	878790
5	1919096	875829
6	1930615	878666
Mean	1924018	877304
SD	4543	1217
%RSD	0.24	0.1

analysis of samples on 3 consecutive days by different analysts using same process. Six replicates of standards preparations were used. The precision of the method for empagliflozin (100 µg/mL) and linagliptin (50 µg/mL) was found to be 0.33 and 0.22, respectively (Table-5).

Accuracy: The accuracy was well-established by studying the recovery experiments as per the International Conference on Harmonization (ICH) guideline. The spiked samples were analyzed and compared with the result of pre-analyze for sample solutions with pure drug at three concentration levels in triplicate. The average recovery for empagliflozin and linagliptin at 3 different concentrations was estimated. Mean recovery of empagliflozin was ranged from 100.96 to 101.48%, while for linagliptin ranged from 100.09 to 101.13% at every level, which was within the specified range of 98.0 to 102.0% (Table-6).

Ruggedness: The ruggedness of method for empagliflozin and linagliptin was evaluated using the six replicate injections of empagliflozin ((150 µg/mL) and linagliptin (75 µg/mL) using different column. The results indicate the method is rugged the intentional changes does not influence the performance of the method the RSD was less than 2% in both cases (Table-7).

Stress studies: Stress studies for stability of the drug were achieved as in the ICH-guidelines Q1A (R2) on stability testing of new drug-substances API and finish product. The analysis was performed as per the ICH guideline and the results are given in Table-8.

Acid degradation studies: The samples of empagliflozin and linagliptin (1 mL each of stock solution) and 1 mL of 2 N HCl were mixed and refluxed for 30 min at 60 ºC. After the hydrolysis the samples were diluted and analyzed using HPLC. The result shows that under acidic exposure the empagliflozin

TABLE-5
INTERMEDIATE PRECISION FOR EMPAGLIFLOZIN AND LINAGLIPTIN

S. No.	Area of empagliflozin (100 µg/mL)				Area of linagliptin (50 µg/mL)			
	Day-1	Day-2	Day-3	Mean	Day-1	Day-2	Day-3	Mean
1	1919332	1925927	1913079	1919446	876871	881647	875509	878009
2	1927659	1939848	1927659	1931722	876313	871857	876418	874863
3	1923605	1928785	1923605	1925332	877356	879224	876938	877839
4	1923801	1923727	1923801	1923776	878790	881018	877241	879016
5	1919096	1913246	1911305	1914549	875829	889705	876136	880557
6	1930615	1929975	1929104	1929898	878666	882459	878210	879778
Mean	1924018	1926918	1921426	1924121	877304	880985	876742	878344
SD	4543	8696	7487	6422.03	1217	5745.8	942	1994.58
%RSD	0.24	0.45	0.39	0.33376	0.1	0.7	0.1	0.22709

TABLE-6
ACCURACY OF HPLC METHOD FOR THE ANALYSIS OF EMPAGLIFLOZIN AND LINAGLIPTIN

Empagliflozin				Linagliptin			
Conc. (µg/mL)	Calculated conc. (µg/mL)	Recovery (%)	SD (%RSD)	Conc. (µg/mL)	Calculated conc. (µg/mL)	Recovery (%)	SD (%RSD)
50	50.4628	100.93		25	24.9183	99.67	
50	51.0382	102.08	0.57 (-0.56)	25	25.4259	101.7	1.19 (1.18)
50	50.7235	101.45		25	24.9008	99.6	
100	101.595	101.6		50	49.9172	99.83	
100	101.389	101.39	0.93 (-0.92)	50	50.0332	100.07	0.27 (-0.27)
100	99.8865	99.89		50	50.1881	100.38	
150	153.919	102.61		75	75.7185	100.96	
150	150.864	100.58	1.05 (-1.03)	75	76.6979	102.26	1.05 (-1.04)
150	151.69	101.13		75	75.1369	100.18	

TABLE-8
STRESS STUDIES RESULTS FOR EMPAGLIFLOZIN AND LINAGLIPTIN UNDER DIFFERENT CONDITIONS

Stress conditions	Empagliflozin				Linagliptin			
	Assay (%)	Degradation (%)	Purity angle	Purity threshold	Assay (%)	Degradation (%)	Purity angle	Purity threshold
Acid degradation (30 min)	97.07	2.93	0.95	1.20	96.89	3.11	0.41	0.54
Base degradation (30 min)	98.20	1.80	0.50	0.80	98.02	1.98	0.39	0.46
Peroxide degradation (30 min)	96.50	3.50	0.77	0.90	95.14	4.86	0.03	0.48
UV degradation (7 days)	99.73	0.27	0.14	0.30	99.36	0.64	0.44	0.62
Thermal degradation (24 h)	99.72	0.28	0.16	0.30	99.23	0.77	0.45	0.54

TABLE-7
RESULTS OF RUGGEDNESS STUDIES

S. No.	Empagliflozin (150 µg/mL)		Linagliptin (75 µg/mL)	
	Column 1	Column 2	Column 1	Column 2
1	148.06	148.12	74.18	74.14
2	149.06	148.2	74.21	74.01
3	148.34	148.09	74.14	74.04
4	148.54	148.22	74.02	74.02
5	148.15	148.11	74.15	74.09
6	148.55	148.24	74.04	74.11
Mean	148.45	148.1633	74.1233	74.0683
± SD	0.359	0.064083	0.07659	0.05269
RSD	0.24183	0.043252	0.10333	0.07114
Accuracy (%)	98.9667	98.77556	98.8311	98.7578

and linagliptin were degraded and the recovery of empagliflozin and linagliptin were 97.07 and 96.89%, respectively. HPLC chromatograph indicate the presence of degraded product which are separated by the present chromatographic technique (Fig. 3).

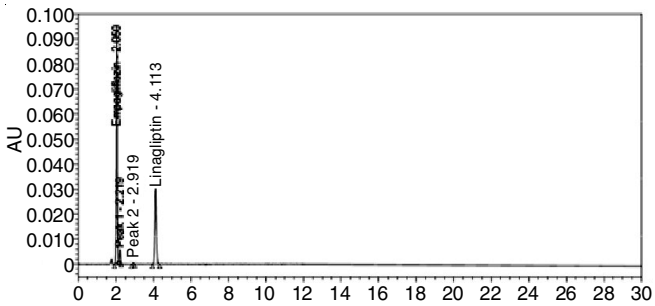


Fig. 3. Chromatograph of analytes showing separation of empagliflozin and linagliptin and its degraded products under acidic stress

Studies of alkali (basic) degradation: The samples of empagliflozin and linagliptin (1 mL each of stock solution) and 1 mL of 2 N NaOH were mixed and refluxed for 30 min at 60 ºC. After the hydrolysis the samples were suitably diluted and analyzed using HPLC. The percentage of empagliflozin and linagliptin remained were 98.2 and 98.02%, respectively. The degraded products are separated efficiently (Fig. 4).

Oxidative degradation: In case of oxidative degradation studies, the samples of empagliflozin and linagliptin (1 mL each) were mixed with H_2O_2 (1%) and refluxed for 30 min. The samples were diluted and analyzed. The percentage of empagliflozin and linagliptin remained were 96.5 and 95.14%, respectively, which showed that the samples are more susceptible to oxidative stress (Fig. 5).

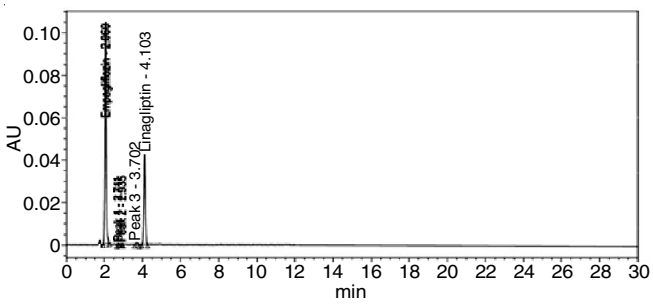


Fig. 4. Chromatograph of analytes showing separation of empagliflozin and linagliptin and its degraded products under alkaline stress

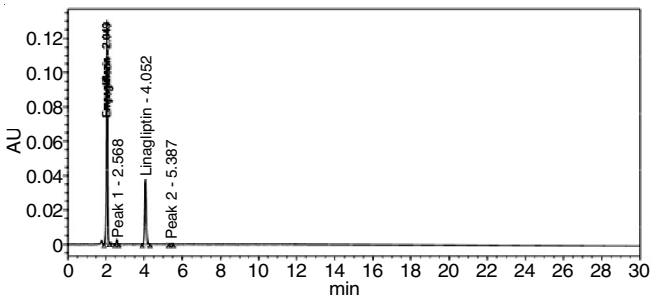


Fig. 5. Chromatograph of analytes showing separation of empagliflozin and linagliptin and its degraded products under oxidative stress

Photostability studies: For photolytic stability, the drug solutions were exposed to ultraviolet radiation for 7 days (200 W/h/m^2) in stability chamber. After 7 days the samples were collected and analyzed. The percentage of empagliflozin and linagliptin remained were found to be 99.73 and 98.36%, respectively (Fig. 6).

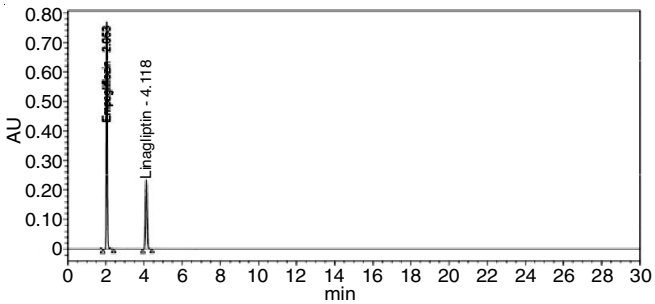


Fig. 6. Chromatograph of analytes showing separation of empagliflozin and linagliptin and its degraded products under photolytic stress condition

Thermal degradation studies: For thermal studies, the samples were stored in sealed tubes with Teflon septum. Samples were exposed to 105 ºC for 24 h. After exposure, the samples were cooled suitability diluted and analyzed. The results indicate

that the percentage of empagliflozin and linagliptin remained were 99.72 and 99.23%, respectively. The degradation of these drugs under thermal exposure was lesser than other stressed conditions (Fig. 7).

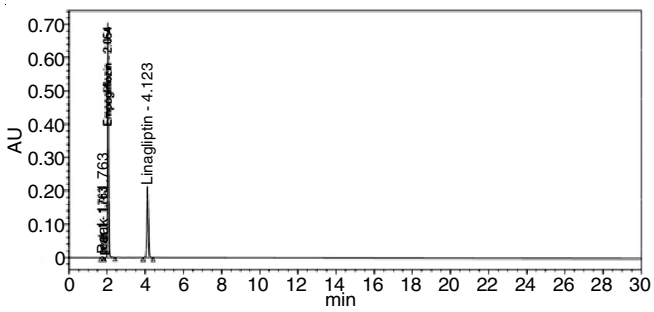


Fig. 7. Chromatograph of analytes showing separation of empagliflozin and linagliptin and its degraded products under thermal stress condition

Empagliflozin and linagliptin produces degradation products in acidic, alkaline, oxidative, thermal and photochemical stress. As per ICH-International guidelines pointers, peak purity angle ought to be but peak purity threshold. The result of assay of empagliflozin and linagliptin in tablets shows that the degradation product does not interfere with the analytical procedure quantitively when these drugs are analyzed. Thus, the planned analytical methodology is additionally helpful for the determination of empagliflozin and linagliptin in sample. The present method is stability indicating and able to separate the degraded product effectively and can be applied for the analysis of these drugs in pharmaceutical quality control.

Conclusion

A simple, precise, accurate, stability indicating and efficient HPLC method was developed and validated as per ICH guideline for the routine analysis of empagliflozin and linagliptin. The present method is capable of analysis the empagliflozin and linagliptin with high accuracy and precision. Since it is stability indicating methods it can be applied for the routine quality control of these drugs in API, formulation and dissolution studies.

ACKNOWLEDGEMENTS

The authors thank to the Faculty of Pharmacy and Medical Sciences at the University of Petra, Amman, Jordan for encouraging scientific research and support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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