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Research Article

Simultaneous Estimation of Gliflozin Derivatives Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin Using RP-HPLC Methods

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Abstract

A reliable, simple, precise, stability indicating reverse phase HPLC method was developed to study simultaneous estimation of Gliflozin derivatives like Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin. Focus of our study is to quantify the derivatives with freely available non expensive solvent systems within a short period of time. The chromatographic separation obtained by using Inertsil ODS column (25 cm x 46 mm) x 5 µm internal diameter with isocratic flow. The mobile phase TEA: ACN pH (50:50) at a flow rate 1 ml/min. The analytes were detected by using a UV detector at 260 nm. Forced degradation studies for gliflozin derivatives conducted as per the ICH guidelines and the resulting degrades were characterized by its peak area at its retention time as per the proposed method. The system suitability results support the proposed method elutes the analytes with good resolution within 10 min in a repeatable manner. The linearity value observed with the concentration range of 30-450 µg/mL for Canagliflozin, 1-15 µg/mL for Dapagliflozin, 2.5 to 37.50 µg/mL for Empagliflozin and 0.5 to 7.5 µg/mL for Ertugliflozin and its calibration curve value found to be y = 25151x + 40130 (r^2 = 0.9991), y = 109655x +8166 (r^2 = 0.9989), y = 73755x + 636 (r^2 = 0.9998), y = 169230x + 8269 (r^2 = 0.9997) respectively. The percentage recovery of the drugs found to be 100.2-100.4, 99.7-100.6, 99.5-99.7, 99.6-100.03 for 50%, 100% and 150% and these results are within acceptable limits. In this developed method the separated analyte peak found to be sharp, specific, reproducible, and robust in all the studies conducted in repeatable process. Forced degradation studies involved in studying the drug stability in different stress conditions and to identify the degradants of the drug products. The validated method found precise in forced degradation studies conditions and found less than 10% degradation products in this study. The developed and validated method proved that the proposed method was simple, reliable, highly specific and stability indicating for gliflozin derivatives based on its statistical data obtained with its peak area and relevant factors. Hence, this method might be applied for simultaneous estimation of all these drugs in pharmaceutical formulation.

Keywords: Canagliflozin; SGLT2; T2DM; Dapagliflozin; Empagliflozin; Ertugliflozin; RP HPLC; Forced Degradation

Introduction

Near to 6.28% number of the population suffered from Type 2 Diabetic mellitus as per the 2017 report which must be considered as a life threatening and cause remarkable changes in social and health care systems of human race. Treating patients suffering with Type 2 diabetes mellitus need drug therapy with improved changes in lifestyle pattern like proper diet, exercise, and prescribed lifestyle [1].

In T2DM drug therapy treating patients with SGLT found to be recurrent and positive in the patients and volunteers participated

in clinical trials. Gliflozin derivatives reduce the blood glucose level in patients by reducing glucose reabsorption by kidney and improve urinary excretion of glucose for the suffering with Type 2 diabetic mellitus. In diabetic type 2 blood glucose level was found more and it is due to 90% absorption of glucose by SGLT, inhibiting them by gliflozin reduces the level which is highly recommended therapy nowadays [2-4].

Canagliflozin ($C_{24}H_{25}FO_5S$) is chemically named as (2S,3R,4R,5S,6R) -2- [3- [[5-(4-fluoro phenyl) thiophen-2-yl] methyl]-4-methylphenyl]-6-(hydroxymethyl) oxane-3,4,5-triol; hydrate with physical appearance of white to off white solid with melting range of 95-105°C [5]. Canagliflozin is a novel oral anti-diabetic agent and the first SGLT2 inhibitor approved for glycemic control in adults with T2DM. U.S. Food and Drug Administration (US FDA) approved this drug in March-2013 for treating the patients having type-II diabetes [9,10].

Dapagliflozin ($C_{21}H_{25}ClO_6$) is chemically named as (1S)-1, 5-anhydro- 1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol, physical appearance of it is a white to off white solid with melting range of 74-78 °C. The molecular weight is 408.873 g/mol [6]. Dapagliflozin is a C-glycosyl comprising beta-D-glucose in which the anomeric hydroxyl group is replaced by a 4-chloro-3-(4-ethoxybenzyl) phenyl group to improve glycemic control in adults with type 2 diabetes along with improved lifestyle. Adults with proper diet and exercise showed improved glycemic control during treatment [11,12].

Empagliflozin ($C_{23}H_{27}ClO_7$) gliflozin derivative, chemically called as (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl] oxy phenyl] methyl] phenyl]-6-(hydroxy methyl) oxane-3, 4, 5-triol [7]. Empagliflozin by its independent hypoglycemic mechanism protects the diabetic patients suffering with cardiac and kidney disorder [13,14]. Empagliflozin with combination therapy produces positive results among T2DM patients.

Ertugliflozin [$C_{22}H_{25}ClO_7$] chemically called as (1S,2S,3S,4R,5S)-5-(4-chloro-3-(4-ethoxybenzyl) phenyl)-1-(hydroxymethyl)-6,8-dioxabicyclo [3.2.1] octane-2,3,4-triol [8]. Ertugliflozin and fixed-dose combinations of Ertugliflozin and Metformin, Ertugliflozin and sitagliptin have recently been approved by the US FDA as an adjunct to diet and exercise to improve glycemic control in adults with T2DM [15-17] The structure of Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin was shown in figure 1.

Various analytical reports submitted for gliflozin derivatives with combination of more than 2 drugs or gliflozin derivatives with Metformin using RP-HPLC methods. Current study focused on identifying the relationship between weakly ionizable gliflozin drugs and its separation properties in buffer pH 3-6 [17-21].

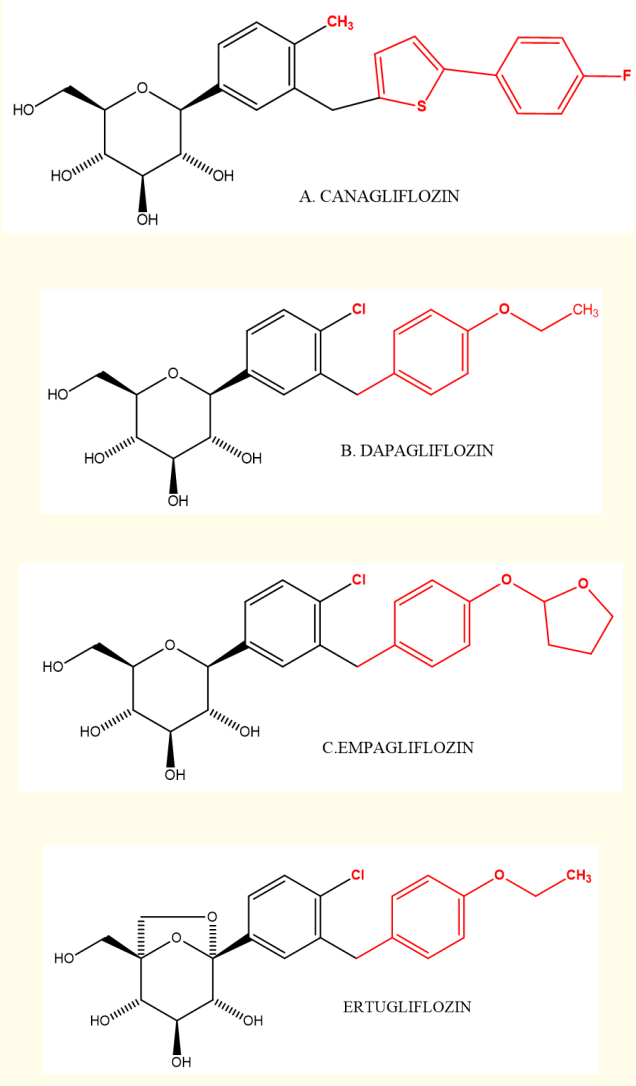

Figure 1: Chemical Structure of A. Canagliflozin, B. Dapagliflozin, C. Empagliflozin and D. Ertugliflozin.

Methods and Materials

Instrumentation

Waters HPLC system consisted of a Quaternary pump, Rheodyne injector with PDA detector used for our developed method.

Agilent HPLC system made up of Quaternary pump, Auto injector with G1315B detector used for Intermediate precision study. Empower 2.0 software, collected, and compiled the chromatographic data obtained for Gliflozin derivatives in both the HPLC systems. Zorbax C18 and Inertsil ODS column is used for separation through isocratic elution.

Chemical and reagent

For this proposed method HPLC grade Acetonitrile, Triethylamine and HPLC grade distilled water procured from Loba Chemie Pvt Ltd, India. Working standards of Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin were purchased from Supriya Life science Ltd, India.

Chromatographic parameters

Equipment: Waters, Alliance Model 2695

Wavelength: 232 nm

Injection volume : 10 µL.

Flow rate: 01 mL/minute.

Run time: 6 Minutes.

Column: ZORBAX C_{18} (250 x 4.6 mm, 5µm particle size)

Mobile Phase: Acetate buffer (pH 3.4): Acetonitrile (60:40)

Oven Temperature: 28°C.

Preparation of mobile phase

Mix Triethylamine with Acetonitrile in the ratio of 60:40 and adjust the pH to 2.5 by using 0.1% Ortho phosphoric acid. Degas the mobile phase before use and sonicate the mobile reservoir for 20 minutes before injection.

Preparation of standard solution

Accurately weighed 300 mg of Canagliflozin, 10 mg of Dapagliflozin, 25 mg of Empagliflozin and 5 mg of Ertugliflozin working standards are taken into 100 mL volumetric flask, add 70 mL of Mobile phase, sonicated for 10 min to dissolve the contents, and made up to the mark with mobile phase. Further dilute 5 mL of above solution to 50 mL volumetric flask with diluent.

Preparation of sample solution

20 Tablets of Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin were crushed into powder form. Accurately weighed tablet equivalent of 372 mg of Canagliflozin sample (each tablet contains 300 mg of Canagliflozin), 39 mg of Dapagliflozin sample (each tablet contains 10 mg of Dapagliflozin), 64 mg of Empagliflozin sample (each tablet contains 25 mg of Empagliflozin) and 58 mg of Ertugliflozin sample (each tablet contains 5 mg of Ertugliflozin) were transferred into 100 ml volumetric flask, added 70 ml of mobile phase, sonicated it for 30min to dissolve the contents. Finally make the volume up to mark using mobile phase as diluent. Further dilute 5 mL of above solution to 50 mL volumetric flask with diluent.

Validation

Validation performed as per the guidelines provided by ICH guidelines. Validation parameters performed for the proposed methods were linearity, precision, accuracy, robustness and forced degradation study [22,23].

Linearity

Linear test reading was conducted by preparing standard solution of different concentrations range 30-450 µg/mL for Canagliflozin, 1-15 µg/mL for Dapagliflozin, 2.5 to 37.50 µg/mL for Empagliflozin and 0.5 to 7.5 µg/mL for Ertugliflozin by transferring 0.5 to 7.5 mL of stock solution into 50 mL volumetric flask. Make the volume with mobile phase and inject 10µL triplicate solutions of different concentrations into the HPLC system.

Precision

Intermediate and Method Precision was conducted as per the procedure and ICH guidelines. In Intermediate and Method precision, the sample of same concentration of different analyte like 300 µg of Canagliflozin, 10 µg of Dapagliflozin, 25 µg of Empagliflozin and 5 µg of Ertugliflozin were injected into 2 different systems at 6 different time intervals within a day and on alternate days.

Accuracy

The percentage recovery study was performed by injecting different concentrations of Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin with 50%, 100% and 150%. The percentage of recovery of gliflozin derivatives was estimated by considering peak area of spiked concentration versus peak area of standard concentration.

Robustness

Deliberate changes or deliberate variation of the proposed method was conducted by introducing minor changes in various

chromatographic parameters such as flow rate, mobile phase composition, detection wavelength and column temperature.

System suitability

System suitability for the proposed method is evaluated by injecting the working standard of Gliflozin derivatives into the system with the same concentration six times and analyzing its peak area.

Forced degradation studies

Acid degradation

Accurately weighed 372 mg of Canagliflozin, 39 mg of Dapagliflozin, 64 mg of Empagliflozin and 58 mg of Ertugliflozin sample and transferred it into a 100 ml volumetric flask. Added 70 mL of diluents sonicated for 15 min to dissolve the contents, diluted to volume with using diluent. To 1 mL of above stock solution added 1 mL of 0.1N HCl. Sonicate it for 15min and then add 1 mL of 0.1N NaOH to neutralize the solution. Same procedures followed for 1N by diluting it with 0.1N HCl utilized 01 N HCl.

Alkali degradation

Accurately weighed 372 mg of Canagliflozin, 39 mg of Dapagliflozin, 64 mg of Empagliflozin and 58 mg of Ertugliflozin sample and transferred into a 100 ml volumetric flask. Added 70 mL of diluents sonicated for 15 min to dissolve the contents, diluted to volume with diluent. To 1 mL of above stock solution added 1mL of 0.1N NaOH. Sonicate it for 15min and then add 1mL of 0.1N HCl to neutralize the solution. Same procedures followed for 1N by diluting it with 0.1N NaOH utilized 01 N NaOH.

Peroxide degradation

Accurately weighed 372 mg of Canagliflozin, 39 mg of Dapagliflozin, 64 mg of Empagliflozin and 58 mg of Ertugliflozin sample and transferred it into a 100 mL volumetric flask. Added 70 mL of diluents sonicated for 15 min to dissolve the contents, diluted to volume with diluent. To 1 mL of above stock solution add 1mL of 10% H_20_2 and diluted to volume with diluent and mixed. Same procedures followed for 1N by diluting it with 30% H_2O_2 instead of 10% H_2O_2.

Reduction degradation

Accurately weighed 372 mg of Canagliflozin, 39 mg of Dapagliflozin, 64 mg of Empagliflozin and 58 mg of Ertugliflozin sample and transferred into a 100 mL volumetric flask. Added 70 mL of diluents sonicated for 15 min to dissolve the contents, diluted to volume with diluent. To 1 mL of above stock solution added 1ml of 10% sodium bi sulphate and diluted to volume with diluent and mixed. Same procedures followed for 1N by diluting it with 30% $NaHSO_4$ instead of 10% $NaHSO_4$.

Thermal degradation

Weighed sample of 500 mg of Canagliflozin, 100 mg of Dapagliflozin, 100 mg of Empagliflozin and 100 mg of Ertugliflozin was exposed at 105 °C for 3 h and the exposed sample was analyzed. Same procedures followed for 1N by exposing it for 6 h instead of 3 h.

Photolytic degradation

Weighed sample of 500 mg of Canagliflozin, 100 mg of Dapagliflozin, 100 mg of Empagliflozin and 100 mg of Ertugliflozin sample was exposed to sunlight for 3 hours. and the exposed sample was analyzed. Same procedures followed for 1N by exposing it for 6h instead of 3h.

Hydrolysis degradation

Accurately weighed 372 mg of Canagliflozin, 39 mg of Dapagliflozin, 64 mg of Empagliflozin and 58 mg of Ertugliflozin sample and transferred into a 100 ml volumetric flask. Added 70 ml of diluents sonicated for 15 min to dissolve the contents, diluted to volume with diluent. After dissolving the content take 1 mL of above stock solution and then add 5 ml of water and diluted to volume with diluent and mixed for properly dissolving the drug. Same procedures followed for 1N by diluting it with 10 mL water instead of 5 mL water.

Results and Discussion

Selection of mobile phase

For this study we utilize different mobile phases with different mobile phase composition. Gliflozin derivatives are found to be weakly ionizable in nature so that we reduce that mobile phase selection by selecting buffer samples solution with pH 2 to pH 5. Buffer samples with pH 2.5 to 3.5 found most promising in eluting the drug with good resolution and peak area and for this study we utilize Triethylamine buffer solution with acetonitrile in ratio of 50:50.

Selection of wavelength

Identification of analytical wavelength for Gliflozin derivatives involved in identifying the wavelength where we will get a good amount of absorption. Trial run conducted at wavelength near to 200 to 400 nm to identify the λmax for gliflozin derivatives. After different trials we found that 260 nm is the λmax for simultaneous estimation of gliflozin derivative.

Selection of column and column temperature

The column with different nature includes C18, C8 with different size 25 cm, 15 cm were studied for this developed method. Column size and its effective column temperature are involved in separating the gliflozin derivatives, with good amount of resolution and even in minimal concentration range of drugs. Column with 25 cm size, internal diameter 5 µm found to separate the analytes with good resolution at ambient temperature.

The proposed method Elute the analytes with good percentage of peak area resolution for various analytical parameters at ideal chromatographic conditions ZORBAX C_{18} (250 x 4.6 mm, 5 µm particle size) ambient temperature, buffer pH adjusted to 2.5 with 0.1% OPA: Acetonitrile 50:50 ratio and the flow rate 1 mL/min conducted as per ICH guidelines.

Method validation

Linearity

The calibration curve found to be linear (Figure 2 and Figure 3) for Canagliflozin Dapagliflozin, Empagliflozin and Ertugliflozin with concentration range of 30-450 µg/mL for Canagliflozin, 1-15 µg/mL for Dapagliflozin, 2.5 to 37.50 µg/mL for Empagliflozin and 0.5 to 7.5 µg/mL for Ertugliflozin. The correlation coefficient of regression value, concentration and intercept value was calculated using the formula y = 25151x+40131 (r^2 = 0.9991), y = 109655x+8166 (r^2 = 0.9989), y = 73756x+6364 (r^2 = 0.9998) and y = 169230x+8269 (r^2 = 0.9997) for Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin respectively and summarized in table 1.

Mean peak area of three replicates

Accuracy

The % Mean recovery for Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin was found to be 100.2-100.4, 99.7-100.6, 99.5-99.7, 99.6-100.03 for 50%, 100% and 150% and these results

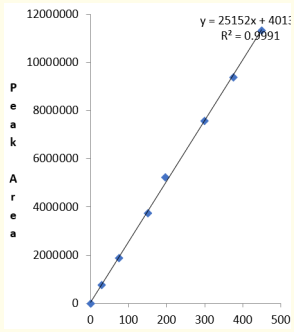

Figure 2: Calibration Curve of Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin.

Figure 3: Linearity chromatogram peaks of Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin (L to R).

are within acceptable limits. The % RSD for Gliflozin derivatives were found within limit of ≤ 2 and its high value of recoveries at 50%, 100% and 150% concentrations indicate the performed method is accurate. The accuracy data of the proposed method is summarized in table 2A.

S. No	*Mean Peak area			
	Canagliflozin	Dapagliflozin	Empagliflozin	Ertugliflozin
1	0	0	0	0
2	768241	107356	199101	87738
3	1861715	277356	454301	229337
4	3763987	562500	929653	432045
5	5237973	841265	1395299	650437
6	7584341	1146456	1878191	852224
7	9387487	1371404	2288539	1075632
8	11332103	1625525	2770639	1265632
Corr Coeff value	0.999573127	0.999430855	0.999887919	0.999841104
Slope	25152	109655	73756	169230
Intercept	40131	8166	6364	8268

Table 1: Linearity Results of Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin.

Spiked Conc (µg/ml)	Canagliflozin	Dapagliflozin	Empagliflozin	Ertugliflozin
	mean peak area ± SD (% RSD)	mean peak area ± SD (% RSD)	mean peak area ± SD (% RSD)	mean peak area ± SD (% RSD)
50 %	3790076 ± 15551 (0.41)	559896 ± 2216 (0.40)	923267 ± 14831 (1.61)	428419 ± 2238 (0.52)
100 %	7593835 ± 10633 (0.14)	1123366 ± 11069 (0.99)	1843084 ± 14566 (0.79)	853104 ± 1693 (0.20)
150 %	11380904 ± 73561 (0.65)	1669808 ± 21293 (1.28)	2767665 ± 19470 (0.70)	1276287 ± 11342 (0.89)

Table 2A: Recovery studies values of Empagliflozin.

Mean of three replicates

Precision

Intermediate Precision and Method precision of gliflozin derivatives such as Canagliflozin Dapagliflozin, Empagliflozin and Ertugliflozin was calculated by injecting 300, 10, 25 and 5 µg/mL samples of triplicate solution into HPLC system respectively, the obtained results were found to be more precise. The % RSD of both the methods was found 0.16 to 0.91 indicates that the method was precise and reproducible.

S. No	Canagliflozin		Dapagliflozin		Empagliflozin		Ertugliflozin	
	Inter	Method	Inter	Method	Inter	Method	Inter	Method
1	7591234	7559274	1113125	1101286	1854786	1855679	850430	852319
2	7571328	7599553	1115871	1120871	1872367	1872432	855320	853247
3	7541384	7544189	1127154	1112783	1841542	1833387	853461	851054

4	7523784	7585510	1116245	1106874	1835143	1827485	852312	855138
5	7583128	7574115	1116871	1117136	1846357	1842066	855761	852055
6	7513354	7583067	1127652	1121784	1882687	1861679	853124	851359
Mean	7554035	7574285	1119486	1113456	1855480	1848788	853401	852529
SD	29607	18132	5721	7414	16916	15858	1796	1362
%RSD	0.39	0.24	0.51	0.67	0.91	0.86	0.21	0.16

Table 2 B: Intermediate and Method Precision studies of Empagliflozin.

Mean of three replicates

Robustness

Deliberate changes in validation parameters didn't alter the robustness of the developed method. Robustness value for different parameters like Flow rate, Temperature and Mobile phase were summarized in table 3. The % RSD was found to be less than 02% for Empagliflozin and no significant changes in the entire procedure which indicates the method is Robust.

S. No	Parameters	Condition	Mean peak area ± SD (%RSD)			
			Canagliflozin	Dapagliflozin	Empagliflozin	Ertugliflozin
1	Flow rate Minus	(0.8 ml/min)	7255060 ± 10163 (0.14)	10555363 ± 12434 (1.18)	1646629 ± 18567 (1.13)	802784 ± 1370 (0.17)
2	Flow rate Plus	(1.2 ml/min)	7756051 ± 22941 (0.3)	1749673 ± 15198 (0.87)	1968917 ± 26790 (1.36)	896526 ± 2001 (0.22)
3	Mobile phase Minus	(48:52)	7372226 ± 19610 (0.27)	949916 ± 8471 (0.89)	1562673 ± 13471 (0.86)	845500 ± 1781(0.21)
4	Mobile phase Plus	(52:48)	7955559 ± 44866 (0.56)	1562110 ± 15315 (0.98)	2169657 ± 16777 (0.77)	875669 ± 2161 (0.25)

Table 3: Result of Robustness Method.

Mean of three replicates

Limit of detection and limit of quantification

The limit of Detection (LOD) and Limit of quantification (LOQ) for gliflozin derivatives was found to be 10.2 µg/mL and 30 µg/mL for Canagliflozin, 0.3 µg/mL and 1µg/mL for Dapagliflozin, 0.85 µg/mL and 2.5 µg/mL for Empagliflozin and 0.17 µg/mL and 0.50 µg/mL for Ertugliflozin respectively. The very lowest value obtained by this method indicates the developed method was more precise and reproducible.

Analysis of marketed formulation

The percentage purity of the six replicate samples found to be within the limit and the amount recovered for the assay method found to be 297.9 mg, 10.13 mg, 25.02 mg, and 5.04 mg for Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin respectively and the % purity was reported between 99.3 - 101.3. Reported % purity of gliflozin derivatives found within the limit as per pharmacopeia.

Drug	Labeled claim (mg)	Amount found * (mg)	Recovery * (%)
Canagliflozin	300	297.9	99.3
Dapagliflozin	10	10.13	101.3
Empagliflozin	25	25.02	100.1
Ertugliflozin	5	5.04	100.8

Table 4: Assay estimation of Empagliflozin.

Mean of three replicates

Stress degradation studies

Stress degradation studies conducted as per the ICH guidelines and the drug few gliflozin derivatives doesn't degrade in acidic, thermal, and photolytic method whereas the some of it degrades in presence of basic and oxidative hydrolysis process (Figure 4). Amount of degraded drug products recovered in acidic, basic, oxidative, Thermal and Photolytic conditions were found less than 8% in 0.1N conditions and less than 20% for 1N stress conditions summarized and reported in table 5.

Figure 4: Chromatogram peaks of Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin (L to R) in Forced degradation Studies A-Blank, B-Control, C-Acidic, D-Alkaline, E-Peroxide, F-Reduction, G-Thermal degradation, H-Photolytic and I-Hydrolysis degradation.

Degradation Type	Degradation condition	Drug recovered (%)			
		Canagliflozin	Dapagliflozin	Empagliflozin	Ertugliflozin
Acid Degradation	0.1N HCl, 15 min., 25°C	98.2	98.7	98	97.8
	01N HCl, 15 min., 25°C	85.6	87.5	83.9	86.4
Alkaline Degradation	0.1N NaOH, 15 min., 25°C	97.8	97.9	96.9	98.5
	01N NaOH, 15 min., 25°C	84.8	88.4	86.1	85.2
Oxidative Degradation	10% H_2O_2, 15 min., 25°C	96.5	97.4	95.2	97.4
	30% H_2O_2, 15 min., 25°C	87	89.3	88.9	83.9
Hydrolysis Degradation	5 ml H_2O, 5 min., 25°C	99.9	99.6	99.5	99.3
	10 ml H_2O, 5 min., 25°C	98.9	98	99.1	97.4
Reduction Degradation	10% $NaHSO_4$, 15 min., 25°C	97.2	95.4	96.6	96.1
	30% $NaHSO_4$, 15 min., 25°C	83.7	85.8	88	81.5
Thermal Degradation	105°C for 3 hrs	99.2	97.2	97.1	98.4
	105°C for 6 hrs	99.3	97.6	97.2	98.4
Photolytic degradation	3 hrs	99.2	97.2	97.1	98.4
	6 hrs	88.8	84.7	89.7	87.8

Table 5: Data of Stress degradation studies Empagliflozin.

Conclusion

Our research work on gliflozin derivatives found promising in all parameters in eluting the analyte in very less time and low organic solvent consumptions. Individual methods were developed and reported for Canagliflozin, Dapagliflozin, Empagliflozin and Ertugliflozin [24]. Intraday precision and Interday precision % RSD values indicates the designed method has less than 2% RSD value without any significant variable changes. In Linearity regression method the peak area increases with the increasing concentration of sample and found linear towards the drug concentration. Our

method can perform estimation of gliflozin derivatives in different laboratory conditions and different analytical conditions. Degradation studies practically proved that weakly ionizable flozin derivatives of few drugs can alter its nature in stress conditions such as basic and oxidative conditions whereas remain stable in those conditions too. Altering forced degradation studies may be useful in identifying the various degraded products and can be analyzed further by using the LCMS method. This simplified method consumes less organic solvent and at the same time it elutes the analyte within a short period of time.

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Conflict of Interest

Author declares No conflict of interest for this research work.

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