Research Article

Assessment of organic impurities in solid oral product of SGLT-2 inhibitors category (empagliflozin) by HPLC.

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Abstract

A simple and robust HPLC method for the determination &estimation of related substances of SGLT-2 Inhibitors Drug (Empagliflozin) Active pharmaceutical ingredient as well as in Marketed formulation. The chromatographic resolution achieved with different percentages of mobile phase by using ACE 5 C18 (octadecyl silane, 25 cm×4.6mm, 5.0µm column) with mobile phase composition of solvent A (phosphate buffer & methanecarbonitrile with ratio of 92:8 %) and solvent B (phosphate buffer and ACN with ratio of 10:90 %) at a flow rate of 0.8 and Column oven temperature 30°C. The main components and its related substances eluted were estimated at 220 nm. The specificity investigated under different forced degradation conditions, mainly hydrolytic, oxidative, photolytic and thermal as recommended by ICH guidelines. The method was developed and quantified in a manner indicating the stability and the sensitivity of the drug substance.

Keywords: RP- HPLC, Diabetes-II, Empagliflozin, Related substances, Method validation.

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Introduction

Empagliflozin IUPAC name is (2S,3R,4R,5S,6R)-2-[4-Chloro-3-[[4-[(3S)-oxolan-3yl]oxyphenyl] methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol.

Empagliflozin is a white to yellowish non hygroscopic crystalline solid with a weight of 450.91 amu. It is slightly soluble in methanol.

It's having chemical formula of C23H27ClO7. Empagliflozin is an inhibitor of sodium-glucose co-transporter-2 (SGLT2), the transporters primarily responsible for the reabsorption of glucose in the kidney.

It is used clinically as an adjunct to diet and exercise, often in combination with other drug therapies, for the management of type 2 diabetes mellitus.

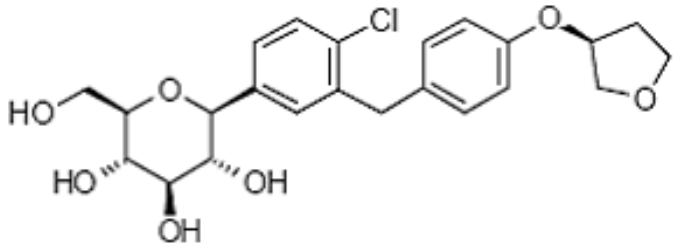


Figure 1. *Chemical structure of Empagliflozin.*

By literature look over it came to know that there is no single related substances analytical method for quantification of Empagliflozin and its organic impurities in product of Empagliflozin. When we searched in pharmacopoeias or any research articles.

Hence, it is necessary to develop a new chromatographic analytical method which is having capability of accuracy, specificity and stability indicating for quantification of Empagliflozin and its organic impurities in product of Empagliflozin by HPLC.

This research article explains about method validation of brief description of Organic impurities method for four potential Organic impurities in solid oral product of Empagliflozin as per Regulatory Guidelines.

To prove our method is stability indicating we need to do mainly forced degradation study as per regulatory guidelines.

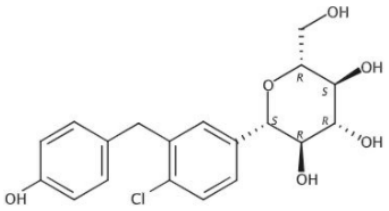
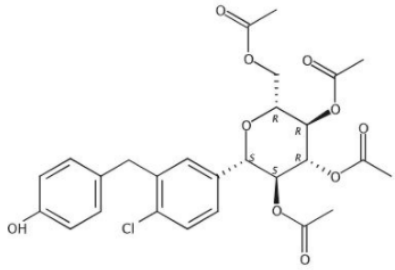
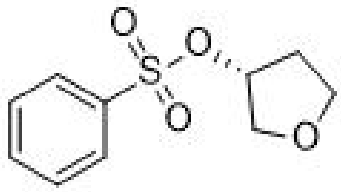
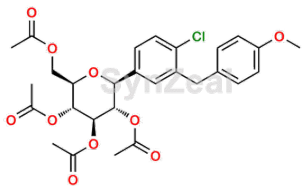
Experimental

Equipment and chemicals

Drug Standard of pure Empagliflozin active pharmaceutical ingredient (API) Sample and Organic impurities has been obtained as gift samples received from Metrochem API Pvt Ltd.

Sodium dihydrogen phosphate, disodium hydrogen phosphate, ortho phosphoric acid and methanecarbonitrile were procured from finer chemicals. The possible Organic impurities that may raise from the Empagliflozin are mentioned below

Table 1. *Possible organic impurities.*

S. No	Organic impurities name	Structure
1	Hydroxy Impurity	
2	Phenolic Impurity	
3	Sulphonic Impurity	
4	Acetylated Impurity	

The HPLC used for method validation was Waters-HPLC, having PDA detector and the stationary phase is ACE 5 C_{18} (octadecyl silane, 25 cm×4.6mm, 5.0µm column). The signal outcome were integrated by empower 3 software.

Chromatographic conditions

ACE 5 C_{18} (octadecyl silane, 25 cm×4.6mm, 5.0µm column) was used as a stationary phase maintained at oven of 30°C. The mobile phase elution having different composition of solvent A solution containing phosphate buffer and methanecarbonitrile in the ratio of 92:8 v/v and solvent B phosphate buffer and methanecarbonitrile in the ratio of 10:90

v/v delivered at a flow rate of 0.8 mL/min. The wavelength finalized is 220 nm which gives high response for all Organic impurities in order to permit concurrent estimation of Organic impurities of Empagliflozin.

The forced degradation samples injected in a system having a PDA detector. Diluentt finalisedwas 70:30 V/V (WATER-ACN) for the dilutions of the drug substances and for their Organic impurities.

Table 2. *Individual organic impurities retention times.*

S. No	Organic impurities name	Retention Times
1	Hydroxyl Impurity	19.3
2	Sulphonic Impurity	24.8
3	Empagliflozin	32.4
4	Phenolic Impurity	52.8
5	Acetylated Impurity	61.5

Table 3. *Gradient composition.*

Duration(min)	Pump A (%)	Pump B (%)
0	85	15
30	64	36
50	22	88
65	22	88
67	85	15
80	85	15

Formation of reference solution: Reference solution of Empagliflozin made with the concentration of 2 ppm by using diluent. To get above 2 ppm we made some series of dilutions.

Formation of sample solution: Take 5 tablets of 10 mg Emapgliflozin in 100ml volumetric flask to give concentration of 500 ppm as sample solution.

Protocol for method validation: Finalized analytical method need to do validation for unique parameters like Specificity, method Precision, Sensitivity, Linearity, Recovery and Robustness as recommended by regulatory guidelines.

Specificity

To perform specificity of this analytical method for Empagliflozin solid oral dosage product, Blank, Master exceipent solution & sample solutions were prepared and injected in to the HPLC system. Next sequence was created by injecting the solutions of Organic impurities-Hydroxy Impurity, Sulfonate Impurity, Phenolic Impurity and Acetylated Impurity of at a concentrations of 0.05% to 0.1% sample concentration. Concentrations of Organic impurities were finalized by considering the area of the Organic impurities.

Forced Degradation Studies

To conduct Stress studies for Empagliflozin solid oral dosage product different forced degradation conditions were planned those are, Acid stress (5 Molar Hydrochloric acid / 70º / 24 hours), Base hydrolysis (2.5 Molar NaoH / 70º / 24 hours), Oxidation (0.05 % H_2O_2 / Room Temperature / 24 hours), Heat (95°C /7Days), Humiliation (95% RH / RT / 120 Hr.) and Photolytic were performed to know the interference's of degraded organic impurities.

Precision

To conduct precision for this related substances analytical method six samples prepared by spiking the solutions of all known organic impurities of Empagliflozin at specification concentration in sample solution.

Response-Limit of Detection/Limit of Quantification

To finalize Limit of Detection and Limit of Quantification, samples were made in the concentration from 1% to150 % level of individual Organic impurities spec level by conducting several dilutions to the Organic impurities primary solution to the needed levels. After injecting this solutions, by taking Organic impurities concentration (on X-Axis) A & Organic impurities area (On Y-Axis). By linearity curves, Limit of Detection and Limit of Quantification will be finalized by using the equation of 3.3□/S and 10□/S respectively.

Linearity

To perform linearity, individual impurities and API were made in the concentration from 1% to 150 % level of individual Organic impurities spec level by conducting several dilutions to the Organic impurities primary solution to the needed levels.

After injecting this solutions, by taking Organic impurities concentration (on X-Axis) AND Organic impurities area (On Y-Axis). By linearity curves, by this curve will get correlation coefficient R & slope calculated for each individual Organic impurities.

Recovery

To perform recovery, sample solutions were made by adding the Organic impurities stock solutions at 50 %, 100 % and 150 % of the spec level. The accuracy of the Organic impurities was determined.

Solution stability

To determine stability of reference solution and sample solutions, solutions were made freshly and injected immediately and injecting at later some time lapse periodically by keeping the solutions at RT and refrigerator conditions.

Robustness

To know robustness of this analytical related substances method, experimental parameters are slightly differed and the output of the change was recorded for individual Organic impurities.

To know the influence of flow rate, ± 0.1 mL/min (±10 %). The change of column heat (± 4°C) is checked. For change in gradient composition ± 2%. PH of buffer for Mobile phase –B by ± 0.2 units. For lambda max change, ± 5 nm was changed from the finalized analytical method. While doing one change in analytical method remaining parameters kept unchanged.

Results and Discussion

SpecificityAfter processing the chromatograms of stress conditions and spiked sample chromatograms, it was observed that there is no inference with any peak. All the known & unknown organic impurities, placebo peaks and diluent peak are well resolute from each other.Based on stress studies Empagliflozin solid oral dosage product on subjected to various stress conditions, while it is found stable to all degradation conditions employed.Further, the evaluation of Peak purity of Empagliflozin and its organic impurity peaks from the analysis of every stress condition sample showed that these are homogeneous and have no co-eluting peaks. In all chromatograms baseline is perfect.

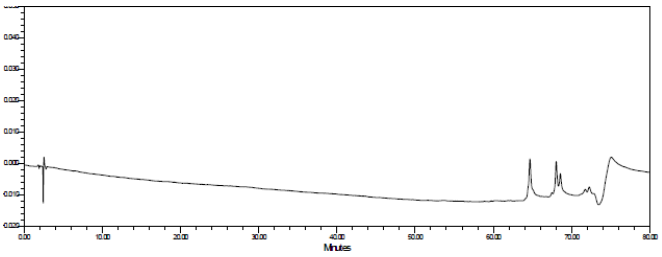


Figure 2. *Typical chromatogram of blank solution.*

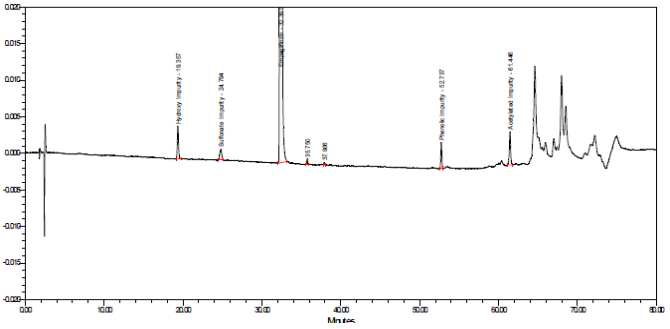


Figure 3. *Typical chromatogram of sample spiked with organic impurities solution.*

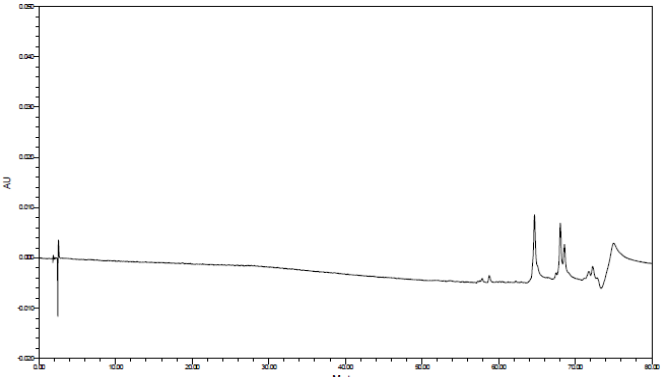


Figure 4. *Typical chromatogram of Placebo solution.*

Table 4. Forced degradation outcome.

Environment kept	% ASSAY	% Deg	Mass balance	Peak Purity
Acid hydrolysis	97.4	0.2	98.3	Pass
Alkali hydrolysis	97.7	0.1	98.5	Pass
Oxidation	97.4	0.1	98.2	Pass
Thermal degradation	95	0.3	96	Pass
Photolytic degradation	97.7	0.1	98.5	Pass

Precision

For precision we got the relative standard deviation of % w/w of Organic impurities-Hydroxy Impurity, Sulphonic Impurity, Phenolic Impurity, and Acetylated Impurities are 5.3, 0.8, 1.9 and 1.5. The % Relative standard deviation for Ruggedness for the same Organic impurities is 3.4, 0.6, 1.0, and 1.0 respectively. By above values it is knowing that this method was well precised and rugged.

Response (Limit of detection and Limit of quantification

The Limit of detection values for Organic impurities- Hydroxy Impurity, Sulphonic Impurity, Phenolic Impurity, Acetylated Impurities is 0.010%,0.01%,0.01%,0.01% and 0.01%respectively . The Limit of quantification values for the same Organic impurities Hydroxy Impurity, Sulphonic Impurity ,Phenolic Impurity, Acetylated Impurities are 0.05%, 0.05% ,0.05%, 0.05% and 0.05 respectively.

Linearity

Linearity graph created with values of concentration and areas of related compounds which was taken in X Axis and Y Axis respectively.

After entering the values it was observed that correlation coefficient values of all related compounds and active compounds found greater than 0.999 for the entire concentrations spiked. The linearity graph drawn from Limit of quantification to 150 % of the organic impurities as well as active compounds.

By seen the results we know that this analytical method was well linear.

Linearity Data

Table 5. *Linearity data of organic impurities.*

Organic impurities	Trend line formula	Correlation Coefficient	Intercept
Hydroxy Impurity	Y=350345X+1196	0.9999	1196
Sulphonic Impurity	Y=253485X+1309	0.9999	1309
Empagliflozin	Y=327776X+708	0.9999	708
Phenolic Impurity	Y=195346X-467	0.9999	-467
Acetylated Impurity	Y=19433X-1025	0.9999	-1025

Recovery

Recovery of this analytical proved by injecting spiked sample solutions at Limit of quantification, 50, 100, & 150 percentage level of the organic impurities specification levels. After processing chromatograms we came to know all recoveryvalues are in between 85-115 with related standard deviation values below 5%. By this result it was proved that this analytical method was accurate.

Table 6. *Accuracy study of Empagliflozin organic impurities.*

Sample spiked level	Hydroxy Impurity		% Accuracy	Sulphonic Impurity		% Accuracy
	mg added (%w/w)	mg found (%w/w)		mg added (%w/w)	mg added (%w/w)	
50 % Spl_1	0.251	0.252	100.3	0.511	0.514	100.5
50 % Spl_2	0.251	0.253	100.8	0.511	0.514	100.5
50 % Spl_3	0.251	0.253	100.7	0.511	0.512	100.2
100 % Spl_1	0.503	0.502	99.8	1.013	1.016	100.3
100 % Spl_2	0.503	0.503	99.7	1.013	1.019	100.5
100 % Spl_3	0.503	0.503	99.9	1.013	1.015	100.2
150 % Spl_1	0.755	0.751	99.5	1.523	1.517	99.5
150 % Spl_2	0.755	0.751	99.4	1.523	1.521	99.8
150 % Spl_3	0.755	0.751	99.5	1.523	1.519	99.7
Sample spiked level	**Phenolic Impurity**		**% Accuracy**	**Acetylated Impurity**		**% Accuracy**
	mg added (%w/w)	**mg found (%w/w)**		**mg added (%w/w)**	**mg added (%w/w)**	
50 % Spl_1	0.538	0.546	101.4	0.511	0.506	99
50 % Spl_2	0.538	0.546	101.4	0.511	0.514	100.7
50 % Spl_3	0.538	0.544	101.2	0.511	0.512	100.3
100 % Spl_1	1.069	1.095	102.4	1	0.992	99.2
100 % Spl_2	1.069	1.093	102.3	1	0.994	99.4

100 % Spl_3	1.069	1.087	101.7	1	0.998	99.8
150 % Spl_1	1.538	1.545	100.4	1.516	1.338	88.2
150 % Spl_2	1.538	1.544	100.3	1.516	1.342	88.5
150 % Spl_3	1.538	1.537	99.9	1.516	1.33	87.7

Solution Stability

After processing chromatograms of spiked sample and reference solution we came to know that there no much difference in areas of all organic impurities and active compounds. By calculation it was find that sample and standard solutions are stable up to 36 hours at RT condition.

Robustness

After processing of spiked sample solution we came to know that all Relative retention times of organic impurities of Empagliflozin were unchanged over the changes of temperature, gradient composition, pH of the buffer and wavelength. By this we proved that this method was robust.

Conclusion

By taking above all results as consideration the above analytical method for related substances of Empagliflozin was specific, linear, accurate, precise, rugged and robust. So finally we can say that this analytical method was stability indicating, so this method we can use for routine sample analysis, stability analysis of Empagliflozin solid oral dosage product.

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