ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 3, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

DEVELOPMENT AND VALIDATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINATION OF DAPAGLIFLOZIN AND ITS IMPURITIES IN TABLET DOSAGE FORM

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Received: 03 November 2018, Revised and Accepted: 09 January 2019

ABSTRACT

Objective: The aim of the present work is the development of new, sensitive, specific, and accurate high-performance liquid chromatographic method for the separation and determination of dapagliflozin and its impurities in tablet dosage form.

Methods: The chromatographic separation of drug and its impurities was achieved using Hypersil BDS C_{18} column (250 mm × 4.6 mm, 5 µ) with mobile phase consisted of mobile phase-A (Buffer pH 6.5) and mobile phase-B (acetonitrile:water 90:10) by gradient program at a flow rate of 1 mL/min with ultraviolet detection at 245 nm.

Results: Dapagliflozin and its impurities A, B, C, D, E, and impurity-F were successfully eluted at the retention time of 16.95, 2.72, 7.82, 10.58, 21.11, 30.37, and 34.36 min, respectively, with good resolution. The method was validated according to the international conference on harmonization guidelines. The validation results showed good precision, accuracy, linearity, specificity, sensitivity, and robustness.

Conclusion: Successful separation and determination of dapagliflozin and its six impurities were achieved by the proposed method. The developed method can be applied for the routine analysis of dapagliflozin and its impurities in pharmaceutical formulations.

Keywords: Dapagliflozin, High-performance liquid chromatography method, Impurity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i3.30853

INTRODUCTION

Dapagliflozin is an antidiabetic drug used for the management of type 2 diabetes mellitus and belongs to a novel class called sodium glucose cotransporter 2 (SGLT2) inhibitor. It is a potent, competitive, reversible, highly selective, and orally active inhibitor of SGLT2, the major transporter protein responsible for the renal glucose reabsorption. By suppressing the SGLT2, dapagliflozin reduces plasma glucose concentration by elevating the renal glucose excretion. Hence, it is used to treat patients with type 2 diabetes. Dapagliflozin's mechanism of action is different from the mechanisms of other antidiabetic drugs as it involves the direct and insulin-dependent elimination of glucose by the kidney. It is a synthetic aryl glycoside contains multiple chiral centers, but the drug is a single enantiomer and it is chemically described as (2S, 3R, 4R, 5S, 6R)-2-(4-chloro-3-[4-ethoxybenzy] phenyl)-6-(hydroxyl methyl) tetrahydro-2H-pyran-3,4,5-triol [1-3].

Impurities are unwanted chemicals present within the formulation and active pharmaceutical ingredient which affects the quality, safety, and efficacy of the medicinal products. A significant aspect of ensuring the safety of drug products is the qualification of impurities [4]. Identification of impurities is done by a variety of chromatographic and spectroscopic techniques, either alone or in combination with other techniques. The objective of the study was to identify and quantify the dapagliflozin and its related impurities A, B, C, D, E, and F [5] (Table 1) in the marketed pharmaceutical formulation. Literature survey revealed that few analytical methods have been reported for the estimation of dapagliflozin alone or in combination with other drugs by ultraviolet (UV) spectrometry [6-9], high-performance liquid chromatography (HPLC) [10-19], and LC-mass spectrometry [20]. However, there is no reported method about the separation and determination of dapagliflozin impurities. Hence, an attempt was made to develop simple, accurate, precise, and sensitive HPLC method for estimation of dapagliflozin in the presence of its above-mentioned impurities.

METHODS

Chemicals and reagents

Dapagliflozin reference standard and impurities were obtained from Veeprho laboratories Pvt. Ltd., Pune. Dapagliflozin tablet, Forziga 10 mg, was purchased from local market. Analytical grade orthophosphoric acid, HPLC grade acetonitrile, methanol, and water were purchased from Merck (Mumbai, India).

Instrumentation

The HPLC system used for the method development and validation composed of Waters alliance system 2695 separation module with autosampler and UV detector. Separation was carried out in a Hypersil BDS column C18 column (250 mm × 4.5 mm, 5 µ). The data analysis was processed with empower software.

Preparation of mobile phase

Mobile phase-A

Two milliliters of 88% orthophosphoric acid was measured and transferred into 2000 ml standard flask, and the volume was made up to the mark with HPLC grade water and adjusted the pH of the solution to 6.50 using triethylamine.

Mobile phase-B

Acetonitrile and water mixture were prepared in the ratio of 75:25 % v/v and degassed by sonication.

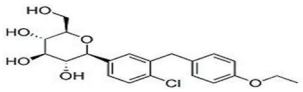
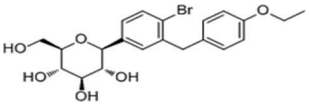
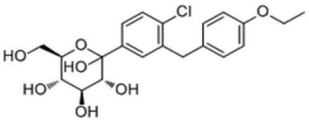
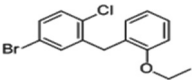
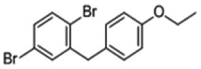
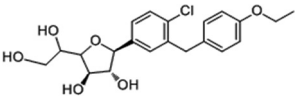
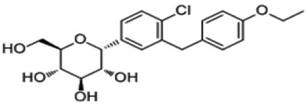
Preparation of diluent

Mobile phase-B was used as diluent.

Preparation of standard solution

Accurately weighed and transferred about 50 mg of dapagliflozin standard into a 50 ml volumetric flask. To this, 30 ml of diluent was

Table 1: Chemical name and structure of dapagliflozin and its related impurities[5]

Name of compound	Chemical name	Structure
Dapagliflozin	(2S,3R,4R,5S,6R)-2-[4-chloro-3-(4-ethoxybenzy) phenyl]-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol	
Impurity-A	(2S,3R,4R,5S,6R)-2-(4-bromo-3-(4-ethoxybenzyl) phenyl)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol	
Impurity-B	(3R,4S,5S,6R)-2-(4-chloro-3-(4-ethoxybenzyl) phenyl)-6-(hydroxymethyl) tetrahydro-2H-pyran-2,3,4,5-tetraol	
Impurity-C	4-bromo-1-chloro-2-(2-ethoxybenzyl) benzene	
Impurity-D	1,4-dibromo-2-(4-ethoxybenzyl) benzene	
Impurity-E	(2S,3R,4R)-2-(4-chloro-3-(4-ethoxybenzyl) phenyl)-5-(1,2-dihydroxyethyl) tetrahydrofuran-3,4-diol	
Impurity-F	(2R,3R,4R,5S,6R)-2-(4-chloro-3-(4-ethoxybenzyl) phenyl)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol	

added and was sonicated with intermittent shaking to dissolve and diluted to the volume with diluents (1000 µg/ml). 5 ml of this solution was diluted to 50 ml with diluent (100 µg/ml). Further 5 ml of this solution was diluted to 50 ml with diluents (10 µg/ml).

Preparation of sample solution

The commercial dapagliflozin 10 tablets (Forxiga tablet 10 mg) were accurately weighed and powdered. From the tablet, powder transferred about 50 mg equivalent dapagliflozin sample into a 50 ml volumetric flask. To this, 30 ml of diluent was added and sonicated with intermittent shaking to dissolve and diluted to the volume with diluent. The solution was filtered through a 0.45-µ-membrane filter. The above solution was appropriately diluted to get the final concentration of 10 µg/ml sample solution.

Impurities working solution

About 10 mg of all the six impurities were accurately weighed and transferred into six different 10 ml volumetric flask, and the volume was made up to the mark with diluent (1 mg/ml). 1 ml each of impurity stock solution was taken separately in six different 100 ml volumetric flask; and the volume was made up to the mark with mobile phase to get the concentration of 10 µg/ml for each; and all the solutions were studied individually on the HPLC system.

Preparation of impurities mixture

Accurately weighed and transferred about 2 mg each of impurity A, B, C, D, E, and F in 10 ml of volumetric flask. To this, 5 ml of diluent was added and was sonicated with intermittent shaking to dissolve and diluted to volume with diluent (200 µg/ml). The solution was filtered through a 0.45-µ-membrane filter.

Preparation of impurities spiked sample solution

Transferred 1ml of the stock solution of the known impurities in 200 ml of volumetric flask made up to volume with Dapagliflozin sample solution (Dapagliflozin 10 µg/ml and impurities 1.0 µg/ml).

Preparation of resolution solution

Accurately weighed and transferred about 5 mg of impurity-D and 50 mg of Dapagliflozin into a 50 ml volumetric flask. To this, 30 ml of diluent was added and was sonicated with intermittent shaking to dissolve and made up to the volume with diluent. From the above solution, 5 ml was diluted to 100 ml with diluents to get the concentration of dapagliflozin, 50 µg/ml and impurity-D, 5 µg/ml.

Chromatographic conditions

The separation and analysis of all compounds were carried out on Hypersil BDS C18 column (250 mm × 4.5 mm, 5 µ) at 50°C, and the analytes were monitored with UV detection at 245 nm. A gradient mixture of mobile phase-A and mobile phase-B was used at a flow rate of 1 ml/min. The LC gradient program was set as follows, Time (min)/mobile phase-A: mobile phase-B percentage (%). It was programmed as 0–8/75:25, 8–12/55:45, 12–25/55:45, 25–35/40:60, 35–65/30:70, 65–66/30:70, and 66–75/75:25.

Method validation

The method validation was performed as per the international conference on harmonization (ICH) guidelines [21]. The parameters such as specificity, linearity and range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and robustness were evaluated.

Specificity

The specificity was demonstrated by injecting blank solution, dapagliflozin standard solution, and sample spiked with impurity solution, and the chromatograms were checked for interferences [22].

Linearity

The linearity was tested in the concentration range of 0.20–13.00 µg/ml for dapagliflozin, 0.26–2.00 µg/ml for impurity-A, 0.21–2.00 µg/ml for impurity-B, 0.52–2.00 µg/ml for impurity-C, 0.26–2.00 µg/ml for impurity-D, 0.37–2.00 µg/ml for impurity-E, and 0.37–2.00 µg/ml for impurity-F.

Precision

The precision was studied by repeatability and intermediate precision (ruggedness). The repeatability was checked by injecting the sample solution spiked with impurities in six replicates, and the intermediate precision was evaluated by different analyst using different columns on different days. The percentage relative standard deviation (%RSD) of % total impurity was calculated.

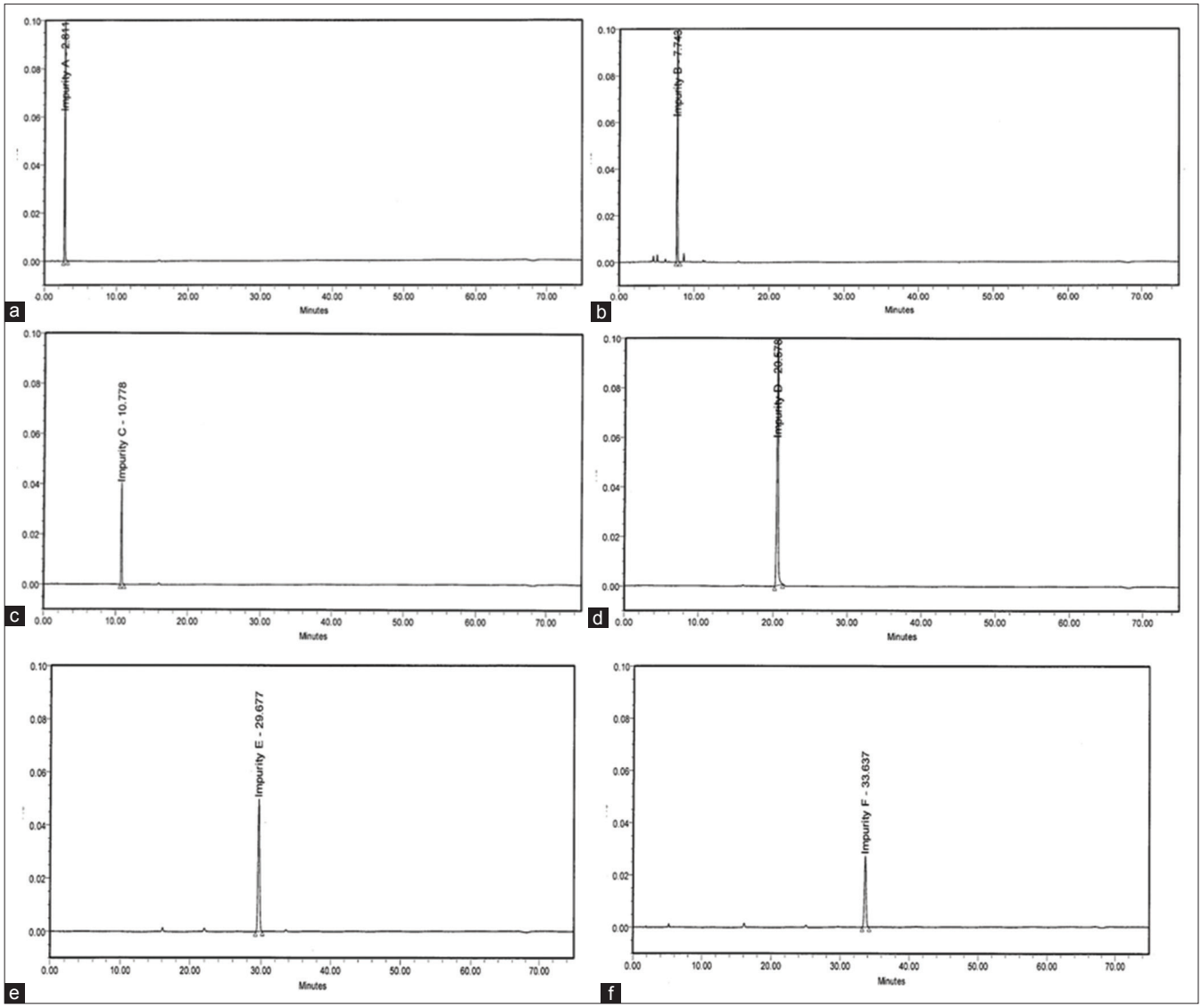


Fig 1: Chromatograms of impurities standard. (a) impurity-A, (b) impurity-B, (c) impurity-C, (d) impurity-D, (e) impurity-E, (f) impurity-F

Accuracy

The accuracy of the method was verified by injecting the sample solution spiked with impurities at different levels ranging from 70% to 130% in three replicates, and % mean recovery of each impurity was calculated.

Limit of detection and limit of quantitation

LOD is defined as the lowest concentration of an analyte that an analytical method differentiates from background levels. The LOQ is defined as the lowest concentration that can be measured with acceptable accuracy, precision, and variability. The LOD and LOQ were calculated from the linearity curve by using the formulae:

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where σ is the standard deviation of the y-intercept and S is the slope of the calibration plot.

Robustness

Robustness of the method was verified by deliberately varying the instrumental conditions by flow rate (±10%), organic phase ratio (±2%), pH of buffer (solution-A) (±0.2), and column oven temperature (±5°C). The %RSD of % total impurity was calculated [21-25].

RESULTS AND DISCUSSION

Method development

Analytical detection wavelength of 245 nm was selected for the proposed HPLC method based on the UV absorption of dapagliflozin and its impurities. Several trials were done to separate dapagliflozin from its impurities using various columns and by changing mobile phase composition and pH. Finally, the separation of all the impurities from the drug peak was achieved with Hypersil BDS column C18 column (250 mm × 4.5 mm, 5 µ) and with a gradient mixture of the mobile phase-A (Buffer pH 6.5) and mobile phase-B (acetonitrile:water 75:25 % v/v), and the peak shape of the drug and the impurities was good. The retention time (RT) of the drug and the impurities were identified by analyzing the chromatograms (Fig. 1) of individual standard solutions of dapagliflozin and the impurities in five replicates under the optimized method conditions.

The chromatographic system performance was checked by injecting the resolution solution prepared with dapagliflozin standard and the closely eluted impurity-D, and the chromatogram was recorded (Fig. 2). The resolution was found to be 9.80. From the chromatograms of resolution, and individual standard solutions of dapagliflozin, and the impurities, the system suitability parameters such as the number of theoretical plates, tailing factor, and the resolution were calculated. The results are listed in Table 2. The chromatographic system is considered suitable when it meets the following criteria. The resolution between the peaks must be >2, the number of theoretical plates should be more than 2000, and tailing factor must be lower than 2.

Method validation

Specificity

The specificity of the method was tested by comparing the chromatograms of blank (Fig. 3), dapagliflozin standard solution (Fig. 4), and sample spiked with impurity solution (Fig. 5). No interference peaks were observed at the RT of dapagliflozin due to the blank, impurities, and placebo.

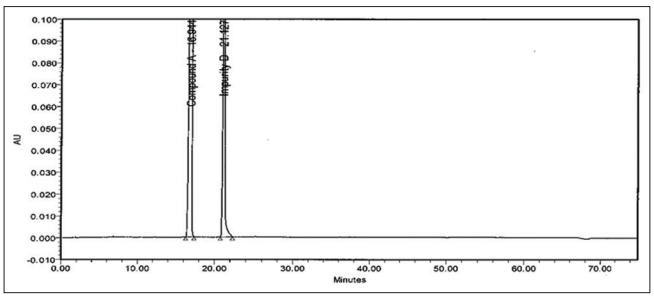


Fig 2: Chromatogram of resolution for dapagliflozin and impurity-D

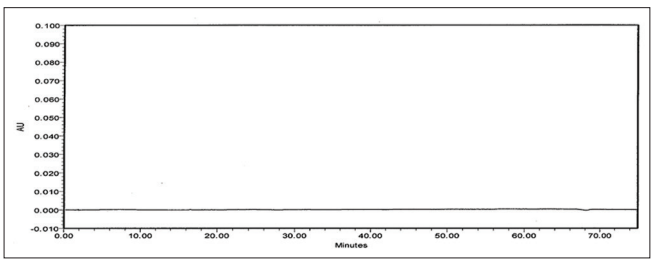


Fig 3: Chromatogram of the blank solution

Linearity

The linearity graph was plotted by taking the concentration in the x-axis and peak area in y-axis over the calibration ranges tested for dapagliflozin and impurities. The data were subjected to linear regression analysis, and the regression statistics such as concentration range, slope, y-intercept, and correlation coefficient are shown in Table 3.

Precision

Precision of the method by repeatability and intermediate precision was assessed by injecting sample solution spiked with impurities six times, and the results were expressed in terms of %RSD of the percentage of total impurities. The results are presented in Table 4.

Accuracy

The accuracy of the proposed method was determined by analyzing the dapagliflozin sample solution spiked with impurities at three concentration levels of 70%, 100%, and 130% of each impurity in triplicate. The mean percentage recovery was calculated and reported in Table 5.

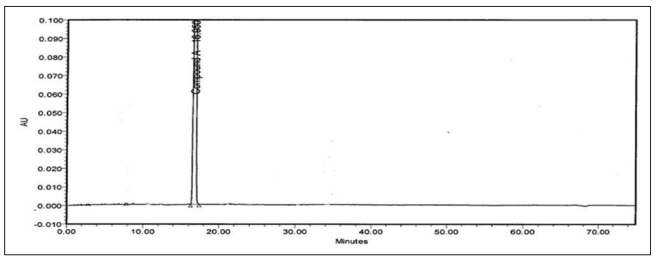


Fig 4: Chromatogram of dapagliflozin standard solution

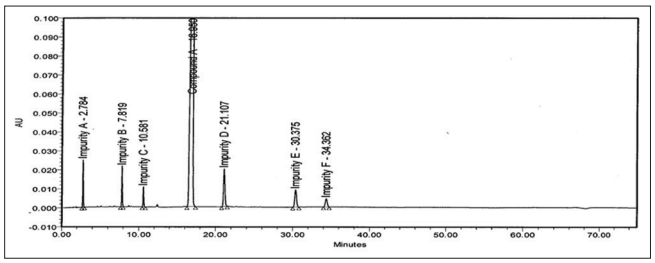


Fig 5: Chromatogram of sample solution spiked with impurities

Table 2: System suitability parameters

System Suitability parameter	Imp-A	Imp-B	Imp-C	Dapagliflozin	Imp-D	Imp-E	Imp-F
Retention time	2.72	7.82	10.58	16.95	21.11	30.37	34.36
Theoretical plates	6168	43,350	64,182	18,298	64,685	82,991	87,950
Symmetry factor	1.12	1.03	1.11	0.64	1.11	1.05	1.04
Peak area	128,579	119,169.6	66,577.8	7,906,417	254,283.6	143,306	73,306.6
Percentage RSD of peak area	0.21	0.15	0.13	0.026	0.12	0.21	0.11
Resolution		34.68	17.27	18.75	9.80	24.15	8.81

Imp: Impurity, RSD: Relative standard deviation

Table 3: Linearity evaluation data for dapagliflozin and impurities

Compound name	Linearity range (µg/ml)	Slope	Intercept	Correlation coefficient
Impurity-A	0.26–2.00	11978	9.2664	0.9981
Impurity-B	0.21–2.00	15070	82.95	0.9996
Impurity-C	0.52–2.00	6497.5	140.47	0.9979
Dapagliflozin	0.20–13.00	8897.7	490.18	0.9999
Impurity-D	0.26–2.00	25152	25.71	0.9988
Impurity-E	0.37–2.00	15795	980.3	0.9952
Impurity-F	0.37–2.00	8742.6	264.97	0.999

Table: 4 Results of the precision study

Sample number	Method precision (total impurities % w/w)	Intermediate precision (total impurities % w/w)	
		Analyst-I, column-I and day-I	Analyst-II, column-II and day-II
1	0.146	0.146	0.142
2	0.148	0.148	0.142
3	0.145	0.145	0.161
4	0.146	0.146	0.135
5	0.148	0.148	0.159
6	0.146	0.146	0.145
Mean	0.146	0.146	0.147
SD	0.0012	0.0012	0.0104
Percentage RSD	0.82	0.82	7.07
Overall mean		0.147	
Overall SD		0.0071	
Overall percentage RSD		4.83	

RSD: Relative standard deviation, SD: Standard deviation

Table 5: Results of accuracy study

Recovery level (%)	Mean recovery* (%)					
	Impurity-A	Impurity-B	Impurity-C	Impurity-D	Impurity-E	Impurity-F
70	103.02	96.08	92.14	96.37	104.27	103.02
100	104.44	101.47	99.99	101.37	98.50	102.22
130	104.42	104.36	98.17	102.25	99.38	102.76
Overall mean	103.96	100.63	96.76	99.99	100.71	102.67
Overall SD	1.940	3.647	4.883	2.788	3.528	1.209
Overall percentage RSD	1.87	3.66	4.96	2.80	3.48	1.18

*n=3. RSD: Relative standard deviation, SD: Standard deviation

Table 6: Limit of detection and limit of quantitation data

Compound name	LOD (µg/ml)	LOQ (µg/ml)	Precision at LOD level		Precision at LOQ level	
			Mean peak area*±SD	Percentage RSD	Mean peak area*±SD	Percentage RSD
Impurity-A	0.084	0.253	944±125.81	13.33	3078±212.25	6.90
Impurity-B	0.042	0.127	749±113.12	15.10	2027±198.75	9.81
Impurity-C	0.217	0.434	1286±99.40	7.73	2676±127.59	4.77
Dapagliflozin	0.065	0.196	949±57.54	6.06	2092±153.63	7.34
Impurity-D	0.071	0.216	1688±213.6	12.66	5151±212.38	4.12
Impurity-E	0.114	0.345	1478±271.3	18.36	5027±243.61	4.85
Impurity-F	0.176	0.352	2046±156.9	7.67	3495±140.28	4.01
Overall mean percentage RSD				11.55		5.97

*n=6. LOD: Limit of detection, LOQ: Limit of quantitation, RSD: Relative standard deviation, SD: Standard deviation

Limit of detection and limit of quantitation

LOD and LOQ were predicted from the linearity curve using the slope and standard deviation of y-intercepts of regression lines, as per the ICH guideline. The predicted LOD and LOQ values were verified by checking the precision of six replicate injections at that concentration for impurity-A, impurity-B, impurity-C, Dapagliflozin, impurity-D, impurity-E, and impurity-F, and the results are shown in Table 6.

Robustness

To test the robustness small but deliberate changes were made in the method conditions, the samples were analyzed in triplicate and the %RSD of percentage of total impurities were calculated for each altered condition. In the varied method conditions, all the analytes are well resolved, and the elution order of analytes was unchanged. The results were summarized in Table 7.

DISCUSSION

According to the literature review, there is no HPLC method development was reported for simultaneous estimation of dapagliflozin and its impurities. In the proposed method under optimized chromatographic conditions, the dapagliflozin and its impurities were well separated with good peak shape and proper RT. System suitability test results confirm that the developed method is suitable for the analysis of impurities in the drug product. The results of method validation parameters are within the acceptance criteria as per the ICH guidelines [21]. The specificity study meets the acceptance criteria [21], as that no interference was observed from the blank at the RT of known impurities. It is evident that from the obtained data that all the peaks were well resolved, and the method is said to be specific. The result of linearity study shows excellent correlation existed between the peak area and concentration of the drug dapagliflozin and impurities, and the correlation coefficient of the drug and impurities complied with the acceptance limit (not ≥0.95) [22]. The obtained precision data represent no significant variation in the measured response and demonstrate that the method is repeatable and rugged with the %RSD value below 4.83 which meets the acceptance limit (<10%) [22]. The mean % recovery of recovery study was in the range of 96.76–103.96. This range complies with the acceptance criteria [22] 85%–115%, thus confirming the accuracy of the method. The mean %RSD of precision at LOD and LOQ level was below 11.55 (acceptance limit – <15%) [22], and the very low LOD and LOQ values when compared to the reported method [25] indicate the sensitivity of the developed method for determination of dapagliflozin impurities. The satisfactory mean %RSD value of robustness study exhibits the proposed method which is robust enough to withstand the small variations of method conditions.

Table 7: Results of robustness

Altered method conditions		Total impurities % w/w*±SD	Percentage RSD
Flow rate (±10%)	0.9 ml/min	0.143±0.0104	2.17
	1.0 ml/min	0.147±0.0031	7.07
	1.1 ml/min	0.141±0.0021	1.49
Mobile phase-B composition-organic phase ratio±2%	Acetonitrile 73%	0.144±0.0111	7.71
	Acetonitrile 75%	0.147±0.0031	7.07
	Acetonitrile 77%	0.145±0.0040	2.76
Column oven temperature±5°C	45°C	0.147±0.0020	1.36
	50°C	0.147±0.0031	7.07
	55°C	0.132±0.0015	1.14
pH of mobile phase-A±0.2	6.3	0.164±0.0025	1.52
	6.5	0.147±0.0031	7.07
	6.7	0.148±0.0010	0.68

*n=3. RSD: Relative standard deviation, SD: Standard deviation

CONCLUSION

The quality and safety of the drug product not only depends on the adopted manufacturing procedure and toxicological properties of an active substance but also depends on the impurities that it contains. Hence, a thorough examination of related impurities plays a significant role in controlling the quality of a drug product. In the current research work, a simple, sensitive, specific, and accurate HPLC method was developed for separation and determination of dapagliflozin in the presence of its related impurities. The developed method was validated as per the ICH guidelines and it was found to be precise, linear, rugged, and robust. Hence, the present method can be adapted to separate, identify, and quantify the related impurities of dapagliflozin in its pharmaceutical formulations. Application of this method in quality control shall improve the safe use of medicinal products which contain dapagliflozin as an active substance.

AUTHORS' CONTRIBUTION

The research work, manuscript preparation, and grammar check using the software Grammarly were done by Mrs. A. CAROLINE GRACE, the research work was guided by Dr. T. PRABHA, and critical revision and final proofreading of the manuscript were done by Dr. T. SIVAKUMAR.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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