SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND ITS IMPURITIES IN TABLET DOSAGE FORM

B. Akhila[1*], B V Ramana[2]

Abstract

A new stability- indicating analytical method by LC-MS/MS was developed for the simultaneous determination of dapagliflozin and its synthesis impurities. A LC-MS/MS model was used for method development and validation. The separation was achieved in a column (50 x 3.0 mm, 1.8 µm), using a mixture of acetonitrile: water (70:30, v/v) as mobile phase in isocratic mode. The method was properly validated according ICH guidelines with respect to linearity, specificity, precision, accuracy and robustness. The calibration curves of each analyte showed determination coefficients (r2) and the method was linear at the concentrations ranges for dapagliflozin and its impurities. Lastly, this method presented low limits of detection (LOD) and quantification (LOQ) for both dapagliflozin and impurities, being a technique with high sensitivity.

Key words: Analytical validation, Dapagliflozin, Drug impurities, LC-MS

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Introduction

SGLT2 Inhibitors are a new class of oral antihyperglycemic agents indicated as an adjunct to diet and exercise to improve glycemic control in adult patients with type 2 diabetes mellitus. The Diabetes Association recently updated the guidelines for pharmacotherapy in type 2 diabetes and added SGLT2 inhibitors as an add-on to metformin or when metformin is not tolerated. [1]SGLT2 Inhibitors can be prescribed in monotherapy or combination with other oral antihyperglycemic agents and Insulin. Pharmacopeia indicated restrictions for active pharmaceutical ingredients (APIs) and formulations for allowable levels of impurities. [2] Moreover, the Food and Drug Administration (FDA) and International Council for Harmonisation (ICH) mentioned strategies for the identification and quantification of impurities along with residual solvent in any in novel dosage forms [3-5]. Moreover, some impurities in trace levels could affect the efficacy and safety of API, as well as be carcinogenic [6]. Hence, monitoring and control of trace impurities in any API turn into a very tough assignment. Therefore, the process of minimizing such carcinogenic substances became important in pharmaceutical toxicology [7-9].

In the present work an analytical method by LC-MS/MS was developed and validated to identify and quantify the drug, dapagliflozin and its related impurities C and D in the marketed tablet formulation. [10-12] The analytical conditions for method development were selected on the basis of chemical nature of the dapagliflozin and its related impurities. By considering the chemical nature of dapagliflozin and its impurities the mobile phase system of phosphate buffer and the mixture of acetonitrile and water were selected for analysis. [13-15] The several trials were done to separate dapagliflozin from its impurities by using various columns and by changing mobile phase composition and pH. To conduct the trials, various columns with different dimensions and particle size were used, gradient mobile phase system with various buffer pH such as 7.0, 6.8 and 6.7 with different ratios of acetonitrile and water were used. [16-17] To get the optimum separation of the compounds the buffer pH was adjusted and the acetonitrile composition in the mobile phase was increased to reduce the longer retention time of main drug peak. Finally the separation of all the impurities from the drug peak was achieved with the following chromatographic conditions.

Materials and Methods: [18-20]

HPLC grades of ammonium acetate and methanol were purchased from Merck (Mumbai, India). Analytes were obtained from synthink research chemicals (Hyderabad, India). A Shimadzu LC-MS/MS- 8050 system associated with the Nexera X2 HPLC and Lab Solutions software v.5.6 was used. Separations were accomplished on a 5 µm particle size of Hypersil C18 column (4.6×250 mm) purchased from Thermo Fisher Scientific.

Method development:

Generally, for any analysis, sample preparation plays an important role; it affects the sensitivity, as well as better recovery of impurities. So, preferable combinations of acetonitrile, water, ammonium acetate, and methanol were used as diluents for chromatographic efficiency. In the present work, 0.01 M ammonium acetate in methanol was chosen as a diluent with column oven at 40º due to good response and recovery for impurities. Also, both isocratic and gradient modes of elution were performed. Nevertheless, from the observations, it was noticed that all the impurities were effectively separated by the gradient method. Similarly, a column of dimensions, namely Zorbax C8, Hypersil C18 column Phenomenox, Kromasil C8 and C18, were also investigated for resolutions. Finally, Hypersil C18 column was selected due to its better response, peak shape, linearity, and reproducibility even at a lower concentration.

Method optimization: [21-22]

Mobile phase A used was prepared by dissolving 0.77 g of ammonium acetate in 1000 ml Milli-Q water by sonication followed by filtration (0.22 µm). Pure HPLC grade methanol was used as a mobile phase B. An LC-MS/MS system, coupled with an 8050 triple quadrupole detector, was used. Separation was achieved on a 5 µm Hypersil C18 column (250×4.6 mm) with injection volume 10 µl, 1 ml/min flow rate, sample cooler temperature at 15° and column oven temperature 40°. **Table-1** summarized conditions of MRM, Valco valve, and source gas parameters for mobile phases A and B under gradient mode against the blank solution (diluent).

Table 1: Gradient Programme

Gradient program		
Time (minute)	**Mobile Phase A**	**Mobile Phase B**
0.01	45	55
8.00	45	55

10.00	20	80
13.50	Total Flow	0.8 ml
15.00	45	55
17.00	45	55
17.00	Total Flow	0.8 ml
20.00	Total Flow	1 ml
21.50	20	80
32.00	45	55
37.00	Controller	Stop

Multiple reactions monitoring conditions

	Parameters				
Impurity	**MRM**	**Q1 Prebias**	**CE**	**Q3 Prebias**	**Dwell Time (milliseconds)**
Dapagliflozin Impurity 1	305.70>69.05	20.0	22.0	24.0	100
Dapagliflozin Impurity 2	364.15>291.25	22.0	22.0	30.0	100
Dapagliflozin	314.15>244.10	20.0	19.0	26.0	100

Valco Valve Condition for sample method

Time (min)	**Command**	**Value**
17.00	FCV2=	1
24.00	FCV2=	0

Standard preparation:

Separately, 2.6 mg of impurity 1, 2 and DAPA were weighed accurately and dissolved completely in 100 ml diluent via sonication. One milliliter of the above impurity/ intermediate standard stock solution was further diluted to 100 ml with diluent. One hundred milligrams of accurately weighed DAPA was diluted to 5 ml. To evaluate the system suitability parameters, 10 µl of the above-prepared solution was separately injected namely blank, standard, and sample preparations and their peak area responses were monitored. As per the pharmacopeias, the average peak area response of % relative standard deviation (RSD) of impurity 1, 2, and DAPA impurities should not be more than 15.0.

Method validation: [23,24]

Method was validated according to the USFDA and ICH guidelines. The appropriateness and efficacy of the chromatographic scheme were obtained from the system suitability test and it is proficient in the investigation without any bias. To guarantee the capacity of the chromatographic systems, these must placate pre-defined acceptance conditions to implement the examination of various samples. In the contemporary experiment, impurity 1, 2, and DAPA impurity solutions were injected into the LC-MS/MS system for determining system suitability parameters such as peak area and its RSD and retention time, which were detailed after data incorporation using software (**Table-2, Fig-2**).

Table 2: Results for System Suitability

System suitability parameters	**Impurity-1**	**DPA**	**Impurity-II**
%RSD of Peak areas obtained from six replicate injections of the standard solution	0.8	1.2	1.6

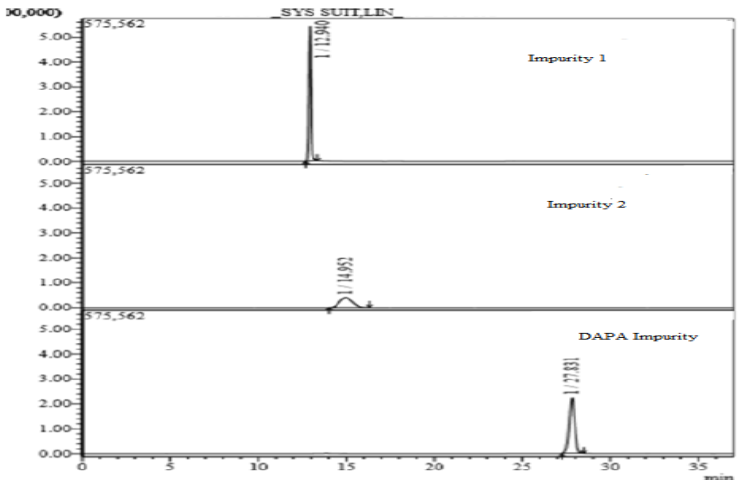

Figure 2: System suitability

The impurity 1, 2, and DAPA impurities were also checked for specificity by injecting them against the blank solution. The outcomes showed that the chosen method is unbiased concerning the presence of further components and interestingly, no nosiness was recorded at the RTs of impurity 1, 2, and DAPA impurities. (**Table-3, Fig-3**).

Table 3: Blank Interference Results for impurity 1, 2, and DAPA impurities

ame	Retention time (min)	Interference found at the retention time of CPP, CPE, and EFI
CPP	12.940	No
CPE	14.952	No
EFI	27.831	No
Blank	NA	No

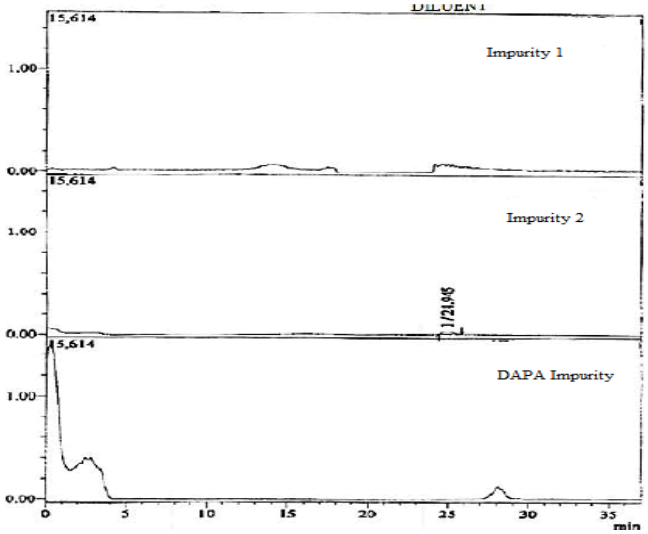

Figure 3: Specificity

Table 4: standard drug and its procurement

Drug standard	Procured from
Dapagliflozin (99.67%) and Impurities	Veeprho laboratories pvt ltd, Pune, India

Table 5: Commercial drug information

Commercial formulations	Components	Manufacturer
FORZIGA Tablet	Dapagliflozin 10 mg	AstraZeneca Pharma India Limited, Bangalore, India.

Results and Discussion

At limit of quantification (LOQ), 4 levels of the precision method, namely system precision, intermediate precision (ruggedness), method precision (repeatability) and precision were evaluated. System precision suggested inconsistency in the dimensions of the analytical system, while repeatability (method precision) indicated the reproducibility of the method. Standard solution was prepared with impurity 1, 2, and DAPA impurities and injected (n=6) into the LC-MS/MS system from which peak area and RSD were derived, whereas form method precision, the % RSD data was obtained (**Table 6**).

Table 6: Method Precision Results for impurity 1, 2, and DAPA impurities

Preparation	Impurity 1 (ppm)	Impurity 2 (ppm)	DAPAI (ppm)
1	2.5	2.8	3.0
2	2.6	2.9	3.1
3	2.7	3.0	3.2
4	2.7	2.9	3.2
5	2.8	3.1	3.4
6	2.5	2.7	3.1
Average	2.6	2.9	3.2
% RSD	4.6	4.9	4.3

The peak area of each sample was noted and plotted against respective concentrations. The Eqn. y=mx+b, defined the linear relation between impurity concentration (x) and respective peak area (y). From this analysis, a correlation coefficient (must be above 0.99) and slope-intercept values were derived (**Table 7**).

Table 7: Linearity of impurity 1, 2, and DAPA impurities

Linearity results						
Levels	**IMP 1**		**IMP 2**		**DAPA IMP**	
	Conc. in ppb	**Area**	**Conc. in ppb**	**Area**	**Conc. in ppb**	**Area**
5 %	2.508	259187	2.531	117538	2.569	305901
10 %	5.224	298024	5.272	137684	5.352	388002
25 %	12.538	1214979	12.653	619137	12.845	1482624
50 %	25.075	2661829	25.306	1341802	25.690	3110678
75 %	37.613	3873591	37.958	1991286	38.534	4712314
100 %	50.046	4817911	50.506	2451722	51.272	5857968
125 %	62.688	6522284	63.264	3349419	64.224	8271495
150 %	75.748	8502919	76.444	4296518	77.604	11062322
Slope	107848		55129		129684	
Intercept	-165458.4		-87086.3		-234051.5	
Correlation	0.9974		0.9994		0.9976	

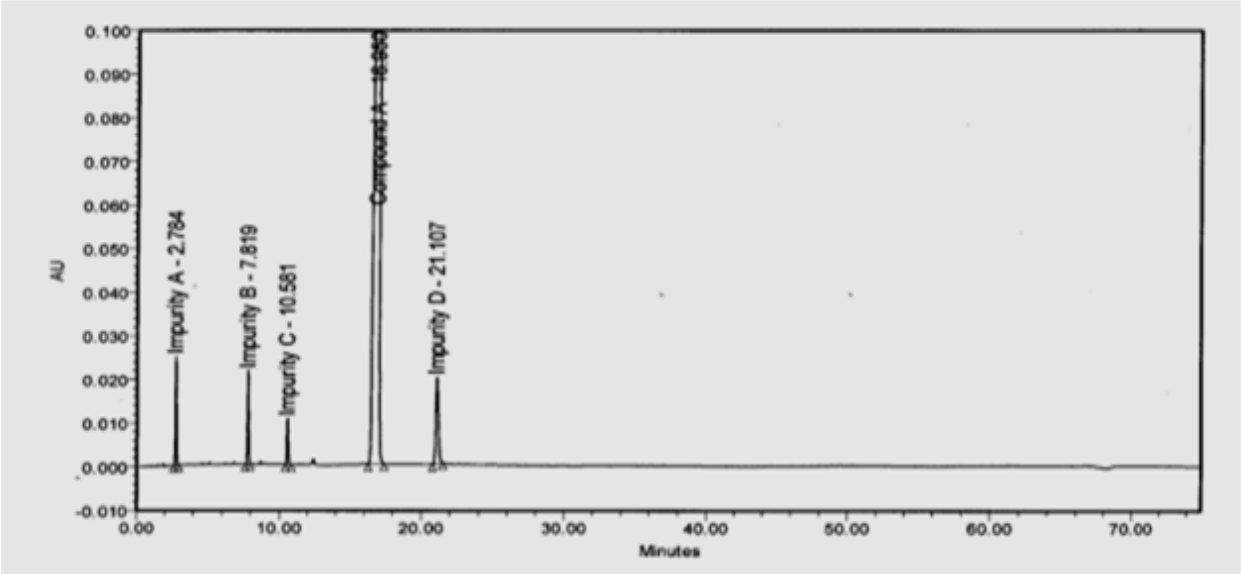

Figure-4: Chromatogram of Dapagliflozin sample solution spiked with impurities

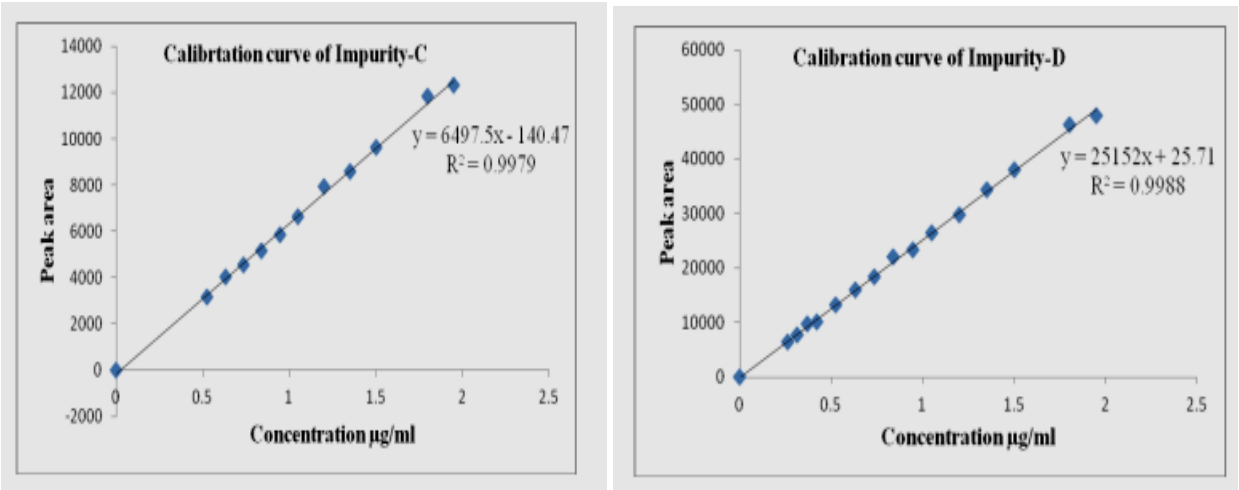

Figure-5: Calibration curves of Impurity C and D

Table-8: Results of accuracy study of impurities

	Mean recovery %	
Recovery level %	Impurity-C	Impurity-D
70%	92.14	96.37
100%	99.99	101.37
130%	98.17	102.25
Overall mean	96.76	99.99
Overall SD	4.883	2.788
Overall % RSD	4.96	2.80

Table-9: LOD and LOQ data of dapagliflozin and impurities

Compound name	**LOD (µg/ml)**	**LOQ (µg/ml)**	**Precision at LOD level**		**Precision at LOQ level**	
			Mean peak area±SD	**%RSD**	**Mean peak area±SD**	**Mean peak area±SD**
Impurity- C	0.217	0.434	1286±99.403	7.73	2676±127.59	4.77
Dapagliflozin	0.065	0.196	949±57.548	6.06	2092±153.63	7.34
Impurity-D	0.071	0.216	1688±213.62	12.66	5151±212.38	4.12
Overall % RSD				**8.81**		**5.41**

Table-10: Results of robustness of impurities

Altered Method Conditions		**Total impurities % w/w±SD**	**% 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

Conclusion:

The calibration curves of each analyte showed determination coefficients (r2) and the method was linear at the concentrations ranges for dapagliflozin and its impurities. Lastly, this method presented low limits of detection (LOD) and quantification (LOQ) for both dapagliflozin and impurities, being a technique with high sensitivity.

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