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A Validated LC-MS/MS Method for Determination of Dapagliflozin in Tablet Formulation

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Abstract: A highly sensitive, precise and accurate LC-MS/MS method is developed and validated for the determination of Dapagliflozin in tablet formulation. Chromatographic separation was carried out on Agilent InfinityLab Poroshell 120 EC-C18 (2.1×100 mm, 2.7 µm) column. Isocratic elution was based on 5mMammonium acetate: acetonitrile (20:80, v/v) as mobile phase, column temperature at 35°C and flow rate at 0.2 mL min^{-1} were utilized. The mass spectrometer was operated under multiple reaction monitoring (MRM) mode using electrospray ionization by monitoring the transition pair (precursor to product ion) of m/z 426.20-107.20 in the positive mode. The method was found linear in the concentration range of 25-500 ng/mL. The limit of detection (LOD) and limit of quantitation (LOQ) were 6.83 ng/mL and 20.70 ng/mL respectively. The optimized method was validated according to the International Conference on Harmonization (ICH) guidelines. The developed method was found suitable for the quantitation of dapagliflozin in tablet dosage form.

Keywords: Dapagliflozin, LC-MS/MS,Validation, Assay, Spectroscopy.

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I. INTRODUCTION

Dapagliflozin (Fig. 1) is a sodium-glucose cotransporter 2(SGLT2) inhibitor used in the therapy of type 2 diabetes mellitus(T2DM) along with dietary and lifestyle changes. It acts by inhibiting the SGLT2 proteins which are responsible for 90% of the glucose reabsorption in the kidneyleading to glucose elimination via urine.This mechanism of action improves glycaemic control along with a limited risk of hypoglycaemia. In addition, it has potential benefit of weight loss due to increased glucosuria,cardiovascular benefitsandreduction in arterial blood pressure associated with the osmotic effect [1]. The pharmacokinetic characteristics of SGLT2 inhibitors show an excellent oral bioavailability and long elimination half-life allowing once-daily administration. Furthermore, these agents share a negligible risk of drug–drug interactions.Dapagliflozin is a safe drug which is well tolerated by major population [2].

Analytical methods available for estimation of dapagliflozin include a couple of RP-HPLC methods for the determination of dapagliflozin in API and tablet dosage form [3, 4], stability indicating HPLC method for tablets [5], ultraviolet spectrophotometric methods [6, 7]for tablets utilizing first and second order derivatization technique and LC-MS/MSmethods [8, 9]for estimation of dapagliflozin in biological fluids utilizing negative ion electrospray ionization mode. LC-MS/MS is a promising technique having benefits of better sensitivity and selectivity as compared to other techniques. No LC-MS/MSmethod is available for estimation of Dapagliflozin in tablet dosage form.Thus, the present study was designed for the determination of dapagliflozin using LC-MS/MS technique fortablet formulation.

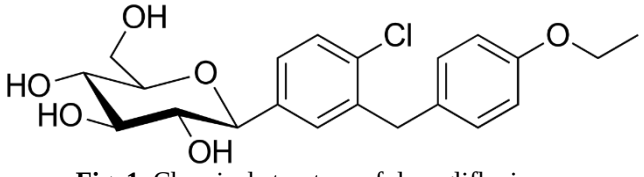

Fig. 1. Chemical structure of dapagliflozin**.**

II. MATERIAL AND METHODS

2.1 Chemicals and reagents

Dapagliflozin (99% purity) was provided as a gift sample by Sun Pharmaceuticals limited (Gurgaon, India) with Certificate of Analysis. HPLC grade acetonitrile was purchased from Merck (Mumbai, India) and analytical reagent grade ammonium acetate was procured from Fluka Analytical(St. Louis, MO, USA). Water used in the entire analysis was prepared usingMillipore Direct-Q 3UV water purification system byMillipore (Bangalore, India). Forxiga (Dapagliflozin 10 mg) tablets manufactured by AstraZeneca were purchased from market.

2.2 Liquid chromatographic and mass spectrometric conditions

The analysis was performed on Agilent 1200 HPLC system equipped with Agilent 6410 triple quadrupole LC-MS and an electrospray ionization (ESI) source. Chromatographic separation was achieved using an Agilent InfinityLab Poroshell 120 EC-C18 (2.1×100 mm, 2.7 µm) column. The isocratic mobile phase consisted of 5mM ammonium acetate: acetonitrile (20:80, v/v) and was delivered at a flow rate of 0.2 mL/min. The column temperature and autosampler temperature were maintained at 35°C and 5°C respectively and injection volume was kept at 10 µL.

Detection was performed using the multiple reaction monitoring (MRM) mode to measure the transition pair (precursor to product ion) of m/z 426.20-107.20 for dapagliflozin-ammonium adduct ions $[M+NH_4]^+$ in the positive mode. The optimized parameters of the mass spectrometer likefragmentor value and collision energy were 140 and 40 respectively and the dwell time was set at 200 ms. Quantitation was carried out using Agilent MassHunter Workstation software version B.06.00.

2.3 Preparation of standard solutions

The standard stock solution was prepared by dissolving 10 mg of dapagliflozin in 10 mL diluent i.e. acetonitrile: water (50:50 v/v) to acquire a concentration of 1000 µg/mL. Working standard solutions containing 25, 50, 100, 250, 500 ng/mL of dapagliflozin were prepared by serial dilutions from the standard stock solution.

2.4 Sample preparation

Ten tablets were weighed and finely powdered, an amount of powder equivalent to 10 mg of dapagliflozin was weighed accurately and transferred to a 10 mL volumetric flask. Sufficient amount of diluent was added to make up the volume, sonicated for 15 min and filteredusing 0.22 µm nylon syringe filter.From the filtrate, measured volume was taken and diluted with the diluent to achieve the final concentration of 100 ng/mL.

2.5 Validation of the analytical method

The proposed LC-MS/MS method was validated according to the International Conference on Harmonization (ICH) guidelines [10]. The parameters evaluated were linearity, precision, accuracy, limit of detection, limit of quantitation, specificity and robustness.

III. RESULTS AND DISCUSSION

The analytical method was developed and optimised for the determination of Dapagliflozin. Based on signal intensity and reproducibility, transition pair (precursor to product ion)of m/z 426.20-107.20 was selected for analysis as shown in Fig. 2 and Fig. 3.

The representative MRM chromatogram of dapagliflozin is depicted in Fig. 4, The retention time was found to be 1.45 min.

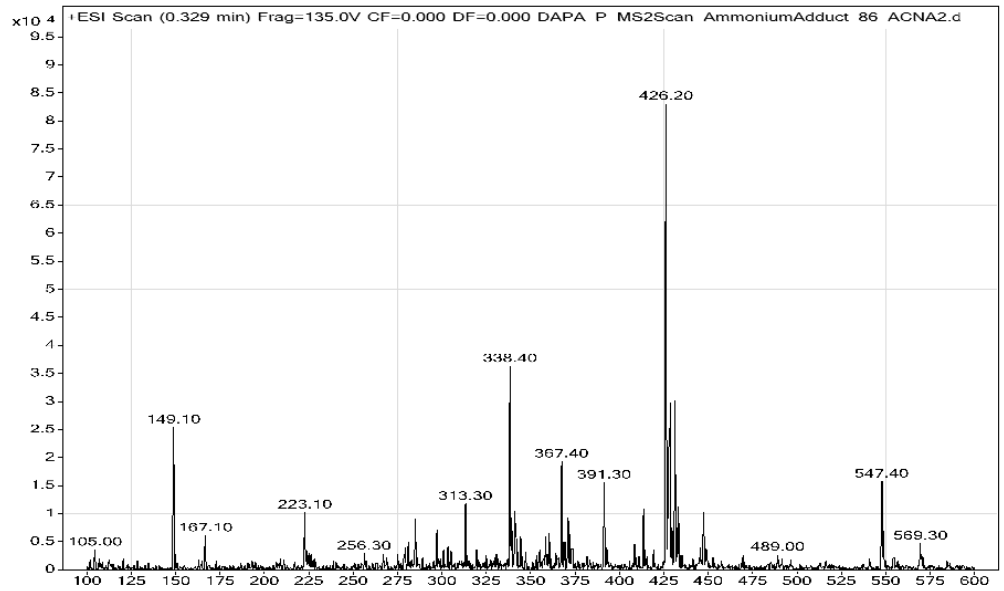

Fig. 2. Full scan mass spectra of dapagliflozin.

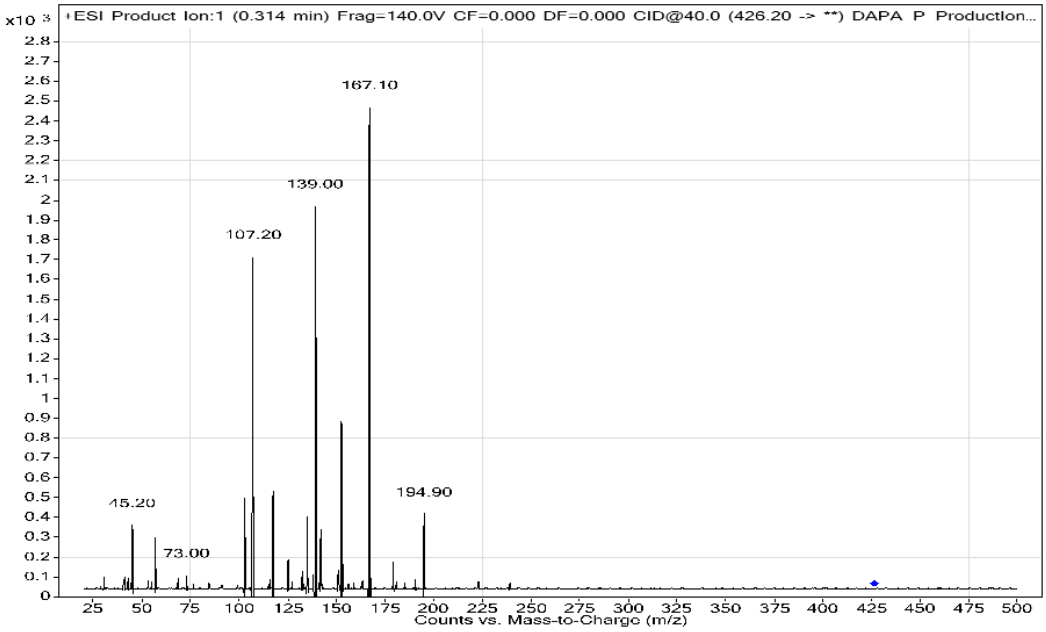

Fig. 3. Product ion mass spectra of dapagliflozin.

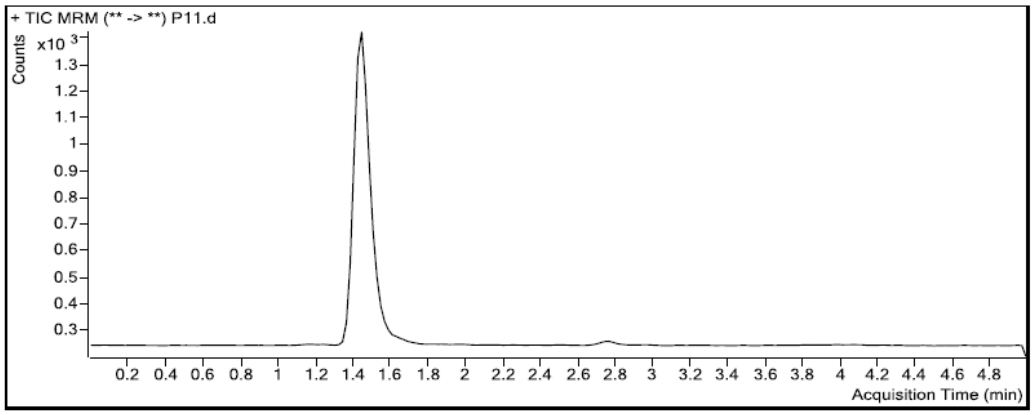

Fig. 4. MRM chromatogram of dapagliflozin**.**

3.1 Method validation

The method was validated as per ICH guidelines and the results of validating parameters are discussed below.

3.1.1 Linearity and range

Calibration curve was obtained by plotting the area under the peak (AUP) against the concentrations. Linearity was found to be acceptable over the concentration range of 25-500 ng/mL.Calibration curve is shown in Fig. 5.

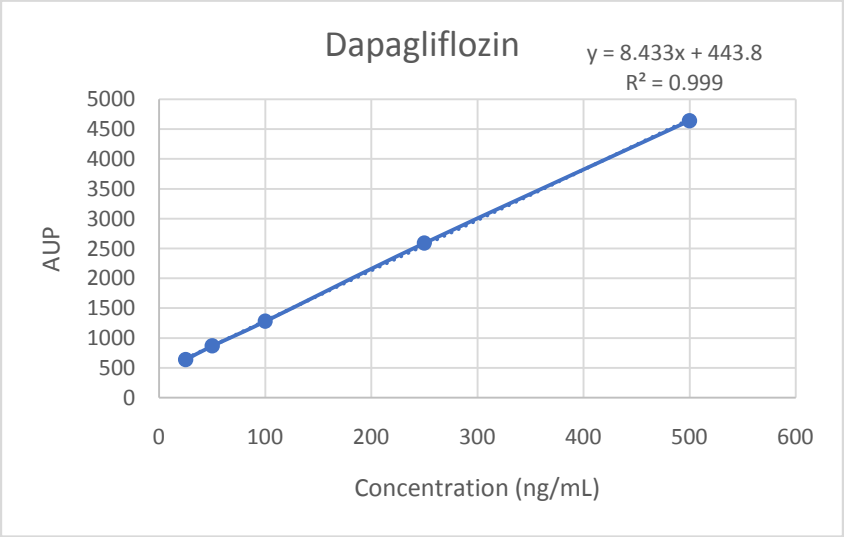

Fig. 5.Calibration curve of dapagliflozin.

3.1.2 Limit of Detection (LOD) and limit of Quantitation (LOQ)

The minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be 6.83 ng/mL and 20.70 ng/mL respectively.The calculation was based on the standard deviation of the response and the slope.

3.1.3 Precision

Precision of the method was determined in terms of repeatability, intra-day and inter-day precision. Repeatability of the method was confirmed by injecting six replicates of 100 ng/mL of Dapagliflozin. Intra-day and Inter-day precision were both performed by selecting three concentrations from the linearity range and analysing them in triplicate, on same day and for three successive days.The required %RSD for precision is ≤2. The method was found to be precise with %RSDmuch lower than 2. Results for repeatability and intra-day and inter-day precision are shown in Table 1 and Table 2respectively.

Table1: Results for repeatability.

S. No.	AUP
1	1278
2	1249
3	1239
4	1287
5	1256
6	1274
Mean	1263.83
Standard deviation	18.65
%RSD	1.48

Table2: Intra-day & inter-day precision results for dapagliflozin.

Conc. (ng/mL)	Intra-day (n=3)		Inter-day (n=3)	
	Avg. area	%RSD	Avg. area	%RSD
50	855.67	1.29	860	1.54
100	1255.33	0.89	1264.14	1.23
250	2567.33	0.85	2572	1.03

3.1.4 Accuracy

Accuracy of the method was studied by spiking the pre-analysed conc. at 50%, 100% and 150%, and then the % recovery was calculated.Acceptable % recovery is 100 ±2% and the accuracy achieved in our study was within the specified range indicating that the method is accurate.Data for accuracy is shown in Table 3.

Table3: Results for accuracy

% level	Amount spiked (ng/mL) n=3	Amount recovered (± SD) ng/mL	% recovery
50	50	50.71 (±0.82)	101.41
100	100	99.20 (±0.23)	99.2
150	150	149.49 (±0.52)	99.66

3.1.5 Robustness

Changes in chromatographic conditions such as flow rate (± 1%), mobile phase ratio (± 2%) and column temperature (± 5°C) were studied to determine the robustness of the methodwhich should have %RSD ≤2%. Developed method was robust with a %RSD <1%.Results are presented in Table 4.

Table4: Results for robustness

Parameter	Variation (n=3)	%RSD
Flow	0.202	0.63
Flow	0.198	0.61
Mobile phase	81.6	0.4
Mobile phase	78.4	0.38
Temperature	40	0.62
Temperature	30	0.64

3.1.6 Application on the pharmaceutical dosage form

Applicability of the method on pharmaceutical formulation was determined by performing the assay on the Forxiga 10 mg tablets.The acceptable % assay should be 100 ±2% and the % assay was found to be 100.13%. Thus, it can be successfully applied for the estimation of dapagliflozin in tablets.Results for the assay are shown in Table 5.

Table5: Results for assay of dapagliflozin tablets

Pharmaceutical formulation	Claimed conc. (ng/mL)	Mean (± SD) amount found (n=6)	%Assay
Forxiga 10 mg tablets	100	100.13 (±0.34)	100.13

IV. CONCLUSIONS

The developed LC-MS/MS method for pharmaceutical dosage form analysis is based on positive ion electrospray ionization technique for theestimation of dapagliflozin in tablet dosage form while the previous methods available for the estimation of dapagliflozin were bioanalytical methods which were developed using negative ion electrospray ionization technique.The proposed method was validated for linearity, LOD & LOQ, precision, accuracy and robustness as per ICH guidelines. The developedmethod wasproved to be highly sensitive, accurate and reproducible serving as a sophisticated analytical tool for estimation of dapagliflozin in tablet dosage form.

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Conflicts of interest

Authors declare that there are no conflicts of interest.

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