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RESEARCH ARTICLE

Development and Validation of Dissolution Test Method for Dapagliflozin using RP-HPLC and UV Spectrophotometer

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ABSTRACT

The present work describes development and validation of a dissolution test method for Dapagliflozin tablets. Dapagliflozin is an anti-diabetic drug involves the direct and insulin independent elimination of glucose by the kidney. For the development of dissolution test method for Dapagliflozin, several conditions were evaluated. The dissolution test was performed using type II apparatus, 900ml of 0.1N Hydrochloric acid as dissolution medium and release was monitored for 25min to verify the immediate release pattern of the drug in acidic pH. The % release of drug was analyzed by using UV spectroscopy at 245.6nm and RP-HPLC method. The release was found maximum with agitation speed of 50rpm as compared to 75rpm. The simple RP-HPLC method was developed using Princeton C18 as stationary phase and ACN-0.1%TEA (50:50) as mobile phase. The dissolution test method for Dapagliflozin was validated for the parameters of accuracy, precision, linearity and robustness. The proposed dissolution test method was found adequate and can be applied for the quality control test of Dapagliflozin tablets.

KEYWORDS

Dapagliflozin (DAPA), Dissolution, Validation HPLC, UV Spectroscopy

INTRODUCTION

The dissolution can be defined in a narrow sense as the process by which a solid substance is incorporated into the solvent to form a solution. However, in a broad sense, it is more than a simple measurement of solubility rate and can be better described as physical test to predict the drug release from a dosage form, for a given area for some precise time. Fundamentally, this process is controlled by the affinity between the solvent and the solid substance and the way by which the pharmaceutical system releases the drug [1, 2].

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According to Mehta and coworkers [3] dissolution test provides an indication of bioavailability of a drug and thus, pharmaceutical equivalence from batch to batch. The dissolution test is an important tool in quality control of drugs and it becomes more important for drugs.

Dapagliflozin (DAPA) is a highly selective, orally active and reversible inhibitor of the human Sodium-Glucose Co-Transporter 2 (SGLT2), the major transporter responsible for the renal glucose reabsorption. It's mechanism of action is complementary to and different from the mechanisms of currently available

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anti-diabetic drugs as it involves the direct and insulin independent elimination of glucose by the kidney.

Dapagliflozin selectively block for SGLT2 over SGLT1. It is chemically known as (1s)-1, 5-anhydro-1-C-[4-chloro-3-[(4-ethoxyphenyl) methyl] phenyl]-D-glucitol. The structure of Dapagliflozin as shown in Fig.1.

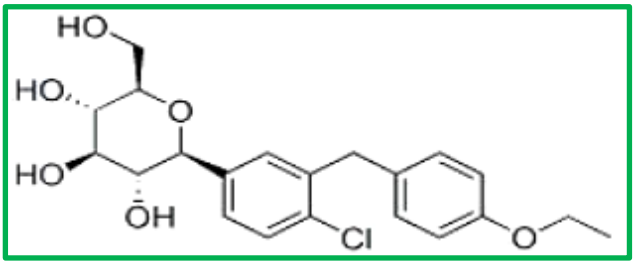

Figure 1: Structure of Dapagliflozin

It has a molecular formula $C_{24}H_{33}ClO_8$ with molecular weight 408.98. Dapagliflozin is a white to half white crystalline powder which is soluble in ethanol, methanol, dimethyl sulfoxide and dimethyl formamide.

The dissolution test is required for various dosage forms for product release testing. It is also commonly used as predictor of the in vivo performance of a drug product. Dissolution testing has emerged in the pharmaceutical field as a very important tool to characterize drug performance.

Literature survey indicated that the drug has been estimated from bulk by RP-HPLC and UV-spectroscopy [11-17]. The proposed work represents application of simple, economical and rapid RP-HPLC and spectroscopic method for the development of dissolution test method of Dapagliflozin. The developed method was validated for accuracy, precision, ruggedness and sensitivity as per ICH guidelines [4,5].

MATERIAL AND METHODS

Chemicals and reagents

Pharmaceutical grade Dapagliflozin (DAPA) standard was obtained as generous gift from Indoco Remedies, Mumbai, India. The commercially available formulation of Dapagliflozin was purchased from local market. Methanol and acetonitrile of HPLC grade, potassium dihydrogen phosphate, ortho phosphoric acid, hydrogen chloride and sodium hydroxide of GR grade were used. The dissolution medium includes 0.1N HCl, Acetate Buffer pH 4.0, Phosphate Buffer pH 6.8 and Phosphate Buffer pH 7.5 were prepared as per the Indian Pharmacopoeia.

Instruments

Dissolution Apparatus: Electrolab, Tablet Dissolution tester: TDT 06P, Lab India Ds1400, HPLC: Shimadzu HPLC series 1100, UV-Spectrophotometer: Jasco V-630, Shimadzu-1700 double beam, Sonicator: PCI Mumbai 3.5L 100H, Spectra lab. UCB-300, pH-meter: GLOBAL Model No. DPH-500, EI, Model No. 1102012 and Weighing Balance: Shimadzu AUX220, RADWAG PS1500.

DEVELOPMENT OF UV-SPECTROPHOTOMETRIC METHOD [11-12,17]

Preparation of Standard stock solution

An accurately weighed about 10.0mg of DAPA was transferred in a 10.0ml volumetric flask, dissolved in sufficient quantity of methanol to prepare a stock standard solution of 1000µg/ml of DAPA.

Preparation of working standard solution

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1.0ml of the standard stock solution was diluted to 10.0ml (100µg/ml of DAPA); from this solution further 3.0ml was diluted to 10.0ml with methanol to prepare working standard solution having concentration about 30µg/ml of DAPA.

Selection of wavelength

The working standard solution of DAPA (30µg/ml) was scanned in the UV range of 200-400nm in 1 cm cell against solvent blank (methanol) and spectrum was recorded. The recorded spectrum of DAPA showed peak maxima at 245.6nm. The obtained UV spectrum of Dapagliflozin is shown in Fig.2.

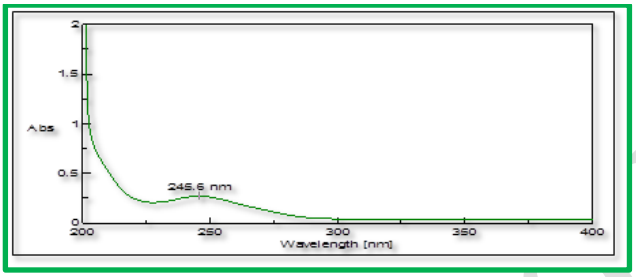

Figure 2: UV spectrum of Dapagliflozin

DEVELOPMENT OF RP- HPLC METHOD [7-10, 16]

Standard stock solution (A)

An accurately weighed quantity of DAPA is equivalent 10.0mg was transferred in a 10.0ml volumetric flask, dissolved in sufficient quantity of diluents to prepare a standard stock solution of 1000µg/ml of DAPA.

Working stock solution (A1)

A 5.0ml of stock solution (A) was transferred in 50.0ml volumetric flask and volume was made upto the mark with mobile phase. (100µg/ml)

Working standard solution (A2)

The working stock solution (A1) was appropriately diluted with mobile phase to get the final concentration of 30µg/ml.

Preparation of mobile phase

The mobile phase was prepared by mixing acetonitrile and 0.1% triethylamine (pH-5.0) in ratio

50:50 %v/v. The prepared mobile phase was sonicated and filtered through 0.45µm membrane filter.

DEVELOPMENT OF DISSOLUTION TEST METHOD

Determination of solubility and sink conditions

Solubility profile was used as the basis for the selection of a dissolution medium for DAPA. Drug solubility was determined at 25°C in different media and expressed as mg/ml. Sink conditions were determined in different media.

Mechanical calibration of dissolution apparatus

Conventionally, for oral solid dosage forms, dissolution Apparatus I or II is suggested by FDA guideline but to satisfy with cGMP requirements mechanical calibration for Apparatus I and II should be carried out.

Optimization of dissolution test

The dissolution studies were performed using a six-station dissolution apparatus by subjecting six commercial formulation in each dissolution medium containing 900ml of dissolution media using both a paddle and basket dissolution

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apparatus and stirring speeds of 50 and 75rpm at temperature 37±0.5°C were tried. Aliquots of 10.0ml were withdrawn manually at intervals of 5, 10, 15, 20 and 25min. The same volume of fresh medium at 37±0.5°C was added to maintain the constant volume. The sample was filtered through whatman filter paper and analyzed by UV and RP-HPLC method.

VALIDATION OF DISSOLUTION METHOD

The proposed dissolution test method was validated for its accuracy, precision, linearity and robustness to demonstrate reproducibility and reliability [11-13, 17].

Linearity

Aliquots of DAPA stock solution (100µg/ml) were diluted to get concentrations in the range of 10-60µg/ml. Each solution was read in triplicate. Calibration curve was plotted as absorbance/AUC Vs concentration.

Precision

Using the optimized dissolution parameters, the test solution was withdrawn from 5min time interval up to 25min. The absorbance and area under curve was noted to estimate the amount of drug release at each time interval using proposed methods. Thus, repeatability was evaluated at the 100% level and the relative standard deviation (RSD) of the data was calculated. The evaluation of intermediate precision was performed by analyzing the sample on different days by different analyst and the %RSD values were calculated.

Accuracy

The accuracy of proposed method was carried out by performing recovery study for DAPA; standard drug substance was added to the dissolution vessels in known amounts at the 80%, 100% and 120% levels. Accordingly, 8, 10 and 12mg of standard drug was added along with 10 mg tablet. Dissolution test was carried out at 25min using 900ml of 0.1N HCl as dissolution medium in paddle type-II apparatus at 50rpm (Temp. 37±0.5°C). Aliquots of 10.0ml were withdrawn at appropriate interval and filtered through whatman filter paper and analyzed by UV and RP-HPLC.

Robustness

The robustness of analytical method is the ability to remain unaffected by small but deliberate variations in method parameters and provide an indication of its repeatability during normal uses. The robustness study was performed for change in flow rate and wavelength.

RESULTS AND DISCUSSION

The saturated solubility of DAPA was determined by using different dissolution media. Using appropriate solvents, working standard solutions of DAPA were prepared of desired concentration.

Optimized chromatographic conditions

In order to achieve the optimized chromatographic condition, one or two parameter modified at each trial and

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chromatograms were recorded with all specified chromatographic conditions. The various mobile phases were tried to select the most suitable one by changing flow rate, buffer and its pH. The optimized chromatographic conditions are shown in Table 1.

System	Shimadzu HPLC series 1100
Stationary Phase	Princeton C-18-4E (5µm), 250 x4. Mm
Mobile phase	Acetonitrile - 0.1Triethylamine (50:50 % v/v)
Detection wavelength	254.6 nm
Flow rate	1ml/min
pH	1.2
Injection volume	20µl

Table 1: Optimized chromatographic parameters

The chromatographic conditions were set as per final chromatographic conditions; mobile phase was allowed to equilibrate with stationary phase indicated by steady baseline. The mobile phase containing Acetonitrile - 0.1Triethylamine (50:50 % v/v) gave well resolved peak and reasonable retention time as shown in Fig.3.

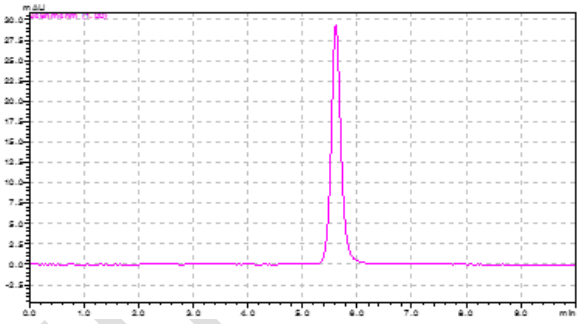

Figure 1: Chromatogram of standard DAPA

System suitability parameters

The system suitability study was performed by injecting six replicate injections of standard DAPA (30µg/ml) were injected and chromatographed. The results obtained indicate that proposed method was suitable for further experimentation. The results of system suitability parameters are shown in Table 2.

Sr. No.	Wt. of Std. drug taken (mg)	Area (µV)
1.	10.03	9813465
2.		9820122
3.		9916110
4.		9819154
5.		9822461
6.		9634056
	Mean	9804221.5
	± SD	9205.54
	% RSD	0.94
	Retention time	5.615
	Tailing factor (Asymmetry)	0.956
	Theoretical Plate	16720.50

Table 2: Results of system suitability parameters

Drug substance solubility study

Solubility study of DAPA was carried out by using different dissolution media. The prepared solution was sonicated, filtered and analyzed by UV spectroscopy to determine the solubility of the drug in respective dissolution media. The graph was plotted between pH of dissolution media and observed solubility. The drug was found highly soluble in 0.1N hydrochloric acid followed by Phosphate buffer of pH 6.8.

Optimization of dissolution parameters

Various dissolutions were performed to optimized dissolution parameter. For maximum percent release of drug, several trials were taken by using USP Apparatus I and II, i.e., Basket and paddle type at different rpm 50 and 75. Based on the solubility of DAPA, 0.1N HCl was selected as suitable dissolution media as compared to Phosphate buffer pH 6.8 and Acetate buffer pH 4.0.

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Dissolution media (0.1 N HCl) at 50rpm	Time points % release (min)				
	5	10	15	20	25
USP type- I (Basket)	6.59	21.59	33.43	56.13	68.06
USP type- II (Paddle)	15.63	39.97	58.05	78.13	89.20

Table 3: The % release of drug using USP type–I basket apparatus

From above results of Table 3, it was observed that the release of drug in USP type-I (Basket) apparatus, shows minimum release at first time point while using type-II apparatus shows maximum drug release. Using type-II apparatus, proper profiling for drug release was observed (Table 4).

Dissolution media (0.1 N HCl) at 75rpm	Time points % release (min)				
	5	10	15	20	25
USP type- II (Paddle) Apparatus	12.37	28.41	34.29	62.78	85.16

Table 4: Results showing effect of change in speed of rotation on DAPA

It was also observed that the release of drug at first time point was found minimum when the speed of rotation was changed from 50 to 75rpm. The following dissolution parameters have been finalized for the estimation of DAPA as depicted in Table 5.

Drug	Dissolution media	Media volume	USP Apparatus	RPM
DAPA	0.1N HCl	900ml	Type-II Paddle	50

Table 5: Final optimized dissolution conditions

Linearity

Pipette out 1.0ml to 6.0ml from the working stock solution (100µg/ml) and diluted upto10.0ml in volumetric flasks. Absorbance of each solution and peak area was noted. Calibration curve was plotted as absorbance/AUC verses concentration. The obtained result shows linearity between concentration and absorbance as shown in Fig. 4.

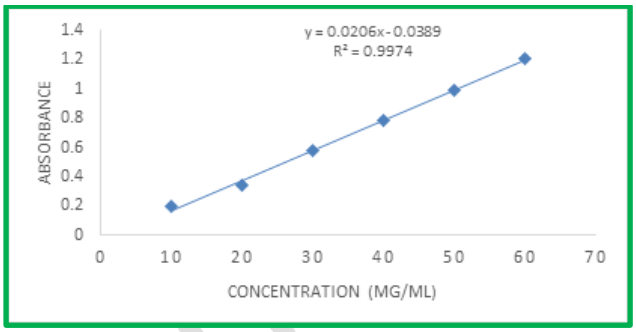

Figure 4: Linearity plot of DAPA

Percent release of Dapagliflozin

The test solution was obtained by performing the dissolution of drug under finalized dissolution parameters. The six replicates of test solution of drug so obtained were chromatographed, % RSD of drug was calculated by proposed method and results are recorded in Table 6.

Test	Peak Area (µV)	% dissolution of DAPA at 25 min	
		RP-HPLC	UV
1.	9524485	89.33	87.89
2.	9522470	85.58	88.26
3.	9523478	87.99	88.06
4.	9523459	86.23	86.50
5.	9523470	86.58	89.11
6.	9521302	86.89	88.12
Average	952311.66	86.25	87.99
%RSD	**0.01**	**1.38**	**1.24**

Table 6: Observations and results of precision study

The proposed methods were found to be precise with %RSD less than 2 and recorded chromatograms as shown in Fig. 5(a), 5(b) and 5(c).

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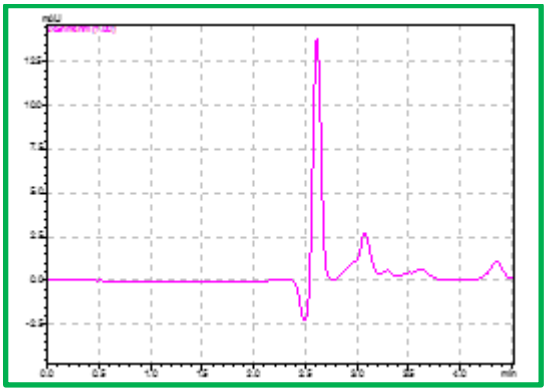

Figure 5 (a): Chromatogram for blank

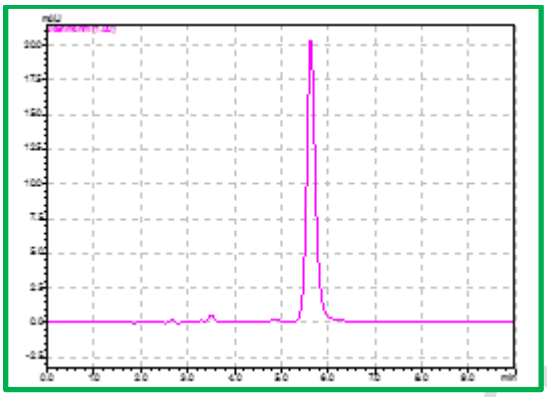

Figure 5 (b): Chromatogram of Standard

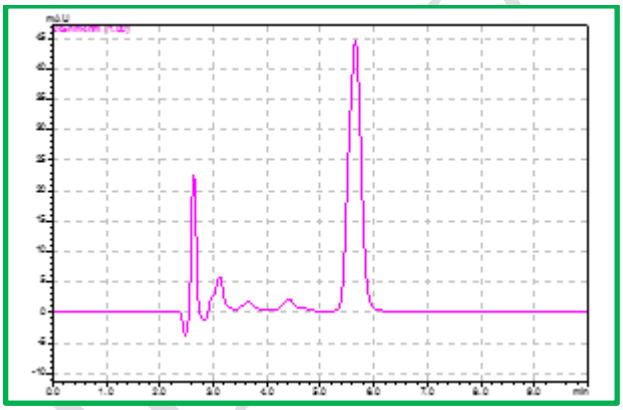

Figure 5 (a): Chromatogram of sample (25min)

Intermediate precision

Estimation of DAPA in marked formulation analyzed by proposed methods had yield quit concurrent results, standard deviation and %RSD of series of measurement were found to be within limit (Not more than 2%). The dissolution test precision (Intraday and interday) results by UV spectroscopic method are shown in Table 7 and 8.

Average % release ± SD (n=3)				
Sr. No.	**Time (min)**	**10:00am**	**01.00pm**	**04.00pm**
1.	5	15.54±0.068	16.56±0.072	17.25±0.074
2.	10	39.24±0.1440	40.15±0.01527	42.54±0.1652
3.	15	58.11±0.2110	60.02±0.2209	59.04±0.2108
4.	20	78.26±0.2811	76.36±0.2820	78.17±0.2845
5.	25	86.26±0.3072	89.11±0.3101	88.06±0.3082
		Average at 25 min		87.31±0.3085
		% RSD at 25 min		1.66

Table 7: Dissolution test precision (Intraday) results

Average % release ± SD (n=3)				
Sr. No.	**Time (min)**	**1st day**	**2nd day**	**3rd day**
1.	5	17.96±0.079	15.54±0.068	16.84±0.074
2.	10	40.33±0.1543	37.66±0.1443	42.13±0.1652
3.	15	60.04±0.2212	58.05±0.2110	57.03±0.2337
4.	20	79.21±0.2852	74.73±0.2701	70.97±0.2512
5.	25	89.27±0.3142	86.14±0.3067	85.38±0.3094
		Average at 25 min		87.86±0.3115
		% RSD at 25 min		1.56

Table 8: Dissolution test precision (Interday) results

The % RSD for dissolution of the test sample of DAPA was found to be 1.66 and 1.56 which is within the acceptance limit.

Accuracy of test method

The accuracy study for Dapagliflozin was demonstrated by adding standard drug substance to the dissolution vessel in known amounts at the 80%, 100% and 120% levels. Accordingly about 11.2mg, 14mg and 16.8mg of reference drug was added along with 10mg tablet. Dissolution test was performed for 25min using 900ml of 0.1N HCl as a

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dissolution medium in a paddle type at 50rpm. Aliquots of 10ml were withdrawn and filtered through whatman filter paper and analyzed by proposed methods. The results of recovery study are shown in Table 9.

**Each value is mean of three observations*

Level (%)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery*
80	8.1	7.94	98
100	10	9.94	99.5
120	12.53	12.73	101.6
		Mean	99.7
		± SD	1.25
		%RSD	1.37

Table 9: Results of recovery study

The data indicated that %RSD values and the mean % recovery was found under acceptance criteria.

Robustness of test method

The robustness of the proposed method was evaluated by change in analyst and instrument using optimized dissolution parameters. The results of robustness study are shown in Table 10-11.

Sr. No.	Time (min)	Analyst I	Analyst II
1.	5	17.96±0.079	16.84±0.074
2.	10	40.33±0.1543	37.66±0.1443
3.	15	60.04±0.2212	63.57±0.2337
4.	20	79.21±0.2852	74.73±0.2701
5.	25	89.21±0.3184	88.37±0.3094
		Avg % release at 25 min	88.79±0.3139
		% RSD	1.25

Table 10: Results of robustness study (change in Analyst)

Sr. No.	Time (min)	Equipment I	Equipment II
1.	5	15.54±0.068	17.25±0.074
2.	10	39.24±0.1440	42.54±0.1652
3.	15	58.11±0.2110	59.04±0.2108
4.	20	78.26±0.2811	78.17±0.2845
5.	25	86.26±0.3072	88.06±0.3082
		Avg % release at 25 min	87.16±0.3077
		% RSD	1.663

Table 11: Results of robustness study (change in Equipment)

The %RSD of dissolution study of DAPA was found to 1.25 and 1.663, which is within acceptance. Also, robustness study by RP-HPLC method was carried out via deliberate change in flow rate (±0.2ml/min) and detection wavelength (±5nm). The overall %RSD for the deliberate variations was found within the range.

CONCLUSION

The proposed dissolution test method was developed and validated as per the ICH guidelines for Dapagliflozin by using UV spectrophotometer and RP-HPLC method. The results obtained by proposed methods were found to be reliable, accurate and precise. Hence, the developed methods can be employed for routine dissolution analysis of Dapagliflozin tablets.

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CONFLICT OF INTEREST

None

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