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METHOD DEVELOPMENT AND VALIDATION OF DAPAGLIFLOZIN IN BULK AND PHARMACEUTICAL DOSAGE FORM BY UV-SPECTROSCOPIC METHOD

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Abstract:

A simple, sensitive, highly accurate spectrophotometric method has been developed for the determination of Dapagliflozin in bulk and pharmaceutical tablet dosage form as per ICH Guidelines. The adequate drug solubility and maximum assay sensitivity was found in sodium citrate. The absorbance of Dapagliflozin was measured at 231 nm in the wavelength range of 200-400 nm. Beer's law was obeyed in the concentration range of 10-50 µg/mL, in the linearity study regression equation was found to be y =0.2125x+0.0085 & correlation coefficient was found to be 0.999. This method was Rugged and Robust in different testing criteria, LOD and LOQ was found to be 2.19µg / ml & 6.641 µg / ml respectively. Accuracy study was done in 3 different concentration levels 50, 100, 150% & % recovery of the method was found to be 99.8%, 99.7%, 99.8% respectively in 3 different levels & mean recovery was 99.7%, so method was accurate. Results of all validation parameter were within the limit as per ICH guideline. Results of percentage recovery shows that the method was not affected by the presence of common excipients. The proposed method has been successfully used for the analysis of the drug in pure and its tablet dosage forms. easily and the method was precise accurate to perform in future

Keywords: *Dapagliflozin, UV Visible Spectrophotometry, Method development, Validation, ICH guidelines, Sodium citrate , Accuracy, Precision.*

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INTRODUCTION:

Dapagliflozin is a Anti -Diabetic , molecular formula $C_{21}H_{25}Cl\ O_6$, Molecular Weight : 408.873 mol , IUPAC Name (2S,3R,4R,5S,6R)-2-(4-Chloro-3-(4-ethoxybenzyl)phenyl)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol Mechanism of Action of drug involves a competitive inhibitor of the sodium-glucose transport subtype 2 protein, dapagliflozin blocks glucose reabsorption into the kidney, resulting in the elimination of blood glucose through the urine.

According to literature review [1-11] there are very few methods reported for the determination of in different Instrumental techniques, out of these methods only 1 method were reported in Single Drug by using UV spectroscopic method

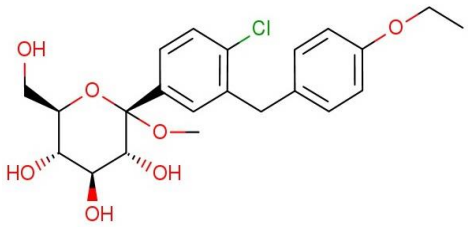

Fig-1 Structure of Dapagliflozin

MATERIALS AND METHODS

Chemicals and Reagents: Methanol, Ethanol, Acetonitrile, Sodium citrate, Potassium Dihydrogen Phosphate, Sodium Phosphate, Ammonium Dihydrogen Phosphate with water.

Instruments:

The Spectroscopic analysis was carried out using Double beam PG Instruments recording UV-Visible Spectrophotometer (SHIMADZU UV-1601**)** with 1mm path length matched quartz cells was used for analytical purpose.

Reagents and Solutions

Diluent preparation: In a 100ml volumetric flask take 50ml Sodium Citrate and make the volume upto the mark.

Preparation of Standard Solutions

Accurately weighed 100mg of Dapagliflozin was weighed accurately and transferred into 100ml volumetric flask. About 10 ml of diluent was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100µg/ml of Dapagliflozin Working standard solution of Dapagliflozin containing 30µg/ml for method. Finally add those above solutions and prepare the final solution is about 30µg/ml.

Preparation of Sample Solutions.

Take 10 Tablets average weight and crush in a mortar by using pestle and weight powder 100 mg equivalent weight of Dapagliflozin sample into a 100ml clean dry volumetric flask, dissolve and make up to volume with diluent. Further dilution was done by transferring 0.3 ml of the above solution into a 10ml volumetric flask and make up to volume with diluent.

Determination of wavelength of maximum absorbance for Dapagliflozin

The absorbance of the final solution scanned in the UV spectrum in the range of 200 to 400nm against solvent mixture as blank.

Optimization Optimization of selection of Solvent

It is well known that the solvents do exerts a profound effect on the quality and the shape of the peak. The choices of solvents for UV method development are: Methanol, Ethanol, , Acetonitrile, Sodium citrate, Potassium Dihydrogen Phosphate, Sodium Phosphate , Ammonium Dihydrogen Phosphate etc. First optimize the different solvents. From that solvents Sodium Citrate satisfied the all the optimized conditions.

Wavelength Selection

The standard solutions are prepared by transferring the standard drug in a selected solvent or mobile phase and finally diluting with the same solvent or diluent. That prepared solution is scanned in the visible wavelength range of 200-400nm.This has been performed to know the maxima of Dapagliflozin. While scanning the Dapagliflozin solution we observed the maxima at 231 nm. The visible spectrum has been recorded on (SHIMADZU UV-1601 make UV – Vis spectrophotometer model UV-1601.The scanned visible spectrum is attached in the following page. The λ_{max} of the Dapagliflozin was found to be 231 nm in diluents as solvent system.

METHOD VALIDATION

1. Accuracy: Recovery study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of pure drug of Dapagliflozin were taken and added to the pre-analyzed formulation of concentration 30µg/ml. From that percentage recovery

values were calculated. The results were shown in Table-1.

2. Precision:
Repeatability
The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Dapagliflozin (API) the percent relative standard deviations were calculated for Dapagliflozin is presented in the Table-2.

Intermediate Precision:
Intra-assay & inter-assay:
The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Dapagliflozin revealed that the proposed method is precise. The results were shown in Table-3 and 4.

3. Linearity & Range: The calibration curve showed good linearity in the range of 10-50µg/ml, for Dapagliflozin (API) with correlation coefficient (r^2) of 0.999 (Fig-2). A typical calibration curve has the regression equation of y = 0.2125x+0.0085 for Dapagliflozin.

Standard solutions of Dapagliflozin in the concentration range of 10 µg/ml to 50 µg/ml were obtained by transferring (,10,20 30,40 and 50 ml) of Dapagliflozin stock solution (100ppm) to the series of clean & dry 10 ml volumetric flasks. The volumes in each volumetric flask were made up with the solvent system and mixed.

The absorbances of the solutions were measured at 231 nm against the solvent system as blank and calibration curve is plotted. The Lambert-Beer's Law is linear in concentration range of 10 to 50 µg/ml at 231 nm for Dapagliflozin the results were shown in Table-5.

4. Method Robustness:
Robustness of the method was determined by carrying out the analysis under different Wavelength i.e. at 229nm,231nm and 233nm.. The respective absorbances of 10µg/ml were noted SD < 2%) the developed UV-Spectroscopic method for the analysis of Dapagliflozin (API). The results were shown in Table-6.

5. LOD & LOQ:
The LOD and LOQ were calculated by the use of the equations LOD = 3.3 × σ / S and LOQ = 10 × σ / S where σ is the standard deviation of intercept of Calibration plot and S is the average of the slope of the corresponding Calibration plot.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 2.19µg/ml and 6.641 µg/ml respectively. the results were shown in table -7

Recovery parameters table -8

6. ASSAY OF DAPAGLIFLOZIN IN DOSAGE FORM:

DAPAGLIFLOZIN 10mg

Assay of marketed tablet formulation Brands:

Dapagliflozin was procured from the local market as tablets of strength having 10mg, marketed with brand names of Forxiga. when referring to the generic drug name Dapagliflozin These marketed formulations were manufactured by the Sun Pharmaceuticals, respectively.

Weighed accurately about ten tablets and calculate the weights of individual tablets and finally calculate the average weight. They were triturated to fine powder by using a mortar and pestle. The powdered tablet equivalent to 5mg of Dapagliflozin was dissolved in 15ml of diluent with the help of sonication process and the final volume was made upto the mark with the diluent in 25 ml volumetric flask. The resulted solution was filtered using whatman filter paper (0.45µm). This final solution was further diluted to obtain 10µg/ml concentration of the solution by using diluents used as a solvent and observed by UV analysis. This procedure was repeated in triplicate.

The data are shown in Table-6.

$$\text{Amount Present} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times \frac{\text{Standard Dilution}}{\text{Sample Dilution}} \times \frac{\text{Potency}}{100} \times \text{Average weight}$$

$$\%\text{Content} = \frac{\text{Amount Present}}{\text{Label Claim}} \times 100.$$

RESULTS AND DISCUSSION:

The standard solutions of Dapagliflozin in Sodium citrate with Water (10µg/ml) subjected to a scan individually at the series of wavelengths of 200 nm to 400 nm. Absorption maximum of Dapagliflozin was found to be at 231 nm. Therefore, 231 nm was selected as λ_{max} of Dapagliflozin for the present study. The calibration curve of Dapagliflozin was found to be linear in the range of 10 to 50µg/ml at 231 nm.

Therefore, it was clear that Dapagliflozin can be determined without interference of any irrelevant substance in single component pharmaceutical products. The used technique was initially attempted on bulk drugs in their synthetic sample and concentrations were estimated.

The % recovery was carried out at 3 levels, 50%, 100% and 150% of Dapagliflozin standard concentration. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were found to be satisfactory within the acceptable limits as per the content of the label claim for marketed tablet dosage form. The newly developed method was validated according to the ICH guidelines and the method validation parameters.

The developed method was subjected to do the various method validation parameters such as specificity, accuracy, precision, linearity and range, limit of detection and limit of quantification, robustness and ruggedness etc.

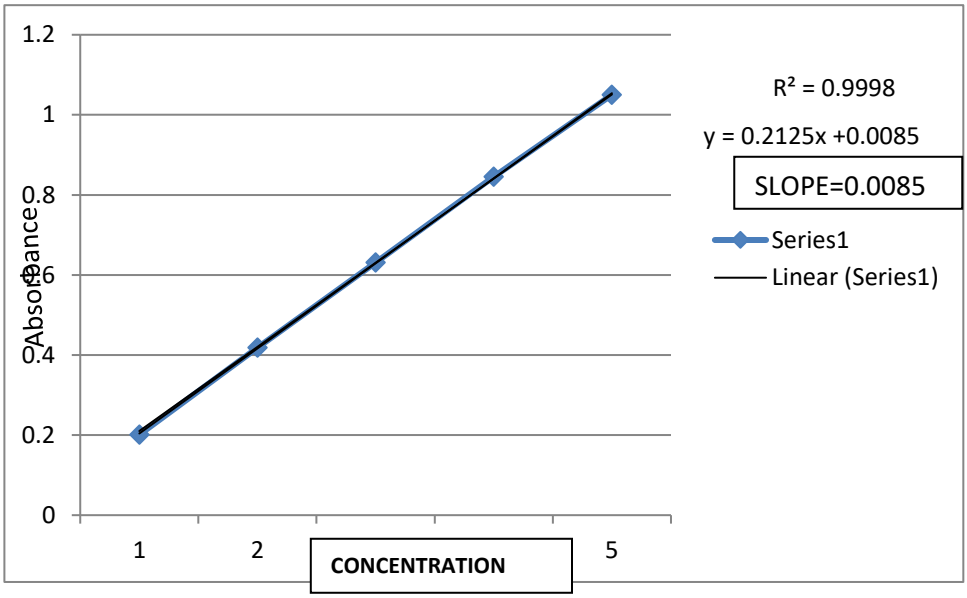

Fig-2: Calibration curve of Dapagliflozin (API).

Table: 1. Shows % Accuracy Recovery of Dapagliflozin

Concentration level	Amount added (mg)	Amount found(mg)	%recovery	Average % recovery
50%	12.5mg 12.5mg 12.5mg	12.51mg 12.48mg 12.48mg	99.9% 99.8% 99.8%	99.8%
100%	25mg 25mg 25mg	24.98mg 24.87mg 24.98mg	99.9% 99.4% 99.9%	99.7%
150%	37.5mg 37.5mg 37.5mg	37.48mg 37.43mg 37.42mg	99.9% 99.8% 99.7%	99.8%

Result: The accuracy for the average of triplicate in each concentration samples are within the limit.

Table: 7. Shows % Recovery of Dapagliflozin

Amount added (mg)	Amount found (mg)	Average % recovery
25 mg	24.98mg	99.7%

2. Precision:

Repeatability: Table-2: Results of Repeatability

Concentration (µg/ml)	Absorbance of Dapagliflozin
30	0.613
30	0.624
30	0.614
30	0.609
30	0.613
30	0.621
Mean	**0.6156**
SD	**0.005645**
%RSD	**0.916991**

INTERMEDIATE PRECISION

a) Intra day

Table: 3. Shows Results of Intra Day

Concentration(µg/ml)	Analyst -1		Anayst-2	
	DAY-1	DAY-2	DAY-1	DAY-2
30	0.613	0.609	0.611	0.603
30	0.619	0.618	0.601	0.609
30	0.611	0.622	0.609	0.621
Mean	0.614	0.616	0.607	0.611
S.D	0.00416	0.00665	0.00529	0.00916
%RSD	0.67	1.07	0.87	1.49

Acceptance criteria: A method is said to be precise if the % RSD is < 2 %, the results show % RSD for the intermediate precision as 0.67-1.49 which are within the limits and hence the method is said to be precise.

b. Inter day

Table: 4. Shows Results of Inter Day

Concentration(µg/ml)	Day -1	
	2hrs	4hrs
30	0.613	0.613
30	0.616	0.614
30	0.618	0.616
Mean	0.615	0.614
S.D	0.0025	0.0015
%RSD	0.406	0.244

LINEARITY

Table-5: Results of Linearity

S.no	Linerty level	Concentration	Area
1	I	10µg	0.201
2	II	20 µg	0.418
3	III	30 µg	0.631
4	IV	40 µg	0.845
5	V	50 µg	1.05
Correlation Coefficient			0.9998
Intercept			Y=0.2125x+0.0085
Slope			0.0085

Acceptance criteria: correlation coefficient should not be less than 0.9990

Table-6: Result of Method Robustness Test
Wavelength

Concentration(µg/ml)	Wavelength	Absorbance	Statistical Analysis
10	229	0.6109	Mean = 0.61091 SD = 0.009168 % RSD = 1.500712
10		0.6102	
10		0.6102	
10	231	0.6114	
10		0.6119	
10		0.6128	
10	233	0.6098	
10		0.6104	
10		0.6106	

Table: 7. Shows LOD & LOQ results of Dapagliflozin

Parameters	Dapagliflozin
LOD	2.19 µg/ml
LOQ	6.641 µg/ml

Table: 8. Shows summary of validation parameter Results

S.NO	Parameter	Acceptance criteria	UV
1	% recovery	92-103%	99.7%
2	Linearity range (µg/ml)	-	10-50(µg/ml)
3	Correlation Coefficient	NLT 0.999	0.999
4	Precision	% RSD (NMT 2%)	0.91
5	Intermediate Precision	% RSD (NMT 2%)	0.67
6	Ruggedness	% RSD (NMT 2%)	0.65
7	LOD	-	2.19(µg/ml)
8	LOQ	-	6.641(µg/ml)

CONCLUSION:

From the experimental studies it can be concluded that best UV-Spectroscopic method is developed for Dapagliflozin in bulk and marketed pharmaceutical dosage form. The developed method for the drug (Dapagliflozin) was found to be accurate and precise.

The great features of spectrophotometric methods are their simplicity, economical and rapidity. The results of method validation showing that the developed analytical procedure is suitable for its intended purpose and meets the Guidelines given by the ICH.

The developed method was successfully applied for the routine analysis of Dapagliflozin in bulk and pharmaceutical dosage form in the future.

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