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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF DAPAGLIFLOZIN IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Dapagliflozin in tablet dosage form. **Methods:** The drug is soluble in Methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in Methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Dapagliflozin in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is soluble in Methanol, slightly soluble in acetonitrile and ethanol and very slightly soluble in water. So, the Methanol is used as a diluent in method. The melting point of Dapagliflozin was found to be 151-152˚C (uncorrected). It showed absorption maxima 240 nm in Methanol. On the basis of absorption spectrum the working concentration was set on 5 µg/ml (PPM). The linearity was observed between 1-9 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 100.9, 101.9 and 98.6% for three levels respectively. The % RSD for precision and ruggedness was found to be 085% and 0.55% respectively. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Dapagliflozin in tablet dosage form. The method could be considered for the determination of Dapagliflozin in quality control laboratories.

KEYWORDS: Dapagliflozin, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Dapagliflozin is supplied as a crystalline solid. Dapagliflozin is inhibiting renal glucose reabsorption through the solid glucose cotranspoter (SGLT) offers an insulin-independent alternative to controlling blood glucose concentrations in patients with type 2 diabetes.[1] Dapagliflozin is a first generation, selective SGLT inhibitor that blocks glucose transport with about 100-fold selective for SGLT2 over SGLT. The Dapagliflozin (DAPA) is an undoable, dynamic and particular inhibitor of sodium-glucose cotransporter 2 (SGLT2).[2] It works by the reabsorption of glucose from the liver, resulting in more glucose excretion in the urine, thereby increasing glycemic control in individual with type 2 diabetes mellitus.[3] It is defined in chemical terms as (1S)-1, 5-anhydro-1-C-[4-chloro-3- [(4-ethoxyphenyl) methyl]-D-glucite. Structure of Dapagliflozin shown in Figure 1. This is an ethanol, methanol, dimethyl-sulfoxide, and dimethyl-formamide soluble white crystalline powder. Dapagliflozin is type as Category III in the Biopharmaceutical Classification System according to the European Medicines Agency being more soluble and almost impermeable.[4,5]

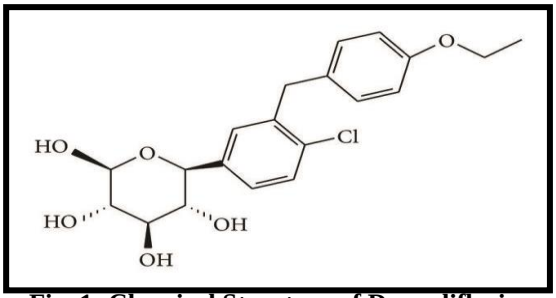

Fig. 1: Chemical Structure of Dapagliflozin.

From literature review it's found that one method was reported on spectrophotometry for simultaneous estimation of Dapagliflozin their combined dosage form.[6, 7, 8] Also the method was reported on LCMS[11] on Dapagliflozin. Lot of work was done on HPLC method development for Dapagliflozin drugs.[9-10] But very few methods were reported on estimation of

Dapagliflozin in tablet dosage form for UV spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Dapagliflozin in tablet dosage forms.

MATERIALS AND METHODS

- **Instruments**

Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance.

Melting point apparatus of Equiptronics were used.

- **Materials**

Dapagliflozin was obtained as a gift sample. Dapagliflozin tablets were procured from local pharmacy. Methanol was used throughout the experiment as a diluent. Freshly prepared solutions were employed.

Method development

A. Determination of ƛ max (8 PPM)[13, 14]

50 mg weighed amount of Dapagliflozin was dissolved into 100 ml of volumetric flask with Methanol. Pipette out 0.8 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with Methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

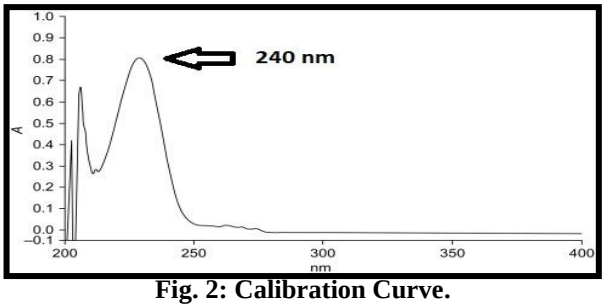

Fig. 2: Calibration Curve.

B. Preparation of Working concentration[14]

Preparation of Standard stock solution

Standard stock was prepared by dissolving 50 mg of Dapagliflozin in 100 ml of Methanol to get concentration of 500 µg/ml (PPM).

Preparation of Standard solution

Pipette out 0.5 ml from standard stock solution and diluted up to 50 ml with Methanol to get concentration of 5 µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with Methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[13]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Dapagliflozin was accurately weighed and transferred into the 100 ml of volumetric flask, added 60 ml Methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with Methanol. Filtered the solution through whatmann filter paper. Pipette out 0.5 ml of the above solution and diluted up to 50 ml with Methanol. The absorbance was measured at 240 nm. The absorbance was recorded:

Table 1: Absorbance of Dosage Form.

MSN Pharmaceutical Limited Empaone® 25 (25 mg)		
Sr. no.	**Sample**	**Absorbance**
1	Blank	0.0001
2	Standard	0.5341
3	Sample	0.5275

Table 2: Dosage Form Specifications.

Type	Company	M.D.	E.D.	Batch No.	Average weight (g)	Assay (%)
1	MSN Pharma LTD Empaone® 25	06/2022	07/2025	CBA 0147	0.124	98.76

E. Method of validation[12-17]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 1 - 9 PPM at 240nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. no.	Sample Concentration	Absorbance
1	1 PPM	0.1514
2	3 PPM	0.3501
3	5 PPM	0.5417
4	7 PPM	0.7624
5	9 PPM	0.9823
Correlation coefficient		**0.999**

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with Methanol and measuring the absorbance at 240nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.81	100.92 ~ 100.9
100	1	1.02	101.86 ~ 101.9
120	1.2	1.18	98.55 ~ 98.6

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the % RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5311
2	Sample Solution 2	0.5245
3	Sample Solution 3	0.5321
4	Sample Solution 4	0.5314
5	Sample Solution 5	0.5225
MEAN		**0.5283**
SD		**0.0045**
% RSD		**0.8463 ~ 0.85**

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.5221	0.5227	97.86	0.5525 ~ 0.55
		0.5232			
2	Analyst 2	0.5251	0.5268	98.62	
		0.5284			

RESULTS

1. Solubility of Dapagliflozin

Solubility test was passed as per criteria.

Table 7: Results for solubility studies.

Sr. no.	Title	Result
1	Methanol	Soluble
2	Acetonitrile and Ethanol	Slightly soluble
3	Water	Very slightly soluble

2. Melting point of Dapagliflozin

The melting point of Dapagliflozin was found to be 151-152˚C (uncorrected).

3. Results for linearity for assay method of Dapagliflozin [Conc Vs Absorbance]

The linearity of method was determined at concentration level ranging from 1 to 9 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.999.**

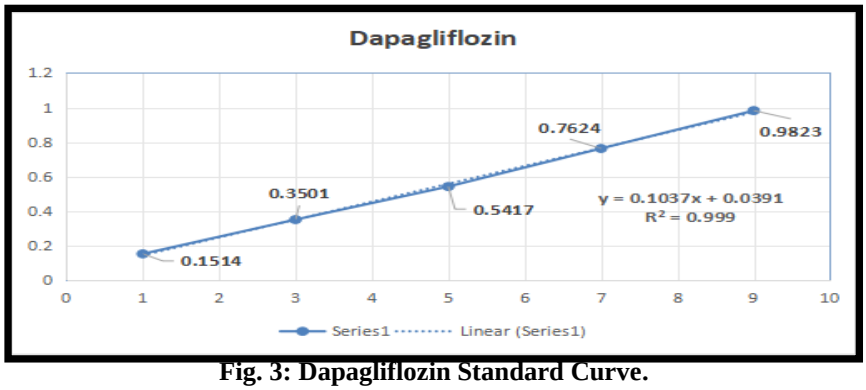

Fig. 3: Dapagliflozin Standard Curve.

4. Results for accuracy for assay method of Dapagliflozin

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Dapagliflozin

The % RSD for different sample of precision was found to be 0.85 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Dapagliflozin

The % RSD for different sample of ruggedness was found to be 0.55 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Dapagliflozin in tablet form has been developed. From the spectrum of Dapagliflozin, it was found that the maximum absorbance was 240 nm in Methanol. A good linear relationship was observed in the concentration range of 1-9 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Dapagliflozin in solid dosage forms. Hence, the method could be considered for the determination of Dapagliflozin in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. SGLT - Solid-glucose Cotranspoter
4. DAPA - Dapagliflozin
5. HPLC - High Performance Liquid Chromatography
6. UV - Ultra violet
7. FDA - Food and Drug Administration
8. NaOH - Sodium Hydroxide
9. ICH - International Council for Harmonization
10. RSD - Relative Standard Deviation
11. SD - Standard Deviation
12. Qty - Quantity
13. C - Celsius
14. M.D. - Manufacturing Date
15. E.D. - Expiry Date

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