ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in
K. Bhavyasri et al /J. Pharm. Sci. & Res. Vol. 12(8), 2020, 1100-1105

A Novel Method Development and Validation of Dapagliflozin and Metormin Hydrochloride using Simultaneous Equation Method by UV– Visible Spectroscopy in Bulk and Combined Pharmaceutical Formulation including Forced Degradation Studies

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Abstract

A new, simple, precise, accurate, reproducible and economic stability indicating spectroscopic method was developed and validated for simultaneous estimation of Dapagliflozin and Metformin in pure and combined pharmaceutical dosage form. The UV spectrophotometric estimation of Dapagliflozin and Metformin was determined using the simultaneous equation method at 222 nm and 232 nm respectively. The linearity ranges for Dapagliflozin and Metformin were 2 – 32 µg/ml and 1 – 20µg/ml respectively with their correlation coefficient values (R2) 0.999. The percentage recovery at various concentration levels varied from 96.82 - 99.8 % for Dapagliflozin and 98.15 to 99.35 % for Metformin confirming that the method is accurate. LOD and LOQ for Dapagliflozin was found to be 0.0241 µg/ml and 0.0293 µg/ml and for Metformin 0.0732 µg/ml and 0.0890 µg/ml. In the precision study, the% RSD value was found to be 0.1845 % and 0.2052 % for Dapagliflozin and Metformin respectively. Degradation studies were performed, both the drugs were found to be degraded in acid, base, peroxide, temperature and light. The proposed method can be applied successfully for the simultaneous estimation of both drugs in quality control laboratories.

Key Words: Dapagliflozin, Metformin, Stability, Simultaneous equation Method.

INTRODUCTION

Metformin (MET) hydrochloride Fig. 1 which chemically known as (3-(diamino methylidene)-1, 1-dimethylguanidine; hydrochloride. It has molecular formula of $C_4H_{11}N_5$ and molecular weight is 165.62 g/mol. Metformin is an oral anti-hyperglycemic agent (Type 2 diabetes) belongs to class of biguanides and useful for treating non-insulin-dependent diabetes mellitus. It decreases blood sugar levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by AMP-activated protein kinase, a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats. [1]

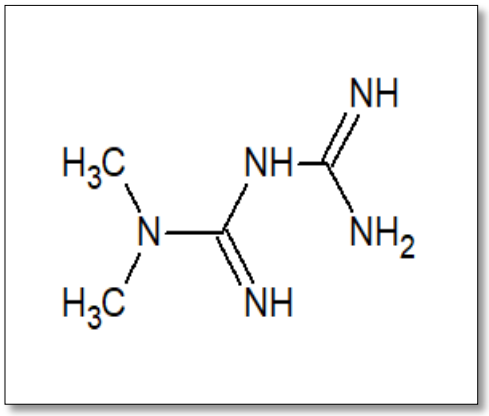

Fig: 1 Structure of Metformin

Dapagliflozin (DAPA) Fig. 2 is an antidiabetic drug. Its chemical name is (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-(4-ethoxybenzy) phenyl]-6- (hydroxymethyl) tetrahydro-2H-pyran-3, 4, 5-triol.It acts as SGLT-2 inhibitor. Inhibition of this enzyme system reduces the rate of digestion of carbohydrates. [2]

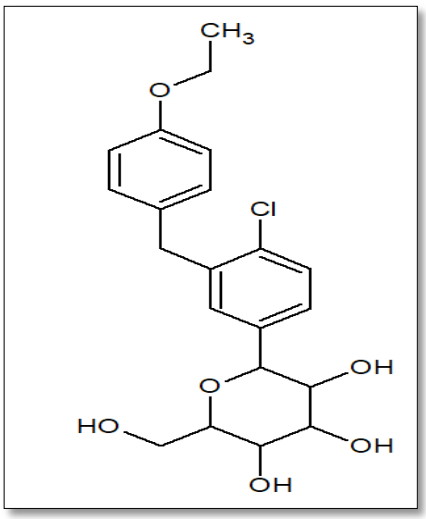

Fig: 2 Structure of Dapagliflozin

A literature survey has revealed that only few articles on UV spectroscopic method for the simultaneous estimation of Dapagliflozin and Metformin. [1-6]

AIM OF THE WORK

The aim of our work was development of new, stability indicating UV method for determination of Dapagliflozin and Metformin HCL which possess the following advantages when compared to the already

existing UV methods which is simple, cost-effective, and economic. The main target for our new developed method is estimation of Dapagliflozin and Metformin HCL in the Pharmaceutical dosage forms.

EXPERIMENT

Material and Method

Spectrophotometric measurements were made in (ELICO) Double beam SL 210 UV-Visible spectrometer with 0.5 cm quartz cells. Drug Dapagliflozin and Metformin HCL were kindly supplied as a gift sample from laboratory.

Solubility

Solubility of drugs 10mg of Dapagliflozin and Metformin HCL of each was weighed and solubility of these samples was checked in water, methanol and acetonitrile. Both the drugs were found to be soluble in water.

Selection of wavelength

Scan the standard solutions in UV spectrophotometer between 200 nm to 400 nm on spectrum mode, using water as a blank. The two drugs show λ max at 222 nm and 232 nm for Dapagliflozin and Metformin HCL respectively.

Preparation of standard drug solution

10 mg of Dapagliflozin and Metformin HCL was accurately weighed separately and dissolved in 5 ml diluent (Water), then transferred into a 10 ml volumetric flask, sonicated it for 10 min, finally, volume was made up to the mark with the same solvent to make 1000 µg/ml stock solution. From this 0.1 ml was again diluted to 10 ml to get a concentration of 10 µg/ml solution. It was scanned in UV range [200-400 nm] in 1.0 cm cell against solvent blank. The spectrum of drugs was recorded. After the study of spectrum of drugs the λ max of Dapagliflozin was found to be 222 nm and the absorbance was found to be 0.6498 and the λ max of Metformin was found to be 232 nm and the absorbance was found to be 0.9679.

METHOD VALIDATION

Method validation is defined as the process that confirms the analytical procedure employed for a particular test is suitable for its intended use. Validation assures that a measurement process produces valid measurements. Results from method validation are used to judge the quality, reliability of analytical results. It is an integral part of any good analytical practice. The proposed method was validated for the parameters like linearity, accuracy and robustness as per ICH guideline. [7]

Accuracy

Accuracy indicates the deviation between the mean and true value. The accuracy is the closeness of agreement between the true value and test result. Accuracy was determined by means of recovery experiments. Solution containing known concentration of Dapagliflozin and Metformin HCL was used for this purpose. The accuracy was assessed from the test results as the percentage of the drug recovered by the assay at 3 levels.

Linearity

The linearity of an analytical method is its ability to elicit that test results are proportional to the concentration of drug in samples within a given range. Linearity of the method was determined by constructing calibration curves by taking. Standard solutions Dapagliflozin and Metformin HCL of different concentrations level (1mcg - 20mcg\ ml) and (2 - 36 mcg/ml) respectively were used for this purpose. Each absorbance was plotted against the concentrations to obtain the calibration curves and correlation coefficients. Characteristic parameters for regression equation (y = mx+ c) of the method were obtained by least squares treatment of the results and these parameters were used to confirm the good linearity of the method.

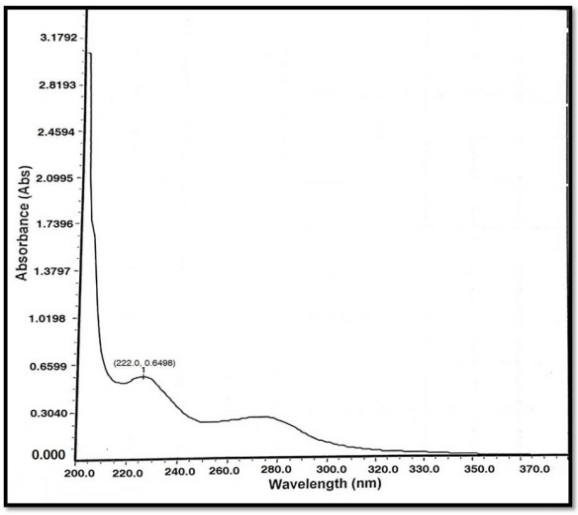

Fig: 3 Spectrum of Dapagliflozin

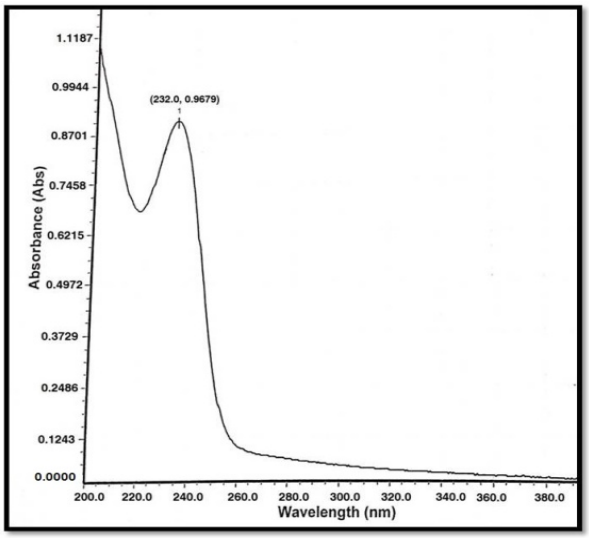

Fig: 4 Spectrum of Metformin

Table: 1 Accuracy of Dapagliflozin

ACCURACY LEVEL	SAMPLE CONCENTRATION (PPM)	STANDARD CONCENTRATION (PPM)	DRUG CONCENTRATION (PPM)	%RECOVERY	% MEAN
50%	10	5	15	102.16% 99.11% 98.13%	99.8%
100%	10	10	20	98.55% 95.44% 96.46%	96.82%
150%	10	15	25	98.70% 99.53% 99.71%	99.31%

Table: 2 Accuracy of Metformin

ACCURACY LEVEL	SAMPLE CONCENTRATION (PPM)	STANDARD CONCENTRATION (PPM)	DRUG CONCENTRATION (PPM)	%RECOVERY	% MEAN
50%	5	2.5	7.5	98.23% 99.20% 100.61%	99.35%
100%	5	5	10	98.03% 98.92% 97.49%	98.15%
150%	5	7.5	12.5	95.08% 94.39% 95.06%	98.85%

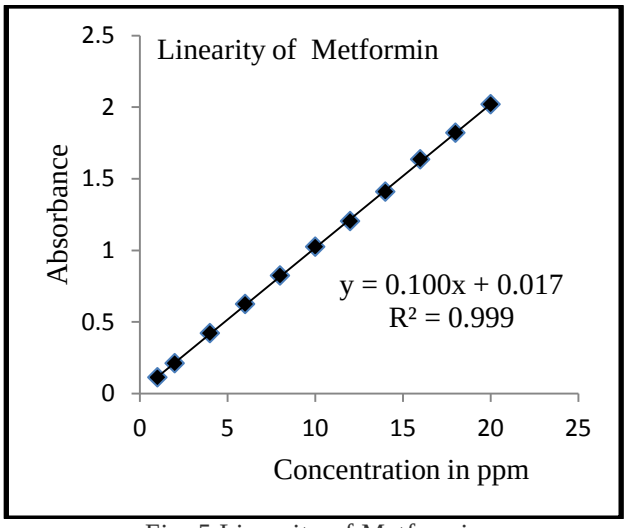

Fig: 5 Linearity of Metformin

Table: 3 Data of Linearity of Metformin

Concentration(ppm)	Absorbance at 232 nm
1	0.1132
2	0.2116
4	0.4215
6	0.6251
8	0.8243
10	1.025
12	1.2035
14	1.4098
16	1.6342
18	1.8213
20	2.0198

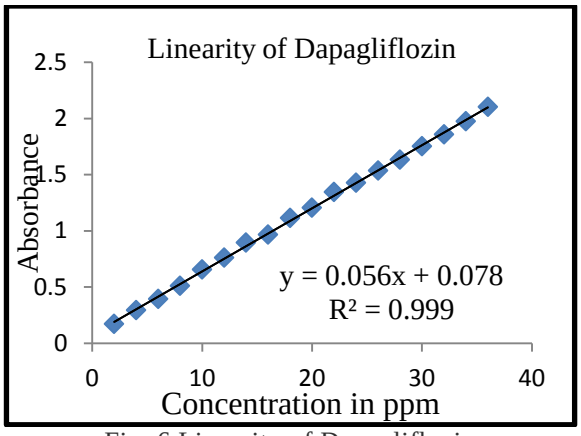

Fig: 6 Linearity of Dapagliflozin

Table: 4 Data of Linearity of Dapagliflozin

Concentration (ppm)	Absorbance at 222 nm
2	0.1725
4	0.2934
6	0.3942
8	0.5123
10	0.6563
12	0.7624
14	0.8946
16	0.9654
18	1.1145
20	1.2065
22	1.3458
24	1.4268
26	1.5368
28	1.6321
30	1.7524
32	1.8564
34	1.9751
36	2.1024

Precision

Precision was estimated by studying repeatability by injecting 10 ppm concentration of Dapagliflozin and Metformin. The results were calculated as standard deviation, relative standard deviation and shown in Table 5.

Table: 5 Data for Precision

S.NO	Dapagliflozin	Metformin
1	0.5264	0.9963
2	0.5268	0.9953
3	0.5263	0.9965
4	0.5269	0.9946
5	0.5265	0.9998
6	0.5266	0.9996
Standard deviation	0.000787	0.00184
%RSD	0.1845	0.2052

Limit of detection (LOD)

LOD is defined as the lowest level of concentration of analyte that can be detected, though not necessarily quantitated. It can be calculated from the below formula, and the results are shown in Table 6.

LOD = 3.3 σ/S

Where,

σ = Standard deviation of the response,

S = Slope of calibration curve.

Limit of quantization (LOQ)

LOQ is defined as the lowest concentration of analyte that can be determined with acceptable accuracy and precision when the specified procedure is applied. It can be calculated from the below formula, and the results are shown in Table 6.

LOQ = 10 σ/S

Where,

σ = Standard deviation of the response,

S = Slope of calibration curve.

Table: 6 Data for LOD & LOQ

Drug Name	LOD	LOQ
Dapagliflozin	0.0241	0.0732
Metformin	0.0293	0.0890

Robustness

It is the capacity of the method to remain unaffected by small but deliberate variations in method parameters. The analysis was performed by slightly changing the wavelength. To determine the robustness at +1 nm and -1nm from the fixed wave length. The results were calculated as % RSD Table 7 & 8 .10 ppm solutions of both the samples are used for the analysis.

Table: 7 Evaluation data for Dapagliflozin Robustness study.

S.NO	221 nm	222 nm	223 nm
1	0.6479	0.6580	0.5499
2	0.6475	0.6582	0.5498
3	0.6478	0.6792	0.5588
Standard deviation	0.0002	0.0005	0.00516
%RSD	0.0008	0.0008	0.0079

Table: 8 Evaluation data for Metformin Robustness study.

S.NO	231 nm	232 nm	233 nm
1	0.9955	0.9952	0.9952
2	0.9950	0.9959	0.9955
3	0.9954	0.9958	0.9954
Standard deviation	0.00083	0.00038	0.00017
%RSD	0.00083	0.0381	00.0017

Assay of tablets formulation

For estimation of drugs in the commercial formulations, twenty tablets were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drugs, quantity of powder equivalent to 10 mg equivalent to Dapagliflozin and Metformin was transferred to 10 ml volumetric flask and dissolved in sufficient quantity of water. It was sonicated for 10 min and volume was made up to obtain a stock solution 1000µg/ml of Sample. Further dilutions were made from this stock solution to get 10µg/ml. The concentration of Dapagliflozin and Metformin was determined by measuring absorbance of sample solutions at 222 nm (λ_{max} of Dapagliflozin) and232 nm (λ_{max} of Metformin) using simultaneous equation. The results of analysis for the marketed tablet formulation (OXRAMET which contains 10 mg of Dapagliflozin and 500 mg of Metformin) are reported in Table 9.The amount of Dapagliflozin and Metformin was calculated using simultaneous equation method given below.

C_x = A_2ay_1-A_1ay_2/ax_2 ay_1-ax_1ay_2

C_y = A_1ay_2- A_2ax_1/ax_2ay_1-ax_1ay_2

A_1 = absorbance of formulation at 222 nm.

A_2 = absorbance of formulation at 233 nm.

ax1 = absorptivity of Dapagliflozin at 222 nm.

ax2 = absorptivity of Dapagliflozin at 233 nm.

ay1 = absorptivity of Metformin at 222 nm.

ay2 = absorptivity of Metformin at 233 nm.

C_X = concentration of Dapagliflozin.

C_y = concentration of Metformin.

To determine both the components

By simultaneous equation method. The information required is:

Ø The absorption

To determine both the components

By simultaneous equation method. The information required is:

Ø The absorptivity

Consider a multicomponent system consisting of two components X and Y, each of which absorbs at the λmax of the other wavelength of maximum absorption

Table: 9 Evaluation data for Assay of tablets.

Drug Name	Label claim (mg)	Amount found
Dapagliflozin	10 mg	0.1957 µg/ml
Metformin	500 mg	9.7913 µg/ml

Forced Degradation Studies

To assess the stability indicating property of the developed UV method stress studies were carried out under ICH recommended conditions. [4,11]

Acid Degradation

From the 100 ppm of drug solution , take 1 ml of the drug solution into 10 ml volumetric flask and 1 mL of 1 N HCL was added and was kept for 24 hours. After 24 hours neutralize with 1 ml of 1N NaOH room temperature, and further dilute with water to get concentration of 10 µg/mL and determine its absorbance.

Alkali Degradation

From the 100 ppm of drug solution , take 1 ml of the drug solution into 10 ml volumetric flask and 1 mL of 1N NaOH was added and was kept for 24 hours. After 24 hours neutralize with 1 ml of 1 N HCL room temperature, and further dilute with water to get concentration of 10 µg/mL and determine its absorbance.

Peroxide Degradation

From the 100 ppm of drug solution, take 1 ml of the drug solution into 10 ml volumetric flask and 1 mL of 30% Hydrogen peroxide solution was added and was kept for 24 hours. After 24 hours dilute with water to get concentration of 10 µg/mL and determine its absorbance.

Photolytic Degradation

The bulk sample was exposed to UV light in UV chamber for 2 hrs by placing 10 mg of drugs in closed petridish. The samples were appropriately diluted to get a final concentration of 10 µg/mL solution and were scanned over a range of 400 to 200 nm by placing respective solvents as blank.

Thermal Degradation

The bulk sample was exposed to dry heat 80^{0}C in oven at for 2 hrs by placing 10 mg of drugs in closed petridish. The samples were appropriately diluted to get a final concentration of 10 µg/mL solution and were scanned over a range of 400 to 200 nm by placing the blank solutions and calculate the percentage of degradation.

Table: 8 Percentage of Degradation studies

S.No	Type of Degradation	Dapagliflozin (Series 1)	Metformin (Series 2)
1.	Acid Degradation (0.1N HCl)	18.35 %	15.32%
2.	Acid Degradation(1N HCl)	13.47%	19.64%
3.	Alkali Degradation(0.1N NaoH)	11.82%	13.98%
4.	Alkali Degradation(1N NaoH)	14.74%	14.04%
5.	Photolytic Degradation	11.68%	9.02%
6.	Peroxide Degradation	12.27%	11.95%
7.	Thermal Degradation	4.65%	5.02%

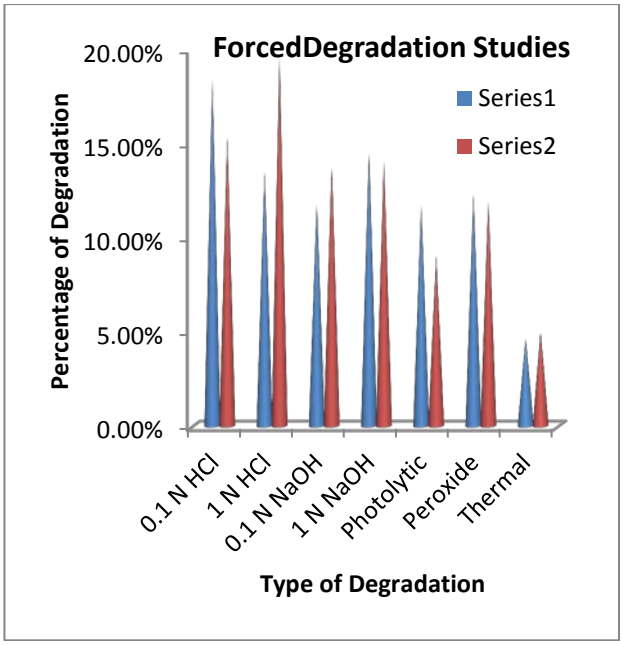

Table: 9 Degradation studies Graph

CONCLUSION

From this validation study we can conclude that the developed UV method is accurate, rapid, precise, reproducible and inexpensive with acceptable correlation co-efficient, accuracy and robustness. The method is versatile for simultaneous determination of Dapagliflozin and Metformin with the use of low cost reagents are the additional benefit of this method. So this method can be used in the quality control department for determination of Dapagliflozin and Metformin.

Acknowledgment

We are very thankful for RBVRR College of Pharmacy, Barkatpura, Narayanguda and Hyderabad for gratis sample of Dapagliflozin and Metformin and also for providing sophisticated equipment and other facilities to complete this work successfully.

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