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Research Article

STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND DAPAGLIFLOZIN IN API AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple and specific stability indicating reversed-phase high-performance liquid chromatography technique has been developed and validated for the concurrent estimation of metformin hydrochloride and dapagliflozin in bulk and pharmaceutical dosage form. The ideal conditions were established for the study or analysis of the drug such as chromatographic separation was carried out on THERMO fisher ODS C_{18} column containing mobile phase of water and acetonitrile 65:35 % v/v of pH 6.8 adjusted with 0.1 % ortho phosphoric acid at a flow rate of 1 ml/minutes detected wavelength at 240 nm. The retention time was found to be 2.13 minutes and 5.41 minutes for metformin hydrochloride (MET) and dapagliflozin (DAPA) respectively. The proposed method was found to be linear in the concentration range of 100-600 ug/ml for MET (R^2=0.9999) and 1-6 ug/ml for DAP (R^2=0.9996), respectively. Method was validated according to ICH guidelines. Co-relation coefficients for both the drugs were found to be less than one. The mean % recoveries obtained were found to be 99.06-100.32% for metformin and 99.1-100.18% for dapagliflozin respectively. Stress testing is carried out for both drugs in acid, base, peroxide, photolytic and thermal degradation. The developed method can be effectively applied for routine analysis in simultaneous determination of metformin hydrochloride and dapagliflozin in bulk and combined tablet dosage form.

KEYWORDS: Dapagliflozin, Reverse-phase high-performance liquid chromatography, 0.1 % OPA.

INTRODUCTION

Strength testing and stress reduction play a very important role in drug discovery and development. Stability is very important in all aspects of a product, and the term “test stability” is basically used to describe a process that provides specific condition of a drug substance in which its products are discounted. The main objective of studying the stability of a drug is to decide the shelf life of the drug. There are various conditions specified for stress degradation studies include acidic, alkaline, oxidation, photolytic and thermal.

Type 2 diabetes mellitus (T2DM) is a chronic progressive metabolic disorder distinguished by absolute or relative insulin deficiency.[2] Expected increase in prevalence of diabetes is mainly due to increased life span because of better healthcare amenities and physical inactivity and obesity due to sedentary lifestyle.

Pancreatic β-cell function is slowly deteriorated in patients of T2DM which is reflected into inadequate glycemic control on an extended run.

Dapagliflozin is selective Sodium Glucose Co carrier 2 inhibitor (SGLT 2). Dapagliflozin is chemically identified as (1s)-1, 5-anhydro- 1- C- [4- chloro- 3- [(4-ethoxyphenyl) methyl] phenyl]-D-glucitol. It has a molecular formula of $C_{24}H_{33}ClO_8$ having molecular weight 408.98 g/mol. It acts by reducing the re absorption of glucose by the kidney which leading to excretion of excess glucose in the urine, thus improving glycemic control in patients with type 2 diabetes mellitus.

Metformin hydrochloride (1,1-dimethylbiguanide hydrochloride with molecular formula of $C_{18}H_{25}N_3O_2$ and drug combination of these two drugs is indicated for the treatment of type-2 Diabetes. The main aim of this study was to develop a stability-indicating method for the simultaneous determination of Metformin HCl and Dapagliflozin in bulk drugs and to apply the developed method for the quantitative determination of these drugs from its tablets. The HPLC technique was chosen because of its earlier mentioned in advantages. The projected method was able to separate the compounds of interest and their degradation products within 10min. Subsequently, this method was validated as per International Conference on Harmonization (ICH) guidelines[1-3].

Literature survey revealed a variety of analytical techniques viz. HPLC, LC-MS and, GC has been reported for quantification of Dapagliflozin and Metformin HCl individually or in combination with other drugs. The reported methods are spectrophotometric, HPLC and LC-MS method are reported for the simultaneous estimation of DAPA and MET in combined pharmaceutical formulation[4-14].

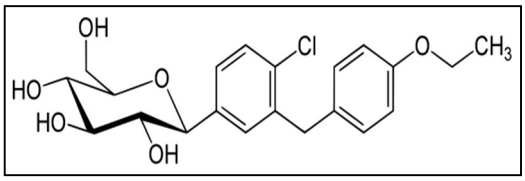

Fig. 1: Chemical Structure of Dapagliflozin

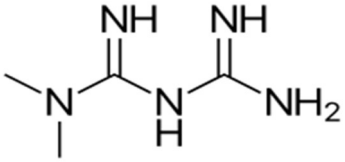

Fig. 2: Chemical Structure of Metformin HCl

MATERIALS AND METHODS

Instruments

The analysis of both the drugs was carried out on Jasco (Japan) Isocratic system with UV detector. Equipped with C_{18} Thermofisher column of dimensions 4.6mm x 250mm; 5µm size, eluting SP940 D pump, a 20µl injection loop and UV740D Absorbance detector and running ChromNAV 2.0 software.

Materials and Reagents

Dapagliflozin and Metformin HCl were obtained in a form of gift samples from Ipca Labs Andheri, Mumbai. O-phosphoric acid were procured from Sigma Aldrich and Acetonitrile were HPLC grade procured from Merck Pvt. Ltd. The pharmaceutical formulation of binary combination of Dapagliflozin and Metformin HCl is Xigduo XR make of Astrazeneca. The commercial formulation of Metformin HCl and Dapagliflozin is available in ratio of 1000:10 mg in tablet.

Chromatographic Conditions

UV Detection wavelength kept at 240 nm using flow rate 1 ml/min. Mobile phase used was acetonitrile: water in the ratio of 35:65 of pH 6.8 adjusted with OPA 0.1% solution.

Preparation of standard stock solution: (Stock I)

100mg of pure powdered Metformin and 10 mg powdered dapagliflozin was separately weighed dissolved in 100 ml conical flask containing methanol. The solution was sonicated for 15 min and filter through Whatman filter paper. The final concentration of this solution was 1000µg/ml of metformin and 100ug/ml of dapagliflozin.

FORCED DEGRADATION STUDY

Acid Hydrolysis

Hydrolysis samples were prepared by weighing the drugs 10mg each and transfer in 10ml volumetric flask. Continue with addition of 2 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add 0.1 N HCl and adjust volume 10ml with it. After complete preparation of solution, store it at 80^0c about 3 hours in water bath. Chromatogram is shown in figure 4.

Alkaline hydrolysis

Hydrolysis samples were prepared by weighing the samples 10mg of metformin and 1mg of dapagliflozin each and transfer in 10ml volumetric flask. Continue with addition of 5 ml HPLC grade methanol in it and dissolve completely. After complete dissolution adjust volume with 10ml of 0.1 N sodium hydroxide. The final solution was stored at 80^0c about 3 hours in water bath.

Oxidation

Hydrolysis samples were prepared by weighing the samples 10mg of metformin and 1mg of dapagliflozin each and transfer in 10ml volumetric flask. Continue with addition of 5 ml HPLC grade methanol in it and dissolve completely. After complete dissolution add hydrogen peroxide (3%) and adjust volume 10ml with it. After complete preparation of solution, store it at 80^0c about 3 hours in water bath. Chromatogram is shown in figure 6. All results were tabulated in table 1

METHOD VALIDATION

Linearity

The solutions were prepared for linearity studies by dilution addition method. From standard stock solution 1, 2, 3, 4, 5 and 6 ml were pipette out in 10 ml conical flask separately containing mobile phase as diluents. The final concentration of these solutions was in the range of 100-600 ug/ml for MET and 1-6 ug/ml for Dapa. The data obtained in the calibration experiments when subjected to linear regression analysis and injected in HPLC and response was measured at 240 nm summarized in Table 2 and 3.

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

Limit Of detection (LOD) is the lowest amount of analyte in a sample that can be detected but not necessarily quantify under the stated experimental conditions. LOQ is the lowest concentration of analyte in a sample that can be determined with the acceptable precision and accuracy under stated experimental conditions. The LOD and LOQ in this work were calculated by using standard deviation of y-intercept in regression line and slope of method as per ICH guidelines**.** Results were given in Table 4.

Accuracy

Accuracy was performed with the help of standard addition of standard solution in pre-analysed tablet solution in different levels of 80%, 100%, and 120%. Three tablet matrix solutions of same concentration (300 ug/ml of MET and 3ug/ml of DAPA) were made. Pure form of mixed drug solution was added in sequence of 2.4 ml for 80 %, 3 ml for 100% and 3.6 ml for 120% to set recovery level.

Percent recovery was evaluated by comparing the area before and after the addition of the standard drug. The solutions were analyzed inform of triplicate at each level as per the proposed method. The percent recovery and % RSD at each level was calculated and presented in following table. The result values are given in Table 5.

Precision

The precision was performed by intraday and interday precision studies. For interday precision sample concentration of 300 ug/ml of MET and 3 ug/ml of DAPA was injected in triplicate form at specific interval of time in a day. For intraday precision above

same concentration was used at different consequence days. The results are shown in Table 6.

Robustness

To estimate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. Each factor was changed at time to study the effect on the chromatographic parameters. The effect of changes in mobile phase composition± 2ml, wavelength ±2 nm and flow rate ± 0.2ml on retention time and tailing factor of drug peak was studied. The results were observed in Table 7.

Assay Preparation for marketed formulation

For analysis of the matrix tablet dosage form, 20 tablets were weighed individually, and their average weight was calculated. After that they were crushed to make fine powders and powder equivalent to 10 mg Metformin and Dapagliflozin into 100 ml volumetric containing methanol. The solution was sonicated for 15 mins to dissolve it completely and filtered through 0.45 µm membrane filters. Further pipette 0.3ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with mobile phase to get final concentration of 300 ug/ml of MET and 3 ug/ml of DAPA. The simple HPLC chromatogram of sample and test Metformin and Dapagliflozin were shown in Table 8.

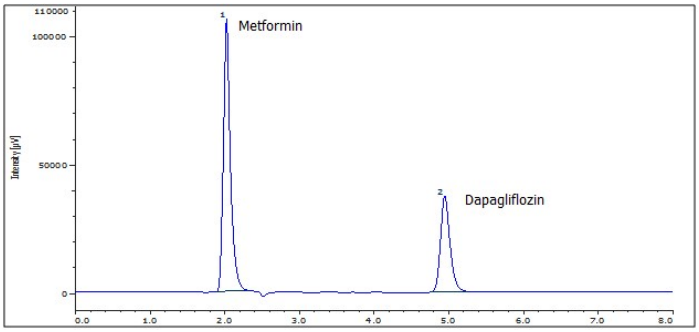

Fig. 3: Chromatogram of standard combination of MET and DAPA

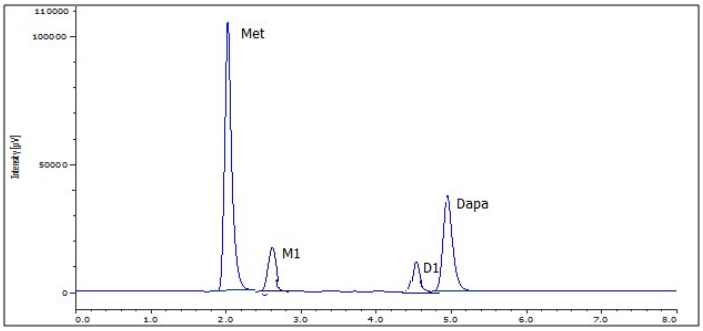

Fig. 4: Acid degradation Study

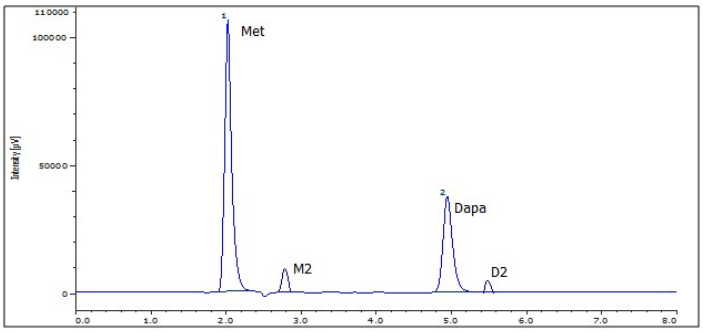

Fig. 5: Alkaline Degradation Study

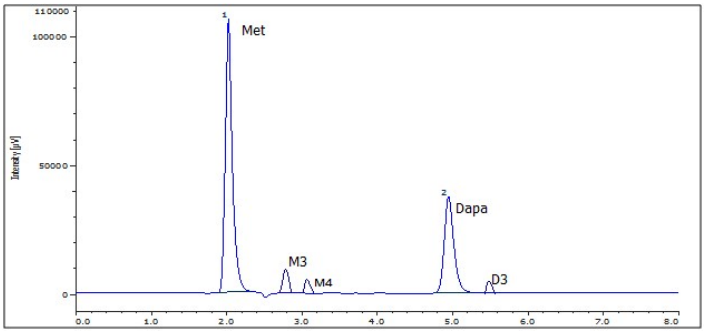

Fig. 6: Oxidative Degradation Study

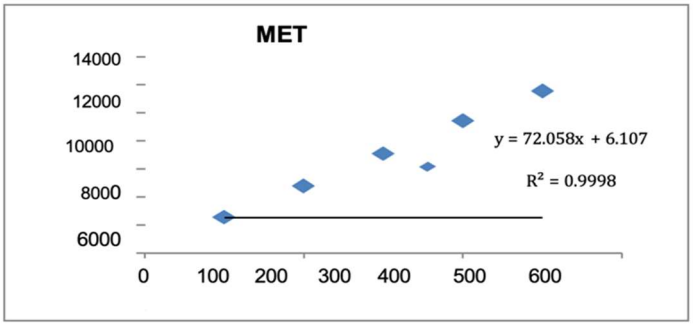

Fig. 7: Calibration curve of Metformin HCl

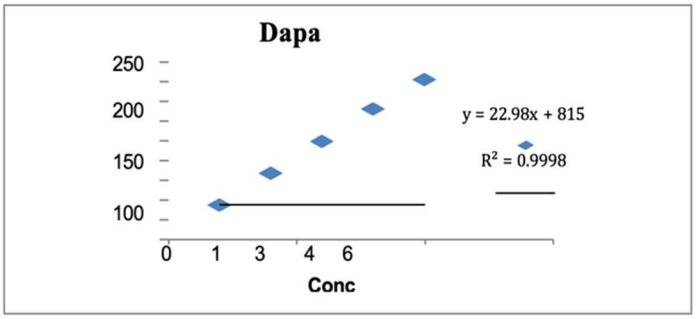

Fig. 8: Calibration plot of Dapagliflozin

Table 1: Amount of Percent degradation

Stress condition	Amount of degradation (%)
Acid hydrolysis	14
Alkaline hydrolysis	8-11
Oxidation	5-8

Table 2: Linearity data for Metformin HCl (*n=3)

Method	Conc µg/ml	Average peak area (µV.sec)	SS.D.	% RSD
	100	202.605	0.36	0.52
	200	385.953	0.78	0.43
	300	607.5	0.88	0.27
Metformin	400	941.207	0.59	0.91
	500	1092.945	0.99	0.4
	600	1284.453	0.53	0.38
	Equation	72.058x+6.107		
	R^2	0.9998		

Table 3: Linearity data for Dapagliflozin

Method	Concentration ug/ml	Average peak area (µV.sec)	SS.D.	%RSD
	1	104.03	0.27	0.48
	2	267.015	0.58	0.13
	3	484.81	0.54	0.2
Dapagliflozin	4	704.845	1.01	0.59
	5	848.425	0.93	0.82
	6	1023.87	0.76	0.5
	Equation	22.98x + 815		
	R2	0.9996		

(*n=3)

Table 4: Limit Studies for MET and DAPA

Parameters	Drug	
	Metformin	**Dapagliflozin**
S.D.	0.87	0.75
SLOPE	72.05	22.96
LOD(ug/ml)	0.04	0.123
LOQ(ug/ml)	0.0123	0.546

Table 5: Result of Recovery data for Metformin and Dapagliflozin

Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Calculated amount	Amt. recovered	%Recovery Mean*± S.D.
MET	80%	300	240	538.32	238.68	99.45
	100%	300	300	589.45	290.96	96.98
	120%	300	360	654.93	294.93	81.92
DAPA	80%	3	2.4	5.19	2.79	101.5
	100%	3	3	5.9	2.9	96.66
	120%	3	3.6	6.45	2.85	97.16

*(n=3)

Table 6: Intraday and Inter day Precision studies on RP-HPLC method for MET&DAPA

Drug	Conc (µg/ml)	Intraday Precision	Interday Precision
		Mean Of area	**Mean of area**
	200	1776.02	1678.96
MET	300	2884.87	2486.87
	400	3378.09	3296.34
	2	464.96	473.96
DAPA	3	945.08	710.48
	4	1040.12	940.91

*(n=3)

Table 7: Robustness study of Metformin and Dapagliflozin

Parameters	Amount of detected (mean ±SD)	%RSD	Amount of detected (mean ±SD)	% RSD
	Metformin		**Dapagliflozin**	
flow change 0.9 ml	1482.33±0.13	0.00877193	509.54 ±0.43	0.084389842
flow change 1.1 ml	1945.5±1.98	0.101799486	432.7±1.07	0.247284493
change in wavelength	1605.8±1.65	0.102752522	534.22±0.7	0.131032159
239nm				
change in wavelength 241nm	1276.21±1.02	0.079924777	437.76±0.68	0.155336257
mobile phase change 33:67	1699.3±1.28	0.075325134	448.8±0.23	0.051247772
Chromatogram of mobile phase change 37:63	1783.33±1.56	0.087476799	481.88 ±0.5	0.103760272

Table 8: Assay results for Metformin and Dapagliflozin

Assay	Drug	Amount found	% label claim	S.D.	% RSD
Xigduo XR 1000mg/10 mg Met/Dapa	MET	400.45	100.17	1.03	0.03
	DAPA	4.71	101.9	0.82	0.98
	MET	400.35	100.88	0.3	0.108
	DAPA	4.67	100.48	0.4	0.12

RESULTS AND DISCUSSION

The current study was aimed at development of stability indicating RP definite, better and resolved method. The retention times of the Metformin and Dapagliflozin were found to be 2.13 and 5.41 min respectively. Chromatogram represented in figure 4. The forced degradation study was conducted for determining the stability indicating power of an analytical procedure. The results of the stress studies are shown in Table 1 and chromatograms were represented in figures 4, 5 and 6.

The respective linear equation for Metformin was 72.058x+6.107 and Dapagliflozin equation 22.98x + 815. The correlation coefficient was 0.9998 and 0.9996. The calibration curve of Metformin and Dapagliflozin is represented in Figure 7and 8. The LOD and LOQ for Dapagliflozin were found to be 0.1230µg/ml and 0.546µg/ml respectively. Metformin LOD and LOQ were found to be 0.04µg/ml and 0.01230 µg/ml respectively which is given in table 2, 3 and 4.

The accuracy and precision for both drugs have been summarized in Table 5 and 6 respectively. The Assay percentage was found to be 99.1-100.18% for Dapa and 99.06-100.32% for Met respectively shown in table 8. The outcomes of precision that is measurements of inter and intra-day and accuracy exhibits excellent reproducibility. The percent relative standard deviation for above was found to be less than 2.00 %. Therefore, the results indicate that the method is highly precise and accurate.

CONCLUSION

A new analytical method has been developed which may routinely applied to simultaneous determination of Metformin and Dapagliflozin in pharmaceutical dosage form. In this study, the stability studies for Metformin and Dapagliflozin in APIs were established according to ICH recommended stress conditions. The developed procedure was validated for linearity, accuracy, precision and robustness parameters. The method was established to be very specific, linear, precise, accurate and robust including stability studies. Hence, the method can be suggested for routine quality control analysis and stability sample analysis.

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