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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND METFORMIN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The Novel, Convenient, sensitive, accurate and precise reverse phase high performance liquid chromatography technique has been established for simultaneous estimation of Metformin and Dapagliflozin in pharmaceutical dosage form. On an Agilent C18 column (250mm x 4.6mm, 5µm), isocratic flow was used to produce the separation. Methanol and 0.05 % orthophosphoric acid buffer (70:30 v/v) make up the mobile phase, which flow rate of 1 ml/min at 233 nm, the wavelength detection was done. The retention times for Metformin and Dapagliflozin utilizing the described approach were determined to be 2.289 and 5.576 minutes, respectively. A linear response was observed over the concentration range 1-5µg/ml for Dapagliflozin and 50-250µg/ml for Metformin. The correlation coefficient was found to 0.999 for both Dapagliflozin and Metformin. The precision of the proposed method was found to be 0.175% for Metformin and 0.835 for Dapagliflozin in intraday precision and 0.083% for Metformin and 0.389% for Dapagliflozin in inter day precision. The percentage recoveries for Metformin and Dapagliflozin were reported to be between 98.21 and 101.06 and 98.45 and 101.68%, respectively. LOD and LOQ for Metformin were found to be 1.1942 µg/ml and 3.6187 µg/ml, and for Dapagliflozin were found to be 0.05651 µg/ml and 0.17126 µg/ml respectively. The % test result of Metformin and Dapagliflozin were found to be 100.47% and 99.38% respectively. All the parameter of validation were in the acceptable range Result of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2R1.

Keywords: Dapagliflozin, Metformin, RP-HPLC, Method Development, Validation, ICH Q2R1

INTRODUCTION:

Dapagliflozin chemically is (2S,3R,4R,5S,6R)-2- {4-chloro-3- [(4-ethoxy phenyl) methyl] phenyl}-6-(hydroxymethyl) oxane-3,4,5-triol. The molecular formula is $C_{21}H_{25}ClO_6$. The molecular weight is 408.873g/mol. It is supplied as a crystalline solid. Dapagliflozin is inhibiting renal glucose reabsorption through the solid glucose cotransporter (SGLT) offers an insulin-independent alternative to controlling blood glucose concentrations in patients with type 2 diabetes. A first-generation, selective SGLT inhibitor called Dapagliflozin prevents the transport of glucose while being around 100 times more selective for SGLT2 than SGLT1 **[1]**. Chemically speaking, Metformin is 1,1-dimethyl biguanide hydrochloride. The chemical structure is C4H11N5. The weight of the molecules is 165.62 g/mol. Originally marketed as Glucophage; the oral diabetes medication Metformin belongs to the biguanide class. It is the first-line therapy option for type 2 diabetes, especially in overweight and obese patients. Despite mounting evidence to the contrary, safety concerns still prohibit its widespread application in this circumstance. AMP-activated protein kinase is turned on (AMPK). Additionally, it has been studied for conditions including polycystic ovarian syndrome, where it is also used as a treatment and where insulin resistance may play a big role, where it may be beneficial **[2]**. Patients with type 2 diabetes mellitus are treated using the therapeutic option of Dapagliflozin and Metformin together (T2DM). Dapagliflozin and Metformin have a particularly effective and safe combination ofmechanisms of action, which supports the idea of using this fixed-dose combination as a treatment for T2DM patients. A review of the literature suggests that a variety of analytical techniques, including UV spectrophotometry **[3-6]** and HPLC **[5-11]**, are reported for the determination of Metformin and Dapagliflozin as well as Metformin with other pharmacological preparations. Dapagliflozin and Metformin have only been tested using a relatively small number of HPLC techniques. Consequently, a sensitive HPLC approach is required. A successful attempt has been made in the current study to design a quick, exact, accurate, and relatively cheap RP-HPLC method for the simultaneous quantification of two medicines. ICH-compliant recovery studies and validation of the developed approach were carried out utilizing a variety of statistical factors guidelines **[12]**.

MATERIALS AND METHODS:

Instruments:

The HPLC System (Agilent1100) was used with Chemstation software. The separation

was done on Agilent C18 column (250mm x 4.6mm x 5µm). With a 20µL. The PDA Detector (SPD-M20A) module is installed. Shimadzu 1600LC UV-Visible Spectrophotometer was utilized. An Elico pH meter. Using a 0.45 membrane filter, solutions were filtered.

Chemicals and Reagents:

The standard of Dapagliflozin and Metformin Drug was procured from micro lab Pvt. Ltd, Jalgaon and Swapnaroop Pharmaceutical with Certificate of Analysis. The Marketed Formulation (xigduo 510mg) were purchased from the local market. HPLC grade water, Ortho phosphoric acid (OPA), methanol was Procured from Fine Chem Industries.

Chromatographic conditions:

The Agilent (1100) HPLC system was used to perform the chromatographic analysis. The analysis was conducted on an Agilent C18 column (250mm x 4.6mm x 5µm), which was kept at room temperature 30 °C. Methanol and 0.05 % OPA (70:30 v/v) make up the mobile phase. Sonicated for 15 min and filtered through a 0.45-inch membrane. Isocratic elution was used as the elution method. With a flow rate of 1.0 ml/min and an injection volume of 20µL mobile phase was transferred from the solvent reservoir to the column. Monitoring the 233 nm elute was done using the PDA detector. Run time was 10 min.

Preparation of Buffer:

Take accurately 0.05ml of Orthophosphoric acid (OPA) and transferred into 100ml volumetric flask containing water and sonicated to dissolve completely. The solution was filtered through 0.45µ filter paper (membrane nylon) and degas it.

Preparation of Mobile phase:

The Mobile phase was prepared by mixing the Methanol and 0.05% OPA in the ratio of 70:30v/v and mixed well and was filtered through 0.45µ nylon membrane filter and degassed by sonication for about 30min

Preparation of standard stock solution:

To make a standard stock solution, 10 mg of Dapagliflozin and 500 mg of Metformin were precisely weighed into two separate 100 ml volumetric flasks, and sonicated to dissolve the contents, made up the volume with methanol, and then passed through a 0.45µ membrane filter. (Concentration of Stock Solution:5000 ppm Metformin and 100 ppm Dapagliflozin)

Preparation of Working standard solution:

A further 0.2 ml of the stock solution was added to the 10 ml volumetric flask, diluted with the appropriate amount of diluent, and thoroughly mixed. (Concentration of Standard Solution: 2 ppm Dapagliflozin & 100 ppm Metformin).

Preparation of sample solution:

20 Marketed tablet formulations weighed accurately and triturated in mortar & pestle. The weight of powder is calculated as

14304 mg. average weight of tablet is 715.2mg then calculated equivalent weight for 500mg which is 715.2 mg. Then it was transfer into a 100 ml volumetric flask. Added 30 ml of methanol, dissolved, shake for 5 min and sonicated for 30 min and made up the volume with methanol, and filtered through 0.45µ membrane filter and collected the sample after discarding the first few ml of solution.

RESULTS:

Determination of maximum wavelength (λ max):

The standard solution of Dapagliflozin & Metformin was scanned separately in the wavelength range of 200-400 nm on a UV-Visible Spectrophotometer and λ max was found to be 236 nm and 223 nm for Dapagliflozin & Metformin. The overlay absorption spectrum of Dapagliflozin and Metformin is shown in **Figure 1** and exhibits maxima at 233 nm (Isosbestic point). Hence wavelength selected for analysis was 233 nm.

Dapagliflozin & Metformin Optimization and method development:

A chromatographic separation of Dapagliflozin and Metformin was achieved with Agilent C18 (250mm×4.6mm, 5µm) column using methanol: 0.05% Orthophosphoric acid (70:30) v/v in isocratic mode at flow rate of 1.0ml/min. Column temperature was maintained at ambient (30ºc) and the detection of the drugs were monitored at 233 nm using PDA detector. The retention time of 5.576 min for Dapagliflozin and 2.289 min for Metformin were determined at 233nm. An optimized peak of Dapagliflozin and Metformin are shown in **Figure 2**.

Linearity and Range:

The analytical curves constructed for Dapagliflozin and Metformin were found to be linear in the 1-5 µg/ml and 50-250 µg/ml range respectively. The results are shown in **Table 1**. The value of correlation coefficient calculated for Dapagliflozin and Metformin were found to be 0.999 and 0.999 respectively. The calibration curves were constructed by plotting absorbance versus concentration, and the linearity was calculated by the least square regression method. A calibration curve for Dapagliflozin and Metformin are shown in **Figure 4 & Figure 5** respectively.

Precision:

A) Repeatability:

The precision of each method was achieved separately from peak areas obtained by actual estimation of 5 injection of fixed homogeneous conc. of Dapagliflozin & Metformin. The results are shown in **Table 2**.

B) Intraday Precision:

Intraday precision study was performed by analyzing standard solution of concentration 50+1 µg/ml on same day but in different time. The results are shown in **Table 3**.

C) Inter day Precision:

Inter day precision study was performed by analyzing standard solution of concentration 50+1 µg/ml on three different consecutive day. The results are shown in **Table 3**.

Accuracy:

Accuracy mainly evaluated at three levels 80%, 100% & 120% of the working concentration level for Dapagliflozin & Metformin. Each level prepared in triplicates. The % recovery was calculated from the amount found and the actual amount added. The results are shown in **Table 4**.

Robustness:

Robustness of the method was verified by applying minor and deliberate changes in the experimental parameters, for e.g flow rate: ±0.1 mL/min, wavelength: ±1 nm, mobile phase composition: ±1ml. Change was made to evaluate its effect on the method. The results are shown in **Table 5**.

LOD and LOQ:

The LOD and LOQ for Dapagliflozin were found to be 0.05651 µg/ml and 0.17126 µg/ml respectively and for Metformin were found to be 1.1942 µg/ml and 3.6187 µg/ml respectively. The results are shown in **Table 6**.

Analysis of Marketed Formulation using developed HPLC method:

The % assay was found to be 99.38 % for Dapagliflozin and 100.47% for Metformin, which is permissible as per the ICH guidelines. The result of assay was shown in **Table 7**. The chromatogram is shown in **Figure 2 and Figure 3**.

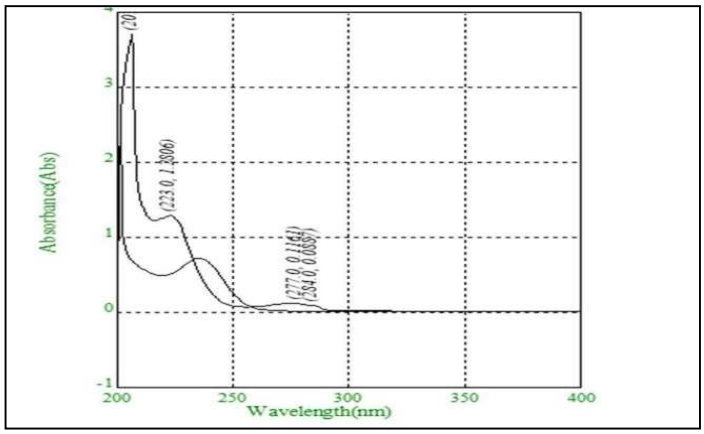

Figure 1: Overlay absorption UV spectra of Dapagliflozin & Metformin

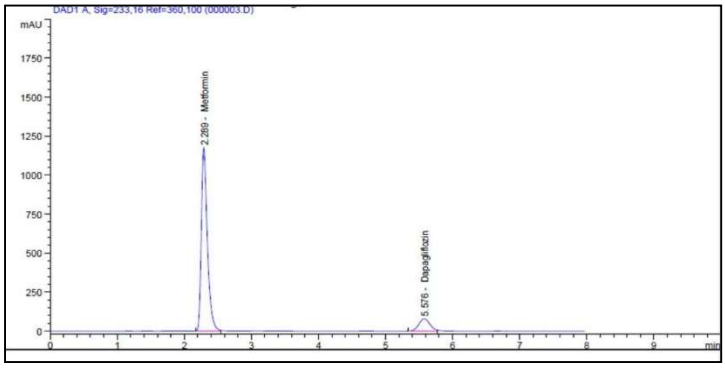

Figure 2: Typical chromatogram of standard Metformin and Dapagliflozin

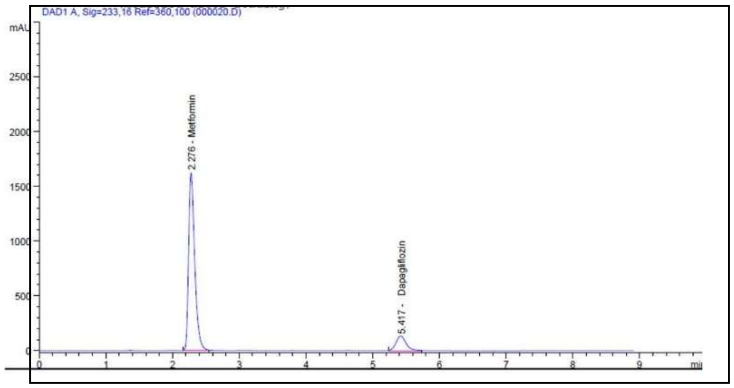

Figure 3: Chromatogram of sample Metformin and Dapagliflozin

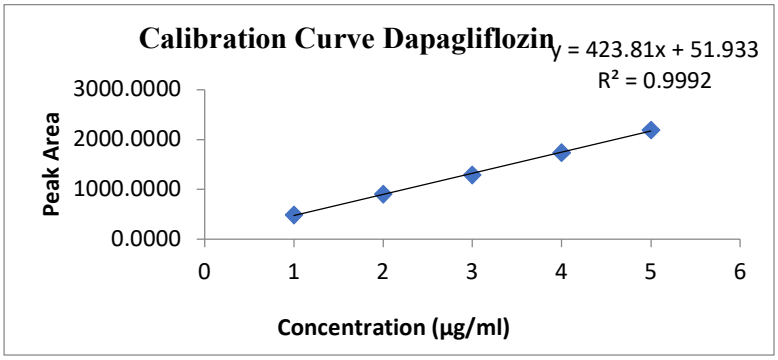

Figure 4: Calibration curve of Dapagliflozin

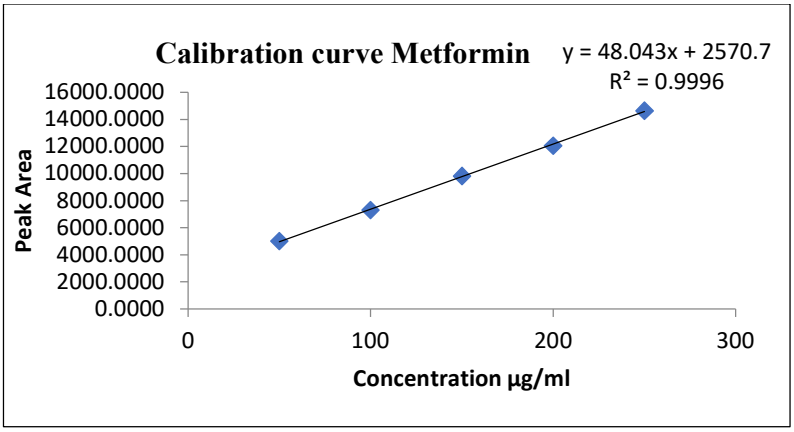

Figure 5: Calibration curve of Metformin

Table 1: Results of Linearity

Dapagliflozin		Metformin	
Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
1	490.8563	50	5045.9746
2	900.4867	100	7316.8740
3	1295.4422	150	9830.0000
4	1730.8438	200	12711.8000
5	2179.5627	250	14645.0000
R^2= 0.999		R^2= 0.999	

Table 2: Data for Repeatability

Sr. No.	Area of Dapagliflozin	Area of Metformin
1	2218.63	14528.8
2	2185.34	14510.5
3	2195.03	14534.4
4	2235.12	14544.3
5	2150.05	14531.5
Mean ± SD	2196 ± 32.65	14529.9 ± 12.32
% RSD	1.49	0.085

Table 3: Data for Precision

Sr. No	Intraday Precision		InterdayPrecision	
	Dapagliflozin	Metformin	Dapagliflozin	Metformin
1	488.743	5025.029	487.1353	5018.5459
2	483.0038	5012.654	484.4618	5012.654
Mean	485.87±4.06	5018.81±8.789	485.80±1.890	5015.60±4.16
%RSD	0.835	0.175	0.389	0.083

Table 4: Data of Accuracy

Drug	% Level	Set	Amount added(µg/ml)	Amount found(µg/ml)	%Recovery	Mean ± SD	%RSD
Dapagliflozin	80%	1	0.8	1.80	100.28	1.81 ± 0.008	0.436
		2	0.8	1.81	101.68		
	100%	1	1	2.0038	100.39	2.01 ± 0.002	0.115
		2	1	2.0071	100.72		
	120%	1	1.2	2.1813	98.45	2.19 ± 0.007	0.326
		2	1.2	2.1914	99.29		
Metformin	80%	1	40	90.35	100.86	90.39 ± 0.056	0.062
		2	40	90.43	101.06		
	100%	1	50	99.952285	99.90	100.02 ± 0.102	0.102
		2	50	100.09688	100.19		
	120%	1	60	108.98232	98.30	108.95 ± 0.042	0.038
		2	60	108.92301	98.21		

Table 5: Data of Robustness

Sr. No	Parameters	Dapagliflozin			Metformin		
		Peak Area	Mean ± SD	%RSD	Peak Area	Mean ± SD	%RSD
1	Lower wavelength (232nm)	2499.665	2500.01 ± 0.50	0.02	15188	15188.7 ± 0.92	0.01
		2500.365			15189.3		
2	Higher wavelength (234nm)	2451.882	2453.77 ± 2.67	0.11	15538.9	15542.4 ± 4.94	0.03
		2455.655			15545.9		
3	Low Flow Rate (0.9 ml/min)	2969.75	2970.94 ± 1.68	0.06	17818.5	17819.35 ± 1.20	0.01
		2972.125			17820.2		
4	High Flow Rate (1.1ml/min)	1950.591	1953.07 ± 3.50	0.18	12697.7	12698.46 ± 1.07	0.01
		1955.547			12699.22		
5	MP Ratio (MeOH: OPA) (69:31)	2511.05	2512.35 ± 1.84	0.07	14970.8	14975 ± 7.07	0.05
		2513.654			14980.36		
6	MP Ratio (MeOH: OPA) (71:29)	2249.153	2249.8± 0.86	0.04	14649.4	14650.3 ± 1.32	0.01
		2250.37			14651.27		

Table 6: Results of LOD & LOQ

Sr. No	Parameter	Dapagliflozin	Metformin
1	LOD	0.05651 µg/ml	1.1942 µg/ml
2	LOQ	0.17126 µg/ml	3.6187 µg/ml

Table 7: Results of Assay

Sr. No	Drug	Label Claim	Amount found	%Assay	SD	%RSD
1	Dapagliflozin	10 mg	9.93 mg	99.38	0.056	0.06
2	Metformin	500 mg	502.34 mg	100.47	0.04	0.05

DISCUSSION:

The Proposed method for simultaneous estimation of Dapagliflozin and Metformin in Tablet dosage form were found to be simple, accurate, economical and rapid. Standard calibration yielded correlation coefficient (r^2) 0.999 and 0.999 for Dapagliflozin and Metformin respectively at all the selected wavelengths and the values were average of three readings. The values of %RSD are within the prescribed limit of 2%, showing high precision of methods and recovery close to 98-101% for both the drugs. Results of the analysis of pharmaceutical formulation reveal that the proposed methods are suitable for their simultaneous determination with virtually no interference of usual additive present in pharmaceutical formulation. Hence, the above method can be applied successfully for simultaneous estimation of Dapagliflozin and Metformin in Formulations.

CONCLUSION:

A rapid, cost effective, precise and accurate RP-HPLC method was developed and validated according to ICH guidelines Q2R1 in terms of Linearity, precision, accuracy, robustness, ruggedness. The developed HPLC method offers several advantages such as rapidity, usage of simple mobile phase and easy sample preparation steps. The proposed method was successfully applied for the quantitative analysis of Metformin and Dapagliflozin in bulk and tablet formulation and we conclude that the developed method is precise, simple, accurate, sensitive, and reproducible and thus may be used for research studies, quality control and routine analysis in Pharmaceutical Industries.

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