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Sneha Madanlal Zanwar
Department of Quality Assurance, School of Pharmacy, Dr. APJ Abdul Kalam University, Indore, Madhya Pradesh, India

Dr. Govind Soni
Department of Quality Assurance, School of Pharmacy, Dr. APJ Abdul Kalam University, Indore, Madhya Pradesh, India

Dr. Rakesh K Jatav
Department of Quality Assurance, School of Pharmacy, Dr. APJ Abdul Kalam University, Indore, Madhya Pradesh, India

Dr. Rakesh Patel
Department of Quality Assurance, School of Pharmacy, Dr. APJ Abdul Kalam University, Indore, Madhya Pradesh, India

Dr. Sanjay Toshniwal
Department of Quality Assurance, School of Pharmacy, Dr. APJ Abdul Kalam University, Indore, Madhya Pradesh, India

Corresponding Author:
Sneha Madanlal Zanwar
Department of Quality Assurance, School of Pharmacy, Dr. APJ Abdul Kalam University, Indore, Madhya Pradesh, India

Development and validation of new analytical method for estimation of form dapagliflozin and metformin HCL drugs pharmaceutical dosage forms

Sneha Madanlal Zanwar, Govind Soni, Rakesh K Jatav, Rakesh Patel and Sanjay Toshniwal

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Abstract
The estimation of Dapagliflozin and Metformin was accomplished with C18 column (4.6×250 mm and 5 µm particle size) with DAD detector. The mobile phase consists of Methanol: Water 74:35%v/v of pH-3 adjusted with 0.05% OPA at flow rate of 1mL/min. The detection wavelength was 233 nm, respectively. In the developed method, the retention time of Dapagliflozin and Metformin HCL was found to be 5.099 min and 2.165 min. The reverse phase high performance liquid chromatography method produces linear response that was found in the range of 100-500 µg/mL. The limit of detection and limit of quantification for Dapagliflozin were found to be 0.06 µg/mL and 0.1855 µg/mL, respectively and for Metformin 6.09 µg/mL and 18.45 µg/mL, respectively. Calculated information acquired for both the preliminaries roughly coordinates with the information given by design expert programming which shows the genuineness of the chromatographic condition. The developed method was validated according to the ICH guidelines. The linearity, precision, range, and robustness were within the limits as specified by the ICH guidelines. Hence, the method was found to be simple, accurate, precise, economic, and reproducible.

Keywords: Dapagliflozin, metformin, RH-HPLC, ICH guidelines, limit of detection

1. Introduction
A number of pharmaceutical drugs are being introduced into the arcade every year in a cumulative phase. Some of these drugs either may be a new entity or a structural modification of existing molecular formulation in multi combination of two or more drug substances. Dapagliflozin and Metformin HCl are two important pharmacological agents used in the management of type 2 diabetes mellitus (T2DM) [1-2]. They are often prescribed individually or as a combination therapy to help achieve better glycemic control in patients with inadequate control through diet and exercise alone. The combination therapy offers complementary mechanisms of action, making it a cornerstone treatment for many individuals with T2DM [3].
Dapagliflozin selectively inhibits the sodium-glucose co-transporter 2 (SGLT2), which is responsible for reabsorbing the majority (About 90%) of glucose filtered by the kidneys. Metformin suppresses glucose production in the liver by inhibiting mitochondrial complex I, thereby reducing hepatic glucose output [4-6].
Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the ICH guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [7-10].
Literature survey revealed a few methods reported for determination of Dapagliflozin and Metformin HCl in pharmaceutical preparation. In this research, a new sensitive and rapid HPLC method was developed for the determination of Dapagliflozin and Metformin HCl in pharmaceutical dosage forms, and this method was validated according to ICH and FDA guidelines.

2. Materials and Methods

2.1. Chemicals and Reagents: Dapagliflozin and Metformin HCl purchased from Macleods Pharma-ceuticals Pvt Ltd. and all chemicals are HPLC grade purchased from purchased from SD fine chemicals, Analyte Pharmajob Training Institute, Kolhapur, Maharashtra.

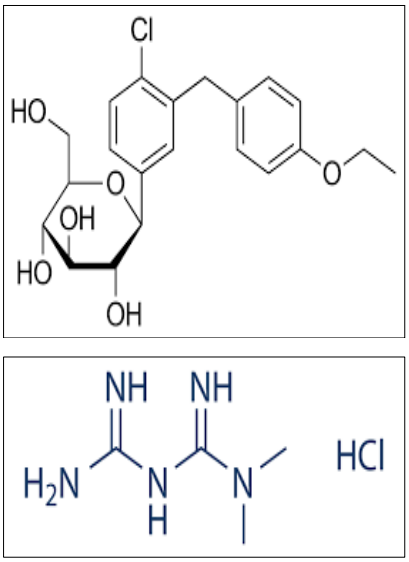

Fig 1: Chemical structure of Dapagliflozin and Metformin HCl

2.3 Selection of wavelength

Selection of wavelength for Dapagliflozin and Metformin HCL were carried out by transferring 10 mg of Dapagliflozin and 10mg of Metformin HCL individually into a 10 ml volumetric flasks and dissolved using mobile phase consist of methanol: phosphate buffer (pH 4) in the ration of 70:30% v/v. The resulting solution was further diluted with the mobile phase to get a concentration 10 µg/ml. The resulting solution was scanned in the UV range from 200-400 nm [11].

2.4 Selection of optimized Chromatographic conditions

For the selection of the optimized condition different trials were performed with the working standards individually (Dapagliflozin and Metformin HCL) with concentration of 10 µg/ ml, the pH of mobile phase was adjusted with Ortho phosphoric acid and system parameters were also fixed during the trail runs such as flow rate, detection wavelength; US tailing factor, resolution, peak symmetry, and US plats were also studied. The standard and mixed standard of Dapagliflozin and Metformin HCL were injected individually for selection of optimized chromatographic condition [12].

2.5 Preparation of Mobile phase A

Consist of Methanol HPLC grade which was sonicated before mixing with buffer solution for 15 minutes [13].

2.6 Preparation of Mobile phase B (Buffer solution)

700 mg of potassium di hydrogen phosphate (KH_2PO_4) was taken into to a clean 1000 ml beaker and dissolve using HPLC grade water and the volume was adjusted to 1000 ml and the pH of the solution was brought to 4.0±0.1 with ortho phosphoric acid. The resulting solution was sonicated for 15 minutes. Then solution was filtered through 0.45µ nylon filter [14].

2.7 Preparation of Mobile phase

700 ml Mobile phase A (methanol) and 300 ml Mobile phase B (buffer solution) were mixed together in a beaker and the sonicated for the 15 minutes and filtered through a nylon filter 0.45µ. The resulting solution was used as mobile phase for the chromatographic separation of Dapagliflozin and Metformin HCL [15].

2.8 Preparation of standard stock solution of Dapagliflozin and Metformin HCL

An accurately weighed quantity of 100mg of Dapagliflozin and Metformin HCL working standard was taken into a 100 ml clean volumetric flask individually and dissolved using the mobile phase consist of methanol: phosphate buffer (pH 4) in the ration of 70:30% v/v (1 mg/ml). Further the solution was diluted to get a concentration of 10µg/ml of Dapagliflozin and Metformin HCL [16].

2.9 Preparation of Mixed stock solution of Dapagliflozin and Metformin HCL

From the above stock solution 0.15ml of Metformin HCL and 0.3 ml of Dapagliflozin were taken into a 10 ml volumetric flask and the volume was adjusted with 10 ml of mobile phase to get a concentration of 0.15 mg/ml of Metformin HCL and 0.3 mg/ml of Dapagliflozin [17].

2.10 Method validation for the simultaneous determination of Dapagliflozin and Metformin HCL by RP-HPLC [18-19]

2.10.1 Linearity

From the standard stock solution of Dapagliflozin and Metformin HCL 10 ml was pipetted out into a 100 ml volumetric flask and the volume was adjusted using mobile phase. For preparation of Metformin HCl linear solution 0.5, 1, 1.5, 2, and 2.5 ml and Dapagliflozin 1, 2, 3, 4, and 5 ml was pipette out into 10 ml volumetric flask individually and volume was made to 10 ml using mobile phase to get of 5 to 25 µg/ml of Metformin HCl and 10 to 50 µg/ml of Dapagliflozin. The resulting solution was injected into the chromatographic system and a linear graph was plotted. From the slop correlation of coefficient were calculated.

2.10.2 Precision

Pipette out 0.15 ml of Metformin and 0.3ml of Dapagliflozin of the standard stock solution into a 10ml volumetric flask and dilute up to the mark with mobile phase. The solution was prepared individually for five times and injected each sample in to HPLC and measured the area for all five injections were measured and the % RSD was calculated using formula (2).

$$\%RSD = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

2.10.3 Inter mediate precision (Ruggedness)

Pipette out 0.15 ml of Metformin and 0.3ml of Dapagliflozin of the standard stock solution into a 10ml volumetric flask and dilute up to the mark with mobile phase. The solution was prepared individually and injected in 2 days for five times and each sample in to HPLC and measured the area for all five injections and the % RSD was calculated using formula (2).

2.10.4 Accuracy

Accurately weigh and transfer 2.68, 5.0, and 7.4 mg of Metformin and 5.48, 10.00 and 14.8mg of Dapagliflozin working standard into a 10 ml clean dry volumetric flask add mobile phase up to the mark and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock Solution). Further pipette out 3 ml of Metformin & Dapagliflozin from the above stock solution into a 10ml volumetric flask individually and dilute up to the mark with mobile phase. To get 50%, 100% and 150% of Metformin and Dapagliflozin. Each level of solution (50%, 100% and 150%) was injected 3 times in to chromatographic system individually and the R recovery was calculated using the formula.

$$\%\,Recovery = \frac{Measured\,Value - True\,Value}{True\,Value} \times 100$$

Result

3.1 Method development for the simultaneous determination of Dapagliflozin and Metformin HCL.

Selection of wavelength the selection of wavelength was performed as per the procedure results are shown in the Fig. 2.

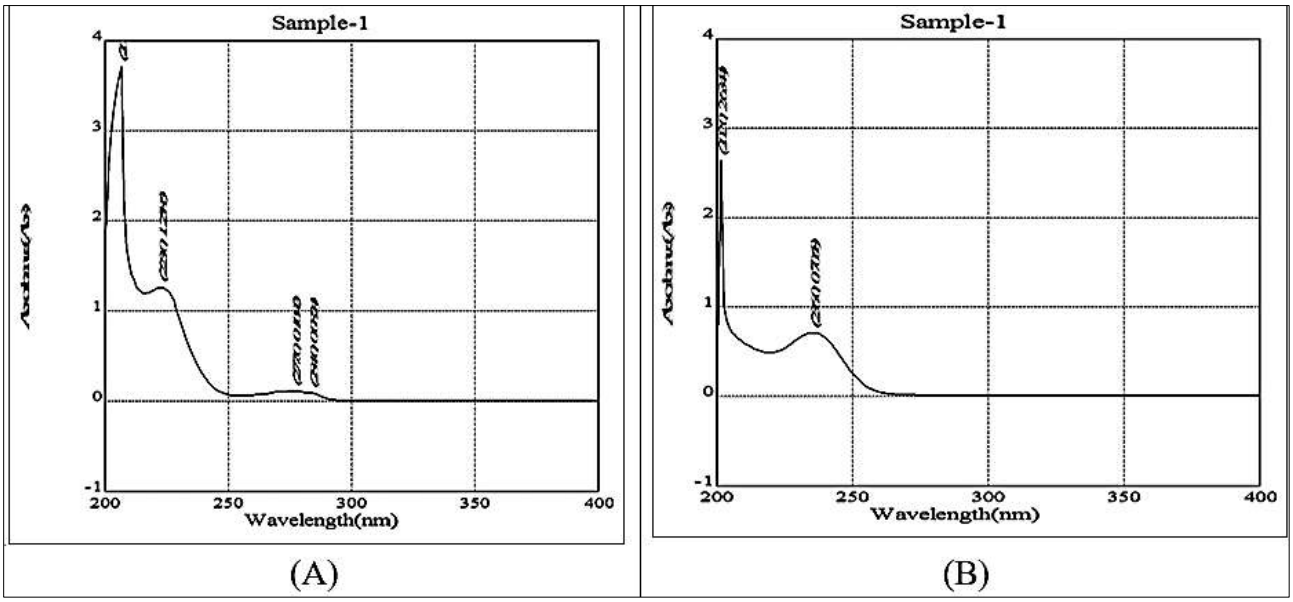

Fig 2: UV-spectra for of Dapagliflozin (A) and Metformin HCL (B).

3.2 Selection of chromatographic condition: For the selection of optimized chromatographic condition many trails were performed. The trail chromatographic conditions are shown in the Table 1.

Table 1: Trails done for the analytical method development for the simultaneous determination of Dapagliflozin and Metformin HCL.

Sr. No	Column	Mobile phase	Flow rate	Wave length	Remarks
Trail 1 Dapagliflozin and Metformin HCL	Symmetry C18 (4.6 x 150 mm, 5µm)	Methanol: Phosphate buffer adjusted to pH5 (60:40)	0.8 mL per min	239 nm	Peak splitting, Improper base line
Trail 2 Dapagliflozin and Metformin HCL	Symmetry C18 (4.6 x 150 mm, 5µm)	Methanol: Phosphate buffer adjusted to pH4 (60:40)	0.8 mL per min	239 nm	Fronting and Peak broadening, Low plate count
Trail 3 Dapagliflozin and Metformin HCL	Symmetry C18 (4.6 x 150 mm, 5 µm)	Methanol: Phosphate buffer adjusted to pH4 (60:40)	0.6 mL per min	239 nm	Peak is good but improper baseline

3.3 Optimized chromatographic condition

From the various trails performed the following optimized chromatographic condition was selected for simultaneous determination of Dapagliflozin and Metformin HCL

- **Equipment:** Waters, Model: Aliance 2695, Detector 2487 with Empower 2 software.
- **Column:** C18 (4.6 x 150 mm, 5µm, Make: XTerra)
- **Mobile phase:** Methanol: Phosphate buffer adjusted to pH 4 with ortho Phosphoric acid (70:30% v/v)
- **Flow rate:** 0.5 mL per min
- **Wavelength:** 239 nm
- **Injection volume:** 10 µl
- **Column oven:** Ambient
- **Runtime:** 10 min

3.4 Method validation for the simultaneous determination of Dapagliflozin and Metformin HCL

3.4.1 Linearity

Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of drug in samples within agiven range. From standard stock solution, 0.05 mL were pipette, out in 10 mL conical flask with diluents was added to makeup volume. The final concentration of these solution was observed in the range of 2-10µg/mL for Dapagliflozin and 100-500µg/mL for Metformin HCL. Calibration curves were plotted with observed peak areas against concentration to obtain the calibration curve and correlation coefficients. Characteristics parameters for regression equation (y=mx+c) of the method and these paramete were used to confirm the good linearity of the method. The results are shown in Table no 2 and Fig. 3.

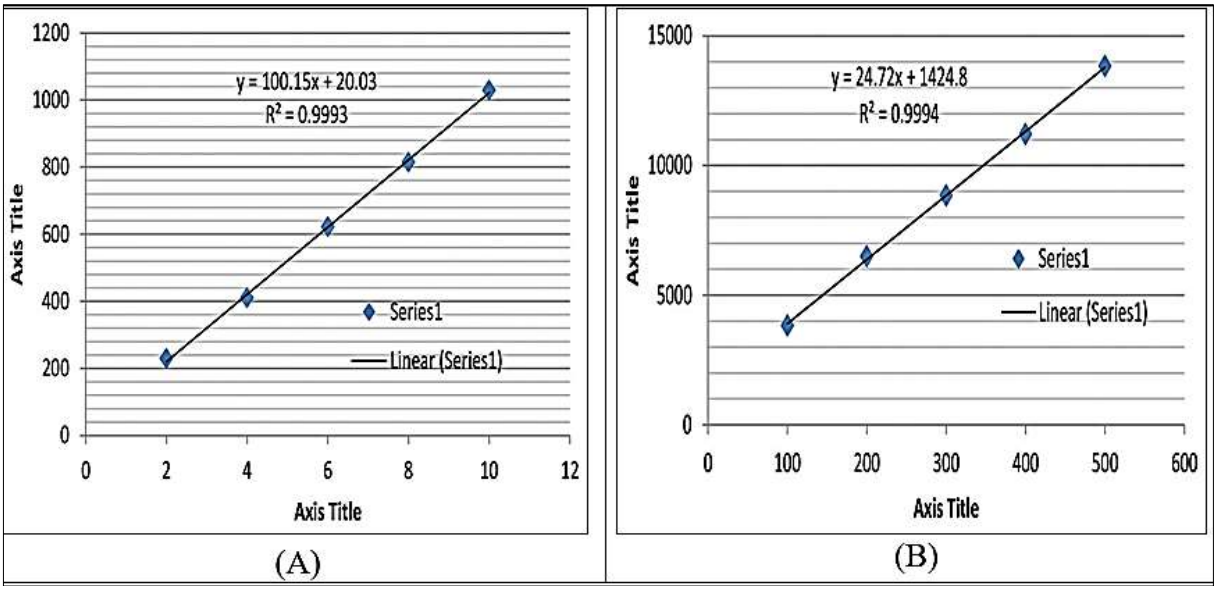

Fig 3: Calibration curved of Dapagliflozin and Metformin HCL

Table 2: Linearity data for Dapagliflozin

Method	Concentration (µg/mL)	Average peak area (µV.s)	SD of peak area	% RSD of peak area
	2	229.49	1.75	0.76
RP-HPLC Method	4	410.18	0.23	0.06
	6	621.64	1.94	0.31
	8	814.86	0.07	0.01
	10	1028.73	5.30	0.52
	Equation	y=100.1x+20.03		
	R2	0.9993		
RP-HPLC: Reverse phase high performance liquid chromatography				

Table 3: Linearity data for Metformin HCL

Method	Concentration (µg/mL)	Average peak Area (µV.sec)	SD of peak area	% RSD of peak area
	100	3828.14	27.64	0.72
RP-HPLC Method	200	6494.95	65.59	1.01
	300	8848.15	67.42	0.76
	400	11206.75	31.18	0.28
	500	13834.85	36.27	0.26
	Equation	y=24.7x+1424		
	R2	0.9994		
RP-HPLC: Reverse phase high performance liquid chromatography				

3.4.2 Accuracy

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as pre sent recovery by the assay of known added amounts of analyte. The accuracy was determined by Dapagliflozin and Metformin HCL (equivalent to 100 mg of Metformin HCL and 40 mg of Dapagliflozin) (80%, 100%, and 120% of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. There sulting mixtures were analyzed in triplicates over 3days. The % recovery of added drug was taken as a measure of accuracy. The results are shown in Table 4.

Table 4: Result of recovery data for Dapagliflozin and Metformin HCL

Drug	Level (%)	Amount taken (µg/mL)	Amount added (µg/mL)	Area mean*±SD	Amt. recovered*±SD	%Recovery Mean*SD
Dapagliflozin	80%	2	1.6	3.60±0.008	1.60±0.008	100.27±0.49
	100%	2	2	4.00±0.004	2.00±0.004	100.22±0.18
	120%	2	2.4	4.41±0.009	2.41±0.009	100.48±0.36
Metformin HCL	80%	100	80	180.20±0.22	80.52±0.22	100.64±0.28
	100%	100	100	198.57±0.76	100.22±0.14	100.22±0.14
	120%	100	120	200.90±0.14	120.90±0.14	100.75±0.12
*Mean of each 3 reading for RP-HPLC method						

3.4.3 Repeatability: Precision of the system was determined with the sample of RP-HPLC method for six replicates of sample solution containing 5mg of Dapagliflozin and 250 mg of Metformin HCL were injected and peak areas were measured and % RSD was calculated. It was repeated for 5 times and results are shown in Table 5.

Table 5: Repeatability studies on RP-HPLC for Dapagliflozin and Metformin HCL

Method	Concentration of Dapagliflozin and Metformin HCL	Peak area	Amount found (mg)	% Amount found
RP-HPLC Method for Dapagliflozin	60	220.244	2.02	101.08
	60	224.548	2.04	101.10
		Mean	2.03	101.09
		SD	3.04	3.04
		%RSD	1.37	1.37
RP-HPLC Method for Metformin HCL	100	3968.786	101.83	101.83
	100	3913.517	101.55	101.55
		Mean	101.70	101.70
		SD	39.08	39.08
		%RSD	0.99	0.99
RP-HPLC: Reverse phase high performance liquid chromatography				

3.4.4 Precision

Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. To determine the precision, intra-day and inter-day precision was performed. For intra-day precision, sample solution containing 250 mg of Metformin HCL of three different concentration (200 µg/mL, 300 µg/mL, and 400 µg/mL) and 5 mg of Dapagliflozin (4 µg/mL, 6 µg/mL, and 8 µg/mL). Dapagliflozin and Metformin HCL were analyzed3 time son the same day. Forinter-day precision above, same concentration was used at different days and % RSD was calculated. The results are shown in Table 6.

Table 6: Intra-day and inter-day precision studies on RP-HPLC method for Dapagliflozin and Metformin

Drug	Conc. (µg/mL)	Intra-day precision		Inter-day precision	
		Mean±SD	%Amt. found	Mean±SD	% Amt. found
Dapagliflozin	4	421.33±0.82	100.22	418.54±2.39	99.53
	6	625.33±0.05	100.78	625.20±0.31	100.76
	8	815.96±3.03	99.39	815.79±0.48	99.37
Metformin HCL	200	6487.07±14.30	102.41	6469.99±0.38	102.06
	300	8817.07±5.69	99.69	299.18±1.16	99.73
	400	11243.2±11.88	99.30	397.28±1.62	99.32
RP-HPLC: Reverse phase high performance liquid chromatography					

3.4.5 Robustness

The robustness of a method is it stability to remainun affected by small deliberate changes in parameters. The effect of changes in mobile phase composition ±1 mL/min-1, wavelength ±1 mL/min-1, and flow rate ±1 mL/min-1 on retention time and tailing factor of drug peak was studied. The results of robustness studies are shown in Table 7. Robustness parameters were also found satisfactory; hence, the analytical method would be concluded.

Table 7: Robustness study of Dapagliflozin and Metformin

Parameter	Amount of detected (mean± SD)	% RSD	Amount of detected (mean± SD)	% RSD
	Dapagliflozin		Metformin	s
Flow change 0.9 mL	453.71±3.06	0.67	7295.50±1.19	0.02
Flowchange1.1mL	491.63±1.29	0.26	5958.91±15.04	0.5
Chromatogram ofmobilephase76:24	444.49±3.20	0.72	6393.09±7.63	0.12
Chromatogram ofmobilephase74:26	404.7±0.97	0.24	6398.0±17.33	0.27
Changeinwavelength232nm	429.3±1.37	0.32	6308.4±7.30	0.12
Changeinwavelength234nm	374.61±1.13	0.30	6954.02±12.58	0.18

3.4.6 Limit of detection (LOD)

The LOD is the lowest limit that can be detected. It is based on the deviation of the response and the slope. The LOD can be calculated from below formula and the results are shown in Table 8.

Table 8: Data for LOD and LOQ

Drugname	LOD	LOQ
Dapagliflozin	0.06µg/mL	0.1855µg/mL
Metformin HCL	6.09µg/mL	18.45µg/mL
LOD: Limit of detection, LOQ: Limit of quantification		

4. Conclusion

Simple, rapid, accurate, and precise RP-HPLC methods have been developed and validated for the routine analysis of Dapagliflozin and Metformin HCL in API and tablet dosage form. Both methods are suitable for simultaneous determination of Dapagliflozin and Metformin in multi-component formulation without interference of each other. In this study, the stability data for Dapagliflozin and Metformin through stress degradation studies under ICH recommended stress condition. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Furthermore, the value of standard deviation and coefficient of variation calculated was satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms. Hence, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Dapagliflozin and Metformin in bulk drug as well as in formulation.

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