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Research Article

STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND METFORMIN TABLET DOSAGE FORM

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ABSTRACT

Objectives: The objective of this study was to developed and validated for the routine analysis of dapagliflozin (DAPA) and metformin (MET) in API and tablet dosage forms.

Methods: The estimation of DAPA and MET was accomplished with C18 column (4.6 × 250 mm and 5 µm particle size) with DAD detector. The mobile phase consists of Methanol: Water 75:25% v/v of pH-3 adjusted with 0.05% OPA at flow rate of 1 mL/min. The detection wavelength was 233nm, respectively.

Results: In the developed method, the retention time of DAPA and MET was found to be 5.099 min and 2.165 min. The reverse phase high performance liquid chromatography method produces linear response that were found in the range of 100–500 µg/mL. The limit of detection and limit of quantification for DAPA were found to be 0.06 µg/mL and 0.1855 µg/mL, respectively and for MET 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.

Conclusion: 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, Reverse phase high performance liquid chromatography, ICH guidelines, Limit of detection, Limit of quantification, Design expert.

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INTRODUCTION

Dapagliflozin (DAPA) (Fig. 1) is a selective sodium glucose cotrasporter subtype-2 (SGLT-2) inhibitor with antihyperglycemic activity. Its chemical name is (2S, 3R, 4R, 5S, 6R)-2[4-chloro-3-(4-ethoxyphenyl) methyl] phenyl-6-(hydroxymethyl)oxane-3,4,5-triol. It has molecular formula of $C_{21}H_{25}ClO_6$ and molecular weight is 408.9 g/mol. DAPA is a SGLT-2 inhibitor indicated for managing diabetes mellitus Type 2. When combined with diet and exercise in adults, DAPA helps to improve glycemic control by inhibiting glucose resorption in the proximal tubule of the nephron and causing glycosuria [1-8].

Metformin (MET) hydrochloride (Fig. 2) is chemically known as (3-(diaminomethylidene)-1,1-dimethylbiguanide; hydrochloride. It was molecular formula $C_4H_{11}N_5$ and molecular weight is 129.16 g/mol. MET is an agent belonging to the biguanide class of antidiabetics with antihyperglycemic activity. MET is the first line agent for the treatment of Type 2 diabetes that can be used alone or in the combination with sulfonylureas, thiazolidinedione, incretin based drugs, sodium glucose cotransporter-2 inhibitor, or other hypoglycemic agent [9-13].

The combination dosage form selected for the present study contains DAPA and MET in solid oral dosage forms, recently this combination has been approved by USFDA. The main aim of this study was to develop a stability indicating method for the simultaneous estimation of DAPA and MET in bulk and to apply the developed method for the quantitative determination of these drugs from its tablets and the reverse phase high performance liquid chromatography (RP-HPLC) method was chosen. This method was validated as per International Conference Harmonization (ICH) guidelines [14-17].

Literature survey revealed that some analytical methods were reported for the estimation of DAPA and MET individually or in combination with other drugs, by HPLC analytical method. No of stability indicating RP-HPLC method was reported for estimation of both these drug. Now a day, stability indicating method as important regulatory and CGMP point of view to assess the drug stability. In the present study, it was tired to develop stability indicating RP-HPLC method to determine possible degradation products of DAPA and MET [18-25].

METHODS

Instrument

The analysis of the drug was carried out on agilent Tech. Gradient System with Auto injector, DAD Detector. Equipped with Reverse Phase (Agilent) C18 Column (4.6 mm × 250 mm; 5 µm) and UV730D Absorbance detector and running chemstation 10.1 software.

Chemicals and reagents

Methanol, Water, and Potassium phosphate buffer of HPLC grade were obtained from Merck Ltd., India. Drug DAPA and MET were kindly supplied as a gift samples from Swapnroop drugs and Pharmaceuticals, Aurangabad.

Chromatographic condition

C18 Column (250 mm × 4.6 mm; 5 µm) was used for the chromatographic separation at a detection wavelength of 233 nm using flow rate 1 mL/min. Mobile phase used was Methanol: Water in the ratio of 75:25 of pH=3 adjusted with OPA 0.5% solution.

Selection of wavelength

Scan the standard solution in UV spectrophotometer between 200 nm and 400 nm on spectrum mode, using methanol as a reference solvent. The two drugs show λ_{max} at 277 nm and 236 nm for DAPA and MET (Figs. 3 and 4).

Preparation of standard stock solution

5 mg of pure powdered DAPA and 250 mg of powdered MET was separately weighted dissolved in 25 mL volumetric flask containing methanol. The solution was sonicated for 15 min and filter through Whatman filter paper. The final concentration of this solution was 200 µg/mL of DAPA and 10000 µg/mL of MET.

Method validation

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 a given range. From standard stock solution, 0.05 mL were pipette, out in 10 mL conical flask with diluents was added to make up volume. The final concentration of these solution was observed in the range of 2–10 µg/mL for DAPA and 100–500 µg/mL for MET. 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 parameter were used to confirm the good linearity of the method. The results are shown in Tables 1 and 2 and Figs. 5 and 6.

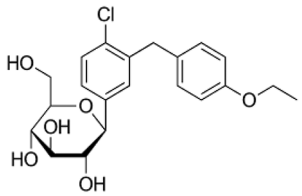

Fig. 1: Dapagliflozin

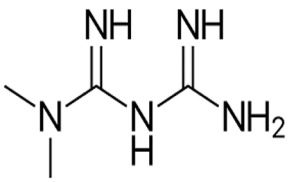

Fig. 2: Metformin

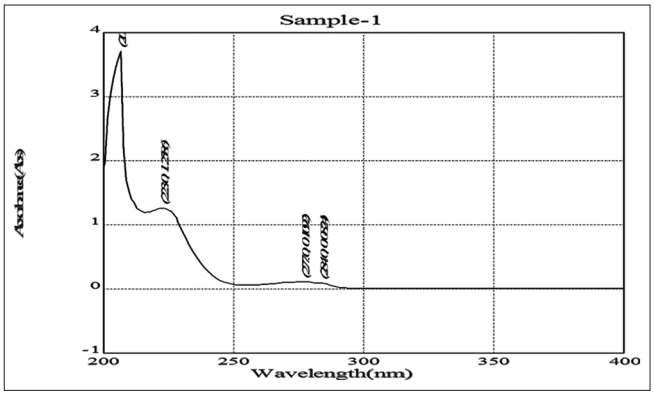

Fig. 3: UV spectrum of dapagliflozin

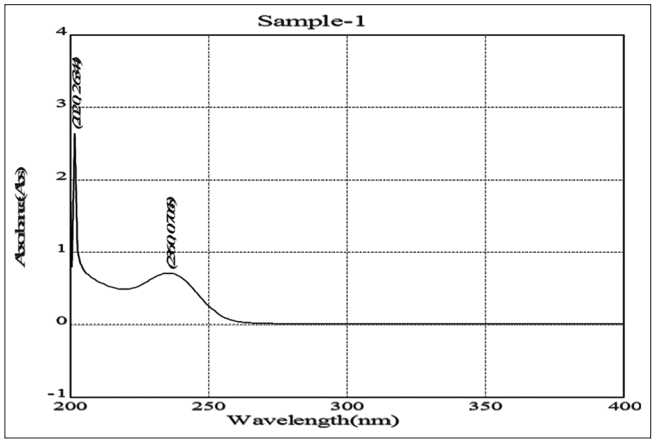

Fig. 4: UV spectrum of metformin

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 present recovery by the assay of known added amounts of analyte. The accuracy was determined by DAPA and MET (equivalent to 100 mg of MET and 40 mg of DAPA) (80%, 100%, and 120% of the label claimed, respectively) to quantity equivalent to average weight of marketed tablets. The resulting mixtures were analyzed in triplicates over 3 days. The % recovery of added drug was taken as a measure of accuracy. The results are shown in Table 3.

Repeatability

Precision of the system was determined with the sample of RP-HPLC method for six replicates of sample solution containing 5 mg of DAPA and 250 mg of MET were injected and peak areas were measured and %RSD was calculated. It was repeated for 5 times and results are shown in Table 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 MET of three different concentration (200 µg/mL, 300 µg/mL, and 400 µg/mL) and 5 mg of DAPA (4 µg/mL, 6 µg/mL, and 8 µg/mL). DAPA and MET were analyzed 3 times on the same day. For inter-day precision above, same concentration was used at different days and %RSD was calculated. The results are shown in Table 5.

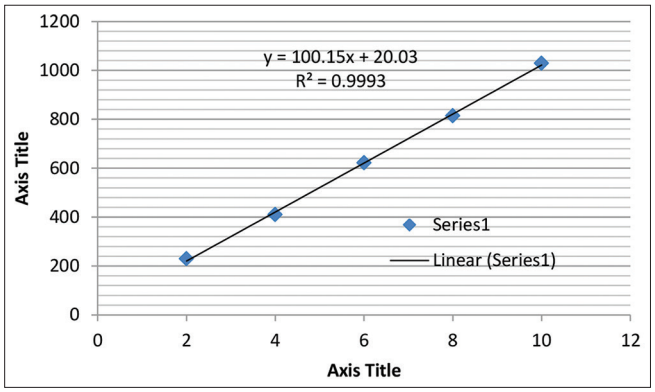

Fig. 5: Calibration curved of dapagliflozin

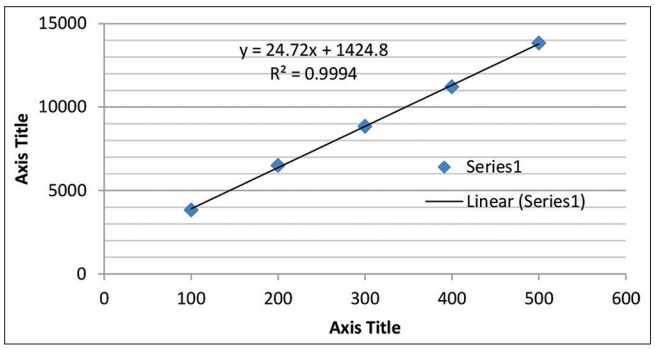

Fig. 6: Calibration curve of metformin

Table 1: Linearity data for dapagliflozin

Method	Concentration (µg/mL)	Average peak area (µV.s)	SD of peak area	%RSD of peak area
RP-HPLC Method	2	229.49	1.75	0.76
	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		
	R^2	0.9993		

RP-HPLC: Reverse phase high performance liquid chromatography

Table 2: Linearity data for metformin

Method	Concentration (µg/mL)	Average peak area (µV.sec)	SD of peak area	%RSD of peak area
RP-HPLC Method	100	3828.14	27.64	0.72
	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		
	R^2	0.9994		

RP-HPLC: Reverse phase high performance liquid chromatography

Table 3: Result of recovery data for dapagliflozin and metformin

Drug	Level (%)	Amount taken (µg/mL)	Amount added (µg/mL)	Area mean* ±SD	Amt. recovered* ±SD	%Recovery Mean* SD
DAPA	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
MET	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

Table 4: Repeatability studies on RP-HPLC for dapagliflozin and metformin

Method	Concentration of DAPA and MET (mg/mL)	Peak area	Amount found (mg)	% Amount found
RP-HPLC Method for DAPA	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 MET	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

Robustness

The robustness of a method is its ability to remain unaffected 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 6. Robustness parameters were also found satisfactory; hence, the analytical method would be concluded.

Limit of detection (LOD)

The LOD is the lowest limit that can be detected. It is based on the SD eviation of the response and the slope. The LOD can be calculated from below formula and the results are shown in Table 7.

LOD=3.3 (SD)/S

Where, SD=Standard deviation of the response

S=Slope

Limit of quantification

The LOQ is the lowest concentration analyte that can be detected quantitatively from sample, with suitable acceptable precision and accuracy under the stated experimental condition. LOQ can be calculated from the below formula and the results are shown in Table 7.

LOQ=10 (SD)/S

Where, SD=Standard deviation of the response

S=Slope

Analysis of marketed formulation

For analysis of the matrix tablet dosage form, 20 tablets were weighed individually and their average weight was calculated. The tablets were triturated to make fine powder and powder equivalent to 10 mg DAPA and MET into 10 mL volumetric containing methanol. It was sonicated for 15 min to dissolve it completely and filtered through 0.45 µm membrane filter. Further pipette 0.4 mL of the above stock solution into a 10 mL volumetric flask and diluent up to the mark with diluents (8 µg/mL DAPA and 400 µg/mL). The results of analysis for the marketed tablet formulation (Xigduo® XR which contain 10 mg of DAPA and 500 mg of MET) are reported in Table 8.

Forced degradation study

Degradation behavior

Forced degradation studies of both the drugs, namely, DAPA and MET were carried out individually and in the combination under

Table 5: 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
DAPA	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
MET	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

Table 6: Robustness study of dapagliflozin and metformin

Parameter	Amount of detected (mean±SD)	%RSD	Amount of detected (mean±SD)	%RSD
	Dapagliflozin		Metformin	
Flow change 0.9 mL	453.71±3.06	0.67	7295.50±1.19	0.02
Flow change 1.1 mL	491.63±1.29	0.26	5958.91±15.04	0.5
Chromatogram of mobile phase 76:24	444.49±3.20	0.72	6393.09±7.63	0.12
Chromatogram of mobile phase 74:26	404.7±0.97	0.24	6398.0±17.33	0.27
Change in wavelength 232 nm	429.3±1.37	0.32	6308.4±7.30	0.12
Change in wavelength 234 nm	374.61±1.13	0.30	6954.02±12.58	0.18

Table 7: Data for LOD and LOQ

Drug name	LOD	LOQ
Dapagliflozin	0.06 µg/mL	0.1855 µg/mL
Metformin	6.09 µg/mL	18.45 µg/mL

LOD: Limit of detection, LOQ: Limit of quantification

Table 8: Analysis of marketed formulation

Assay	Drug	Amount found	% Label claim	SD	%RSD
Xigduo XR 10 mg/500 mg DAPA/MET	DAPA	7.93	99.16	0.93	0.11
	MET	397.01	99.25	2.94	0.02
	DAPA	7.94	99.31	0.09	0.11
	MET	25.85	99.30	0.11	0.03

Table 9: Degradation of different stress condition

S. No.	Degradation parameter	DAPA Amt. found	MET Amt. found
1	Acid degradation (0.1 N HCl) after 1 h 6+300 mcg	8.21	7.00
2	Alkali degradation (0.1 N NAOH) after 1 h 6+300 mcg	9.61	11.01
3	3% H_2O_2 degradation – After 1 h 6+300 mcg	8.11	3.48
4	Neutral after 1 h 6+300 mcg	0.82	0.04
5	Photolysis study after 24 h 6+300 mcg	0.79	0.05

DAPA: Dapagliflozin, MET: Metformin

different stress condition such as acid hydrolysis, alkaline hydrolysis, hydrogen peroxide oxidation, and photolysis. The results are shown in Table 9.

Acid hydrolysis

The acid hydrolysis was performed using 0.1 N hydrochloride at 70°C for 1 h. The major degradation product for DAPA and MET was observed amount found at 70°C for 1 h. The degradation product was observed at RRT of 15 min. Chromatogram is shown in Fig. 7.

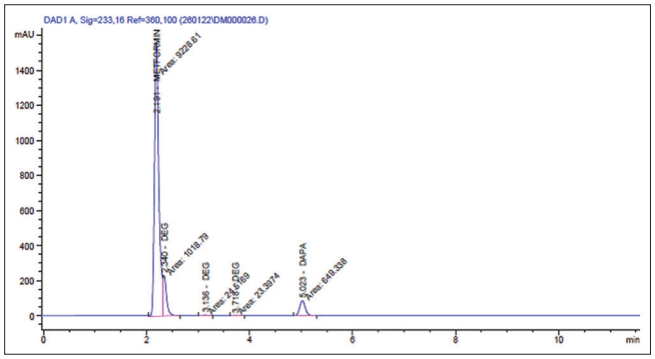

Fig. 7: Degradation for 0.1 N HCl at 1 h

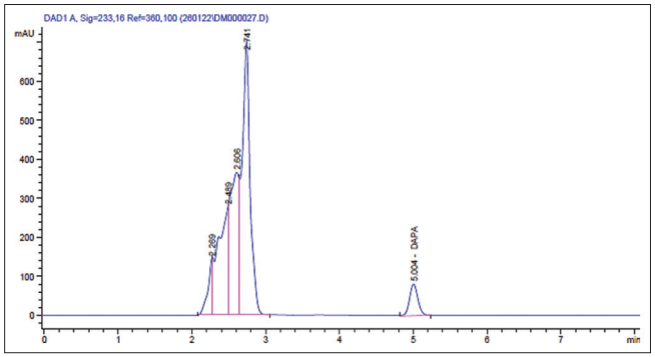

Fig. 8: Degradation for 0.1 N NAOH at 1 h

Alkaline hydrolysis

The alkaline hydrolysis condition was performed using 0.1 N Sodium hydroxide at 70°C for 1 h both DAPA and MET. Degradation of MET was found to occur profusely than DAPA. The major degradation product for DAPA and MET was observed. Chromatogram is shown in Fig. 8.

Oxidation

In the oxidation condition with 3% hydrogen peroxide for 1 h, both DAPA and MET show any oxidative stress degradation peak in the chromatogram (Fig. 9).

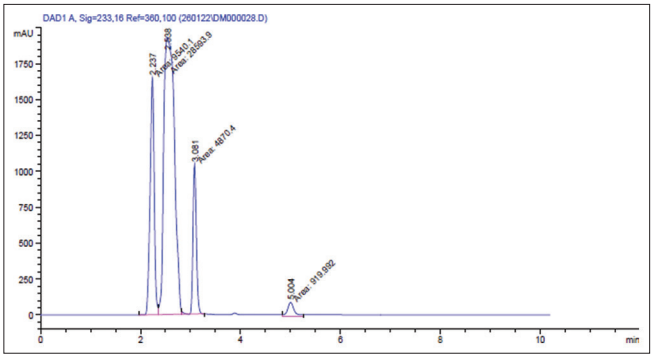

Fig. 9: Degradation for 3% H_2O_2 at 1 h

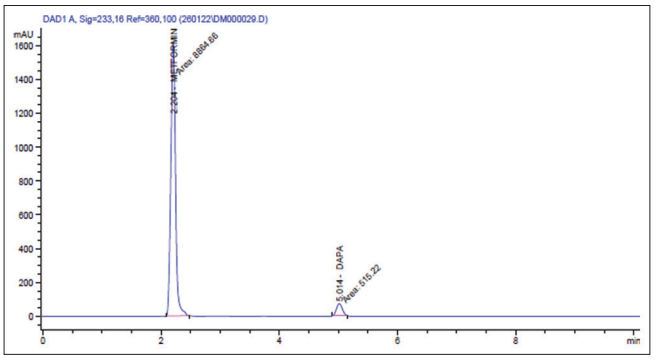

Fig. 10: Degradation for neutral at 1 h

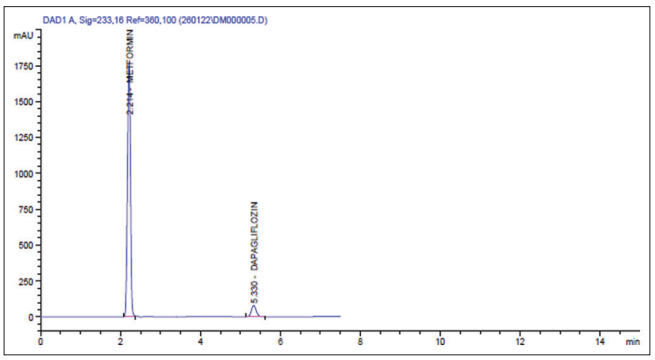

Fig. 11: Degradation for photolysis at 24 h

Neutral

There was no major degradation observed for both DAPA and MET and, hence, they were not sensitive to light at 70°C for 1 h (Fig. 10).

Photolysis studies

For photolysis stress, DAPA and MET – API, tablet powder and solution of both were prepared and exposed to a controlled temperature oven at 70°C for 24 h (Fig. 11).

RESULTS AND DISCUSSION

The goal of this study was to develop and validate a simple, precise, accurate, and economical RP-HPLC method. The retention time of DAPA and MET was found to be 5.099 min and 2.165 min, respectively. The forced degradation study was conducted for determining the stability indicating power of an analytical procedure. The results of the stress studies in Table 9 and chromatograms were represented in Figs. 7-11. The respective linear equation for DAPA was y=100.1x+20.03 and MET was y=24.72x+1424.8 and the correlation coefficient was 0.9993 and 0.9994. The calibration curve of DAPA and MET is represented in Figs. 5 and 6. Accuracy of RP-HPLC is ascertained by recovery studies performed at different levels of concentration (80%, 100% and 120%). The % recovery was found to be within 98% to 102% (Table 3). The precision and repeatability for both drugs are summarized in Tables 4 and 5, respectively. The assay percentage was found to be 99-101% for both drugs, respectively, as shown in Table 8. The percent relative standard deviation for above was found to be <2%. Therefore, the results indicate that the method is highly accurate, precise, and reproducible.

CONCLUSION

Simple, rapid, accurate, and precise RP-HPLC methods have been developed and validated for the routine analysis of DAPA and MET in API and tablet dosage form. Both methods are suitable for simultaneous determination of DAPA and MET in multi-component formulation without interference of each other. In this study, the stability data for DAPA and MET 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 MET in bulk drug as well as in formulation.

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AUTHORS' CONTRIBUTION

Dhanshri S. Nandre has performed the work presented here. Aejaz Ahmed contributed to the analyzing and collecting data. G. J. Khan has guided this research.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

AUTHORS FUNDING

Nil declared by all authors.

REFERENCES

1. Tripathi KD. Essential of Medical Pharmacology. 8th ed. New Delhi: Jaypee Brothers Medical Publishers; 2018. p. 294, 299-301.
2. Suma BV, Deveshwaran R. An overview of analytical methods for dapagliflozin. An antibiotic drug. Int J Pharm Sci Res 2018;10:2688-92.
3. Kasture AV, Wadodkar SG, Mahadik KR, More HM. A Textbook of Pharmaceutical Analysis. Vol. 2. 10th ed. Pune: Nirali Prakashan; 2004. p. 49-50.
4. Grace AC, Prabha T, Sivakumar T. Development and validation of high performance liquid chromatographic method for determination of dapagliflozin and its impurities in tablet. Asian J Pharm Clin Res 2019;12:447-53.
5. Beckett AH, Stenlake GH. Practical Pharmaceutical Chemistry. Vol. 2. 4th ed. New Delhi: CBS Publishers and Distributors; 2004.
6. Urooj A, Sundar PS, Raja A, Dutt KR, Rao KN, Ramana H. Development of validation of RP-HPLC mehtod for simultaneous estimation of dapagliflozin and metformin in bulk and in synthetic mixture. World J Pharm Pharm Sci 2017;6:2139-59.
7. Sanagpati M, Dhanalakshmi K, Nagarjunreddy G, Sreenivasa S. Development and validation of a RP-HPLC method for the estimation of dapagliflozin in API. Int J Pharm Sci Res 2014;2:53-64.
8. Dhale RJ. Degradation pathway proposal, structure elucidation and *in silico* toxicity evaluation studies for dapagliflozin. J Chromatogr 2019;13:54-67.
9. Gadipally S. Method development of metfromin hydrochloride RP-HPLC. World J Pharm Pharm Sci;3:1149-59.

10. Chadalawada PK, Divya V, Adilakshmi C, Sreevidya P, Khan I. RP-HPLC analytical method development and validation of metformin hydrochloride tablets assay. Int J Pharm Biol Sci 2019;9:505-19.
11. Gopal NM, Sridhar C. A validated stability indicating ultra-performance liquid chromatographic method for simultaneous determination of metformin hydrochloride and empagliflozin bulk drug and tablet dosage form. Int J Appl Pharm 2017;9:45-50. doi: 10.22159/ijap.2017v9i3.17441
12. British Pharmacopoeia. British Pharmacopoeia Commission. Vol. 2. London: British Pharmacopoeia; 2015. p. 1292.
13. The Indian Pharmacoporia. Government of India, Ministry of Health and Family Welfare. Vol. 4. Ghaziabad: The Indian Pharmacopoeia; 2018. p. 2544.
14. International Council for Harmonisation. Guideline Q2. A Validation of Analytical Procedures: Definations and Terminology. 2005 Incorporated in Q2 (R1). Geneva: International Council for Harmonisation. Guideline; 1995.
15. International Council for Harmonisation. Guideline Q2. B, Validation of Analytical Procedures: Text and Methodology. In: 2005 incorported in Q2 (R1). Geneva: International Council for Harmonisation; 1996.
16. International Council for Harmonisation. Stability Testing of New Drug Substance and Products, International. Conference on Harmonization, International Federation of Pharmaceutical Manufacturers Associations. Geneva: International Council for Harmonisation; 1993.
17. International Council for Harmonisation. Impurities in New Drug Products, International. Conference on Harmonization, International Federation of Pharmaceutical Manufacturers Associations. Geneva: International Council for Harmonisation; 1996.
18. Madhavi S, Rani AP. Development and validation of a method for simultaneous determination of dapagliflozin and saxagliptin in a formulation by RP-HPLC. World J Pharm Res 2017;6:2277-7105.
19. Nidhi K, Jayvadan P. Development and validation of a stability indicating RP-HPLC method for the simultaneous estimation of metformin and dapagliflozin presence of their degradation products. A review. Int J Pharm Sci Rev Res 2018;56:1-6.
20. Goday S, Shaik AR, Avula P. Development and validation of LC-ESI-MS based bio analytical method for dapagliflozin and saxagliptin in human plasma. Indian J Pharm Educ Res 2018;52:S277-86.
21. Bhavyasri K, Surekha T, Sumakanth M. A novel method development ADN validation of dapagliflozin and metformin hydrochloride using simultaneous equation method by UV-visible spectroscopy in bulk and combined pharmaceutical formulation indicating forced degradation studies. J Pharm Sci Res 2020;12:1100-5.
22. Parmar SH, Luhar SV, Narkhede SB. Development and validation of UV-spectroscopic first derivative and high performance thin layer chromatography analytical methods for simultaneous estimation of dapagliflozin propanedial monohydrate and saxagliptin hydrochloride in synthetic mixture. Eur J Biomed Pharm Sci 2018;5:669-84.
23. Patel PD, Pandya SS. Valiated RP-HPLC method for simultaneous estimation of Dapagliflozin and metformin hydrochloride in tablet dosage form. Int J Pharm Res Scholars 2018;7:9-15.
24. Khalil GA, Salama I, Gomaa MS, Helal MA. Validated RP-HPLC method for simultaneous determination of canagliflozin, dapagliflozin, empagliflozin and metformin. Int J Pharm Chem Biol Sci 2018;8:1-13.
25. Nikam N, Maru A, Jadhav A, Malpure P. Analytical method development and validation of metformin hydrochloride by using RP-HPLC with ICH guidelines. Int J Trend Sci Res Dev 2019;3:415-9.