IJPSR (2023), Volume 14, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 May 2022; received in revised form, 15 July 2022; accepted, 03 August 2022; published 01 February 2023

DEVELOPMENT AND VALIDATION OF QBD-ASSISTED RP-HPLC METHOD FOR DAPAGLIFLOZIN AND METFORMIN HCL IN BULK AND ITS COMBINED DOSAGE FORM

Vidhi Dave * and Paresh U. Patel

Department of Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva - 384012, Gujarat, India.

Keywords:

Dapagliflozin, Metformin HCl, Quality by design, Validation

Correspondence to Author:
Vidhi Dave

Research Scholar,
Department of Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat Vidyanagar, Kherva - 384012, Gujarat, India.

E-mail: vidhidave1988@gmail.com

ABSTRACT: Dapagliflozin and Metformin HCl Combination is the novel SGLT2 inhibitor used to control Hyperglycemia in type 2 diabetes mellitus. A literature survey reveals that various Analytical methods have been developed for Dapagliflozin in single and combined with other drugs. No QbD-assisted RP-HPLC method is available to estimate Dapagliflozin and Metformin HCl in combination. So, present work describes the development and validation of QbD driven RP-HPLC method for Dapagliflozin and Metformin HCl. The RP HPLC method has been developed using Agilent 1200 series HPLC with Intersil ODS column (250 x 4,6mm, 5 µm). Based on the RP-HPLC method, development Flow rate and pH were selected as CAA and Resolution, Retention time and Tailing of Drug were monitored using Design Expert@13.00. By applying CCD, 9 trials, 2 factors and 3 responses method had been selected for method development for Dapagliflozin and Metformin HCl. The optimum method development was selected based on the criteria of attaining appropriate Resolution, Tailing, and retention time. The overlay plot of responses generates an optimized area as per desired criteria of Resolution 12.65, Tailing 1.09 and the retention time of 2.48 min using ACN: KH_2PO_4 pH 4.5 (65:35%v/v) at a flow rate of 1ml/min. The developed method was successfully validated as per ICH Q2(R1) guidelines.

INTRODUCTION [1-9]: Dapagliflozin, chemically (2S, 3R, 4R, 5S, 6R)-2-[4-chloro-3-[(4ethoxyphenyl) methyl] phenyl] – 6 -(hydro-xymethyl) oxane-3,4,5-triol [1-3], is an oral selective Sodium-Glucose co-transporter 2 (SGLT2) inhibitor used for the management of type 2 Diabetes Mellitus as shown in **Fig. 1**. Metformin is chemically N, N-Dimethylimidodicarbonimidic diamide hydrochloride. It is a biguanide derivative, as shown in **Fig. 2.**

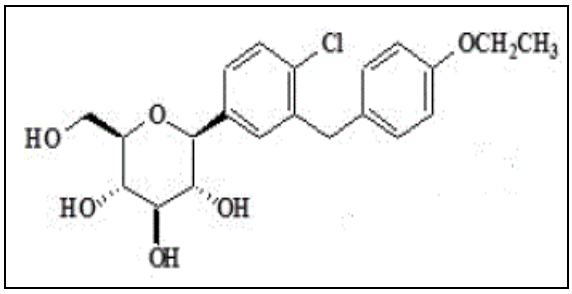

FIG. 1: STRUCTURE OF DAPAGLIFLOZIN

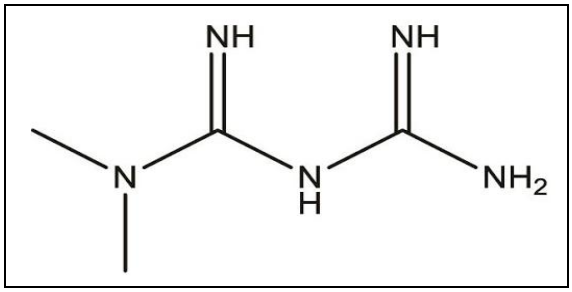

FIG. 2: STRUCTURE OF METFORMIN HCl

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(2).788-94

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.14(2).788-94

From literature survey it was found that various UV, HPLC and stability indicating methods were developed for Dapagliflozin and Metformin HCl, these reported methods are marked with limitations like high retention time (RT), high operational costs and low value of sensitivity, accuracy, and precision [16-23]. This calls for developing a more sensitive, economical and robust analytical method for estimating the drug molecule by applying the QbD approach [24-26]. For the last couple of years, quite a few scientific research have effectively established the Applicability of the analytical quality by design (AQbD) approach for developing efficient, cost-effective and robust chromatographic methods for quantitative estimation of analytes from bulk drugs as well as biopharmaceutical samples. AQbD embarks upon a systematic understanding of the associated interaction(s) among the diverse variables involved during the analysis, where preliminary risk assessment studies are conducted to earmark the potential key critical process parameters (CPPs).

Subsequently, Factor screening studies are carried out to identify the influential factors, followed by method optimization for obtaining the desirable chromatographic solution. The application of AQbD paradigm offers distinct advantages to improving the method performance and facilitates the holistic understanding of the chromatographic process. The present work was, therefore, undertaken to develop a cost-effective, simple, sensitive, and robust analytical method using reversed-phase HPLC for quantification of Dapagliflozin and Metformin HCl with high accuracy and precision, coupled with fast elution while adapting to the principles of AQbD and ICH guidelines Q2 (R1) for subsequent validation [31].

MATERIALS: Dapagliflozin working standard was procured as a gift sample from Amneal Pharmaceuticals Ltd. Tablet formulation of Dapagliflozin and Metformin HCl (Xigduo Dapagliflozin10 mg and Metformin HCl 100mg) was purchased from the local market.

Chemicals and Reagents: All chemicals were of analytical grade.

Instrumentation: Agilent 1200 series HPLC with Intersil ODS column (250 x 4.6mm, 5 µm) and by ChemStation software was used during Method Development and Validation.

Factor Screening Studies: A 2-factor nine-run fractional factorial design was employed for factor screening studies to identify the CMPs/CPPs critically affecting the method CAAs (*i.e.*, Flow rate and pH). A total of nine experimental runs were performed, and the design was analyzed under the influence of studied factors on the CAAs, as shown in **Table 1.** Mathematical data analysis was carried out by fitting the obtained experimental data to the linear polynomial model by obviating the interaction term(s). CAAs associated with this method were selected as per **Table 1.**

TABLE 1: SELECTION OF CAA AND RESPONSE

CAA	Response monitored
Flow Rate	Resolution
pH	Retention time of drug
	Tailing of drug

Selection of CAA and Response Monitored using CCD Model:

Design Matrix as Per the CCD for Optimization of the HPLC Method:

TABLE 2: DESIGN MATRIX FOR DAPAGLIFLOZIN AND METFORMIN HCl

Run	pH	Flow Rate (ml/min)
1	+1	+1
2	+1	-1
3	+1	0
4	0	+1
5	0	-1
6	0	0
7	-1	+1
8	-1	0
9	-1	-1

pH: 0 coded for 4.5, +1 coded for 5, -1 coded for 4.3.

Flow Rate: 0 coded for 1 ml/min, +1 coded for 1.2 ml/min, -1 coded for 0.8 ml/min.

Optimization of Mobile Phase: Aliquot portions of standard stock solutions were injected into the HPLC system. Different solvents with varying polarity as well as a combination of solvents were tried to get well-separated peaks of the drugs.

After trying several arrangements and combinations, the solvent system ACN: Phosphate buffer 6.5 pH 4.5 (65:35%v/v) gave good resolved separated Peaks of Met and Dapa Respectively.

Optimization Data Analysis and Model Validation: The optimization data analysis was carried out by multiple linear regression analysis (MLRA) using Design Expert® ver. 13.0.1 software (M/s Stat-Ease Inc., MN, USA) for fitting the experimental data to the second-order quadratic polynomial model for estimating both the main and interaction effects. The model coefficients with statistical significance <0.05 were considered in framing the polynomial equation. The model aptness was finally ratified by analyzing various parameters like the coefficient of correlation (r^2), predicted error sum of squares (PRESS), and lack of fit analysis. Response surface analysis was carried out from the 2D-contour and 3D-response surface plots to discern the factor–response relationship and plausible interaction effect.

Analytical Method Validation: The optimized method was ratified for linearity, system suitability, the limit of quantification (LOQ), the limit of detection (LOD), precision, accuracy, selectivity, and robustness, as per the guidelines recommended by ICH Q2 (R1).

Preparation of Stock Solution: The standard stock solution of Dapagliflozin and Metformin HCl was prepared by dissolving 10 mg drug in 10 ml methanol, giving 1000 µg/ml concentration. From that 100 µg/ml solution of Dapagliflozin and Metformin HCl were prepared by taking 5ml of the above solution in 50 ml volumetric flasks and were diluted up to the mark with Methanol respectively for Dapa and Met.

Preparation of Calibration Curve [4, 5]: Different aliquots were taken from the 100 µg/ml of Dapagliflozin and Metformin HCl stock solution in separate 50ml volumetric flask and finally diluted with Methanol solution to prepare a series of concentration ranging from 2-6 µg/ml of DAPA and 100-300 µg/ml MET.

Linearity: Linearity of the developed method was investigated for the drug concentrations ranging between 2-6 µg/ml of DAPA and 100-300 µg/ml for MET. A calibration curve is plotted, and the correlation coefficient was calculated per ICH guidelines.

Precision: Precision was measured by performing Repeatability (4 µg/ml for DAPA and 200 µg/ml for MET), Interday precision (3.2 µg/ml, 4 µg/ml and 4.8 µg/ml for DAPA and 160 µg/ml, 200 µg/ml and 240 µg/ml for MET) and Intraday precision (3.2 µg/ml, 4 µg/ml and 4.8 µg/ml for DAPA and 160 µg/ml, 200 µg/ml and 240 µg/ml for MET). % RSD should be less than 2%

Accuracy: Accuracy as determined over the range 50% w.r.t. to lowest sample concentration to 150 % w.r.t. to highest sample concentration Prepared triplicate preparations for each level and HPLC chromatogram were taken and calculated the amount found, % recovery at each level, the mean % recovery and % RSD**.**

Limit of Detection and Limit of Quantification: As per ICH Q2 (R1) guidelines, the sensitivity of the developed method was evaluated by the mean of LOD and LOQ, which are dependent on the Standard deviation of intercept and mean of a slope.

$$LOD = 3.3 * \sigma / s$$
$$LOQ = 10 * \sigma / s$$

σ = standard deviation of intercept and s = mean of slope

System Suitability Parameters: Various system suitability parameters such as Theoretical plates, Resolution, Retention time, asymmetry are calculated and obtained results are compared with ICH guidelines specifications.

Robustness: Robustness was performed by changing Mobile phase (±3), pH ((±3) and Flow rate (±0.1).

Analysis of Dapagliflozin in Tablet Formulation: Twenty tablets were weighed and powdered finely. A quantity of tablet powder equivalent to 10 mg of Dapagliflozin and 100mg Metformin HCl was accurately weighed and Transferred to 10 ml volumetric flask and sonicated for 10 min.

RESULTS: Using Design expert @13, Response surface methodology is used to optimize the developed method. ANOVA quadratic models were applied for resolution, retention time, and Tailing. P value and Model F values are calculated for mentioned three responses. Fit Statistics were also checked by mean of predicted $R^{2,}$ adjusted R^2 and Adequate R^2.

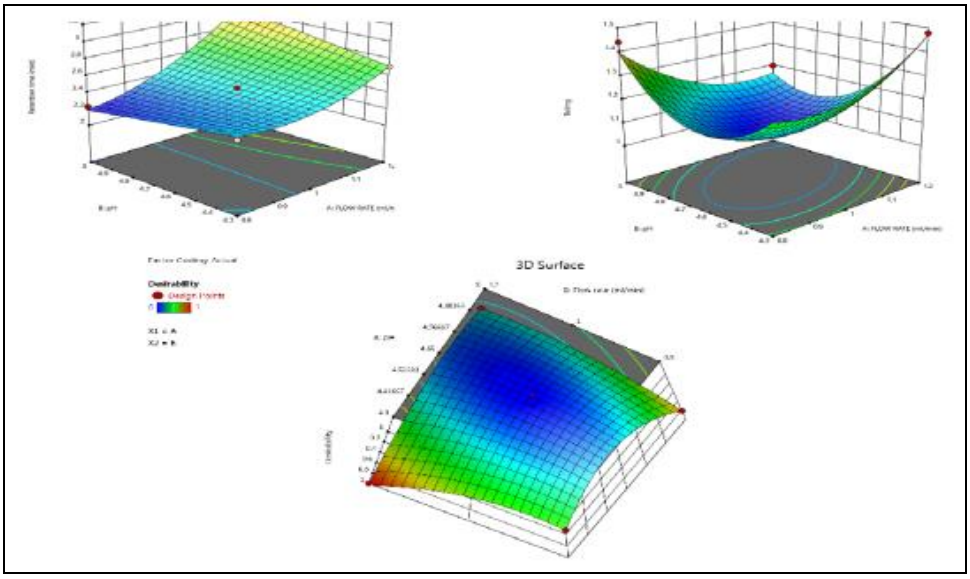

FIG. 3: 3D PLOTS

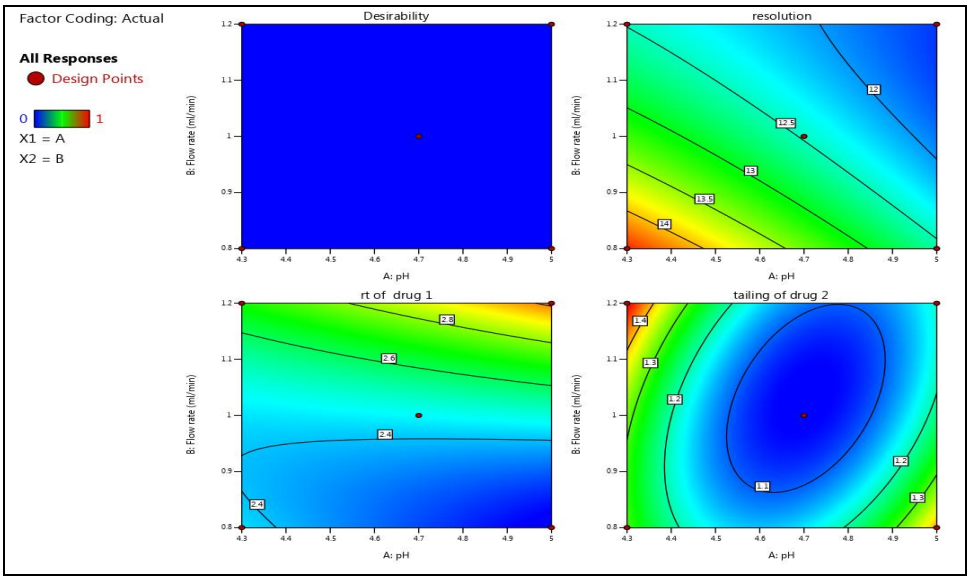

FIG. 4: DESIRABILITY PLOTS

Checkpoint Analysis: Checkpoint analysis was performed by taking three batches from 100 solutions given by DOE Software. Run no. 22, 35, and 98 were selected from input variables. Responses were measured by putting responses coded values in a polynomial equation. Predicted and measured values found for selected runs as per **Table 3.**

TABLE 3: CHECK POINT ANALYSIS

Run No.	X1 Flow Rate	X2 pH	Y1(Resolution)		Y2 (Retention Time)		Y3 (Tailing)	
			Measured	Predicted	Measured	Predicted	Measured	Predicted
26	1.012	4.56	12.82	12.831	2.43	2.478	1.12	1.085
35	1.043	4.74	12.98	12.400	2.43	2.538	1.12	1.056
98	0.833	4.75	12.76	13.132	2.66	2.29	1.39	1.3932

When measured Resolution, Retention Time and Tailing values were compared with Predicted Resolution, Retention Time and Tailing, the values were found to be significant. Thus, it can be concluded that the obtained mathematical equation is valid for the predicted value.

Optimization: An optimization technique using a desirable approach to develop a new method for the desired responses. This was the most important part of the response surface Methodology. The optimum method development was selected based on the criteria of attaining appropriate Resolution, Tailing,

and retention time. The overlay plot of responses generates an optimized area as per desired criteria of Resolution 12.65, Tailing 1.09 and the retention time 2.48 min. So, it can be concluded that by adopting a systemic formulation approach, one can reach an optimum Resolution, Tailing and retention time. The % Predicted error was calculated using the formula;

% Error = (Experimental – Predicted) / Predicted *100.

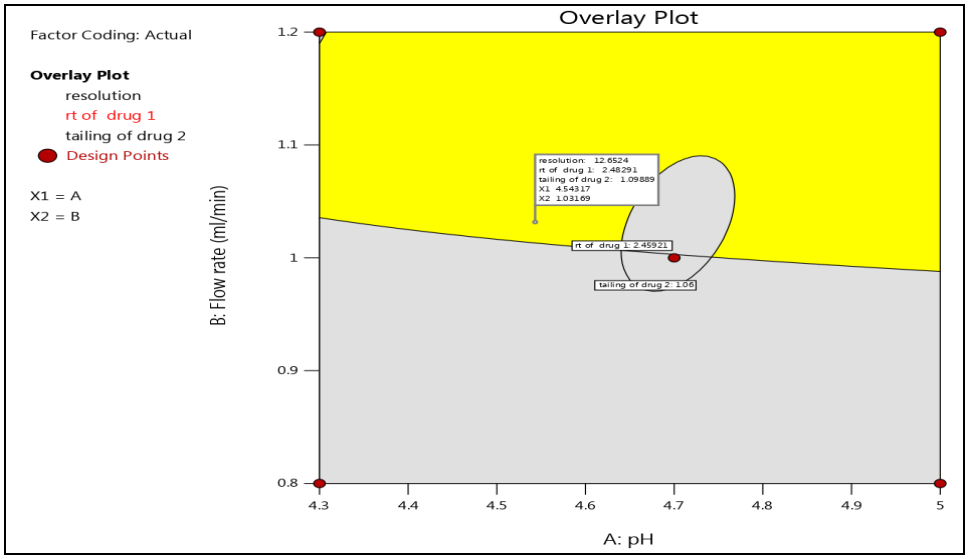

FIG. 5: OVERLAY PLOT FOR DAPAGLIFLOZIN AND METFORMIN HCl

TABLE: 4 PREDICTED ERROR

Optimum Condition	pH	Flo Rate	Resolution	Retention Time	Tailing of Dapa
1	4.56	1.012			
		Predicted	12.83	2.478	1.085
		Experimental	12.82	2.452	1.12
		Predicted error (%)	1.00	0.26	0.04

Very low %predicted error value was observed. This indicates reproducibility and reliability of model and can be successfully employed for the determination of Drugs.

Method Validation:

Linearity: Linearity was performed by taking five drug concentrations, injected in HPLC system and from area calibration curves were obtained as per **Fig. 6** and **Fig. 7**. Overlay spectra of Dapagliflozin and Metformin HCl was shown in **Fig. 8.**

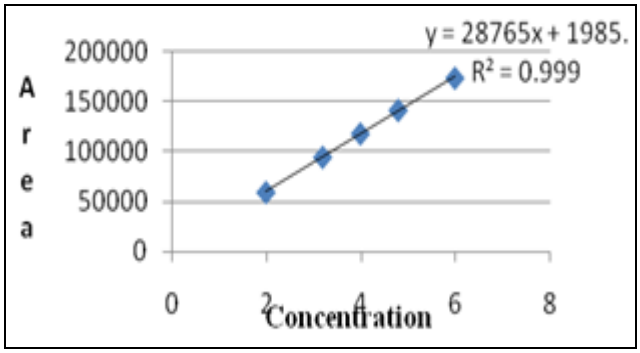

FIG. 6: CALIBRATION CURVE OF DAPAGLIFLOZIN

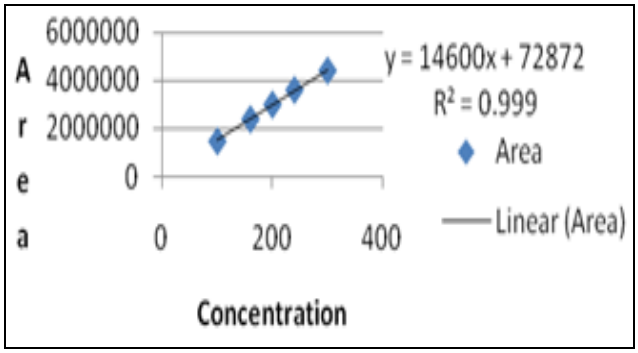

FIG. 7: CALIBRATION CURVE OF METFORMIN HCl

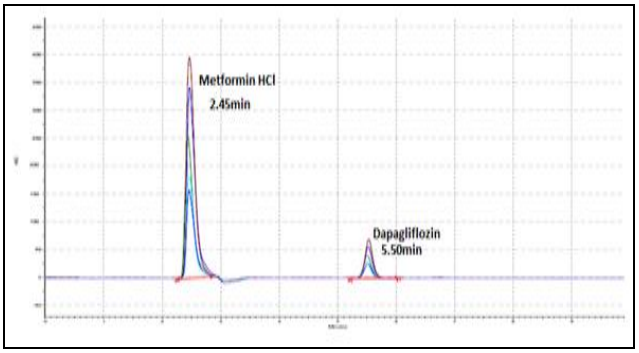

FIG. 8: OVERLAY OF DAPAGLIFLOZIN AND METFORMIN HCL IN RP- HPLC

Precision: Results of the precision study are shown in **Table 5** for Dapagliflozin and Metformin HCl.

TABLE 5: PRECISION OF DAPA AND MET

	Dapagliflozin	Metformin HCl
Repeatability (RSD, n=6)	117366 ± 0.33	3015444 ± 0.03
Precision (RSD)% Interday (n=3) Intraday (n=3)	94155 ± 0.06, 117431 ± 0.249, 1411593 ± 0.039 94180 ± 0.064, 1173673 ± 0.341 141672 ± 0.330	2412226 ± 0.02, 3016541 ± 0.008, 3615392 ± 0.0092 2411225 ± 0.043, 3014964 ± 0.022, 3616059 ± 0.080

Accuracy:

TABLE 6: ACCURACY OF DAPAGLIFLOZIN AND METFORMIN HCl

	Dapagliflozin	Metformin HCl
Accuracy (%)	99.932 ± 0.6927, 100.77 ± 0.044, 99.88 ± 0.185	100.53 ± 0.11, 100.33 ± 0.25, 99.68 ± 0.39

Assay: Results of assay was obtained as per **Table 7.**

TABLE 7: ASSAY OF DAPAGLIFLOZIN AND METFORMIN HCl

	Dapagliflozin	Metformin HCl
Assay	99.09± 0.4817	99.71 ± 0.2397

LOD and LOQ:

TABLE 8: LOD AND LOQ VALUES FOR DAPA AND MET

	Dapagliflozin	Metformin HCl
LOD (µg/ml)	0.062	0.7515
LOQ (µg /ml)	0.1889	2.2777

System Suitability Parameters: The system suitability studies showed a lack of significant difference in the method after making injections of DAPA and MET in hexaplicate. Moreover, the values of % RSD were found to range within the specified limits, thus indicating a high degree of accuracy of the chromatographic process parameters.

DISCUSSION: A simple, precise, and accurate RP-HPLC method has been developed to estimate Dapagliflozin and Metformin HCl in the formulation. This method's standard deviation of the slope and intercept were low. The correlation coefficient exceeded 0.9990. The mean percent label claims estimated for the formulation was close to 100%, indicating the proposed method's accuracy. The mean percent recovery was within the range of 99.68% to 100.53%; thus, it is concluded that the proposed method of analysis is new, simple, accurate and reproducible, successfully applied in the routine analysis of DAPA and MET in Tablet formulation.

CONCLUSION: A new, simple and sensitive RP-HPLC method was developed to analyze Dapagliflozin and Metformin HCl in bulk and in its tablet dosage form. At 227 nm, Dapagliflozin and Metformin HCl were analyzed after applying the QbD approach. It was found that the method was accurate, precise, reproducible, and successfully applied to the pharmaceutical formulation as per ICH guidelines.

ACKNOWLEDGEMENT: The authors are thankful to K. B. Raval College of Pharmacy, Kasturinagar, Gandhinagar, for providing the Research facilities

CONFLICTS OF INTEREST: None

REFERENCES:

1. Gerich JE: Role of the kidney in normal glucose homeostasis and in the hyperglycaemia of diabetes mellitus: therapeutic implications. Diabet Med 2010; 27(2): 136-42.
2. http://www. Drug Bank.com/dapagliflozin. Canagliflozin accessed on February 2017.
3. Beckett AH and Stenlake JB: Practical Pharmaceutical Chemistry; part-ii. 4th Edn; CBS publishers and distributors. New Delhi India 1997; 275-337.
4. Abbott SR: Sample Preparation for Normal and Reversed Phase Analysis. J of Chromatographia 2001; 21: 203-206.
5. Lindsay sandy, HPLC by open learning, Johan wiley and sdons, London 1991; 30-45.

6. Shankar SR: Text book of Pharmaceutical Analysis. 3rd Edn. Rx publication 18.10.
7. Skoog DA, Holler FJ and Nieman TA: In principle of instrumentation analysis; 5th Edn; Thomas Asia Pvt ltd., Singapore 2005; 580.
8. Sharma BK: In Instrumental Method of Chemical Analysis 25th Edn Krishna Prakashan Media Ltd Meerut 2006; 183-186.
9. Jani BR and Shah KV: Development and validation of UV spectroscopic method for simultaneous estimation of Dapagliflozin and metformin hydrochloride in synthetic mixture. International Journal of Research and Development in Pharmacy and Life Sciences 2015; 4(3): 1569-1576.
10. Jani BR and Shah KV: Development and validation of UV spectroscopic first derivative method for simultaneous estimation of Dapagliflozin and metformin hydrochloride in synthetic mixture. Journal of Bioequivalence Studies 1.
11. Yunoos M and Sankar G: A validated stability indicating high-performance liquid chromatographic method for simultaneous determination of metformin HCl and Dapagliflozin in bulk drug and tablet dosage form. Asian Journal of Pharmaceutical and Clinical Research 2015; 8(3):
12. Rafaela ZC and Aline MB: Development and validation of analytical methodology and dissolution profile study for dapagliflozin tablets.
13. Urooj A and Sundar PS: Development of a new stability indicating RP-HPLC method for simultaneous estimation of Dapagliflozin and metformin in bulk and synthetic mixture. World Journal of Pharmacy and Pharmaceutical Sciences 6(7): 2139-2150.
14. Debata J and Kumar S: A New RP-HPLC method development and validation of Dapagliflozin in bulk and tablet dosage form. Int J Drug Dev & Res 2017; 9(2): 48-51.
15. Vermaa MV and Patel CJ: Development and stability indicating hplc method for Dapagliflozin in api and pharmaceutical dosage form. Int J App Pharm 2017; 9(5): 33-41.
16. Patel KJ and Chaudhary AB: Stability indicating RP-HPLC method development and validation for estimation of Dapagliflozin and metformin HCl. World Journal of Pharmacy and Pharmaceutical Sciences 6(9): 796-809.
17. Urooj A and Sundar PS: Development and validation of RP-HPLC method for estimation of Dapagliflozin and metformin in bulk and in synthetic mixture. World Journal of Pharmacy and Pharmaceutical Sciences 6(7): 2139-2150.
18. Deepan T and Dhanaraju MD: Stability indicating HPLC method for the simultaneous determination of Dapagliflozin and saxagliptin in bulk and tablet dosage form. Curr Issues Pharm Med Sci 31: 39-43.
19. Suma BDR and Shenoy P: A new high performance thin layer chromatographic method development and validation of Dapagliflozin in bulk and tablet dosage form. International Journal of Pharmacy and Pharmaceutical Sciences 2019; 11(8): 58-63, Doi:10.22159/Ijpps.2019v11i8.34339.
20. Abdelrahman, Afnan E, Maher and Hadir M: Alzoman, Nourah. HPTLC method for the determination of metformin hydrochloride, saxagliptin hydrochloride, and Dapagliflozin in pharmaceuticals. Current Analytical Chemistry 2020; 16(5): 609-619.
21. Hassib ST, Taha EA, Elkady EF and Barakat GH: Validated liquid chromatographic method for the determination of (canagliflozin, Dapagliflozin or empagliflozin) and metformin in the presence of (1-cyanoguanidine). J Chromatogram Sci 2019; 57(8): 697-707. Doi: 10.1093/Chromsci/Bmz042.
22. Juran JM: Juran on quality by design: the new steps for lanning quality in to goods and services. New York the Free Press 1992.
23. ICH Q8 (R1): Pharmaceutical Development, Revision 1, ICH Harmonized Tripartite Guidelines, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use 2009.
24. ICH Q9: Quality Risk Management. ICH Harmonized Tripartite Guidelines. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2009.
25. ICHQ10: Pharmaceutical Quality System, ICH Tripartite Guidelines. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2009.
26. ICH, Q2 (R1), "validation of analytical procedures: text and methodology:" 2005.

How to cite this article:
Dave V and Patel P: Development and validation of QBD assisted RP-HPLC method for dapagliflozin and metformin hcl in bulk and its combined dosage form. Int J Pharm Sci & Res 2023; 14(2): 788-94. doi: 10.13040/IJPSR.0975-8232.14(2).788-94.