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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DAPAGLIFLOZIN AND METFORMIN HCl

Khyati J. Patel*, Ankit B. Chaudhary, Shweta M. Bhadani and Ruchi J. Raval

Saraswati Institute of Pharmaceutical Sciences, Dhanap, Gandhinagar, Gujarat, India.

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***Corresponding Author**
Khyati J. Patel
Saraswati Institute of Pharmaceutical Sciences, Dhanap, Gandhinagar, Gujarat, India.

ABSTRACT

A simple, specific, accurate, precise and reproducible and robust method have been developed and validated for the Simultaneous Estimation of Dapagliflozin and Metformin HCl in RP-HPLC Method. The separation was carried out using Inertsil ODS C18 column (250mm x 4.6 mm, 5µ) as stationary phase and UV detector set at 227 nm, in conjunction with a mobile phase of 0.05M Potassium Dihydrogen ortho Phosphate buffer (pH- 3.5, adjusted with 0.1% Orthophosphoric acid) and Acetonitrile in the ratio of 50:50% v/v at a flow rate of 1.0 ml/min. The method is linear over concentration range of 5-15 and 25-75µg/ml for Dapagliflozin and Metformin HCl respectively. The retention time of Dapagliflozin and Metformin HCl were 2.633 min and 5.620 respectively. The % recoveries of Dapagliflozin and Metformin HCl were found to be 100.40%-101.27%, 100.18%-100.63% respectively. Method was statistically validated for accuracy, precision, specificity, LOD, LOQ and robustness according to ICH guidelines. The propose method enables rapid quantification and simultaneous analysis of both the drugs from synthetic mixture without any interference of excipients. So, the method can be used for routine analysis.

KEYWORDS: Dapagliflozin, Metformin HCl, RP-HPLC, Force Degradation, Method Validation.

INTRODUCTION

Dapagliflozin, is chemically designated as (2S,3R,4R,5S,6R)-2-(4-Chloro-3-(4-ethoxybenzyl)phenyl)-6- (hydroxylmethyl)tetrahydro-2H-pyran-3,4,5-triol and has the molecular wt. 408.873gm/mol (figure-1).[1]

A competitive inhibitor of the sodium-glucose transport subtype 2 protein, Dapagliflozin blocks glucose reabsorption into the kidney, resulting in the elimination of blood glucose through the urine. Various analytical methods have been reported for the estimation of Dapagliflozin as alone. They include spectrophotometric methods,[2] HPLC.[3]

Metformin HCl is designated chemically as 1-carbamimidamido-N, N-dimethylmethanimidamide (figure-2).[4] Mechanism of action of Metformin differs from other classes of oral antihyperglycemic agents. Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization.[3] Various analytical methods have been reported for the estimation of Metformin HCl as alone as well as in combination with other drugs. They include spectrophotometric methods.[5] RP-HPLC.[6-13]

However an extensive literature search didn't reveal any estimation method for all drugs in their combined dosage form. Therefore, attempt was made to develop and validate simple, precise and accurate, RP-HPLC method for simultaneous determination of all four drugs in their combined dosage form.

MATERIALS AND METHOD

Reagents and Chemicals

Dapagliflozin and Metformin HCl were obtained as gift samples from Jigs Chemical and Tuton Pharma Ahmadabad. The Synthetic mixture was prepared for the combined dosage form. HPLC grade Acetonitrile, Water and Ortho-phosphoric acid and Potassium Dihydrogen Phosphate of analytical grade were obtained from Finar Chemicals Ltd.

Instruments and Chromatographic Conditions

The analysis was performed on Young linn (YL 9100) software. The separation was achieved on Intersil (250 x 4.6 mm, 5µ) column. The column was maintained at room temperature and the eluent was monitored at 227nm using PDA detector. The mixture of Phosphate Buffer pH 3.5 and Acetonitrile in proportion of 50:50%v/v at a flow rate of 1.0 ml/min was used as mobile phase. The injection volume is 10µl.

Preparation of Metformin Hydrochloride standard stock solution (500 µg/ml): 50 mg of metformin hydrochloride was weighed and transferred into 100 ml volumetric flask. 50 ml of HPLC grade water was added and shaken to dissolve. And then diluted up to the mark with HPLC grade water and mixed thoroughly.

Preparation of Dapagliflozin standard stock solution (500 µg/ml)

50 mg of metformin hydrochloride was weighed and transferred into 100 ml volumetric flask. 50 ml of HPLC grade water was added and shaken to dissolve. And then diluted up to the mark with HPLC grade water and mixed thoroughly.

Working standard solution of Dapagliflozin and metformin hydrochloride (10:50 µg/ml)

1.0 ml of Dapagliflozin standard stock solution and 5.0 ml of metformin hydrochloride standard stock solution was tranferred in to 50 ml volumetric flask diluted up to the mark with diluent and mixed thoroughly.(Figure-3).

Peak ID solution of metformin hydrochloride (50 µg/ml)

5.0 ml of metformin hydrochloride standard stock solution was transferred in to 50 ml volumetric flask and diluted up to the mark with diluent and mixed.

Peak ID solution of Dapagliflozin (10 µg/ml)

1.0 ml of dapagliflozin standard stock solution was pipette out in to 50 ml volumetric flask and diluted up to the mark with diluent and mixed.

Forced Degradation Study

Forced Degradation Studies of the drugs, in combination, were performed under different stress conditions as mentioned in ICH guideline Q1A (R2).[14] The standard solution containing 500µg/ml Dapagliflozin and 500µg/m/l Metformin HCl was subjected to acidic, alkaline, oxidative, thermal condition. Acidic and alkaline degradation were performed up to 2N strength of acid/base at different temperature. Oxidative stress studies were carried out for using 3-10% H2O2The sample solution containing Dapagliflozin and Metformin HCl was subjected to degrade in Acid and Alkali condition.

Acid Degradation

1.0ml of Dapagliflozin standard stock solution and 5.0 ml of metformin hydrochloride standard stock solution was pipette out into 50 ml volumetric flask. 1 ml of 0.1N hydrochloric acid heated for 30 minutes at 80ºC temperature and it allowed to cool to room

temperature and 1 ml of 0.1 N NaOH was added and diluted up to the mark with diluent and mixed and injected into HPLC system. Similarly solutions were prepared for respective conditions mentioned in Table.3, while Fig: 5 represent the graph.

Alkali Degradation

1.0 ml of Dapagliflozin standard stock solution and 5.0 ml of metformin hydrochloride standard stock solution was pipette out into 50 ml volumetric flask and 1 ml of 0.1 N NaOH heated for 30 minutes at 80ºC temperature and it is allowed to cool to room temperature and 1 ml of 0.1 N HCl was added and diluted up to the mark with diluent and mixed and injected into HPLC system. Similarly solutions were prepared for respective conditions mentioned in Table. 3, Fig: 6

Oxidative Degradation

1.0 ml of Dapagliflozin standard stock solution and 5.0 ml of metformin hydrochloride standard stock solution was pipette out into 50 ml volumetric flask. 1 ml 6 % H2O2 was added and then it is allowed to kept at room temperature at 25°C and kept for 4 Hours and injected into HPLC system.Similarly solutions were prepared for respective conditions mentioned in Table.3, While Fig: 7 represents the graph.

Thermal Degradation

50mg of Dapagliflozin was weighed and transferred into 100ml of flask and it kept in the oven at 80°C similarly 50mg of Metformin HCl was taken into the another flask and it was also kept in the oven then it was allowed to cool at room temperature and then sample were made. 1.0 ml of Dapagliflozin standard stock solution and 5.0 ml of metformin hydrochloride standard stock solution was pipette out into 50 ml volumetric flask and then it was kept and then diluted up to the mark with diluent and mixed and injected into HPLC system.Similarly solutions were prepared for respective conditions mentioned in Table.8 while fig:3 shows graph.

METHOD VALIDATION

1) Specificity: Specificity of method can be termed as absence of any interference at retention times of samples. Specificity was performed by injecting blank and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes. Fig: 9-11.

2) Linearity and Range: A series of standard solutions 5-15µg/ml, 20-75µg/ml of Dapagliflozin and Metformin HCl respectively were prepared. An aliquot of 10µl of each solution was injected 3 times for each standard solutions and peak area was observed. Plot of average peak area versus the concentration is plotted and from this the correlation coefficient and regression equation were generated. The calibration data of Dapagliflozin and Metformin HCl is given in Table 5-6, while Figure 12-13 represents linearity graphs of Dapagliflozin and Metformin HCl.

3) Precision

1. Repeatability

The data for repeatability of peak area measurement for Dapagliflozin (10µg/ml), Metformin HCl (50µg/ml) based on six measurements of same was found to be 0.85 and 0.49 respectively. The repeatability data of Dapagliflozin and Metformin HCl is given in table- 7.

2. Intraday precision

Combined solution containing the mixture of Dapagliflozin (5, 10, 15 µg/mL) and Metformin hydrochloride (40, 50, 60 µg/mL) were analyzed for 3 times on the same day, peak areas were determined and %RSD was calculated. The result given in Table no-7.

3. Interday precision

Combined solution containing the mixture of Dapagliflozin (5, 10, 15 µg/mL) and Metformin hydrochloride (40, 50, 60 µg/mL) were analyzed for 3 different days, peak areas were determined and %RSD was calculated. The Result is given in Table no.7.

4). Accuracy

Accuracy was carried out by the recovery studies at 3 different levels of concentration (50%, 100%, and 150%). To a fixed amount of pre analyzed sample of Dapagliflozin (10µg/ml) and Metformin Hydrochloride (50 µg/ml), an increasing amount of the standard stock solution of binary mixture of Dapagliflozin and Metformin Hydrochloride were added at a concentration (50%, 100%, 150%) of pre analyze sample. The Result is given in Table-8.

5). LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3* SD/slope of calibration.

curve LOQ = 10 * SD/slope of calibration.

curve

Where, SD = Standard deviation of intercepts.

The LOD and LOQ data of Dapagliflozin and Metformin HCl is given in Table-9.

6). Robustness

Following parameters were changed one by one and their effect was Observed on system suitability for standard preparation. The Robustness data of Dapagliflozin and Metformin HCl is given in Table-10.

1) Buffer pH 3.4 and 3.6

2) Flow rate 0.98 ml/minutes and 1.02 ml/minutes

3) Mobile phase composition: Buffer: ACN (48:52,v/v) and Buffer: ACN (58:42, v/v)

7). Assay of Synthetic mixture.

Applicability of proposed method was tested by analyzing Physical mixture. The Results are shown in table 11.

RESULT AND DISCUSSION

System Suitability Study

The detection was carried out in the UV region at 227nm. The different composition of mobile phase was testing and the composition giving retention time of 2.633 min for Metformin Hydrochloride and 5.620 min for Dapagliflozin with good resolution and theoretical plates and optimized Mobile phase was Phosphate buffer (pH adjusted 3.5 using 1%v/v of ortho phosphoric acid) :ACN (50:50%v/v). A Chromatogram of the Mixture in optimised conditions is shown Figure and the system suitability parameters are shown in Table 1.

Table 1: Result for System Suitability Parameters.

	Metformin Hydrochloride	Dapagliflozin
Retention time (min)(n=3)	2.633 ±0.004	5.615±0.004
Theoretical plate(n=3)	3561 ± 78.824	6910 ± 30.454
Average area ±SD (n=3)	8725173 ± 7468.42	12211924 ±2625.12
Asymmetry(n=3)	1.99 ± 0.007	1.11 ±0.080
Resolution(n=3)	13.33 ± 0.38	

Table 2: Synthetic Mixture of Dapagliflozin and Metformin Hydrochloride.

Ingredients	Quantity
Dapagliflozin	10 mg
Metformin Hydrochloride	500 mg
Lactose Anhydrous	133 mg
Talc	7 mg
Magnesium Stearate	14 mg
PVPK Dry	35 mg
Total	700 mg

Table 3: Results for Force Degradation.

Degradation method	Optimized Condition	% Degradation	
		Dapagliflozin	Metformin Hydrochloride
Acid	5 ml 2N HCl (30 min 80ºC)	9.25	5.02
Alkali	5 ml 1N NaOH (30 min 80ºC)	0.50	36.25
Oxidation	1 ml 10 % H2O2 (30 min 80ºC)	12.11	16.25
Thermal		No degradation	No degradation

Table 4: Preparation of calibration curve.

Linarity solution number	Linearity solution		Volume of standard stock solution of Dapa (200 µg/mL) in mL	Volume of standard stock solution of Met (500 µg/mL) in mL	Dilute up to the mark with diluent
	Dapa (µg/mL)	Met (µg/mL)			
1	5	25	2.5	5	100
2	8	40	4	8	100
3	10	50	5	10	100
4	12	60	6	12	100
5	15	75	7.5	15	100

Table 5: Linearity Study for Dapagliflozin.

Sr. No.	Dapagliflozin			
	Conc. (in µg/ml)	Average Area (n=3)	SD	%RSD
1	5	613165.33	4241.189	0.692
2	8	972777.67	6580.026	0.676
3	10	1220042.00	4420.869	0.362
4	12	1453671.00	5076.531	0.349
5	15	1822449.33	852.956	0.047

Table 6: Linearity of Study Metformin HCl.

Sr. No	Metformin Hydrochloride			
	Conc.(in µg/ml)	Average Area n=3	SD	%RSD
1	25	3817415.33	14934.277	0.391
2	40	6307003.00	10097.338	0.160
3	50	7505102.33	43196.304	0.576
4	60	9174211.33	14275.931	0.156
5	75	11574175.00	60347.846	0.521

Table 7: Precision Study For Dapagliflozin and Metformin Cl.

Parameters	Concentration (µg/ml)		%RSD	
	Dapagliflozin	Metformin HCl	Dapagliflozin	Metformin HCl
Repeatability	10	50	0.85	0.49
Intraday	5	40	0.68	0.67
	10	50	0.21	0.30
	15	60	0.44	0.50
Interday	5	40	0.48	0.60
	10	50	1.31	0.42
	15	60	0.32	0.91

Table 8: Recovery Data for Dapagliflozin and Metformin HCl.

Drug	% Accuracy Level	Amount of Drug Taken (µg/mL)	Amount of Drug added (µg/mL)	Total Amount of Drug (µg/mL)	Amount of Drug Found (µg/mL) ±SD (n=3)	Mean % Recovered ±SD (n=3)
Dapagliflozin	50	2.5	2.5	5	15.18 ±0.07	101.22 ± 0.44
	100	2.5	7.5	10	20.41 ±0.15	102.02 ± 0.76
	150	2.5	12.5	15	25.21 ±0.11	100.84 ± 0.42
Metformin HCl	50	10	15	25	25.06 ±0.11	100.23 ± 0.43
	100	10	40	50	50.09 ±0.16	100.17 ± 0.32
	150	10	65	75	75.11 ±0.12	100.15 ± 0.15

Table 9: LOD and LOQ for Dapagliflozin and Metformin HCl.

	Dapagliflozin (µg/ml)	Metformin hydrochloride (µg/ml)
Limit of Detection (µg/ml)	0.28	0.78
Limit of quantification (µg/ml)	0.85	2.37

Table 10: Robustness Study for Dapagliflozin and Metformin Hydrochloride.

Parameters		Area (n=3)	
		Dapagliflozin	Metformin Hydrochloride
pH (±0.1)	3.4	1211971	7571909
	3.5	1214109	7521319
	3.6	1211412	7621601
	Mean ± SD	1212497 ± 1423.454	7571610 ± 50141.67
	% RSD	0.11	0.66
Flow Rate (±0.02ml/min)	0.98 ml/min	1225412	7565874
	1.0 ml/min	1246894	7465891
	1.02 ml/min	1227845	7521456
	Mean ± SD	1233384 ± 11763.36	7517740 ± 50094.96
	% RSD	0.95	0.66
Mobile Phase Composition Buffer:ACN (± 2 mL)	48:52	1216981	7589741
	50:50	1227584	7498756
	52:48	1234685	7524812
	Mean ± SD	1226417 ± 8909.54	7537770 ± 46856.09
	% RSD	0.72	0.62

Table 11: Analysis of Physical mixture of Dapagliflozin and Metformin Hydrochloride by Proposed Method.

Dapagliflozin			Metformin Hydrochloride		
Labelled Amount Mg	Amount Found (mg)	% Assay	Labelled Amount Mg	Amount Found (mg)	% Assay
10 mg	10.20	100.40	500mg	50.02	100.04
	10.49	98.56		49.94	99.88
	10.15	101.52		49.89	99.78
Mean ± SD	10.28±0.18	100.16±1.49		49.95±0.06	99.9±0.13
% RSD	1.78	1.49		0.13	0.13

Figures

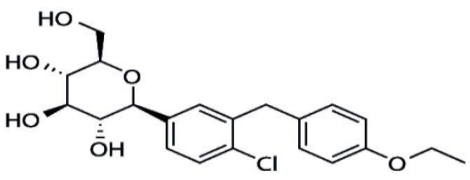
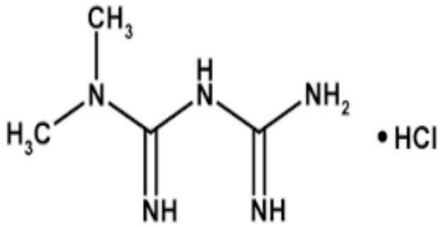


Fig. 1: Chemical Structure of Dapagliflozin



Metformin Hydrochloride

Fig. 2: Chemical Structure of Metformin HCl.

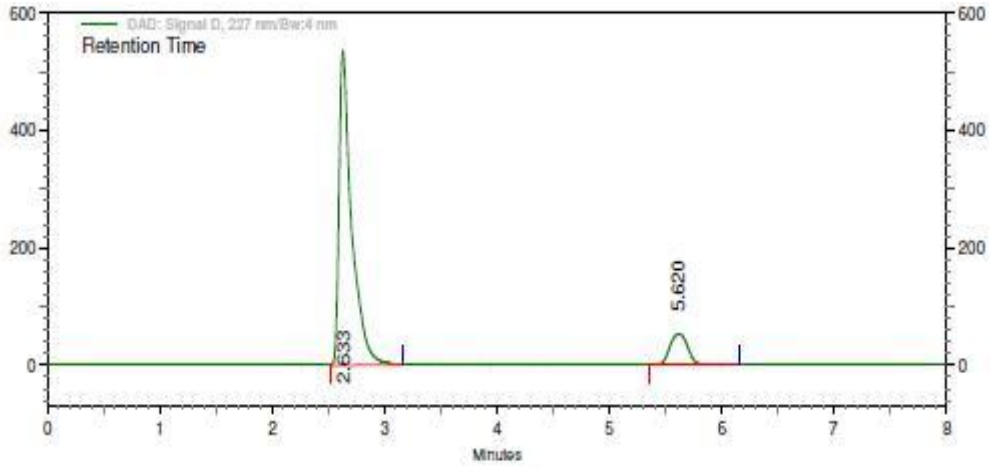


Fig. 3: Chromatogram of Dapagliflozin and metformin hydrochloride (10: 50 µg/ml) Retention time 2.63 and 5.62 minutes.

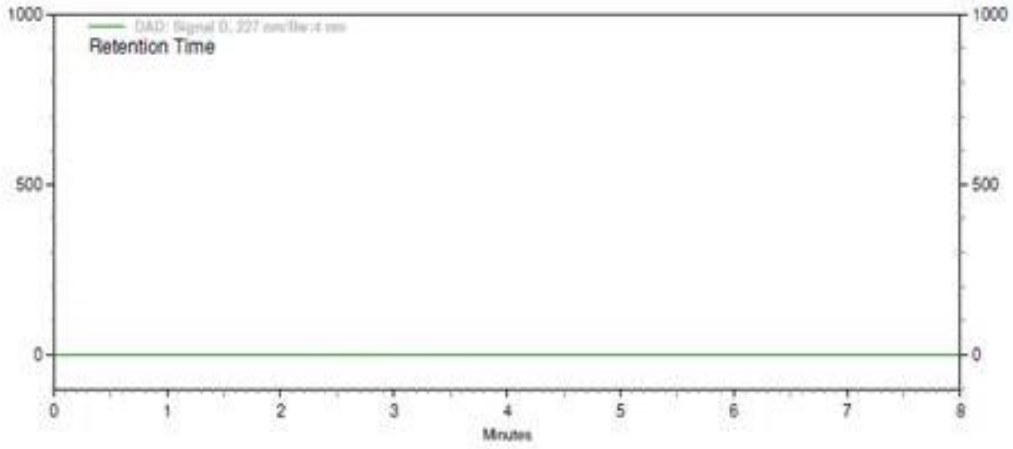


Fig. 4: Chromatogram of Blank for Degradation of Dapagliflozin and Metformin HCl.

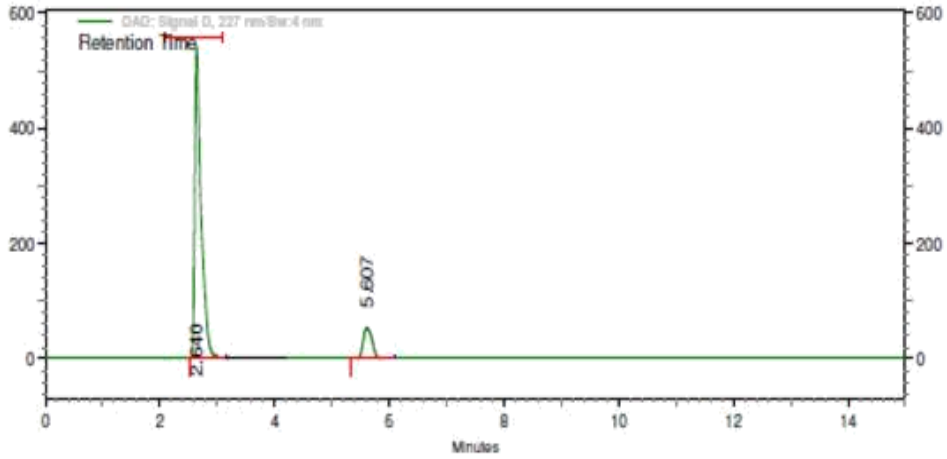


Fig. 5: Chromatogram of optimized condition of Acid Degradation for Dapagliflozin and Metformin HCl.

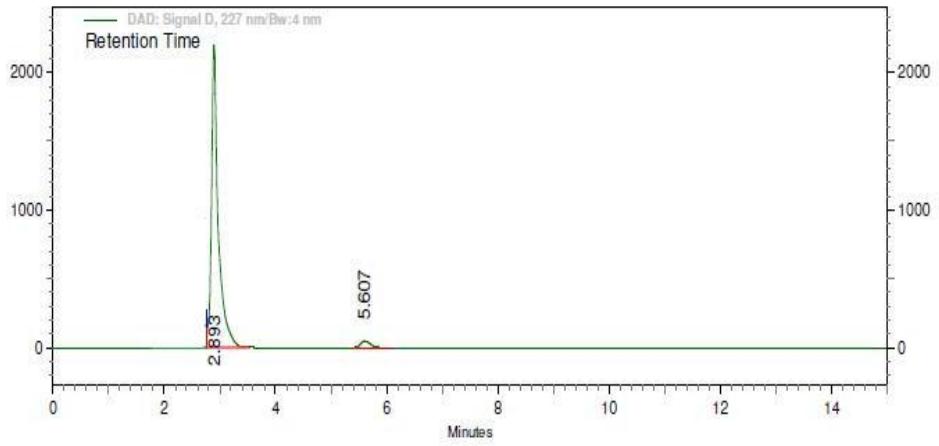


Fig. 6: Chromatogram of optimized condition of Alkali Degradation for Dapagliflozin and Metformin HCl.

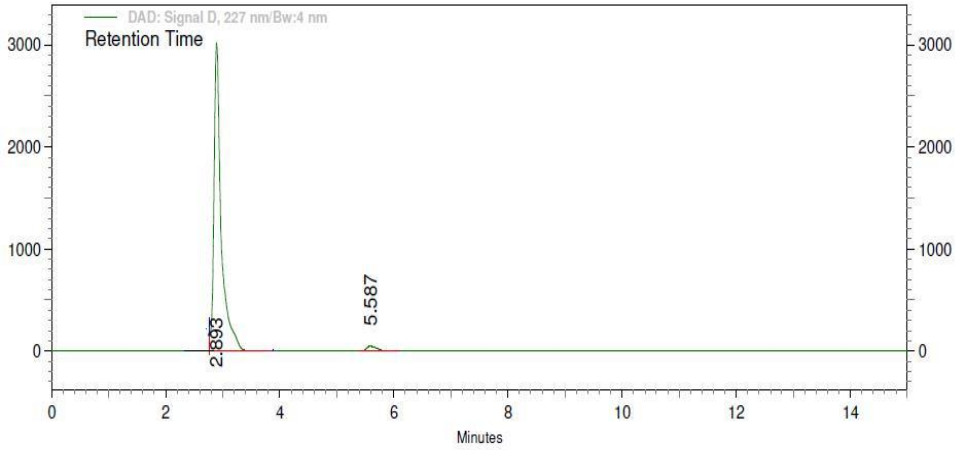


Fig. 7: Chromatogram of optimized condition of Peroxide Degradation for Dapagliflozin and Metformin HCl.

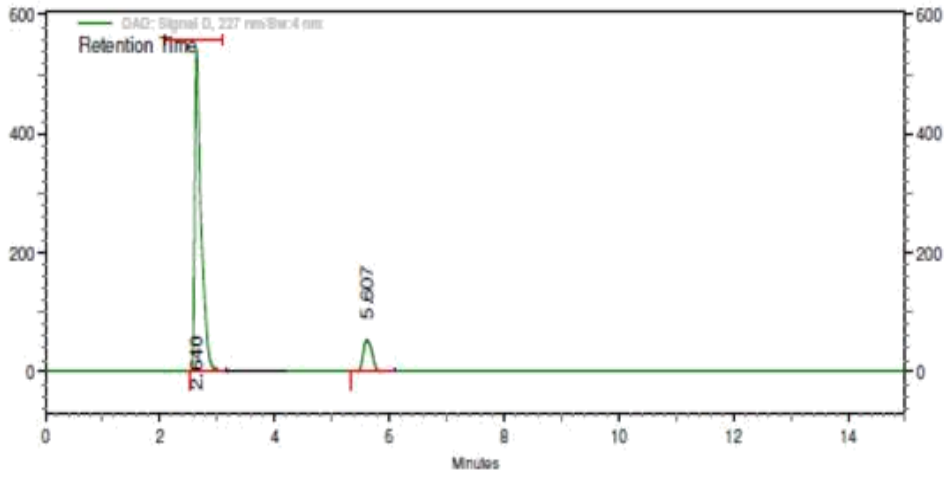


Fig. 8: Chromatogram of optimized condition of Thermal Degradation for Dapagliflozin and Metformin HCl.

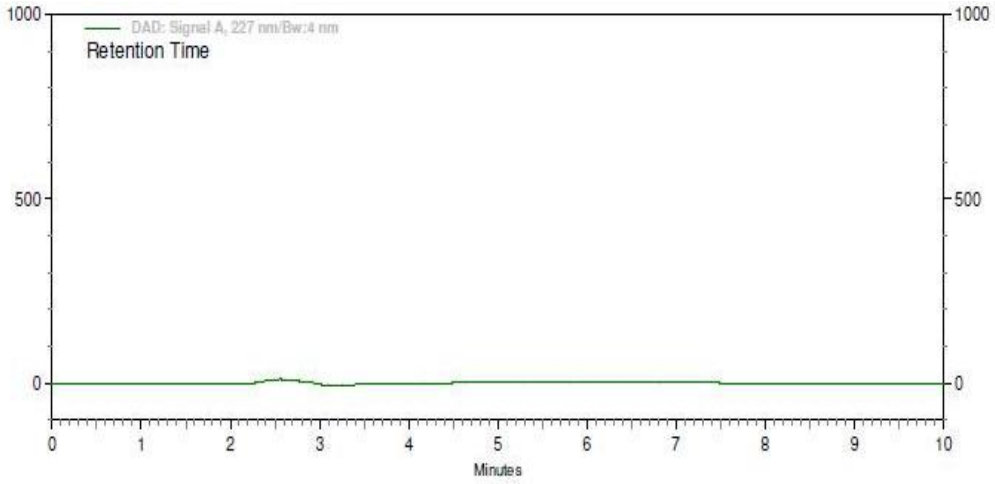


Fig. 9: Specificity Chromatogram of Blank.

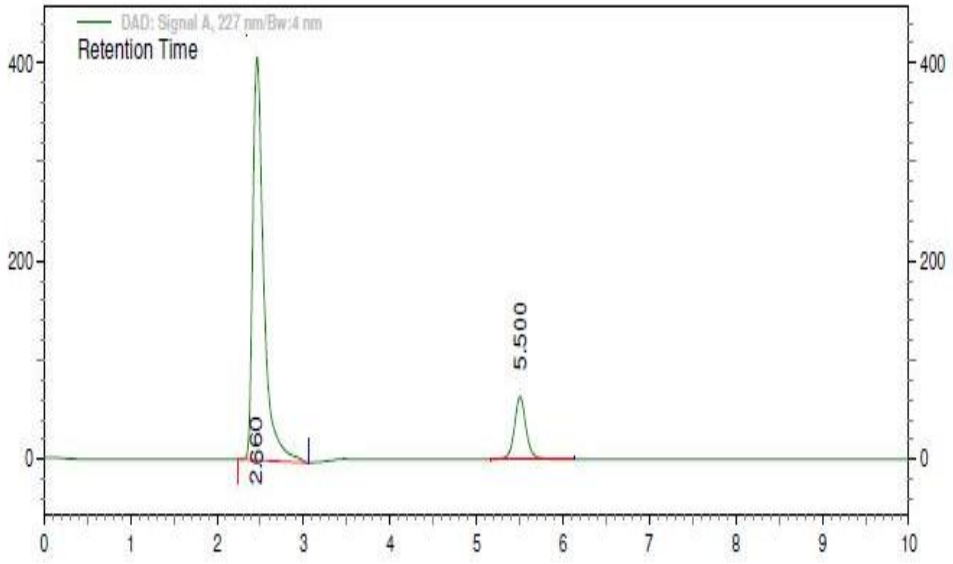


Fig: 10 Specificity Chromatogram of Standard Dapagliflozin and Metformin HCl.

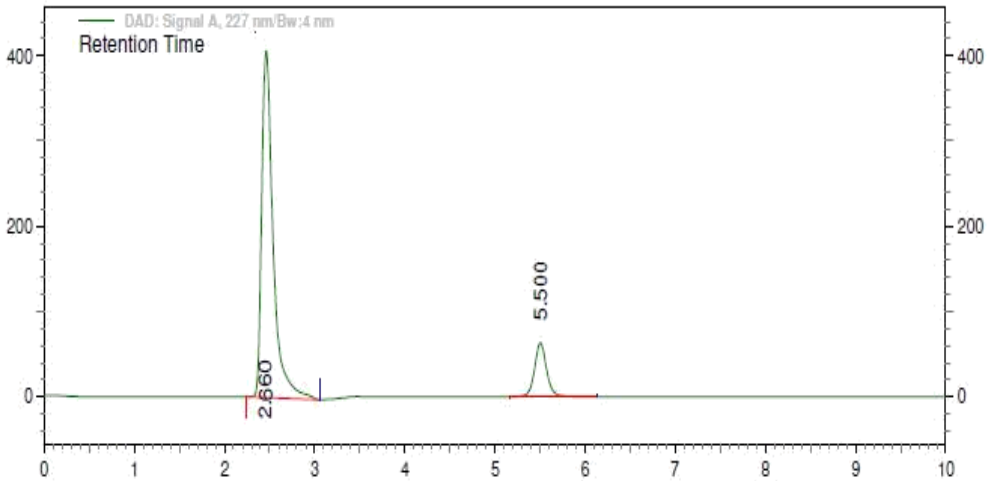


Fig. 11: Specificity Chromatogram of Sample Dapagliflozin and Metformin HCl.

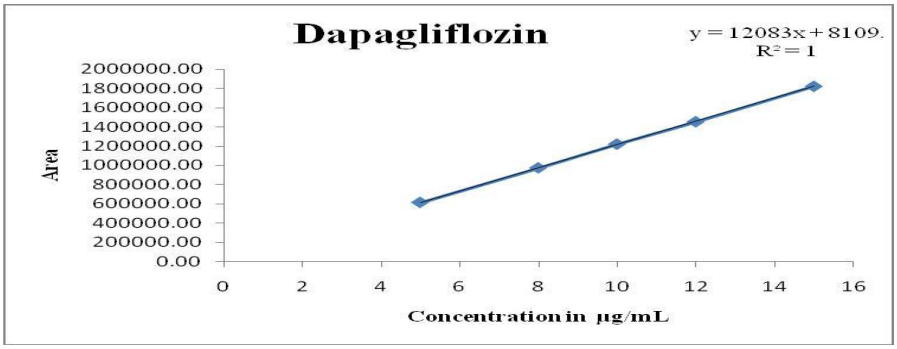


Fig. 12: Linearity Graph for Dapagliflozin.

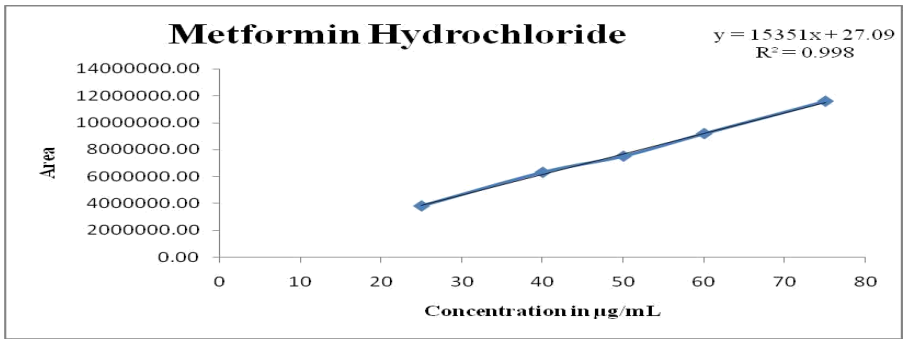


Fig. 13: Linearity Graph for metformin HCl.

CONCLUSION

In the present study we have developed a new, rapid RP-HPLC method and Validated for different parameters (System suitability, linearity, accuracy, precision, LOD, LOQ Robustness). By studying all these we have concluded that the method was linear, accurate, precise, robust and rapid for determination of Dapagliflozin and Metformin Hydrochloride. Hence the method was successfully applied for the estimation of Dapagliflozin and Metformin Hydrochloride in Synthetic Mixture.

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REFERENCES

1. "Drug profile for Dapagliflozin", October http://www.drugbank.ca/drugs/DB06292, 2016.
2. Jani BR, Shah KV and Kapupara PP, "Development and Validation of UV-spectroscopic first derivative estimation of Dapagliflozin and Metformin HCl." *J. Bioequivalence Study.*, 2015; 1(1): 1-9.

3. Dhanlakshmi K, and Reddy NG, "Development and Validation of stability indicating RP-HPLC method for determination of Dapagliflozin." *J. Advanced Pham Edu and Res.,* 2014; 4(3): 350-353.
4. "Drug profile for Metformin", October, 2016, http://www.drugbank.ca/drugs/DB00331 Mubeen G, Khalika N, and Vimala MN, "Spectrophotometric Method for Estimation of Metformin HCl". *Int. J. Chem Tech. res.,* 2009; 2(2): 1330-1331.
5. Mishra K, Soni H, Nayak G, Patel SS and Singhai AK, "Method Development and validation of Metformin Hydrochloride in Tablet Dosage Form". *E-journal of chem.,* 2011; 8(3): 1309-1313.
6. Mahadik PS, Senthikumar GP, Dahiya D, Tamizmani T, Gaikwad PK and Gavali SA. "Method development and validation of Metformin in bulk and pharmaceutical dosage forms by spectrophotometric method." *American J of pharma Res.,* 2012; 2(1): 350-354.
7. Usharani N, Keerthana K, Sarika M, Revathi D, Kusuma D and Kumari VY, "Development and validation of RP-HPLC and UV derivative spectrophotometric methods for determination of Metformin HCl and Pioglitazone," *World J of Pharma.Res.,* 2015; 4(12): 1117-1125.
8. Loni AB, Ghante MR and Sawant SD,"Simultaneous UV spectrophotometric method for estimation of Sitagliptin phosphate and Metformin HCl in bulk and Tablet dosage form".*Scholar Res Lib.,* 2012; 4(3): 854-859.
9. Chirag and Parle A," Simultaneous UV spectrophotometric method for estimation of Metformin HCl and Alogliptin Benzoate in bulk drugs and Combined dosage forms". *Scholar Res Lib.,* 2014; 6(1): 303-311.
10. SM. Sandhya, UF Beevi and G. Babu, "Validated Spectrophotometric Methods for simultaneous analysis of Glimepiride and Metformin in Pharmaceutical dosage forms." *J of chem. And Res.,* 2013; 5(11): 721-725.
11. Gadipally S, Method Development of Metformin Hydrochloride RP-HPLC. *World J of Pharmacy and Pharma Sci.,* 2014; 3(3): 1149-1159.
12. Madhukar A, Prince A, Vijay kumar R, Sanjeeva Y, Jagdeeshwar K and Raghupratap D,
13. "Simple and sensitive analytical Method Development and Validation of Metformin Hydrochloride by RP-HPLC." *Int J of Pharmacy and Pharma Sci,* 2011; 3(3): 117-120.
14. ICH, Validation of Analytical Procedures; Methodology, Q2 (R1), International Conference on Harmonization, IFPMA, Geneva, 1996.