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Research Article

A VALIDATED STABILITY INDICATING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN HCL AND DAPAGLIFLOZIN IN BULK DRUG AND TABLET DOSAGE FORM

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ABSTRACT

Objective: A simple and precise stability indicating reversed-phase high-performance liquid chromatography method was developed and validated for the simultaneous determination of metformin (MET) hydrochloride and dapagliflozin (DAP) in bulk and pharmaceutical dosage form.

Methods: Chromatography was carried out on hypersil BDS C_{18} (250 mm × 4.6 mm, 5 µ particle size) column containing mobile phase of buffer (0.1% orthophosphoric acid) adjusted to pH 6.8 with triethylamine:acetonitrile in the ratio of 50:50%/v/v at a flow rate of 1 ml/minutes. The analyte was monitored using photodiode array detector at 240 nm.

Results: The retention time was found to be 2.791 minutes and 3.789 minutes for MET hydrochloride and DAP respectively. The proposed method was found to be having linearity in the concentration range of 85-510 µg/ml for MET (r^2=0.99995) and 0.5-3.0 µg/ml for DAP (r^2=0.99978), respectively. The mean % recoveries obtained were found to be 99.66-100.23% for MET and 99.61-100.38% for DAP respectively. Stress testing which covered acid, base, peroxide, photolytic and thermal degradation was performed on under test to prove the specificity of the method and the degradation was achieved. The developed method has been statistically validated according to ICH guidelines.

Conclusion: Thus, the proposed method can be successfully applied for the stability indicating the simultaneous determination of MET hydrochlorideand DAP in bulk and combined tablet dosage form and in the routine quality control analysis.

Keywords: Dapagliflozin, Metformin hydrochloride, Reversed-phase high-performance liquid chromatography, Forced degradation, Method validation.

INTRODUCTION

Metformin (MET) hydrochloride

Chemically, it is as shown in Fig. 1, (3-(diamino methylidene)-1,1-dimethylguanidine; hydrochloride. It has molecular formula of $C_4H_{12}ClN_5$ and molecular weight is 165.62 g/mol. MET is an oral anti-hyperglycemic agent (Type 2 diabetes) belongs to class of biguanides and useful for treating non-insulin-dependent diabetes mellitus. MET 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. These effects are mediated by the initial activation by MET of AMP-activated protein kinase, a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats.

Dapagliflozin (DAP)

Chemically, it is as shown in Fig. 2, (2*S*,3*R*,4*R*,5*S*,6*R*)-2-[4-chloro-3-(4-ethoxybenzyl) phenyl]-6-(hydroxymethyl) tetrahydro-2*H*-pyran-3,4,5-triol. It has a molecular formula of $C_{21}H_{25}ClO_6$and molecular weight of 408.873 g/mol. DAP is indicated for the management of diabetes mellitus Type 2, and functions to improve glycemic control in adults when combined with diet and exercise. DAP is an inhibitor of sodium-glucose cotransporter 2 (SGLT2) responsible for the majority of the reabsorption of filtered glucose from the tubularlumen. By inhibiting SGLT2, DAP reduces reabsorption of filtered glucose and lowers the renal threshold for glucose, and thereby increases urinary glucose excretion [1,2].

Literature survey reveals that few analytical methods were reported like liquid chromatography-mass spectrometry method in biological fluids [3], reversed-phase high-performance liquid chromatography (RP-HPLC) methods [4-9] and spectrophotometric methods [10] in alone or in combination with other drugs in pharmaceutical dosage forms, but no simple stability indicating RP-HPLC method for the simultaneous estimation of MET HCL and DAP in pharmaceutical dosage forms have been reported so far. Hence author has planned to develop a simple, accurate, precise and sensitive stability indicating RP-HPLC method for the simultaneous estimation of MET hydrochlorideand DAP in bulk and combined tablet dosage forms and in routine quality control analysis.

METHODS

Chemicals

MET hydrochloride and DAP were obtained as gift samples from Intas Pharmaceuticals Ltd., Ahmadabad and Avanscure Life Sciences

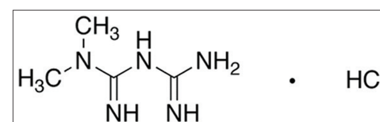

Fig. 1: Chemical structure of metformin hydrochloride

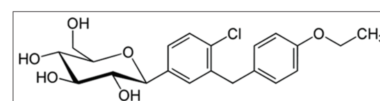

Fig. 2: Chemical structure of dapagliflozin

Pvt. Ltd., Haryana. HPLC grade water, methanol and acetonitrile were purchased from E.Merck. Chem. Ltd., Mumbai. All the chemicals used were of analytical reagent grade (E.Merck). Fixed dose combination tablet formulation (Xigduo) containing 5 mg of DAP and 850 mg of METhydrochloride was procured from US market.

Instrumentation

Quantitative HPLC was performed on waters technologies 2690 series separation module and 2996 series photodiode array (PDA) detector equipped with auto injector using empower software 2. A RP hypersil BDS C_{18} (250 mm × 4.6 mm, 5 µ particle size) column was used. Ultraviolet (UV)-visible spectrophotometer PG Instruments T60 with bandwidth of 10 mm matched quartz cell was used for all spectral measurements. Weighing was done on shimadzu balance (AX 200) and pH adjustments done using pH meter (ELICO LI615) was used.

Chromatographic conditions

Separation and analysis was carried out on hypersil BDS C_{18} (250 mm × 4.6 mm, 5 µ particle size) column. The optimized mobile phase consisting buffer (0.1% orthophosphoric acid) adjusted to pH 6.8 with triethylamine:acetonitrile in the ratio of 50:50%/v/v at a flow rate of 1 ml/minutes. Prior to injection, column was saturated with mobile phase for 30 minutes and injection volume of 20 µl was injected into the chromatographic system using auto sampler mode. The detection response was measured at 240 nm and maintained column at temperature of 30°C.

Preparation of o-phthalaldehyde (OPA) buffer

1 ml of concentrated ortho phosphoric acid (85%) was diluted to 1000 ml with HPLC grade water in a 1000 ml volumetric flask and then volume was made up to the mark with water. Again pH was adjusted to 6.8 with TEA and solution was filtered through 0.45 µ Millipore nylon filter.

Preparation of mobile phase

0.1% ortho phosphoric acid (adjusted to pH 6.8 with triethylamine) and acetonitrile (HPLC grade) were taken in the ratio of 50:50% v/v and mixed well and then degassed by subjecting to sonication for 15 minutes and filtered under vaccum filtration.

Preparation of diluent

Acetonitrile and water were mixed in the ratio 50:50% v/v, sonicated for 10 minutes and used as diluent.

Preparation of standard solution

Accurately weighed and transferred 34 mg of MET and 2 mg of DAP working standards into a 10 ml clean and dry volumetric flasks separately, $3/4^{th}$ volume of diluent was added, sonicated to dissolve for 5 minutes and then made up to the final volume with diluent. From the above stock solutions, each 1.0 ml was pipette out in to a 10 ml volumetric flask and then made up to the final volume with mobile phase to obtain final concentration of 340 µg/ml of METhydrochloride and 2 µg/ml of DAP working standard solution.

Preparation of sample solution

Five tablets were accurately weighed and crushed into fine powder and average weight of each tablet was determined. The powder weight equivalent to five tablets was transferred into a 250 ml volumetric flask, 200 ml of diluent was added and sonicated for 15 minutes, further the volume was made up with diluent and filtered. From the filtered solution, 0.2 ml was pipette out into a 10 ml volumetric flask and made up to volume with mobile phase to obtain final concentration of 340 µg/ml and 2 µg/ml of METhydrochloride and DAP, respectively. Then injected 20 µl of filtered portion of the sample and standard preparation into the chromatograph. Recorded the responses for the major peaks. Calculated the content of METhydrochloride and DAP present in each tablet.

Method validation

System suitability

System suitability was carried out by injecting standard solutions 5 times into the chromatographic system. The system suitability parameters were then evaluated for tailing factor, retention time and theoretical plates of standard chromatograms.

Accuracy

The accuracy of the test method was demonstrated by % recovery across its range by making three different concentrations at 50%, 100% and 150 levels using standard addition method where pre-analyzed sample solutions were spiked with known amount of standard solution and then each concentration was injected three times into the chromatographic system.

Intraday precision (repeatability)

Intraday precision was performed by injecting standard preparations 6 times into the chromatographic system on same day by maintaining the optimized chromatographic conditions and calculated relative standard deviation (% RSD) of retention time and peak areas for both MET and DAP.

Inter-day precision

Inter-day precision was performed by injecting standard preparations 6 times into the chromatographic system on 2 different days by maintaining the optimized chromatographic conditions and calculated %RSD of retention time and peak areas for both MET and DAP.

Specificity

Specificity is the ability to assess unequivocally the analytes in the presence of compounds that may be expected to present, such as impurities, degradation products, and matrix components. The specificity of the method was assessed by comparing the chromatograms obtained from standard and sample solutions. The retention times of the analytes in standard and the sample solutions were found to be same, so the method was specific and free from interference from excipients present in the tablets.

Linearity

The linearity of an analytical method was carried out to check its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different concentrations of linearity solutions were prepared by diluting aliquots (0.25-1.5 ml) of standard stock solution (3400 µg/ml for MET and 20 µg/ml for DAP) in to 10 ml volumetric flasks and diluted to final volume with mobile phase to obtained concentrations in the range of 85-510 µg/ml for MET and 0.5-3.0 µg/ml for DAP respectively to demonstrate linearity for assay and then injected each concentration into the chromatographic system and the chromatograms were recorded. A calibration graph was plotted between amount of drug concentration (µg/ml) and chromatographic peak area (mV).

Robustness

The robustness of the proposed method was determined by analyzing aliquots from homogenous lots by differing physical parameters such as mobile organic phase composition, flow rate, and column temperature. The standard and sample solutions were injected into the chromatograph at varied conditions of flow rate ±0.1 ml/minutes, mobile organic phase ±10%, mobile phase buffer pH ±0.2 units and temperature by ±5°C.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined by using the formula based on the standard deviation of the response and the slope. LOD and LOQ were calculated by using equations:

LOD = 3.3 σ/S
LOQ = 10 σ/S
Where, σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

Forced degradation

Stress testing of the drug substance can help in identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule.

Acid degradation studies

To 1 ml of stock solution of MET HCL and DAP, 1 ml of 2N hydrochloric acid solution was added and refluxed for 30 minutes at 60°C and then neutralized the solution with 1 ml of 2N NaOH solution. The resultant solution was diluted with a mobile phase in a 10 ml volumetric flask to obtain a concentration of 340 µg/ml and 2 µg/ml solution of MET HCL and DAP respectively. Then 20 µl solutions were injected into the chromatographic system, and the chromatograms were recorded to assess the stability of the sample.

Base degradation studies

To 1 ml of stock solution of MET HCL and DAP, 1 ml of 2N sodium hydroxide solution was added and refluxed for 30 minutes at 60°C and then neutralized the solution with 1 ml of 2N HCL solution. The resultant solution was diluted with a mobile phase in a 10 ml volumetric flask to obtain a concentration of 340 µg/ml and 2 µg/ml solution of MET HCL and DAP respectively. Then 20 µl solutions were injected into the chromatographic system, and the chromatograms were recorded to assess the stability of the sample.

Oxidation (peroxide) studies

To 1 ml of a stock solution of MET HCL and DAP, 1 ml of 20% hydrogen peroxide (H_2O_2) was added and kept for 30 minutes at 60°C. The resultant solution was diluted with a mobile phase in a 10 ml volumetric flask to obtain a concentration of 340 µg/ml and 2 µg/ml solution of MET HCL and DAP respectively. Then 20 µl solutions were injected into the chromatographic system, and the chromatograms were recorded to assess the stability of the sample.

Photolytic studies

It is carried out by exposing 1 ml of stock solution of MET HCL and DAP to UV light, by keeping the beaker in UV chamber for 7 days or 200 W hrs/m^2 in photostability chamber. The resultant solution was diluted with mobile phase in a 10 ml volumetric flask to obtain concentration of 340 µg/ml and 2 µg/ml solution of MET HCL and DAP respectively and 20 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Thermal studies

Stress testing under neutral conditions was studied by refluxing on a water bath for 6 hrs at 60°C. For HPLC study, the resultant solution was then diluted with mobile phase in a 10 ml volumetric flask to obtain concentration of 340 µg/ml and 2 µg/ml solution of MET HCL and DAP respectively and 20 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

From this study, it was found that a simple, precise, accurate, sensitive and efficient stability indicating RP-HPLC method has been developed and validated for simultaneous estimation of MET HCL and DAP in bulk and pharmaceutical dosage form. Chromatographic separation was carried out using mobile phase composed of buffer (0.1% orthophosphoric acid) adjusted to pH 6.8 with triethylamine:acetonitrile in the ratio of 50:50%/v/v on hypersil BDS C_{18} (250 mm × 4.6 mm, 5 µ particle size) column at a flow rate 1.0 ml/minutes using PDA detection at 240 nm. The retention times were found to be 2.791 minutes and 3.789 minutes for MET HCL and DAP respectively. System suitability chromatogram as shown in Fig. 3 and results are shown in Table 1. Linearity was evaluated in the concentration range of 85-510 µg/ml for MET and 0.5-3.0 µg ml for DAP. The calibration curves were described by the equation y = 20317.6x+8024 and y = 121282.1x+1591.5 with correlation coefficient of 0.99995 for MET and 0.99978 for DAP respectively as shown in Figs. 4 and 5 respectively. The standard and sample chromatograms in the specifity studies are shown in Figs. 6 and 7. The LOD and LOQ are shown in Figs. 8 and 9. The % RSD in precision, accuracy and robustness studies were found to be <2.0%, indicating that the method was precise, accurate and robust. Accuracy data as shown in Table 2. The validation summary parameters and assay results obtained from the marketed formulation is shown in Tables 3 and 4. The results of robustness studies are shown in Table 5. The stress testing chromatograms for both MET HCL and DAP are shown from Figs. 10-14 and results are shown in Tables 6 and 7.

Linearity

R^2 values were found to be 0.99995 and 0.99978 and regression equation y = 20317.6x+8024 and y = 121282.1x +1591.5 for MET n HCL and DAP respectively.

Specificity

The chromatograms of standard and sample were identical to each other. The blank and placebo injections were also identical without any interference from the excipients.

Table 1: System suitability results

S. no	System suitability parameters	Results	
		MET	DAP
1	USP tailing	1.74	1.20
2	USP resolution (Rs)	5.47	
3	Rt minutes	2.791	3.789
4	USP plate count	3072	8846

MET: Metformin, DAP: Dapagliflozin, USP: United States Pharmacopeia, Rt: Retention time

Table 2: Accuracy study

Sample	Level (%)	Peak area*	Amount recovered (µg/ml)	Mean % recovery*±SD
MET	50	3424951	170.39	100.23±0.24
	100	6803222	339.46	99.84±0.72
	150	10185939	508.27	99.66±0.59
DAP	50	124219	0.996	99.61±0.33
	100	250357	2.01	100.38±0.22
	150	374571	3.00	100.13±0.82

*Mean of three determinations. MET: Metformin, DAP: Dapagliflozin, SD: Standard deviation

Table 3: Summary of validation parameters of the proposed RP-HPLC method

Parameter	MET	DAP
Linearity range (µg/ml)	85-510	0.5-3.0
Regression equation	y=20317.6x+8024	y=121282.1x+1591.5
Correlation coefficient (r)	0.99995	0.99978
LOD (µg/ml)	1.32	0.43
LOQ (µg/ml)	3.95	1.43
Inter-day precision (% RSD)	0.52	0.26
Intraday precision (% RSD)	0.83	0.37

MET: Metformin, RP-HPLC: Reversed-phase high-performance liquid chromatography, DAP: Dapagliflozin, LOD: Limit of detection, LOQ: Limit of quantification

Table 4: Results of assay in marketed formulation

Brand	Drug	Standard peak area	Sample peak area	Labelled amount (mg/tablet)	Amount found (mg/tablet)	% Assay	% RSD
Xigduo	MET HCL	6809226	6812213	850	850.34	100.04	0.38
	Dapagliflozin	248441	247850	5	4.98	99.76	0.42

RSD: Relative standard deviation, MET: Metformin

Table 5: Results of robustness study

S. no	Parameter	Change level	MET			DAP		
			Rt (minutes)	Peak area	Tailing factor	Rt (minutes)	Peak area	Tailing factor
1	Flow rate (±0.1 ml/minutes)	0.9	3.094	6812654	1.66	4.206	252745	1.02
		1.1	2.546	6487420	1.58	3.441	228942	1.17
2	Mobile organic phase composition (±10%v/v/v)	45:55	809	6248925	1.47	3.749	276224	1.13
		60:40	2.691	6459347	1.72	3.856	234721	1.08
3	Temperature (±5°C)	25°C	2.799	6662417	1.55	3.783	249225	1.13
		35°C	2.727	6428691	1.64	3.787	252478	1.02

MET: Metformin, DAP: Dapagliflozin, Rt: Retention time

Table 6: Degradation study of metformin HCL

S. no	Condition	Rt (minutes)	Purity angle	Purity threshold	% Drug degraded
1	Acid degradation hydrolysis	2.777	0.145	0.322	7.54
2	Base hydrolysis	2.779	0.134	0.316	6.90
3	Oxidation (peroxide)	2.787	0.169	0.339	5.78
4	Thermal degradation	2.790	0.094	0.277	4.80
5	UV exposure	2.788	0.135	0.326	1.01

UV: Ultraviolet, Rt: Retention time

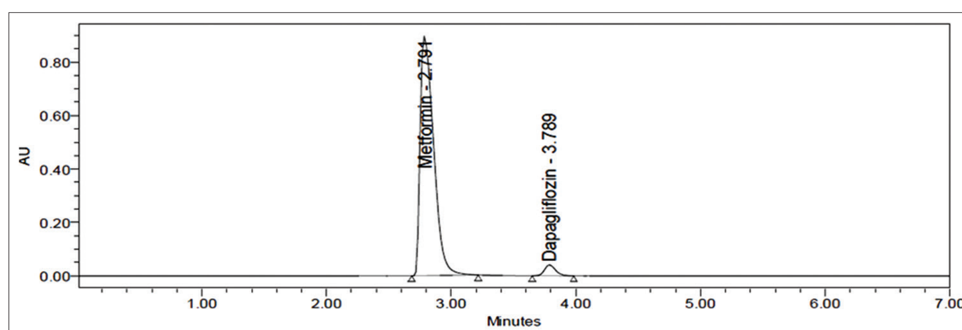

Fig. 3: Typical chromatogram of system suitability solution

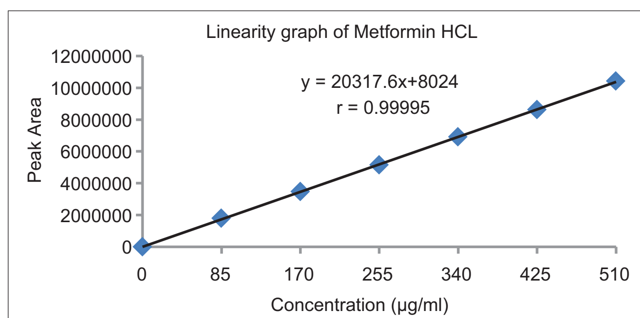

Fig. 4: Linearity graph of metformin HCL (85-510 µg/ml)

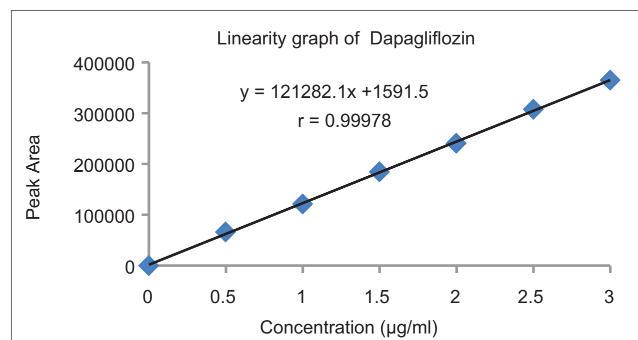

Fig. 5: Linearity graph of dapagliflozin (0.5-3.0 µg/ml)

Table 7: Degradation study of DAP

S. no	Condition	Rt (minutes)	Purity angle	Purity threshold	% Drug degraded
1	Acid degradation hydrolysis	3.770	1.094	1.301	7.49
2	Base hydrolysis	3.773	0.928	1.354	6.37
3	Oxidation (peroxide)	3.776	1.790	2.410	5.66
4	Thermal degradation	3.773	1.817	2.260	4.99
5	UV exposure	3.787	1.240	1.533	1.74

DAP: Dapagliflozin, UV: Ultraviolet, Rt: Retention time

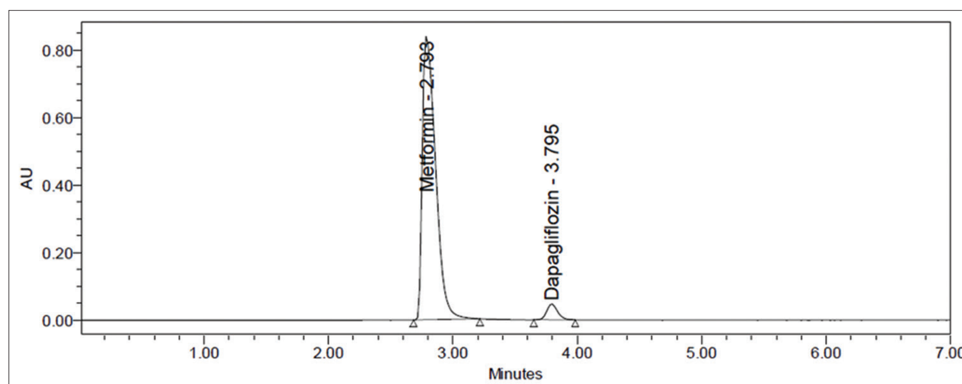

Fig. 6: Typical chromatogram of standard

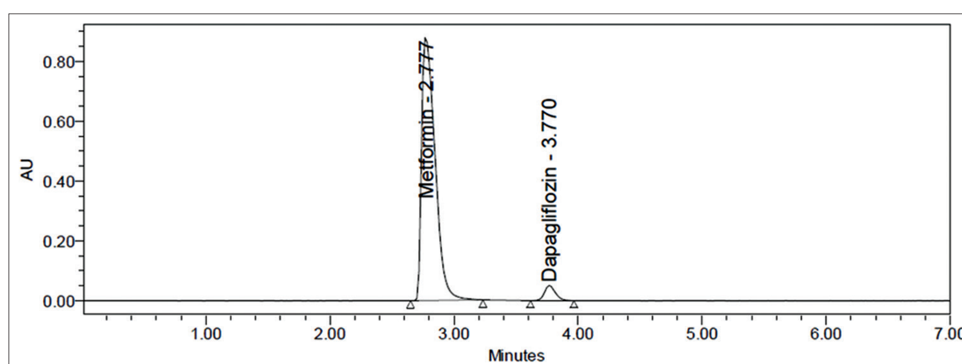

Fig. 7: Typical chromatogram of sample

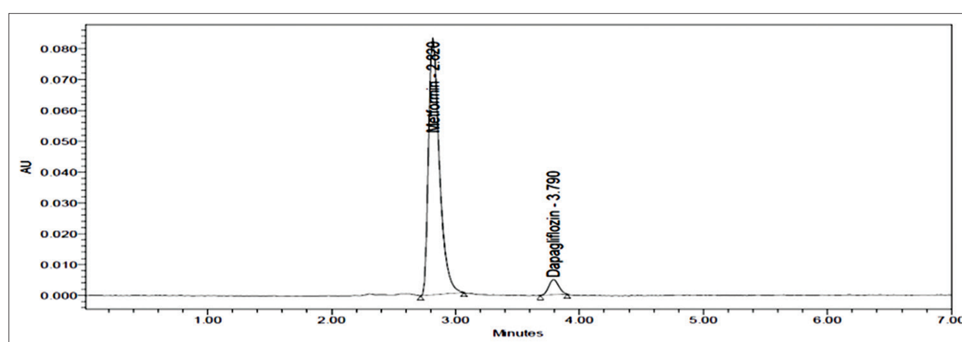

Fig. 8: Typical chromatogram of limit of detection

LOD and LOQ

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by the calibration plot method. A specific calibration plot was constructed using samples containing amounts of analytes in the range of LOD and LOQ. The values of LOD were 1.32 µg/ml and 3.95 µg/ml and LOQ were 0.43 µg/ml and 1.43 µg/ml for MET and DAP, respectively. LOD and LOQ were calculated by using the equations: LOD=Cd×Syx/b and LOQ=Cq×Syx/b where Cd and Cq are the coefficients for LOD and LOQ, Syx is the residual variance of the regression, and b is the slope. Calculations were performed by using values of Cd and Cq of 3.3 and 10.

Forced degradation studies

ICH degradation was attempted to various stress conditions such as acid hydrolysis (using 2N HCl), base hydrolysis (using 2 N NaOH), oxidative hydrolysis (using 20% H_2O_2), thermal degradation (heated at 60°C for 6hours) and photolytic degradation (overall illumination of ≥210Wh/m^2 at 25°C for 7 days with UV radiation at 320-400 nm),

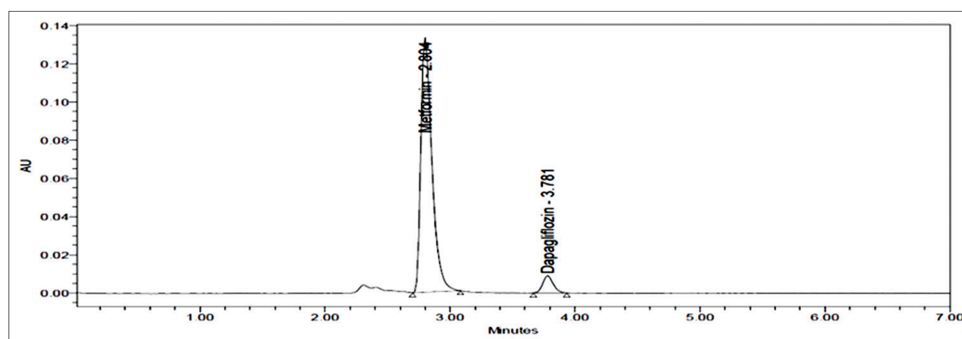

Fig. 9: Typical chromatogram of limit of quantification

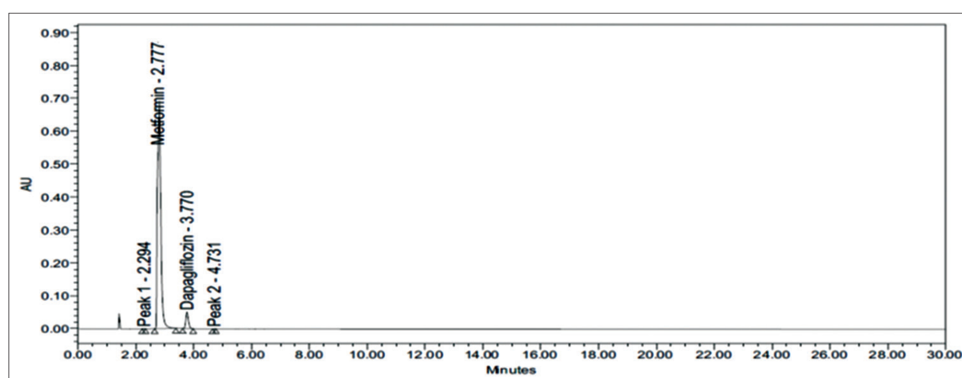

Fig. 10: Chromatogram of acid hydrolysis

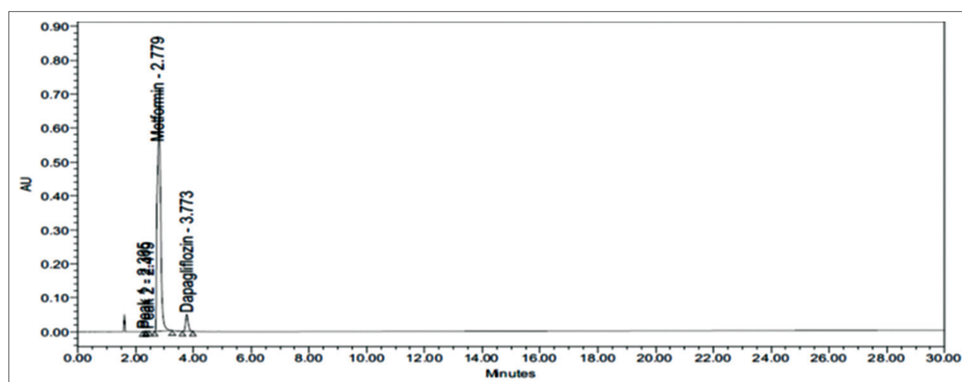

Fig. 11: Chromatogram of base hydrolysis

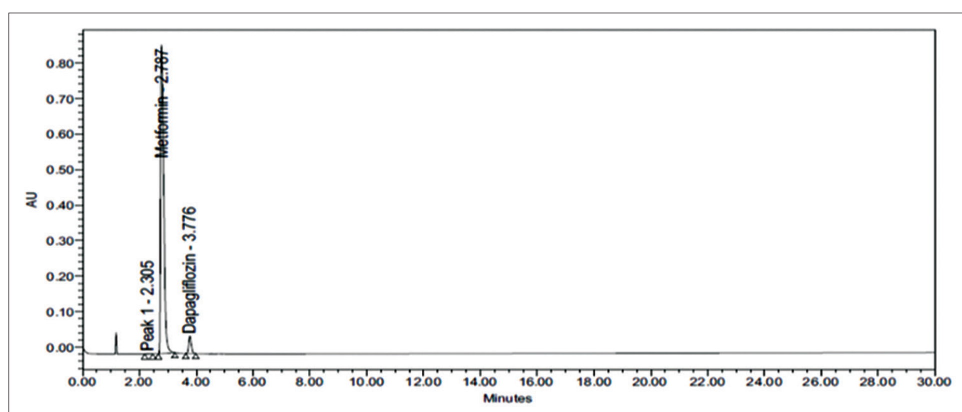

Fig. 12: Chromatogram of oxidation (peroxide) degradation

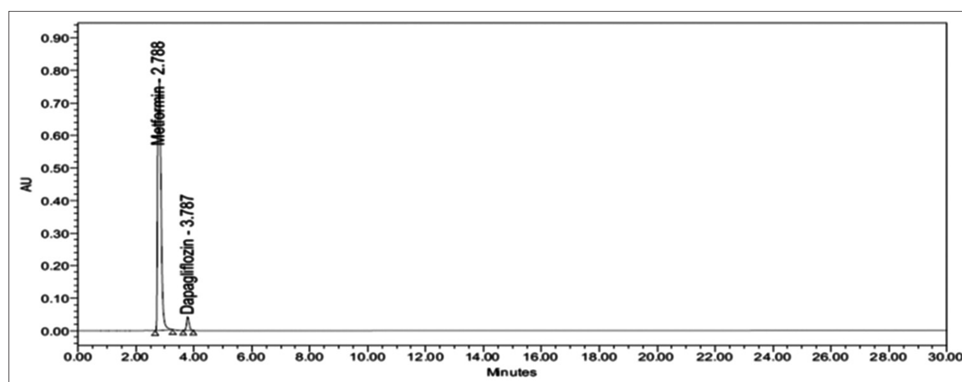

Fig. 13: Chromatogram of ultraviolet exposure

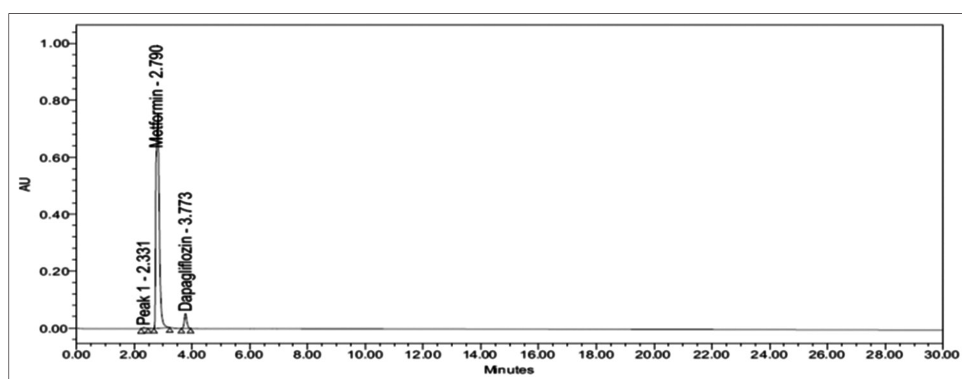

Fig. 14: Chromatogram of thermal exposure

to evaluate the ability of the proposed method to separate MET and DAP from its degradation products. It was observed that MET and DAP degrades with acidic, basic, oxidative and thermal stress conditions

CONCLUSION

From this study it is concluded that the proposed stability indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of MET HCL and DAP in bulk and pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

REFERENCES

1. Tatarkiewicz K, Polizzi C, Villescaz C, D'Souza LJ, Wang Y, Janssen S, *et al*. Combined antidiabetic benefits of exenatide and dapagliflozin in diabetic mice. Diabetes Obes Metab 2014;16(4):376-80.
2. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab 2013;15(9):853-62.
3. Aubry AF, Gu H, Magnier R, Morgan L, Xu X, Tirmenstein M, *et al.* Validated LC-MS/MS methods for the determination of dapagliflozin, a sodium-glucose co-transporter 2 inhibitor in normal and ZDF rat plasma. Bioanalysis 2010;2(12):2001-9.
4. Sanagapati M, Lakshmi DK, Reddy NG, Sreenivasa S. Development and validation of stability - Indicating RP-HPLC method for determination of dapagliflozin. J Adv Pharm Edu Res 2014;4(3):350-3.
5. Madhukar A, Prince A, Vijay Kumar R, Sanjeeva Y, Jagadeeshwar K, Raghupratap D. A simple and sensitive analytical method development and validation of metformin hydrochloride by RP-HPLC. Int J Pharm Pharm Sci 2011;3(3):117-20.
6. Rajesh T, Lakshmi KS, Sharma S. Simultaneous determination of metformin and pioglitazone by reversed phase HPLC in pharmaceutical dosage forms. Int J Pharm Pharm Sci 2009;1(2):162-6.
7. Akula A, Prajwala N, Sandhya M. Development and validation of RP-HPLC method for simultaneous estimation of metformin hydrochloride and Gliclazide in bulk and combined dosage form. Int J Pharm Pharm Sci 2013;5(4):511-7.
8. Bhoomaiah B, Shree JA. Development and validation of RP-HPLC method for simultaneous determination of metformin and miglitol in bulk and pharmaceutical Formulation. Int J Pharm Pharm Sci 2014;6(6):135-41.
9. Prathap GM, Muthukumaran M, Krishnamoorthy B. Development and validation of simultaneously estimation of vildagliptin and metformin hydrochloride by RP-HPLC in bulk and oral dosage form. Int J Adv Pharm Gen Res 2014;2(1):24-33.
10. Manasa S, Dhanalakshmi K, Reddy NG, Sreenivasa S. Method development and validation of dapagliflozin in API by RP-HPLC and UV-spectroscopy. Int J Pharm Sci Drug Res 2014;6(3):250-2.