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STRESS STUDIES OF METFORMIN AND GLICLAZIDE BY HPLC METHOD AND EXTENSION OF METHOD APPLICATION FOR ELUTION OF SOME ANTIVIRAL, ANTI-BACTERIAL AND ANTI-INFLAMMATORY DRUGS

Kanchan Chauhan [*1, 2] and Vishnu Choudhari [2]

Department of Pharmaceutical Chemistry [1], MCE Society's Allana College of Pharmacy, Azam Campus, Camp, Pune - 411001, Maharashtra, India.
School of Pharmacy [2], MIT World Peace University, MIT Campus, Kothrud, Pune - 411038, Maharashtra, India.

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Correspondence to Author:
Kanchan Chauhan

Assistant Professor,
Department of Pharmaceutical Chemistry, MCE Society's Allana College of Pharmacy, Azam Campus, Camp, Pune - 411001, Maharashtra, India.

E-mail: kc7876@gmail.com

ABSTRACT: A simple reverse phase SI-HPLC method was developed for the simultaneous estimation of antidiabetic combination of metformin and gliclazide. The method was based on HPLC separation on a reversed-phase C18 column using a mobile phase consisting of phosphate buffer: acetonitrile (40:60, v/v) at a flow rate of 0.8 ml/min. UV determination was achieved at 240 nm. The analytes were subjected to various forced degradation following ICH guidelines. Degradation of metformin was observed under acidic, basic, and peroxide stress. Separation of all the degraded products was achieved. Method was linear in a concentration range of 100-700 µg/ml for metformin and 20-140 µg/ml for gliclazide. The limit of detection and quantitation for metformin was 43.59 µg/ml and 132.09 µg/ml and for gliclazide 8.96 µg/ml and 27.15µg/ml. According to ICH guidelines, the developed method was validated. Metformin and gliclazide show no interference with the degradation product formed from stress studies. In view of the extended applications of the proposed method for *in-vivo* drug-drug interaction studies, the method was also studied for elution of few more antidiabetic drugs and also drugs from three other categories possibly co-administered with titled analytes. Drugs from anti-inflammatory, antibacterial, antiviral categories were used to achieve sufficient elution within run time of ten minutes with acceptable system suitability parameters.

INTRODUCTION: Diabetes mellitus (DM) is a disorder of irregularity in carbohydrate, lipid, and protein metabolism. The main cause of DM is an abnormality in insulin production. Diabetes mellitus is a common disease affecting seriously to human health.

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Metformin [3-(diaminomethylidene)-1, 1-dimethyl-guanidine], **Fig. 1**, is a biguanide that is approved for hypoglycemic treatment in type 2 diabetes mellitus [1] and is used in the treatment of type 2 DM. Metformin works as antidiabetic agent by causing hepatic uptake of glucose and by inhibiting gluconeogenesis [2].

Gliclazide (GLZ) chemically is 1-(3-Azabicyclo (3.3.0)oct-3-yl)-3-(p-tolylsulfonyl) urea. **Fig. 1** shows the chemical structure of GLZ. The drug is a second-generation sulphonylurea of antidiabetic category. It is used in the treatment of type II diabetes mellitus [2].

It is also used to treat hyperglycemia in gliclazide-responsive type 2 DM, which is stable, mild and non-ketosis prone [1].

Various methods for the determination of metformin [3-6] were found in literature individually and in combination. Methods are also reported in combined dosage form with different drugs [7-13]. No references on stress studies were reported in the literature for the said combination. Metformin and gliclazide combination are commonly prescribed for diabetes mellitus. Therefore, the development and validation of SI assay method study for the said analytes were planned.

Forced degradation studies provide valuable input for determining the degradation products. It also helps to determine the pathways of drug API. As there were no stress degradation studies reported so far for metformin and gliclazide combination, an attempt was made to develop the method.

Further, the extension of the method was studied to elute some different categories of drugs in view of drug-drug interactions (DDIs), which is one of the common causes of a medication error, particularly in elderly patients due to poly-therapy. On average, patients take 4 to 5 medications daily for their diabetes and comorbidities. Thus additional medication increases the risk of drug-drug interactions.

Many times more than two antidiabetic drugs are prescribed, which invites drug-drug interactions. Also, diabetic patients may suffer from peripheral neuropathy and cardiovascular disease where aspirin and paracetamol are commonly prescribed. Further diabetic population is vulnerable to various microbial infections and also to pathophysiological conditions. Therefore co-administration of drugs from various categories may be needed. In this view it is necessary to know the possible drug-drug interactions (DDI), which can be better known by evaluation of the pharmacokinetic parameters of these drugs in preclinical and clinical studies.

To attain this objective, we have studied the same method for the identification of different analytes, which are co-administered with antidiabetic therapies. As these analytes having diverse physicochemical properties, it is important to separate all the analytes by a single method. Thus a simple, accurate, fast LC method was validated with respect to ICH guidelines. The proposed method is also reproducible, efficient, and sensitive. Applicability of method also studied for the determination of antidiabetic drugs; *viz*. metformin, pioglitazone, vildagliptin, canagliflozin, and gliclazide from antidiabetic category, aspirin and paracetamol from anti-inflammatory, cefixime from antibacterial and tenofovir and emtricitabine from antiviral categories of drugs.

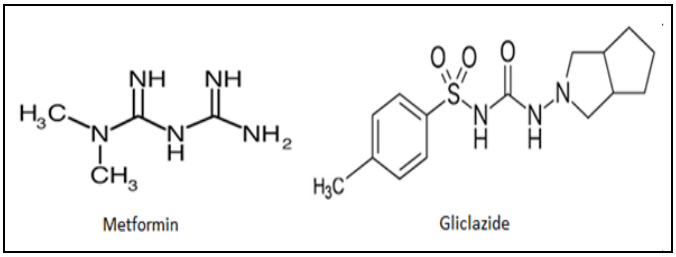

FIG. 1: STRUCTURE OF METFORMIN AND GLICLAZIDE

MATERIALS AND METHODS: Metformin and Gliclazide analyte standards were provided by Emcure Pharmaceuticals Ltd. Pune, India. Tablet Glizid-M containing gliclazide (80 mg) and metformin (500 mg); Batch no. 08316080, Mfg date-2/2017 manufactured by Panacea Biotec Ltd. was purchased. Other chemicals were purchased from Research Lab. Fine Chemicals, Mumbai. Throughout the study, HPLC grade water was used.

HPLC Instrumentation: The stability-indicating assay method was performed on Jasco pump PU-2080, with injecting vol. 20 µl, a detector was set to 240 nm. Jasco Borwin version 1.5, LC-Net II/ADC system was used for data integration. Separation achieved on ODS-3, 250 mm × 4.6 mm, 5µm thermosil column at 0.8 ml/min flow rate of mobile phase (MP) Phosphate buffer: ACN (40:60, v/v). The other instruments used were Ultrasonic Bath, pH-Meter, Digital Balance (Metller Toledo, Japan), UV visible spectrophotometer Jasco V630. Peak purity was assessed by using Waters LC system with PDA detector.

Standard Stock Solutions Preparation: Standard stock solutions (SSS) of MET and GLZ (1mg/ml) was prepared. Suitable Aliquots from SSS of metformin and gliclazide were transferred using pipettes to volumetric flasks and diluted suitably made with MP. To determine the calibration curve, the peak area of each concentration was plotted and compare with the corresponding concentrations.

Method Validation: Method validation was performed using different parameters such as linearity, accuracy, and precision. Also, parameters like specificity and robustness were also performed according to ICH guidelines [14-16]. Similarly, parameters such as method sensitivity parameters, limit of detection (LOD), and limit of quantitation (LOQ), along with solution stability, were also studied.

Linearity: Calibration curve was determined by performing linearity in which analyte response is directly proportional to the concentration. Linearity was established by using seven concentrations of metformin and gliclazide in range of 100µg/ml to 700µg/ml and 20µg/ml to 140µg/ml, respectively. From weighing of analytes to the preparation of SSS, the procedure was repeated thrice. Linearity was further confirmed by residual plots and Fischer ratio methods. For the first method, plots of relative response against amount were plotted and observed for trending. The experimental Fischer ratio value was compared with the critical value found in statistical tables as part of the F test; to exhibit linearity, experimental F ratio values should be less than the tabulated value at the 95% confidence level.

Method Sensitivity: The LOD/LOQ values were calculated by using regression equation, and these parameters were calculated using the formulae as LOD/LOQ=#x(σ/S), where σ is standard deviation and S is the slope. Values of σ and S were used from the calibration plot, the value of # used were 3.3 for LOD and 10 for LOQ.

Estimation of Drugs from Pharmaceutical Dosage Form (Assay) and Assay Specificity: Assay of the marketed product (label claim: metformin 500 mg and gliclazide 80 mg) was carried out using weighed and powdered 20 tablets. Powder equivalent to 100 mg of sample was accurately weighed and transferred to a 100 ml volumetric flask. To it, 80 ml of acetonitrile was added. Flask was sonicated for 30 min volume was adjusted to the mark. The solution passed through a 0.45 µ nylon filter. Suitable aliquots of the filtered solution were diluted with mobile phase to get desired concentrations. Chromatograms were obtained by using the proposed method, and concentrations were compared with the area of the sample with an area of the standard. Specificity of method was determined by measuring the analyte unequivocally in the presence of its stressed degraded products.

Precision and Recovery: For calculating the drug precision, repeatability, intraday, and interday precision were performed. For repeatability, the area of sample peak was measured repeatedly (n=3). To determine intraday precision, metformin peak area at three different concentrations 300, 400, 500µg/ml and gliclazide peak area at 60, 80, 100µg/ml were measured three times (n=3) on the same day. Using three different concentration levels, the interday precision study was performed on three different days, and the % relative standard deviation (%RSD) was calculated.

The recovery studies were done by spiking known concentrations of pure drugs at 80%, 100%, and 120% different levels. The base-level concentration of the drug from formulation used was 200µg/ml and 40µg/ml for metformin and gliclazide, respectively. The spiked sample solutions were assayed in triplicate and the results were compared; % RSD and the mean recovery were calculated.

Method Robustness: For evaluation of robustness, some parameters were varied deliberately, such as a different variant of C18 columns from two manufacturers such as Thermosil column from Thermo scientific and Perfectsil column from MZ-Analysentechnik, acetonitrile percentage in the MP and flow rate. Each factor was changed at three levels (-1, 0, and 1) and examined.

Forced Degradation Studies of Metformin and Gliclazide: [17-18] To evaluate the SI properties and SI method specificity, stressed studies were performed. Metformin and gliclazide pure drug were degraded forcefully under different conditions to conduct the degradation studies. A stock solution of 1mg/ml each of metformin and gliclazide was prepared in methanol. The samples were diluted using mobile phase to made the concentration of 400 µg/ml and 80 µg/ml for metformin and gliclazide, respectively.

Acid and Alkali Hydrolysis: For acid and alkali hydrolysis, 2 ml of 0.1NHCl / 0.1NNaOH was added to the stock solution of metformin and gliclazide separately. The solution was kept for 2 and 1 h at R.T. for acid and alkali hydrolysis,

respectively. The resultant solutions were neutralized with 0.1N NaOH/ 0.1N HCl and were injected into the system, and the chromatograms were recorded.

Oxidation Studies: For the peroxide study, 2ml of 3% H_2O_2 was added to the stock solutions. The solutions were set aside for 2 h at R.T. Stressed solutions were diluted suitably and used to acquire chromatographic data.

Dry and Wet Heat Degradation Studies: The standard drug was placed in an oven at 80 ºC for 6 h to determine dry heat degradation. Refluxing the stock solution for 3.0 h determines the effect of wet heat degradation.

Photo Stability Studies and Neutral Degradation Studies: The effect of Neutral degradation was studied by refluxing the drug solutions of analytes in water for 3 hrs at 70 ºC. Stressed solutions were diluted suitably with the mobile phase and used to acquire chromatographic data.

The drug was studied for photostability by keeping 50mg of each drug spread as a thin film in two separate petri dishes and exposed to UV light up to illumination of 200 Watt h/m^2 for 24 h. The drugs were diluted to the mobile phase to obtain 400 µg/ml of metformin and 80µg/ml of gliclazide. 20 µl were injected into the system to assess the sample stability, and the chromatograms were recorded.

RESULTS AND DISCUSSION:

Analytical Wavelength Selection: HPLC method sensitivity depends on the proper selection of detection wavelength that can be determined by recording UV spectra of the analyte. The overlay spectrum of metformin and gliclazide shows the absorbance of both drugs at 240 nm when scanned under UV spectrometer with a range of 200 to 400 nm.

HPLC Method Optimization: Stability indicating HPLC assay method was optimized using different mobile phases. Initially, methanol-water and acetonitrile-water were tried in different ratios. None of these gives good peak shape and symmetry. An attempt was made for the improvement of peak symmetry by the addition of phosphate buffer to the mobile phase. The use of phosphate buffer in mobile phase composition led to improve in the chromatographic performance. Different trials on the composition of organic solvents and buffer were made to finalize the mobile phase composition. The use of acetonitrile as an organic modifier in the mobile phase leads to improvement in peak tailing and symmetry. Thus ACN was selected as an organic modifier instead of ACN. Finally, the mobile phase was optimized with a composition of phosphate buffer: ACN (40:60 v/v ratio) for validation purposes and stability studies. At pH 3.6, theoretical plates and peak shape were found to be satisfactory; hence, the mobile phase pH was decided as pH 3.6. In view of determining the reproducibility of proposed methods, system suitability parameters (SSP) such as asymmetry factor, retention time, plate number were investigated. The metformin and gliclazide peak shows retention times of 3.1 and 7.00 min, respectively **Fig. 2**.

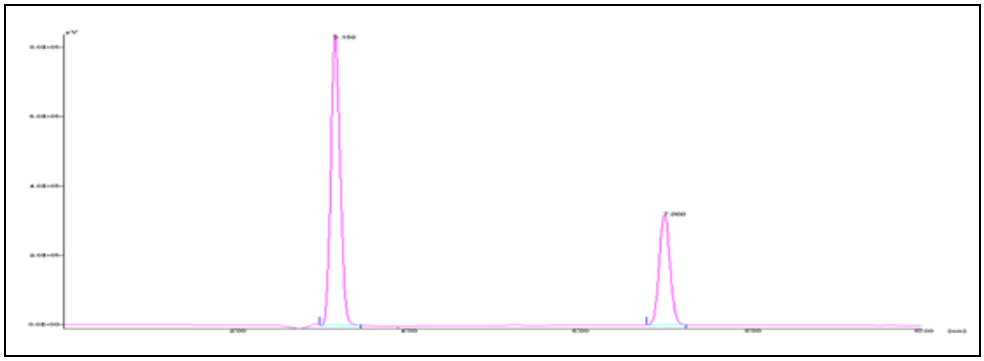

FIG. 2: CHROMATOGRAM OF STANDARD DRUG METFORMIN AND GLICLAZIDE

System Suitability Parameters: The chromatograms and the peak responses were measured for metformin and gliclazide, and the system suitability parameter was evaluated with respect to peak area, theoretical plate retention time, tailing factor, resolution and capacity factor.

Method Validation:

Linearity and Method Sensitivity: Metformin and gliclazide calibration curve were determined as described in the experimental section, from which a correlation coefficient was determined. Results of linearity was found in the range of 100-700 µg/ml for metformin and 20-140 µg/ml for gliclazide **Fig. 3**. The r^2 values were found to be 0.9986 and 0.9984 for metformin and gliclazide, respectively. The results show good linearity between analyte concentration and chromatographic response. Values of standard deviation and the slope were determined from calibration curve data. The values of LOD, LOQ were 43.59 µg/ml 132.09 µg/ml for metformin and for gliclazide were 8.96 µg/ml and 27.15 µg/ml, respectively. To further confirm linearity, the residual plots of relative response against concentration were plotted for metformin and gliclazide, which shows no trending **Fig. 4**. F test was applied to check the linearity, where the experimental Fischer ratio value was compared against the critical value found in statistical tables for both analytes. Experimental F ratio values and tabulated value were compared where the tabulated value was found to be less than the 95% confidence level. The summary of linear regression and method sensitivity study was given in **Table 1**.

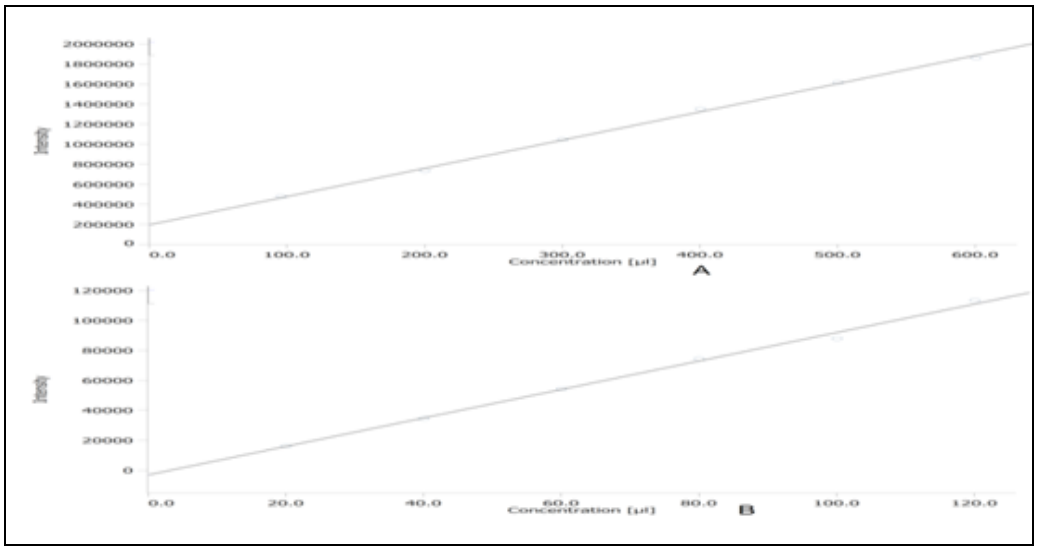

FIG. 3: CALIBRATION CURVE A)-METFORMIN AND B)-GLICLAZIDE

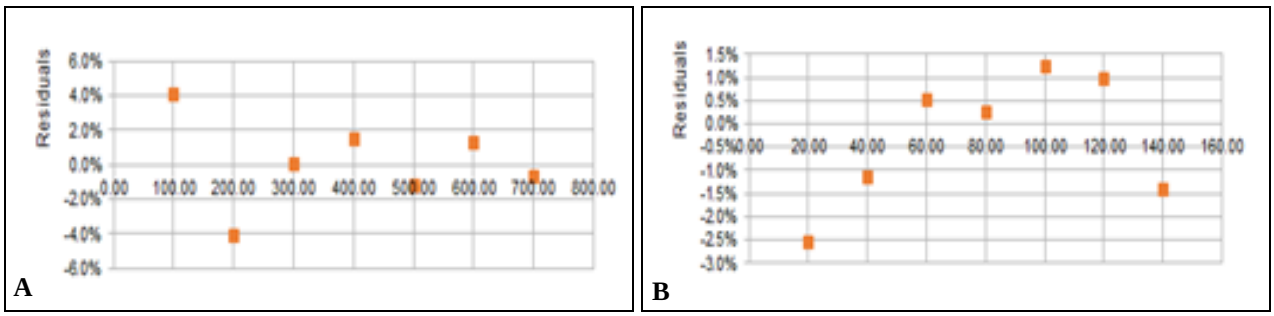

FIG. 4: RESIDUAL PLOT A) METFORMIN AND B) GLICLAZIDE

TABLE 1: SUMMARY OF LINEAR REGRESSION AND METHOD SENSITIVITY STUDY, n=3

Parameter	Metformin	Gliclazide
Linearity Range	100-700 µg/ml	20-140 µg/ml
Linear regression equation	y = 2973x+12383	y = 901.4x - 648
Slope and its standard error	2973, 74.218	901.4, 23.1314
Intercept and its standard error	12383, 4489.2642	-648, 2068.9368
Correlation coefficient and its standard error	0.9986, 0.02492	0.9984, 0.02561
Limit of detection (LOD)	43.59 µg/ml	8.96 µg/ml
Limit of quantitation (LOQ)	132.09 µg/ml	27.15 µg/ml
Tabulated Fischer Variance (F)	2.71	2.71
Experimental Fischer Variance (F)	2.32	2.43

Estimation of Drugs from Pharmaceutical Dosage Form (Assay) and Assay Specificity: Assay of MET and GLZ in marketed tablets was applied for the method. The outcome of the assay yielded 100.46% (% RSD=0.68) for metformin and 99.88% (% RSD 0.75) for gliclazide of the label claim of the tablets. The assay results of MET and GLZ indicate that excipients did not interfere with drug peak, which proves that the method was selective. The method was found to be specific as there was complete separation of metformin, its degradation products, and gliclazide, and the resolution was found to be greater than 2. Assay results are given in **Table 2**.

TABLE 2: ASSAY OF METFORMIN AND GLICLAZIDE IN COMMERCIAL TABLETS (n=6)

Drug label claim	Conc. taken in µg/ml	Avg. % Assay	% RSD
Metformin 500 mg	400	100.46	0.68
Gliclazide 80 mg	80	99.88	0.75

Precision and Recovery: For calculating the method, precision %RSD of peak area of intermediate precision and repeatability was determined. The assay results were evaluated by taking sample stock solution of MET as 300, 400, 500 µg/ml, and for GLZ, it was 60, 80, and 100 µg/ml at three concentration levels. The interday precision study at three different concentration levels was measured on three different days. The repeatability, intra-, and inter-day variation of the results for MET and GLZ were within the acceptable range. Low values of %RSD indicate good precision. Precision study results are given in **Table 3**. Accuracy study was done at three levels of the test concentration according to ICH guidelines. MET and GLZ The %recovery at three levels was found to be satisfactory. The % recovery was found in the range of 99.76 to 100.23 for metformin and 98.82 to 100.65 for gliclazide given in **Table 4**.

TABLE 3: RESULTS OF PRECISION STUDY (n=3)

Analyte name	Intraday Precision			Interday Precision		
	Conc. (µg/ml)	Average Peak Area	% RSD	Conc. (µg/ml)	Average Peak Area	% RSD
Metformin	300	1481423	0.28	300	1478121	0.33
	400	2319867	0.61	400	2331106	0.76
	500	3151463	0.86	500	3151252	0.31
Gliclazide	60	303988	0.72	60	304489	0.89
	80	456697	0.45	80	456798	0.53
	100	603046	0.82	100	602478	0.42

TABLE 4: RESULTS OF RECOVERY STUDY (n=3)

Level (%)	Metformin				Gliclazide			
	Base level Conc. (µg/ml)	Amount added Conc. (µg/ml)	% Recovery	% RSD	Base level Conc. (µg/ml)	Amount added Conc. (µg/ml)	% Recovery	% RSD
80	200	160	99.81	1.18	40	32	98.82	1.41
100	200	200	100.23	0.96	40	40	99.54	0.88
120	200	240	99.76	1.24	40	48	100.65	1.07

Robustness Studies: Percentage (%) RSD of the test results of the selected parameters at different conditions was calculated and found within the ICH limit (% RSD NMT 2%), indicating that the method is sufficiently robust to analyze metformin and gliclazide in the pharmaceutical dosage form. Results are given in **Table 5**.

TABLE 5: RESULTS OF ROBUSTNESS STUDIES (n=3)

Study parameters and its level		Metformin		Gliclazide	
Parameters	Level	% Assay	% RSD	% Assay	% RSD
Flow rate (ml/min)	0.6	99.2	0.98	99.5	0.55
	0.8	99.8	1.23	100.1	0.98
	1.0	100.2	0.89	99.8	1.07
	Mean	99.73	1.03	99.8	0.86
Wavelength (nm)	241	99.5	1.03	99.89	0.86
	243	100.2	1.24	100.3	1.21
	242	99.4	0.98	100.2	0.95

	Mean	99.7	1.08	100.13	1.00
Mobile phase	61+39	98.5	0.98	99.46	0.79
volume	60+40	101.4	0.86	98.98	0.59
(ACN: Buffer	59+41	99.6	0.95	100.5	0.81
v/v)	Mean	99.83	0.93	99.64	0.73
Columns from	Perfectsil	98.76	0.89	100.3	0.96
different	Thermosil	99.81	0.88	99.71	0.56
manufacturers	Mean	99.28	0.885	100.00	0.76

Stability in Analytical Solution: No significant variation was found in the %assay of both drugs before and after storing in refrigerator and room temperature. This confirms the stability of the drugs in solutions. The mean percentage assay and %RSD are presented in **Table 6**.

TABLE 6: METHOD SOLUTION STABILITY DATA OF SAMPLE

Storage conditions	% Assay, % RSD MET	% Assay, % RSD GLZ
Exposure of solution to lab shelf for 2, 4, 6, 12, 24 hrs	99.58, 0.54	99.86, 0.64
Fridge storage of stock solution for 2, 4, 8, 12, 24 and 30 days	99.87, 0.63	99.42, 0.77

Forced Degradation Studies: The percentage assay, percentage degradation at each condition for metformin and gliclazide were tabulated in **Table 7**. Peak purity confirms no interference at the retention time of main peaks. Resolution data shows no interference of analyte and the degradation products from main peaks. The standard drug chromatograms and the representative chromatograms of acid, base, peroxide and neutral degradation of sample are shown in **Fig. 5**.

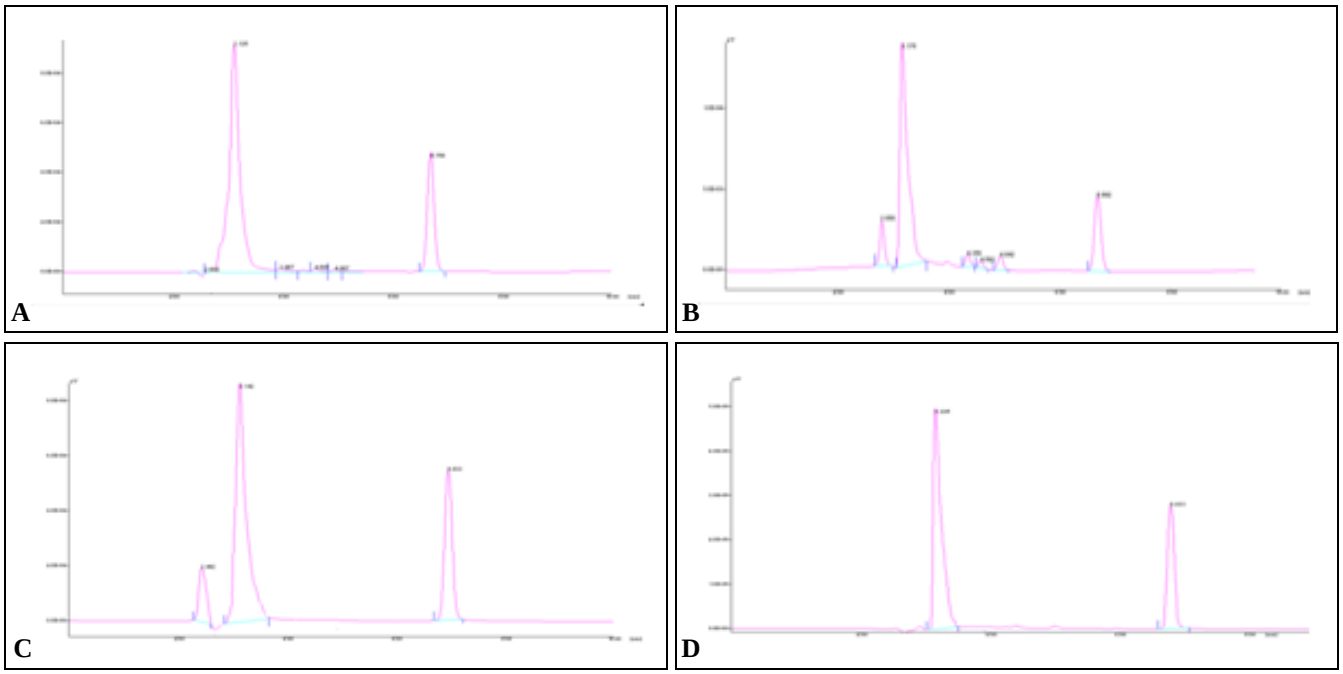

FIG. 5: A) CHROMATOGRAM OF ACID HYDROLYSIS, B) CHROMATOGRAM OF BASE HYDROLYSIS, C) CHROMATOGRAM OF HYDROGEN PEROXIDE DEGRADATION, D) CHROMATOGRAM OF NEUTRAL HYDROLYSIS

Acid Degradation Studies and Base Degradation Studies: Under acidic stress conditions of 0.1NHCl at R.T. for 2 h, the major degradation products of MET are observed at t_R= 2.60, 3.96, 4.6, 4.96 min.

Under basic stress condition of 0.1N NaOH at R.T. for 1 h, the major degradation products of metformin are observed at t_R= 2.658, 4.35, 4.59, 4.94 min

Oxidative Degradation Studies: Under oxidative stress condition at R.T for 2 h using 3% w/v H_2O_2, the major degradation product was found at t_R - 2.442 min given in **Table 7**.

Dry Heat and Wet Heat Degradation Studies: Under these degradation studies, samples showed no extra peak, and the peak area of the standard remains unchanged.

Photostability and Neutral Degradation Studies: Under photochemical degradation, the drug exposed to photolytic conditions for 24 h under a UV lamp. No additional peaks and no change in the area of the analyte drugs were found. The summary of stability studies is given in **Table 7**. Under neutral conditions, no additional peaks were seen when the drug sample was kept for 3 h refluxing with water at 70 ºC.

TABLE 7: SUMMARY OF STRESS STUDY DATA OF MET AND GLZ

Stress Condition	Exposure Time	% Assay MET	t_R (min) of degradation products MET	% Assay GLZ	t_R (min) of degradation products GLZ
Acid 0.1 N HCl	2 hrs R.T	93.14	2.6,3.9,4.6,4.9	99.23	---
Base 0.1 N NaOH	1 hr R.T.	88.46	2.6,4.3,4.5,4.9	98.56	---
H_2O_2 3 %w/v	2 hrs R.T.	93.57	2.442	98.88	---
Neutral hydrolysis (70ºC)	3 hrs	98.94	---	99.14	---
Dry Heat (80 ºC)	6 hrs	99.12	---	98.89	---
Wet Heat, reflux (70 ºC)	3 hrs	98.26	---	99.47	---
UV Photostability 200 Watt hrs/m^2	24 hrs	98.67	---	99.12	---

Extention of Method Application: Multi-drug antidiabetic therapy is currently in need of time and prescribed by physicians to control diabetics' complications. Further drugs from anti-inflammatory, antibacterial, and antiviral categories are also needed to treat co-morbidities. Efforts were directed to the proposed method for seven different antidiabetic drugs such as metformin, pioglitazone, vildagliptin, canagliflozin, and gliclazide. Analytes solutions were prepared in ACN and were suitably diluted and injected into the system to record chromatograms. The chromatogram shows that all the **seven** antidiabetic drugs were resolved from each other with a resolution greater than two, and their SST parameters were found within the limit. The chromatogram of well-resolved peaks achieved within 10 min for all antidiabetics drugs is shown in **Fig. 6A**.

A mixture of anti-inflammatory drug aspirin and antidiabetic drugs, pioglitazone, vildagliptin, and gliclazide were suitably diluted with MP and injected, and the chromatogram was recorded. The chromatogram shows that aspirin and antidiabetic drugs were well resolved from each other with resolution greater than two with acceptable SST parameters within 10 min shown in **Fig. 6B**. The parameters are given in **Table 8A** and **8B.**

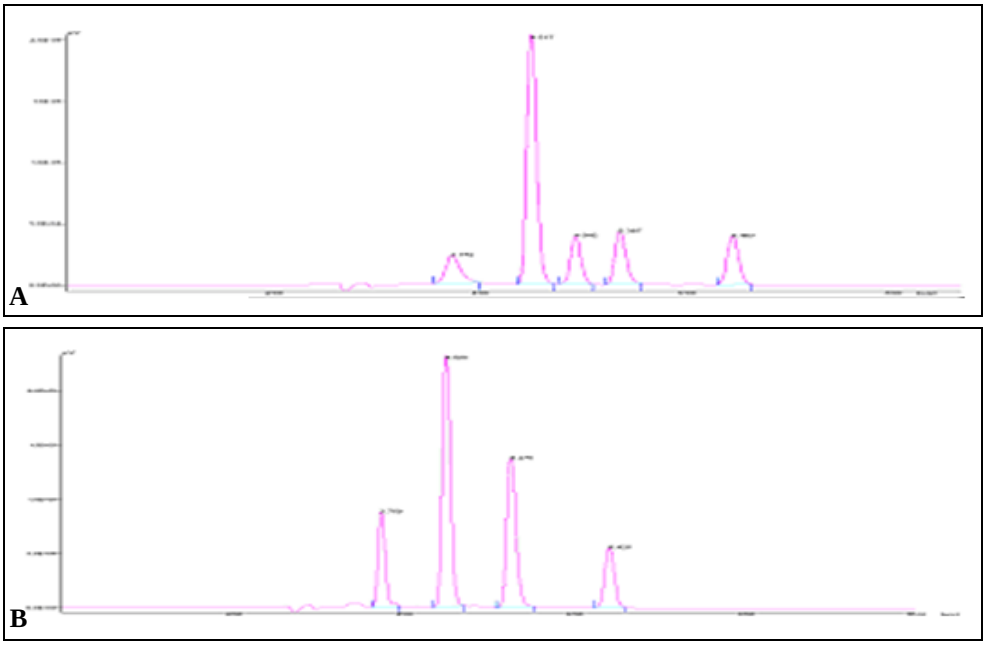

FIG. 6: A-CHROMATOGRAM OF ANTIDIABETICS DRUGS. (PEAK 1)- METFORMIN, (PEAK 2)- VILDAGLIPTIN, (PEAK 3)- CANAGLIFLOZIN, (PEAK 4) – PIOGLITAZONE, (PEAK 5)- GLICLAZIDE. B- CHROMATOGRAM OF ANTI-INFLAMMATORY AND ANTIDIABETIC DRUGS. (PEAK 1)- ASPIRIN, (PEAK 2)-VILDAGLIPTIN, (PEAK 3) - PIOGLITAZONE- (PEAK 4) –GLICLAZIDE

TABLE 8A: SYSTEM SUITABILITY PARAMETERS OF ANTIDIABETIC DRUGS RESOLVED IN FIG. 6A

Analyte name	Retention time	Area	Theoretical plates	Asymmetry factor	Resolution
Metformin	3.742	210250	4119	1.268	0.00
Vildagliptin	4.517	1254187	12470	1.125	3.933
Canagliflozin	4.942	253476	13756	1.077	2.579
Pioglitazone	5.367	299837	13978	1.232	2.433
Gliclazide	6.467	285351	18442	1.049	5.962

TABLE 8B: SST PARAMETERS OF ANTI-INFLAMMATORY (ASPIRIN) AND ANTIDIABETIC DRUGS RESOLVED IN FIG. 6B

Analyte name	Retention time	Area	Theoretical plates	Asymmetry factor	Resolution
Aspirin	3.750	459847	13009	1.168	0.000
Vildagliptin	4.508	1399575	13591	1.149	5.311
Pioglitazone	5.275	947604	14238	1.147	4.635
Gliclazide	6.425	403152	17797	1.051	6.239

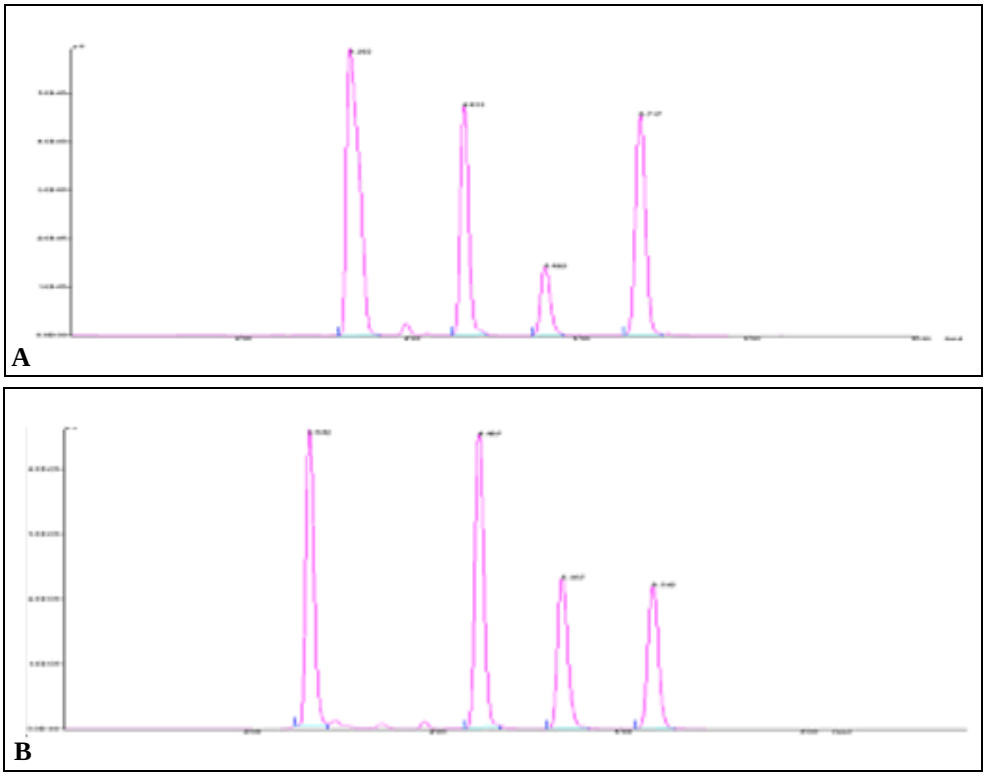

FIG. 7: (A): CHROMATOGRAM OF ANTI-INFLAMMATORY AND ANTIDIABETIC DRUGS. (PEAK 1)- PARACETAMOL, (PEAK 2)-VILDAGLIPTIN, (PEAK 3)-PIOGLITAZONE- (PEAK 4)–GLICLAZIDE. (B)- WELL RESOLVED PEAKS OF ANTIBACTERIAL AND ANTIDIABETIC DRUGS. (PEAK 1)- CEFIXIME, (PEAK 2) - GLIPIZIDE, (PEAK 3)-PIOGLITAZONE- (PEAK 4)–GLICLAZIDE

TABLE 9A: SST PARAMETERS OF ANTI-INFLAMMATORY AND ANTIDIABETICS RESOLVED IN FIG. 7A

Analyte name	Retention time	Area	Theoretical plates	Asymmetry factor	Resolution
Paracetamol	3.292	5676259	2185	1.753	0.00
Vildagliptin	4.633	2940182	12847	1.187	6.041
Pioglitazone	5.592	1008741	14062	1.178	5.455
Gliclazide	6.717	3460114	18331	1.047	5.826

TABLE 9B: SST PARAMETERS OF ANTIBACTERIAL AND ANTIDIABETICS RESOLVED IN FIG. 7B

Analyte name	Retention time	Area	Theoretical plates	Asymmetry factor	Resolution
Cefixime	2.642	2461367	5647	1.355	0.00
Glipizide	4.467	2701054	13573	1.155	12.444
Pioglitazone	5.367	1638046	13364	1.199	5.321
Gliclazide	6.342	1591849	18252	1.071	5.233

A mixture of anti-inflammatory drug paracetamol and antidiabetic drugs such as vildagliptin, pioglitazone, and gliclazide were suitably diluted with MP and injected, and the chromatogram was recorded. The chromatogram shows that paracetamol and the antidiabetic drugs were well resolved. The well-resolved peaks of aspirin and antidiabetics drugs were achieved within 10 min with acceptable SSPs as shown in **Fig. 7A**, and the SSPs are given in **Table 9A**.

Similarly, antibacterial drug cefixime and antidiabetic drugs were studied by the method. The chromatogram shows that cefixime and three antidiabetic drugs were well resolved with acceptable system suitability parameters. The chromatogram of well-resolved peaks of aspirin and antidiabetic drugs is shown in **Fig. 7B,** and the SSPs are given in **Table 9B**.

CONCLUSION: The developed validated Isocratic HPLC method of metformin and gliclazide is simple, specific, accurate, precise, sensitive, and robust. The advantages of the method are short run time and high throughput. This SI method is very convenient to use for MET and GLZ estimation. The method is very useful for the control of pharmaceuticals, stability studies.

DDI may lead to various complications. DDI involving the mentioned molecules needs to be quantitatively evaluated *in-vivo* for more precise predictions. In view of this, the proposed method was extended for analysis of different categories of drugs in a single method. Thus the method has been found to be useful for the simultaneous determination of antidiabetic drugs *viz*. pioglitazone, vildagliptin, canagliflozin glipizide, and gliclazide from antidiabetic category, aspirin and paracetamol from anti-inflammatory, and cefixime from antibacterial categories.

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