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Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide in Bulk and Pharmaceutical Dosage Forms

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Abstract: *A simple, selective and precise Stability indicating RP-HPLC method was developed for the simultaneous estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide in the Bulk and Pharmaceutical Dosage Forms using glimepiride as an internal standard. The chromatographic separation of the three drugs was achieved on a reverse phase Inertsil-ODS, C18, 100X 4.6 mm, 5µm column using 0.1 M Ammonium acetate buffer (pH 4.5adjusted by using formic acid) and Acetonitrile in the ratio of 45:55 v/v with flow rate of 0.8 ml/min with injection volume 20 µL and the detection was carried out at 254 nm. The retention time of metformin hydrochloride, pioglitazone hydrochloride and glibenclamide and glimepiride were found to be 1.1, 4.5, 5.9, 6.5min respectively. The drug products were subjected to stress conditions of acidic, alkaline, oxidation, UV and Thermal conditions. The degradation products were well resolved from Metformin Hydrochloride, Pioglitazone Hydrochloride and Glibenclamide peaks, thus indicating the stability-indicating nature of the method. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range of 62.5-375.00 µg/ml for metformin hydrochloride, 3.75-22.5 µg/ml for pioglitazone hydrochloride and 1.25-7.50 µg/ml for glibenclamide. The developed method was successfully validated in accordance to ICH guidelines. Hence, this method can be conveniently adopted for the routine analysis in quality control laboratories.*

Key words: *Metformin Hydrochloride, Pioglitazone Hydrochloride, Glibenclamide, RP-HPLC*

I. Introduction

Metformin HCl (MET) is an anti diabetic drug. Chemically, it is 1,1-dimethyl biguanide hydrochloride (Fig. 1). Metformin decrease the gluconeogenisis and increases the glucose uptake by muscles and fat cells. It is indicated for the treatment of type II diabetes mellitus, used alone or in combination with sulfonylurea's, alpha-glycosidase inhibitors, or insulin.

Pioglitazone (PIO) is a thiazolidinedione antidiabetic agent, chemically it is (RS)-5-(4-[2-(5ethylpyridin 2-yl) ethoxy] benzyl) thiazolidine-2,4-dione (Fig. 2). It selectively stimulates the peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α. It is used for the treatment of type II diabetes mellitus either alone or in combination with other oral anti diabetic drugs.

Glibenclamide (GLI) is a second generation sulphonyl urea oral hypoglycemic agent, chemically it is 1-[4-[2-(chloro 2-methoxybenzamido) ethyl]-benzene sulphonyl]-3cyclo hexyl urea, 5-chloro-N-[2-[4-[[[(cyclohexyl (amino) carbonyl]-amino] sulphonyl] phenyl] ethyl-2- methoxy benzamide (Fig. 3). It is used to assist to control mild to moderately severe type II diabetes mellitus that does not require insulin that can be adequately controlled by diet alone [1].

Literature survey revealed HPLC, RP-HPLC, LC-MS, Spectrofluorimetric and simultaneous UV spectrophotometric methods are reported for the estimation of metformin hydrochloride [2-5], pioglitazone hydrochloride [6-9] and glibenclamide [10] alone or in combination with other anti-diabetic agents. So the present study aim to develop a simple, selective and precise RP-HPLC method for the simultaneous estimation of MET, PIO and GLI in bulk drug samples and in combined dosage formulation.

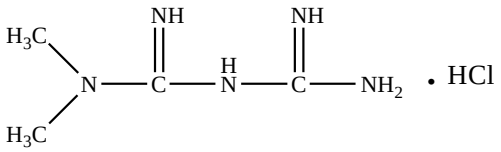

Fig. 1 Structure of Metformin HCl

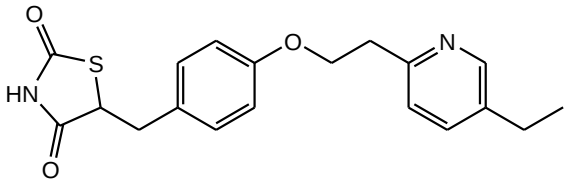

Fig. 2 Structure of Pioglitazone HCl

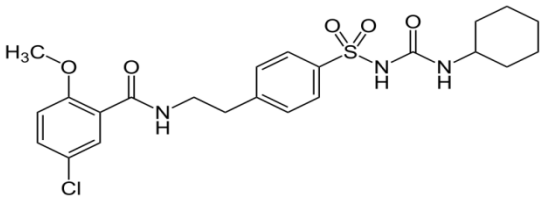

Fig. 3 Structure of Glibenclamide

II. Materials And Methods

2.1 Chromatographic Conditions

The WATERS HPLC with PDA detector and Empower2 software was employed for the present study. The chromatography determination performed at ambient temperature by using Inertsil BDS_{18} (100× 4.6, 5µm) column, with a mobile phase composed of mixture of 0.1 N ammonium acetate buffer of pH 4.5 and Acetonitrile in the ratio of 45:55. The chromatography run time was maintained up to 10.0 min with flow rate at 0.8mL / min with injection volume 20µL and the eluent was monitored at 254 nm.

2.2 Reference Standards, Reagents

Working standards, Metformin, Glibenclamide, Pioglitazone and Glimepiride was obtained from Ranbaxy laboratories, New Delhi, India. Acetonitrile and water employed for the preparation of mobile phase were of HPLC grade was obtained from Merck limited, Mumbai. The pharmaceutical dosage form containing 500 mg MET, 15 mg PIOG and 5 mg GLIB, Triglycomet 520 mg, 20 tablets (USV Pharmaceuticals Ltd.) purchased from a local drug store.

2.3 Preparations of standard solution

500 mg of metformin hydrochloride, 15 mg of pioglitazone hydrochloride and 5 mg of glibenclamide were weighed and transferred into a 100 ml volumetric flask and 50 ml of diluents was added. This solution was sonicated to dissolve and final volume was made with diluent. 10 ml of the above solution was transferred into the 100 ml volumetric flask and diluted to final volume with diluent.

2.4 Sample preparation

20 tablets were weighed and finely powdered. Sample quantitatively equivalent to 500 mg Metformin, 15 mg of Pioglitazone and 5 mg of glibenclamide was transferred in to 100 mL volumetric flask and 50 ml of diluent was added, sonicated to dissolve the sample for 10 minutes and dilute to volume with diluent. Further the solution was filtered through 0.45 µ membrane filter and 10 ml of this solution was diluted to 100 ml with diluent.

2.5 Assay procedure

In case of marketed formulations, twenty tablets were taken and finely powdered and an accurate amount of powder was transferred into a 100ml volumetric flask. The stock solution was further diluted with mobile phase. The column was equilibrated atleast 30min, with the mobile phase flowing through the system with a flow rate of 0.8 ml/min and detector was set at a wavelength of 254nm. The retention times of metformin hydrochloride, pioglitazone hydrochloride, gilbenclamide and internal standard glimepiride in bulk drug were found to be 1.1, 4.5 5.9 and 6.5 mins (Fig. 4) and the retention times of metformin hydrochloride, pioglitazone hydrochloride, gilbenclamide and internal standard glimepiride were found to be 1.1, 4.6, 5.9 and 6.5 mins (Fig. 5). Blank chromatogram is shown (Fig. 6). The % purity of metformin hydrochloride, pioglitazone hydrochloride and glibenclamide in tablet dosage form were compiled and reported in Table1.

Table: 1 Determination of MET, PIO and GLI in Tablet dosage form

Drug	Label claim (mg)	Amount found (mg)	Drug Content (%)
Metformin hydrochloride	500.0	498.33	99.67
Glibenclamide	5.02	5.02	100.48
Pioglitazone hydrochloride	15.15	15.15	101.00

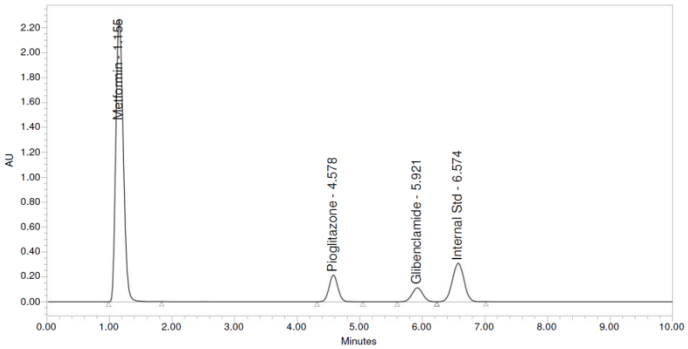

Fig. 4 Standard chromatogram of metformin hydrochloride, pioglitazone, gilbenclamide and internal standard glimepiride

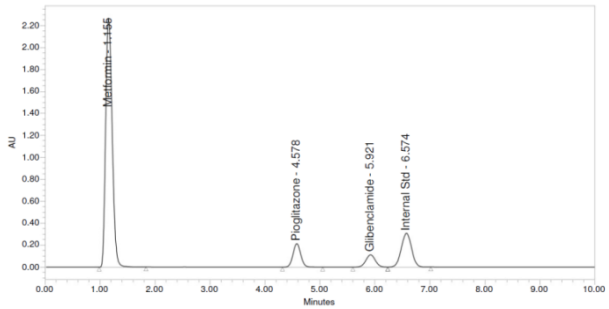

Fig. 5 Sample chromatogram of metformin hydrochloride, pioglitazone, gilbenclamide and internal standard glimepiride

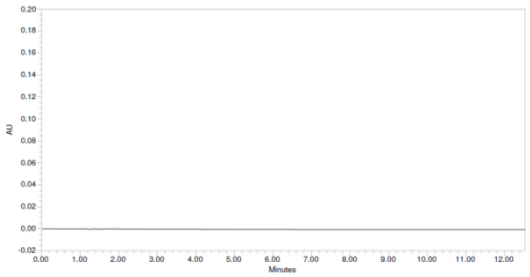

Fig. 6 Chromatogram of blank

III. Validation

After the method conditions were established as described above, method was validated as per ICH guidelines. [12-14] The accuracy, precision, Linearity, limit of detection (LOD) and quantification (LOQ) were determined.

3.1 Linearity

The linearity of the method was established by preparing the series of dilutions from the standard stock mixture to get the concentrations of metformin hydrochloride (62.5-375.00 µg/ml) pioglitazone hydrochloride (3.75-*22.5* µg/ml) and (1.25-7.50 µg/ml) for glibenclamide and the above solutions were injected into the HPLC system. The standard Calibration curve for metformin hydrochloride (Fig. 7), pioglitazone hydrochloride (Fig. 8) and glibenclamide (Fig. 9) was constructed by plotting their response ratios (ratios of the peak area of the analytes) against their respective concentrations. Linear regression was applied and Slope (a), intercept (b), correlation coefficient (r) was determined. The values are summarized in Table 2.

Table 2: Linearity

Sl. No.	Metformin conc	Area	Pioglitazone conc	Area	Glibenclamide conc	Area
1.	62.50	939193	3.75	19681	1.25	5698
2	125.00	1806576	7.50	39811	2.50	11564
3	187.50	2661965	11.25	58036	3.75	17304
4	250.00	3469258	15.0	76016	5.00	23189
5	312.50	4291492	18.75	94525	6.25	29415
6	375.00	5137550	22.5	110557	7.50	34928

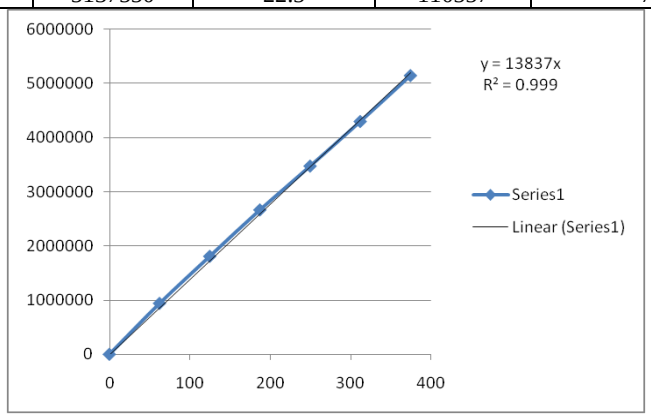

Fig. 7 Standard Calibration Graph of Metformin Hydrochloride

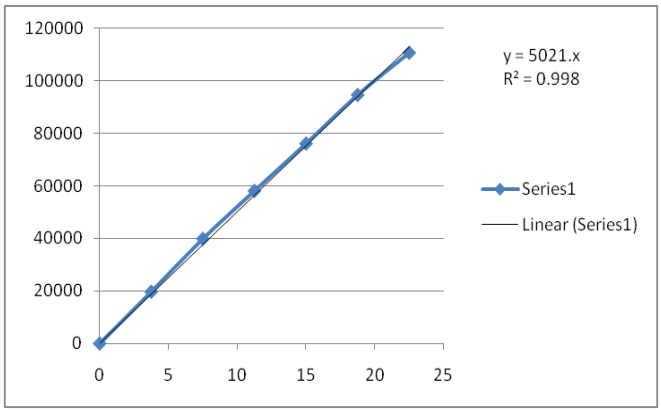

Fig. 8 Standard Calibration Graph of Pioglitazone Hydrochloride

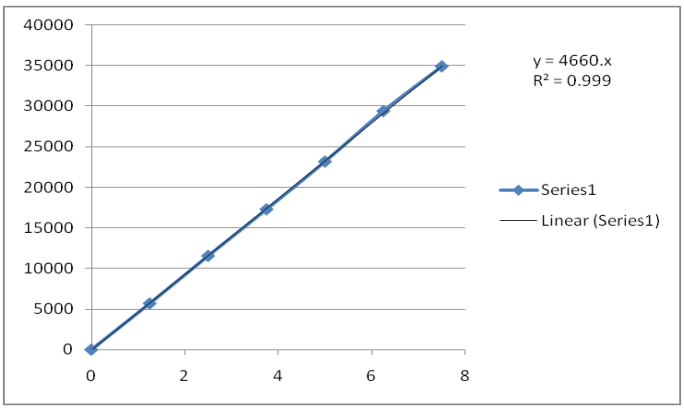

Fig. 9 Standard Calibration Graph of Glibenclamide

3.2 Accuracy

Accuracy was determined in terms of percentage recovery. Sample solution spiked with the analytes at three different concentration levels 62.5-375.0 µg/ml of metformin hydrochloride,3.75-22.5 µg/ml of pioglitazone hydrochloride and1.25-7.50 µg/ml of glibenclamide. Another set of standard mixtures at the same

concentration levels was also prepared with the diluents. Sample and standard solutions are injected into the HPLC system in triplicate. Percentage recoveries of metformin hydrochloride, pioglitazone hydrochloride and glibenclamide were calculated. The values are summarized in Table 3.

Table 3: Recovery studies

Drug	Conc	Peak area (avg)	Amount of drug added	Amount of drug found	% recovery
Metformin hydrochloride	80%	2766617	200.01	200.97	100.48
	100%	3428658	250.00	249.06	99.62
	120%	4136170	300.05	300.46	100.14
Pioglitazone hydrochloride	80%	42116	12.00	11.99	99.91
	100%	52908	15.00	15.06	100.4
	120%	63434	18.02	18.06	100.21
Glibenclamide	80%	18488	4.00	3.99	99.72
	100%	23169	5.00	5.00	99.97
	120%	27977	6.02	6.04	100.26

3.3 Precision

Method precision was determined both in terms of repeatability (injection and analysis) and intermediate precision (intra-day and inter-days reproducibility). In order to determine injection repeatability, samples spiked with metformin hydrochloride, pioglitazone hydrochloride and glibenclamide were injected 6 times into HPLC system and repeatability of the retention time and peak area were determined and expressed as mean and %RSD calculated from the data obtained. The values are summarized in Table 4, 5, 6 and 7.

Table: 4 System Precision

Name	Metformin		Pioglitazone		Glibenclamide	
	RT	Area	RT	Area	RT	Area
System Precision-1	1.153	3390269	4.598	50264	5.999	22594
System Precision-2	1.152	3391873	4.588	51645	5.997	22789
System Precision-3	1.151	3402394	4.590	50611	5.996	22738
System Precision-4	1.152	3396645	4.591	51444	5.997	22765
System Precision-5	1.154	3398545	4.587	50244	5.995	22884
System Precision-6	1.152	3395773	4.586	51145	5.997	22952
Avg	1.152	3395917	4.590	50892	5.997	22787
Std Dev	0.001	4419.56	0.004	604.81	0.001	123.91
RSD	0.090	0.130	0.094	1.188	0.022	0.544

Table: 5 Method Precision

Name	Metformin		Pioglitazone		Glibenclamide	
	RT	Area	RT	Area	RT	Area
Method Precision-1	1.152	3395773	4.589	51145	5.997	22952
Method Precision-2	1.151	3395865	4.585	51246	5.998	22965
Method Precision-3	1.153	3396856	4.584	51151	5.998	22972
Method Precision-4	1.152	3396545	4.592	51344	5.997	22765
Method Precision-5	1.150	3396745	4.582	51145	5.995	22856
Method Precision-6	1.150	3397535	4.595	51421	5.996	22768
Avg	1.151	3396553	4.588	51242	5.997	22880
Std Dev	0.001	659.52	0.005	117.95	0.001	97.18
RSD	0.105	0.019	0.110	0.230	0.019	0.425

Table 6: Intraday Precision

Name	Metformin		Pioglitazone		Glibenclamide	
	RT	Area	RT	Area	RT	Area
Injection -1	1.153	3390269	4.598	50264	5.999	22594
Injection –2	1.152	3391873	4.588	51645	5.997	22789
Injection –3	1.151	3402394	4.590	50611	5.996	22738
Injection –4	1.152	3396645	4.591	51444	5.997	22765
Injection –5	1.154	3398545	4.587	50244	5.995	22884
Injection –6	1.152	3395773	4.586	51145	5.997	22952
Avg	1.152	3395917	4.590	50892	5.997	22787
Std Dev	0.001	4419.56	0.004	604.81	0.001	123.91
RSD	0.090	0.130	0.094	1.188	0.022	0.544

Table 7: Interday Precision

Name	Metformin		Pioglitazone		Glibenclamide	
	RT	Area	RT	Area	RT	Area
Injection-1	1.155	3380243	4.592	51455	5.996	22695
Injection -2	1.155	3391475	4.598	51262	5.992	22656
Injection -3	1.154	3372596	4.592	51173	5.998	22842
Injection -4	1.153	3396747	4.596	51264	5.985	22575
Injection -5	1.155	3388646	4.585	51358	5.991	22476
Injection -6	1.153	3395877	4.593	51472	5.988	22865
Avg	1.154	3387597	4.593	51331	5.992	22685
Std Dev	0.001	9460.78	0.004	118.49	0.005	150.84
RSD	0.085	0.279	0.097	0.231	0.081	0.665

3.4 Robustness

As defined by ICH, the robustness of an analytical procedure describes to its capability toRemain unaffected by small and deliberate variations in method parameters. Robustness wasPerformed by small variation in the chromatographic conditions and found to be unaffected by small variations like flow rate (±10%), column oven temperature (±5°c) and wave length (±5 units). The values are summarized in Table 8.

Table 8: Robustness

Drug	Flow rate	Column oven temperature	Wavelength
Metformin hydrochloride	0.7 ml 0.9 ml	25°c 35°c	249nm 259nm
Pioglitazone hydrochloride	0.7 ml 0.9 ml	25°c 35°c	249nm 259nm
Glibenclamide	0.7 ml 0.9 ml	25°c 35°c	249nm 259nm

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3.5 Ruggedness

The method is rugged by different analyst; different time intervals and the method did not significantly affect the recoveries, peak area and retention time of all the above drugs indicating that the proposed method is rugged. The values are summarized in Table 9.

Table 9: Ruggedness

Name	Metformin		Pioglitazone		Glibenclamide	
	RT	Area	RT	Area	RT	Area
Injection -1	1.153	3390269	4.598	50264	5.999	22594
Injection –2	1.152	3391873	4.588	51645	5.997	22789
Injection –3	1.151	3402394	4.590	50611	5.996	22738
Injection –4	1.152	3396645	4.591	51444	5.997	22765

Injection –5	1.154	3398545	4.587	50244	5.995	22884
Injection –6	1.152	3395773	4.586	51145	5.997	22952
Avg	1.152	3395917	4.590	50892	5.997	22787
Std Dev	0.001	4419.56	0.004	604.81	0.001	123.91
RSD	0.090	0.130	0.094	1.188	0.022	0.544

Table 10: Validation Parameters of the Method

Method Parameters	Metformin	Pioglitazone	Glibenclamide
Linearity range (µg/ml)	62.5-375.00	3.75-22.5	1.25-7.50
Correlation coefficient	0.999	0.998	0.999
LOD (ng/ml)	2.98	3.008	2.98
LOQ (ng/ml)	9.94	9.886	9.996
Retention time	1.15	4.578	5.921
Theoretical plates	2241	5041	5577
Tailing factor	1.15	1.05	1.01
Precision(%RSD)	0.130	1.188	0.544
Intra-day (n=3)	0.09	0.094	0.022
Inter-day (n=3)	0.085	0.097	0.081
% Recovery (n=6)	100.08	100.14	99.98

IV. Forced Degradation Studies (Stress Testing)

Forced degradation studies were carried out for all the three drugs. The bulk drugs were subjected to alkaline studies by adding 1.0 ml of 0.1M NaOH for 4hrs, 8hrs and 12hrs neutralized with 1.0 ml of 0.1M HCl acid. Similarly, the acidic studies were performed by adding 1.0 ml of 0.1 M HCl for 4hrs, 8hrs and 12hrs and neutralized with 1ml of 0.1M NaOH. Oxidation studies were performed on bulk drug by adding 1.0 ml of 3% H2O2,thermalstudies were performed by keeping the drug at 100˚Cand UV studies were performed with UV-Lamp for 4hrs, 8hrs and 12 hrs respectively (Figure10- 14). All samples were taken in different 10 ml volumetric flask and dissolved in mobile phase. Final assay drug concentration was made up with mobile phase and injected in the chromatographic system. For all the stability study, the formation of degradable product was confirmed by comparing to chromatogram of the solution kept under normal conditions. All stressed samples were analyzed by developed HPLC method. The degradation data for metformin hydrochloride, pioglitazone hydrochloride, glibenclamide was shown in Table 11, 12, 13 respectively.

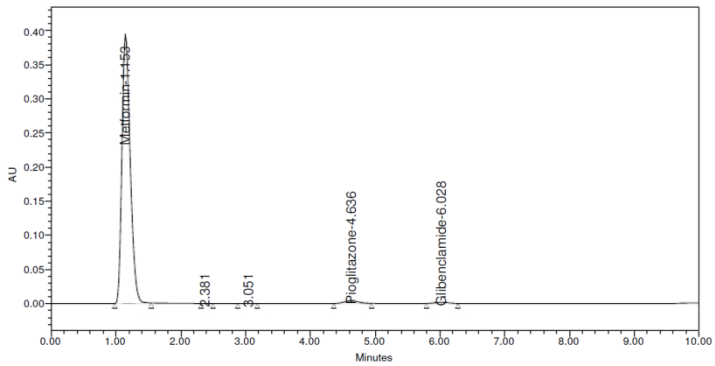

Fig. 10 Typical Chromatogram of Acid Hydrolysis

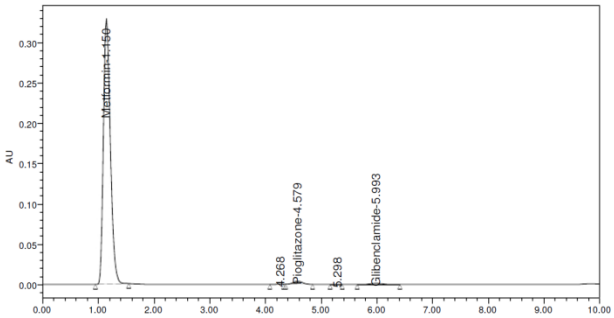

Fig. 11 Typical Chromatogram of Alkaline hydrolysis

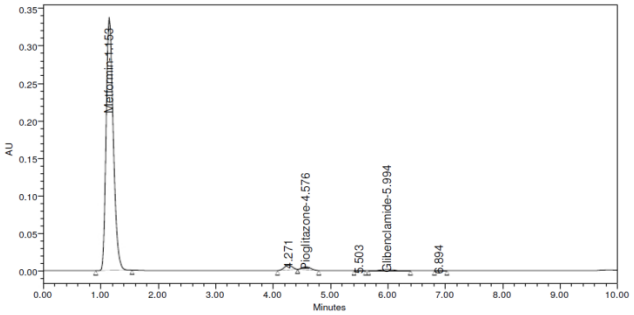

Fig. 12 Typical Chromatogram of Thermal Degradation

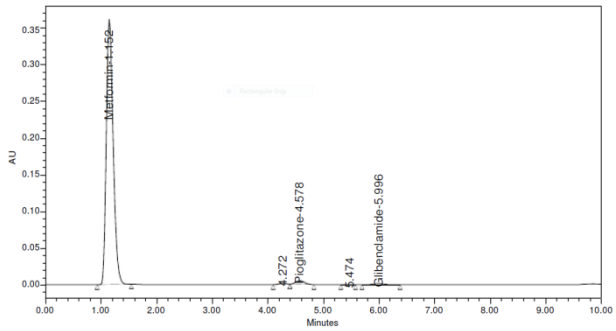

Fig. 13 Typical Chromatogram of UV Degradation

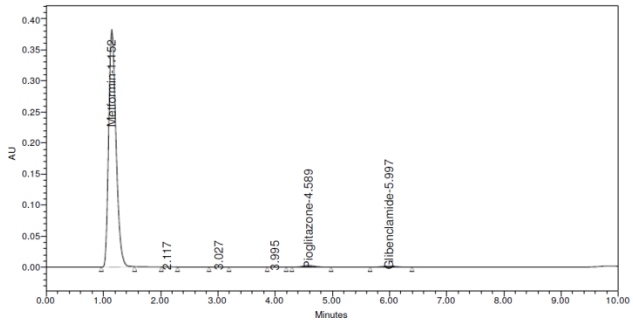

Fig. 14 Typical Chromatogram of Oxidation

Table 11: Degradation data for Metformin Hydrochloride

Stress condition	Degradation time	Degradation (%)
Acidic (0.1Hcl)	4hrs	97.39
	8hrs	95.71
	12hrs	93.47
Alkaline (0.1 NaOH)	4hrs	94.56
	8hrs	89.80
	12hrs	86.56
Oxidation (H_2O_2)	4hrs	92.17
	8hrs	86.63
	12hrs	82.7
UV	4hrs	96.99
	8hrs	94.48
	12hrs	91.52
Thermal	4hrs	92.36
	8hrs	88.73
	12hrs	85.87

Table 12: Degradation data for Pioglitazone Hydrochloride

Stress condition	Degradation time	Degradation (%)
Acidic (0.1Hcl)	4hrs	99.01
	8hrs	98.96
	12hrs	98.04
Alkaline (0.1 NaOH)	4hrs	95.12
	8hrs	90.36
	12hrs	87.59
Oxidation (H_2O_2)	4hrs	93.05
	8hrs	87.63
	12hrs	82.91
Thermal	4hrs	96.03
	8hrs	91.48
	12hrs	86.99
UV	4hrs	99.41
	8hrs	98.72
	12hrs	97.38

Table 13: Degradation data for Glibenclamide

Stress condition	Degradation time	Degradation (%)
Acidic (0.1Hcl)	4hrs	97.13
	8hrs	95.89
	12hrs	93.51
Alkaline (0.1 NaOH)	4hrs	96.73
	8hrs	94.27
	12hrs	91.42
Oxidation (H_2O_2)	4hrs	93.98
	8hrs	87.13
	12hrs	83.43
Thermal	4hrs	92.37
	8hrs	89.28
	12hrs	86.86
UV	4hrs	97.63
	8hrs	95.17
	12hrs	92.36

V. Conclusion

A novel, simple, rapid and cost effective RP-HPLC method was successfully developed for simultaneous determination of metformin hydrochloride, pioglitazone hydrochloride and glibenclamide. The proposed method was optimized and validated for the various experimental parameters. Influence of pH of the mobile phase, column oven temperature and various particulate columns on the analysis of metformin hydrochloride, pioglitazone hydrochloride and glibenclamide was evaluated. All the analytes were well resolved and separated in less than 10 min. The developed method is a stability indicating method and can be conveniently used by quality control outfits to determine the contents of metformin hydrochloride, pioglitazone hydrochloride and glibenclamide simultaneously in routine and stability samples. This method could be used for the analysis of the drugs in pharmaceutical preparations and routine laboratory analysis with slight modification in the extraction procedure. Overall, the proposed method provides high throughput for simultaneous determination of metformin hydrochloride, pioglitazone hydrochloride and glibenclamide with excellent accuracy, precision, selectivity and reproducibility.

Aknowledgement

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