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Development and Validation of a precise single stability indicating HPLC method for determinations of Metformin hydrochloride and Fenofibrate, in Pure form and in Pharmaceutical tablets

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Abstract: A selective, precise, isocratic and accurate stability indicating reverse phase high performance liquid chromatography method have been developed for the simultaneous determination of Metformin hydrochloride and Fenofibrate present in multicomponent dosage forms. The HPLC method was carried out on Inertsil octadecylsilane C_{18} (250 mm x 4.6 mm i.d., 5 µm particle size) column. A mobile phase composed of acetonitrile - water (adjusted to pH 3 using orthophosphoric acid) in proportion of 70:30 v/v, at flow rate of 1 ml/min was used for the separation. Detection was carried out at 250nm. Method was validated statistically and recovery studies were carried out. The proposed method have been applied successfully to the analysis of cited drugs either in pure form or in pharmaceutical formulations with good accuracy and precision. The method herein described can be employed for quality control and routine analysis of drugs in pharmaceutical formulations.
Key words: Metformin, Fenofibrate, Forced degradation, HPLC.

1. Introduction

Metformin Hydrochloride (MET) is chemically 1, 1-dimethyl biguanide monohydrochloride. Fenofibrate (FNB) is Isopropyl 2-[4-(4 chlorobenzoyl) phenoxy]-2-methylpropanoate. Metformin is oral hypoglycemic agent while Fenofibrate is indicated for the treatment of hypercholesterolemia and mixed dyslipidemia. A combination of 500 mg of Metformin and 160 mg of Fenofibrate is available commercially as tablets. This combination is used in treatment of non-insulin dependent diabetes mellitus (NIDDM)[1][2]. MET is official in IP, BP, USP, while FNB is official in BP, USP[3][4][5].

Several spectrophotometric methods have been used for the qualitative and quantitative determination of Metformin. These are Near infra-red reflectance spectroscopy[6], simultaneous spectrophotometric method for synthetic mixture of MET and FNB[7][8], UV-Visible spectrophotometry[9], Multivariate technique[10]. Metformin was also determined in human plasma, urine, breast milk and pharmaceutical formulatios using HPLC, LC-MS-MS and also by potentiometry, spectrofluorimetry[11-19]. The complexity of the multicomponent dosage forms includes multiple entities and excipients poses considerable challenge the analytical chemist during the development of assay procedure. Estimation of the individual drugs in these multicomponent dosage forms becomes difficult due to cumbersome extraction or isolation procedures.

In this study, quantitative determination of Metformin Hydrochloride and Fenofibrate in tablets was

performed using high performance liquid chromatographic method. There was no stability indicating HPLC study about the determination of Metformin Hydrochloride and Fenofibrate in literature. For this reason, it was considered that stability indicating HPLC method would be applicable in routine analysis since it did not require any pretreatment procedure.

2.Experimental work

2.1. Apparatus

Agilent HPLC 1100 series chromatograph equipped with binary pump, UV and 2695 Photodiode Array Detector with data processing capacity was used. An Inertsil ODS column (250 mm x 4.6 mm i.d., 5 µm particle size) was used. The pH measurement was performed by using Labindia controlled pH analyzer equipped with pH electrode. Mobile phase filtration was performed by vaccum pump using 0.45 µm filter paper. As a degasser, Trans-o-sonic ultrasonicator was used. Typical operating conditions include flow rate, 1 ml/min; injection volume, 20 µl; wavelength, 250nm; column compartment temperature, 30ºc; and operating condition, room temperature.

2.2. Reagents and solutions

All solutions were prepared with milli-Q-water. Acetonitrile was HPLC grade, Merck. Orthophosphoric acid was AR grade, Merck. Hydrochloric acid and sodium hydroxide pellets were GR grade, Merck. Hydrogen peroxide solution (30%w/v), Qualigens.

2.3. Optimization of mobile phase

In order to separate Metformin Hydrochloride and Fenofibrate, optimization started with acetonitrile - water (adjusted to pH 3 using orthophosphoric acid) in proportion of 80:20 v/v on Wakosil II RS C_{18} Column, on which fenofibrate was retained with poor peak shape and metformin peak was tailed, later on by changing ratio of acetonitrile - water (adjusted to pH 3 using orthophosphoric acid) in proportion of 70:30 v/v on Hypersil BDS C_8 Column, Metformin was tailed with asymmetry of 3.13, while on Zorbax CN Column, metformin peak was not retained due to it's highly polar nature as compared to fenofibrate. So finally on Inertsil ODS C_{18} column, both peaks were separated with asymmetry of 1.3 for Metformin hydrochloride and 0.88 for Fenofibrate. For details refer Table 1.

Table 1:Optimization of the chromatographic conditions

Sr. No.	Mobile phase	Ratio (v/v)	Column Description	Retention time		Remark	
				Metformin	Fenofibrate	Metformin	Fenofibrate
1	Acetonitrile : Water acidified with O.P.A. to pH 3	80:20	Wakosil II RS C_{18}, 250x4.6mm, 5µ	1.26	16.92	Peak Tailing	Poor peak shape
2	Acetonitrile : Water acidified with O.P.A. to pH 3	70:30	Wakosil II RS C_{18}, 250x4.6mm, 5µ	1.66	20.34	Asymmetry factor was 1.46 (Tailing)	Asymmetry factor was 0.87 (Fronting)
3	Acetonitrile : Water acidified with O.P.A. to pH 3	70:30	Hypersil BDS C_8, 250x4.6mm, 5µ	19.15	9.06	Asymmetry factor was 3.13 (Tailing)	Asymmetry factor was 1.2
4	Acetonitrile : Water acidified with O.P.A. to pH 3	70:30	Zorbax CN, 250x4.6mm, 5µ	-	3.24	Not retained	Asymmetry factor was 2.68 (Tailing)
5	**Acetonitrile : Water acidified with O.P.A. to pH 3**	**70:30**	**Inertsil ODS C_{18}, 250x4.6mm, 5µ**	**1.62**	**19.68**	**Asymmetry factor was 1.3**	**Asymmetry factor was 0.88**

2.4. Preparation of mixed standard solutions

In order to prepare stock solution, 125 mg Metformin hydrochloride and 40 mg Fenofibrate was accurately weighed, dissolved in mobile phase using sonication and diluted to 100 ml with the mobile phase. Standard solution was prepared by further diluting 2 ml stock solution with 100 ml mobile phase.

2.5. Application of proposed method to tablets

Average mass of 10 tablets was determined. Tablets were powdered and accurately weighed. A definite amount of powdered tablet equivalent to 250 mg Metformin hydrochloride was transferred to 200 ml volumetric flask, 80 ml mobile phase was added, sonicated for 15 minutes with intermittent shaking and then adjusted to the mark with mobile phase. Filtered through 0.45µ filter, further 2 ml of solution diluted to 100 ml with mobile phase. Tablet solution, 20 µl, was injected, and the detection was at 250 nm. Percentage assay for MET was found to be 99.76% and for FNB it was 101.58%.

2.6. Method validation

The proposed method was validated as per ICH guidelines. The drug solutions were prepared as per the earlier adopted procedure given in the experiment.

2.6.1. System precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Mixed standard solutions of Metformin hydrochloride (25µg/ml) and Fenofibrate (8µg/ml) were prepared as per test method and injected for 6 times. Results are shown in Table 2.

Table 2: System precision study

Injection	Area of MET	Area of FNB
1	1361.100	439.074
2	1363.827	440.861
3	1367.546	438.058
4	1362.300	439.478
5	1360.855	439.185
Mean±S.D.	**1363.126±2.737**	**439.331±1.009**
% RSD	**0.2**	**0.23**

Table 3: Method precision study

Sample no.	Area		% Assay	
	MET	FNB	MET	FNB
1	1321.28	435.32	98.98	101.02
2	1317.38	436.055	98.99	101.51
3	1313.71	434.839	98.76	101.27
4	1312.75	435.362	98.67	101.38
5	1309.85	435.983	98.42	101.48
6	1335.11	442.439	100.37	103.04
Mean±S.D.			**99.03 ±0.689**	**101.62 ±0.719**
% RSD			**0.7**	**0.71**

2.6.2. Method precision

Six samples were Prepared and analyzed as per the test method and calculated the % RSD for Assay of six preparations. Results are shown in Table 3.

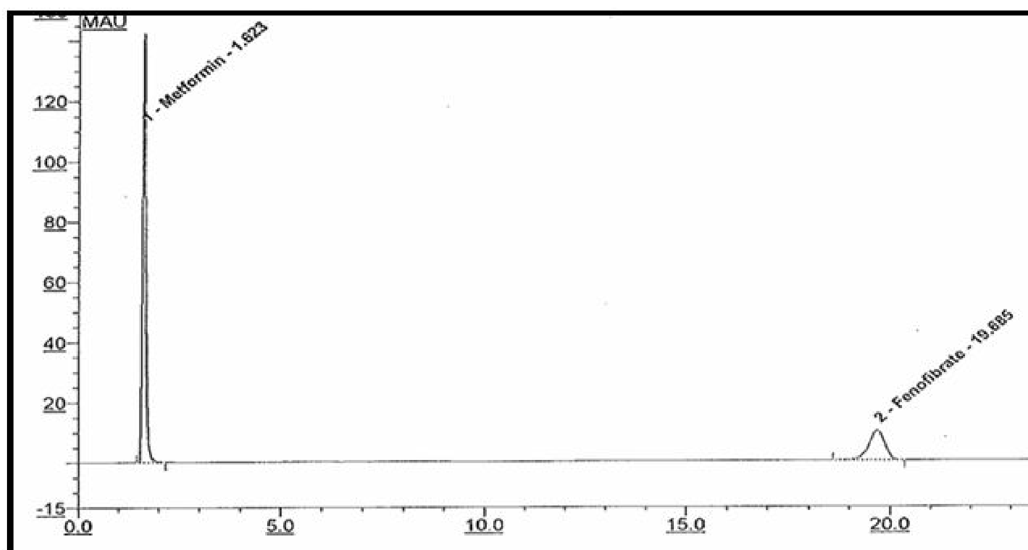

Fig 1: Standard chromatogram (Retention time versus peak height)

2.6.3. Linearity study

Linearity was performed in the range of 70 to 130 % of standard concentration. From stock solution aliquots of 1.4, 1.6, 1.8, 2.0, 2.2, 2.4, and 2.6 ml were taken in 100ml volumetric flasks and diluted upto the mark with mobile phase such that the final concentration of Metformin hydrochloride in the range of 17.5 to 32.5 µg/ml and Fenofibrate in the range of 5.6 to 10.4 µg/ml. Volume of 20 µl of each sample was injected in duplicate for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration. The observations and calibration curve is shown in Table 4, Fig.2a and Fig. 2b.

2.6.4. Analytical solution stability

Standard and sample were prepared and injected into HPLC at initial and different time intervals up to 25 hrs and cumulative % RSD for peak areas was determined. Results are shown in Table 5.

2.6.5. Intermediate precision (Ruggedness)

Six samples were prepared by different analyst by using different column, different system on different day. The system suitability criteria was evaluated. % RSD of % assay for above 6 preparations was calculated and the overall % RSD for % assay of above experiment results and method precision results was also calculated. Results are shown in Table 6.

2.6.6. Accuracy as recovery

It was done by recovery study. Sample solutions were prepared by spiking at about 70 %, 100 % and 130 % of specification limit to Placebo and analyzed by the proposed HPLC method. Results are shown in Table 7a and 7b.

Table 4: Linearity study

Spike level %	Concentration (mcg/ml)		Area	
	MET	FNB	MET	FNB
70	17.49	5.59	893.09	289.940
80	19.99	6.39	1030.26	330.865
90	22.48	7.18	1149.41	374.595
100	24.98	7.98	1257.24	406.855
110	27.48	8.78	1402.94	453.135
120	29.98	9.58	1570.57	495.415
130	32.48	10.38	1670.24	539.830
Slope			**52**	**51.8**
Intercept			**-27.2**	**-0.4**
Correlation coefficient			**0.9984**	**0.9994**

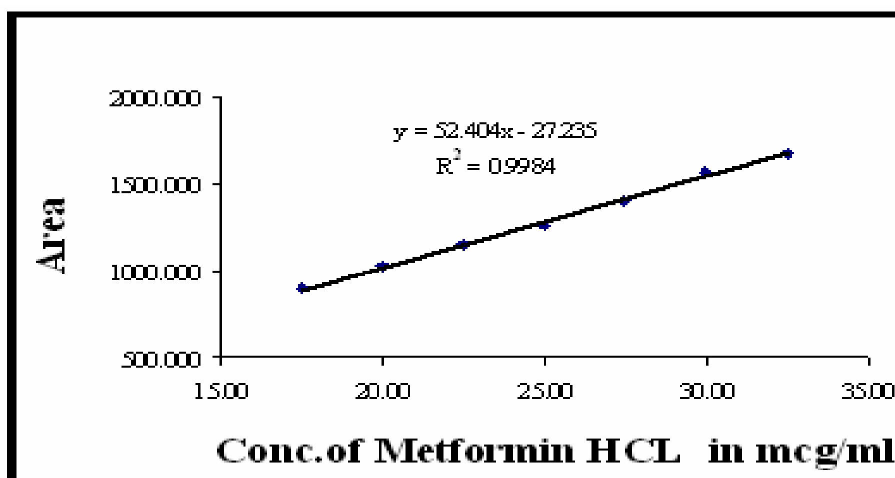

Fig 2a : HPLC Calibration curve for Metformin hydrochloride

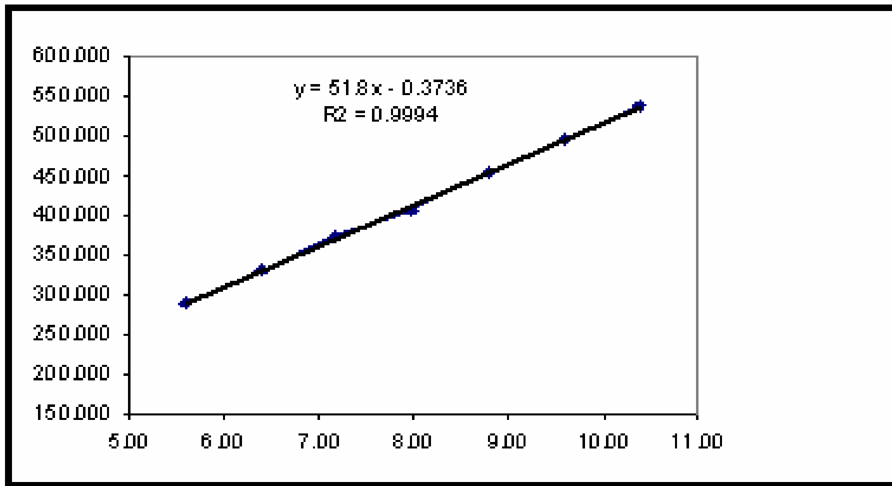

Fig 2b: HPLC Calibration curve for Fenofibrate

Table 5: Analytical solution stability study

Drug		Cumulative mean ± Cumulative SD	Cumulative % RSD
Standard	MET	1375.652±20.579	1.5
	FBN	444.768±8.052	1.81
Sample	MET	1325.121±5.531	0.42
	FBN	438.35±4.202	0.96

Table 6: Ruggedness study

Parameter	% Assay	
	MET	FNB
Ruggedness	99.00	101.48
Method Precision	99.03	101.62
Overall Mean±SD	**99.02±0.480**	**101.57±0.488**
Overall % RSD	**0.48**	**0.48**

Table 7a: Recovery study of Metformin hydrochloride

Spike level %	Actual Amount added in mg	Amount found in mg	% Recovery	Mean±SD	% RSD
70	174.89	172.54	98.66	**99.07±0.355**	**0.36**
70	174.82	173.57	99.28		
70	174.50	173.23	99.27		
100	249.43	249.66	100.09	**99.72±0.344**	**0.34**
100	249.19	248.35	99.66		
100	249.37	247.91	99.41		
130	324.14	320.44	98.86	**98.97±0.127**	**0.13**
130	324.45	321.05	98.95		
130	324.01	321.12	99.11		

Table 7b: Recovery study of Fenofibrate

Spike level %	Actual Amount added in mg	Amount found in mg	% Recovery	Mean±SD	% RSD
70	55.91	55.51	99.28	**99.92±0.782**	**0.78**
70	55.60	56.04	100.79		
70	55.74	55.56	99.68		
100	80.06	80.00	99.93	**100.48±0.867**	**0.86**
100	79.55	80.73	101.48		
100	79.95	79.97	100.03		
130	103.84	102.56	98.77	**98.90±0.114**	**0.11**
130	103.72	102.67	98.99		
130	103.67	102.56	98.93		

2.6.7. Range

Range to be inferred from the data of linearity, recovery and precision experiments.

2.6.8. Specificity and selectivity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is the procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method is quite selective. There was no other interfering peak around the retention time of Metformin Hydrochloride and Fenofibrate; also the baseline did not show any significant noise.

2.6.9. Robustness

The Robustness of the method was evaluated by changing the flow rate by ± 10%, by changing the column oven temperature by ± 5°C, by changing the wavelength by ± 2nm, by changing the organic content of mobile phase by 2% absolute, by changing the pH by ± 0.1, System suitability was done for each condition. The results are shown in Table 8a, 8b, 8c.

2.6.10. Filter paper selection study

Test solutions were prepared in triplicate as per the test method. Filtered test solution with 0.45µm PVDF, 0.45µ Nylon 66 filters and analyzed against the standard. The overall % RSD was calculated for above results and method precision results for the two filters. The results are shown in Table 9.

2.6.11. Forced degradation

A sample was stressed at the following conditions and the peak purity was evaluated for Metformin Hydrochloride and Fenofibrate peak. Degradation by 1 N hydrochloric acid, degradation by 1 N sodium hydroxide, degradation by 3 % w/v solution of hydrogen peroxide, degradation by thermal energy at 105ºc for 12 hours, degradation by exposing UV light for about 7 days cycle. Observations and results are shown in Fig 3a, 3b, 3c, 3d, 3e and Table 10a,10b.

According to USP XXIV (621)[20], system suitability tests are an integral part of chromatographic method. They are used to verify reproducibility of the chromatographic system. To ascertain it's effectiveness , system suitability tests were carried out and it's results are shown in Table 11.

Table 8a: Robustness data for MET (for HPLC method)

S.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
1	98.98	99.75	99.55	99.84	100.3	100.2	100.34	99.20	99.95	100.4	98.60
2	98.99	101.82	101.8	100.77	102.1	102.2	102.26	99.75	99.96	100.6	99.00
3	98.76	100.66	100.6	100.55	100.9	100.8	100.87	99.24	99.51	100.8	99.20
4	98.67	-	-	-	-	-	-	-	-	-	-
5	98.42	-	-	-	-	-	-	-	-	-	-
6	100.37	-	-	-	-	-	-	-	-	-	-
Over all		99.42	99.41	99.48	99.73	99.72	99.74	99.15	99.29	99.57	99.00
Over all SD		1.130	1.137	0.903	1.265	1.281	1.293	0.595	0.681	0.978	0.568
Over all %RSD		1.14	1.14	0.91	1.27	1.28	1.30	0.60	0.69	0.98	0.57

Table 8b: Robustness data for FNB (for HPLC method)

S.	I	II	III	IV	V	VI	VII	VII	IX	X	XI
1	101.0	101.36	100.19	101.23	101.43	100.77	100.96	101.22	100.47	103.3	101.49
2	101.5	102.25	102.73	101.77	102.48	102.79	102.63	101.42	101.92	103.1	101.29
3	101.2	101.11	101.57	101.29	101.70	102.53	101.71	100.66	101.40	102.7	101.59
4	101.3	-	-	-	-	-	-	-	-	-	-
5	101.4	-	-	-	-	-	-	-	-	-	-
6	103.0	-	-	-	-	-	-	-	-	-	-
Over all		101.63	101.60	101.58	101.73	101.78	101.69	101.47	101.52	102.1	101.59
Over all SD		0.628	0.85	0.581	0.624	0.800	0.694	0.647	0.690	0.918	0.565
Over all %RSD		0.62	0.83	0.57	0.61	0.79	0.68	0.64	0.68	0.90	0.56

Table 8c: Robustness study (for HPLC method)

S.No	Experiment (Actual value)
I	Method precision data
II	Flow rate (0.9 ml/min)
III	Flow rate (1.1 ml/min)
IV	Column oven temperature (25°C)
V	Column oven temperature (35°C)
VI	Wavelength (248 nm)
VII	Wavelength (252 nm)
VIII	Mobile phase (-2%)organic
IX	Mobile phase (+2%)organic
X	Mobile phase pH(2.9)
XI	Mobile phase pH(3.1)

Table 9: Filter paper selection study

Assay of MET (%)		Assay of FNB (%)	
0.45µ PVDF membrane filters	100.34	0.45µ Nylon 6,6 membrane filters	101.49
	99.76		100.58
	99.61		100.65
Method precision	98.98	Method precision	98.98
	98.99		98.99
	98.76		98.76
	98.67		98.67
	98.42		98.42
	100.37		100.37
Over all mean±SD	**100.26±1.176**	Over all mean±**SD**	**100.01±0.898**
Over all % RSD	**1.17**	Over all % RSD	**0.90**

Table 10a: Forced degradation for Metformin hydrochloride

Condition	% Assay of MET	% Degradation	Peak Purity
			(Match Factor)
Control sample	99.04	-	-
Acid degradation	96.16	2.91	**999.95471**
Base degradation	88.37	10.77	**999.93564**
Oxidative degradation	91.87	7.24	**999.95027**
Thermal Degradation	105.57	-6.59	**999.95266**
Photolytic Degradation	100.45	-1.42	**999.95317**

Table 10b: Forced degradation for Fenofibrate

Condition	% Assay of FNB	% Degradation	Peak Purity (Match Factor)
Control sample	101.66	-	-
Acid degradation	97.45	4.14	**996.51066**
Base degradation	95	6.55	**998.97963**
Oxidative degradation	93.19	8.33	**995.68541**
Thermal Degradation	95.6	5.96	**996.77612**
Photolytic Degradation	91.29	10.2	**996.36565**

Table 11: HPLC System suitability parameters

System suitability parameters		
Parameters	MET	FNB
Plate count	**1980**	**12434**
% RSD	**0.20**	**0.23**
Asymmetry factor	**1.3**	**0.88**
Resolution	**42.51**	

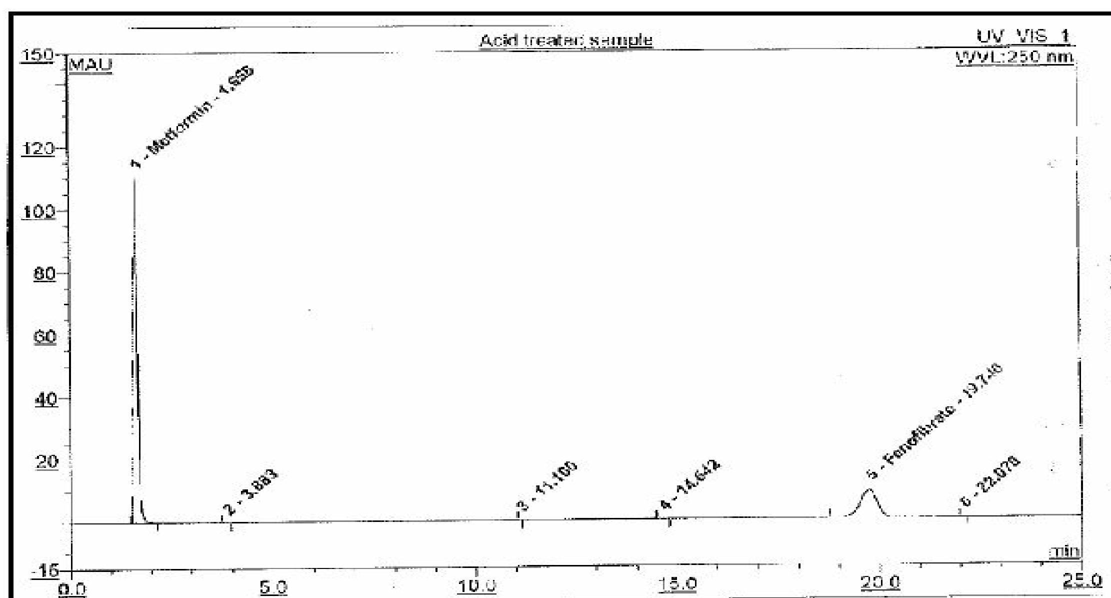

Fig 3a: Acid treated sample (1N HCL for 5 hrs)

Results and discussions

Metformin Hydrochloride and Fenofibrate, indicated for the treatment of non-insulin dependent type II diabetes mellitus. Literature scan revealed no stability indicating HPLC was developed for the determination of Metformin Hydrochloride and Fenofibrate. Fig 1 shows typical chromatograms of Metformin Hydrochloride and Fenofibrate. The retention times of Metformin Hydrochloride and Fenofibrate were 1.6 and 19.6 min, respectively. The calibration curve was linear over the range 17.49-32.48 μg/ml and 5.59-10.38 μg/ml for the determination of Metformin Hydrochloride and Fenofibrate, respectively. The linearity of method was statistically confirmed. The correlation coefficients (r) for calibration curves were not less than 0.99. The relative standard deviation (R.S.D.) values of the slope were not more than 2%. The analytical recovery at three different concentrations of Metformin Hydrochloride and Fenofibrate was determined. Forced degradation study was also carried out. In that, Acid, Base, Peroxide, Heat, UV treatment given to Metformin Hydrochloride and Fenofibrate , refer Fig 3a,3b,3c,3d,3e. Therefore proposed validated method was successfully applied to determine Metformin Hydrochloride and Fenofibrate in tablet dosage form.

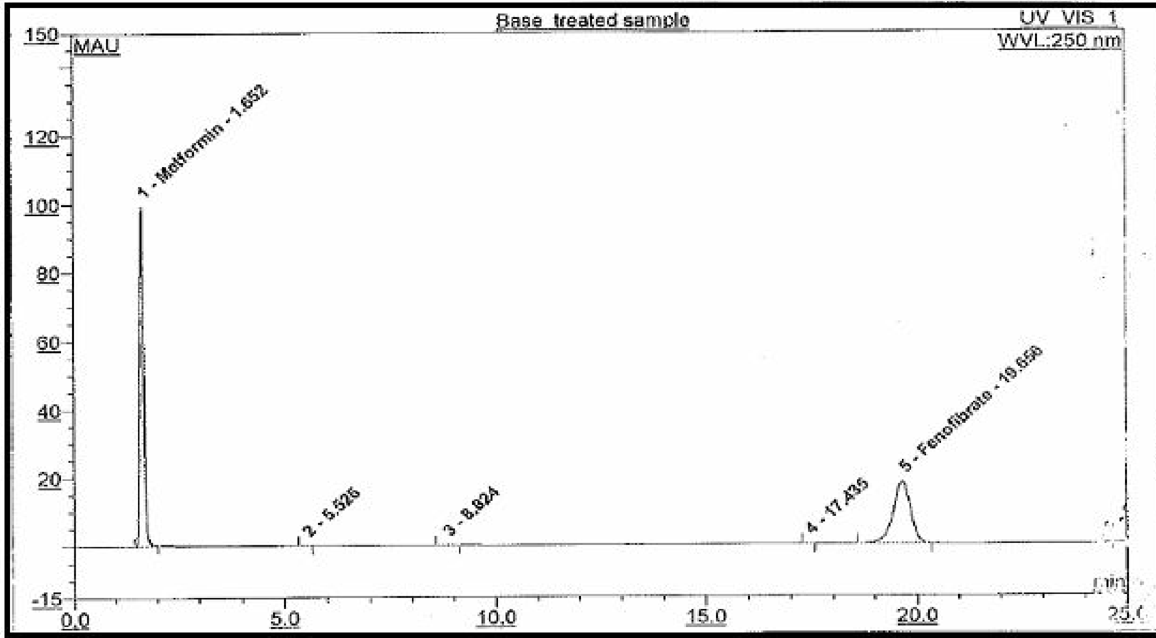

Fig 3b: Base treated sample (1N NaoH for 5 hrs)

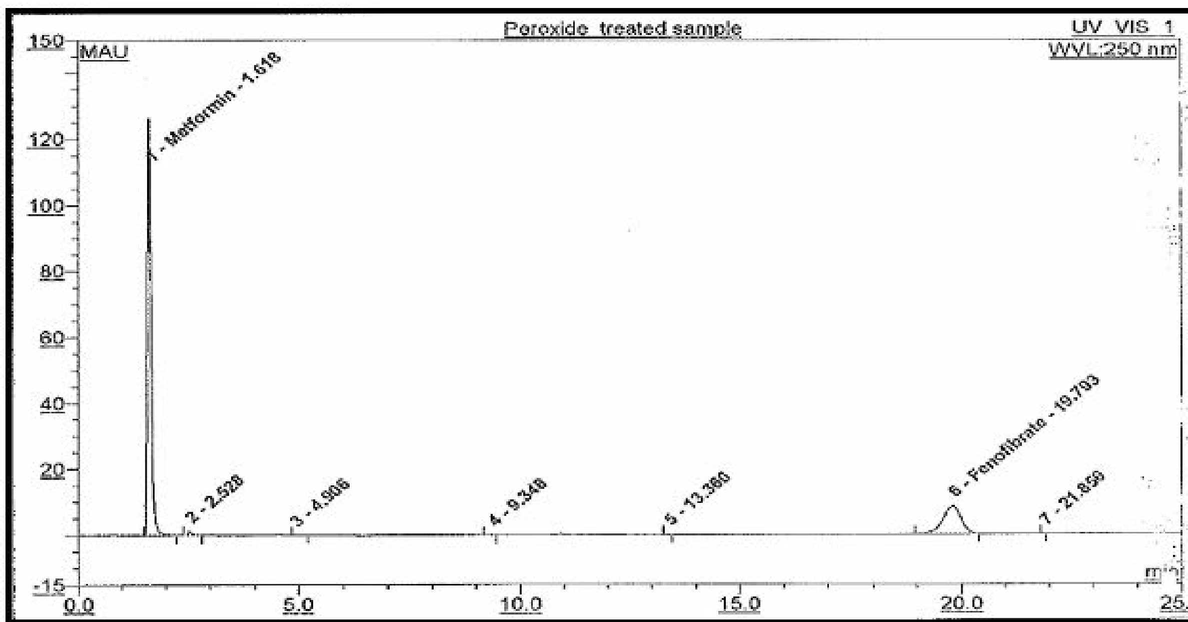

Fig 3c: Peroxide treated sample (3% solution for 5 hrs)

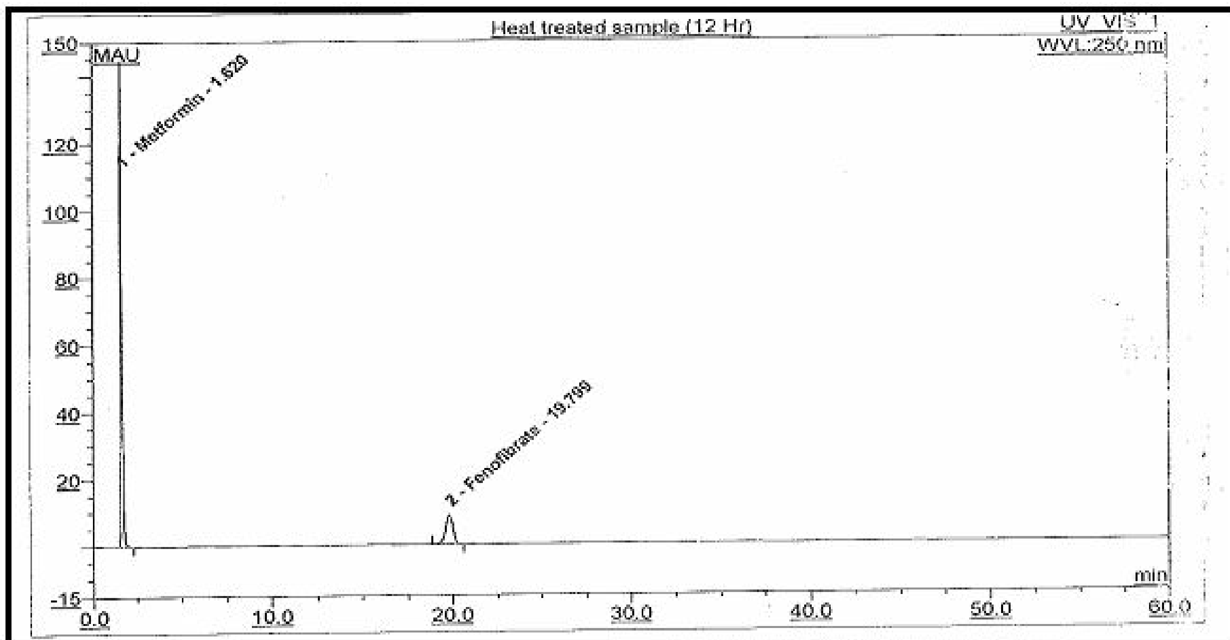

Fig 3d: Heat treated sample (at 105ºc for 12hrs)

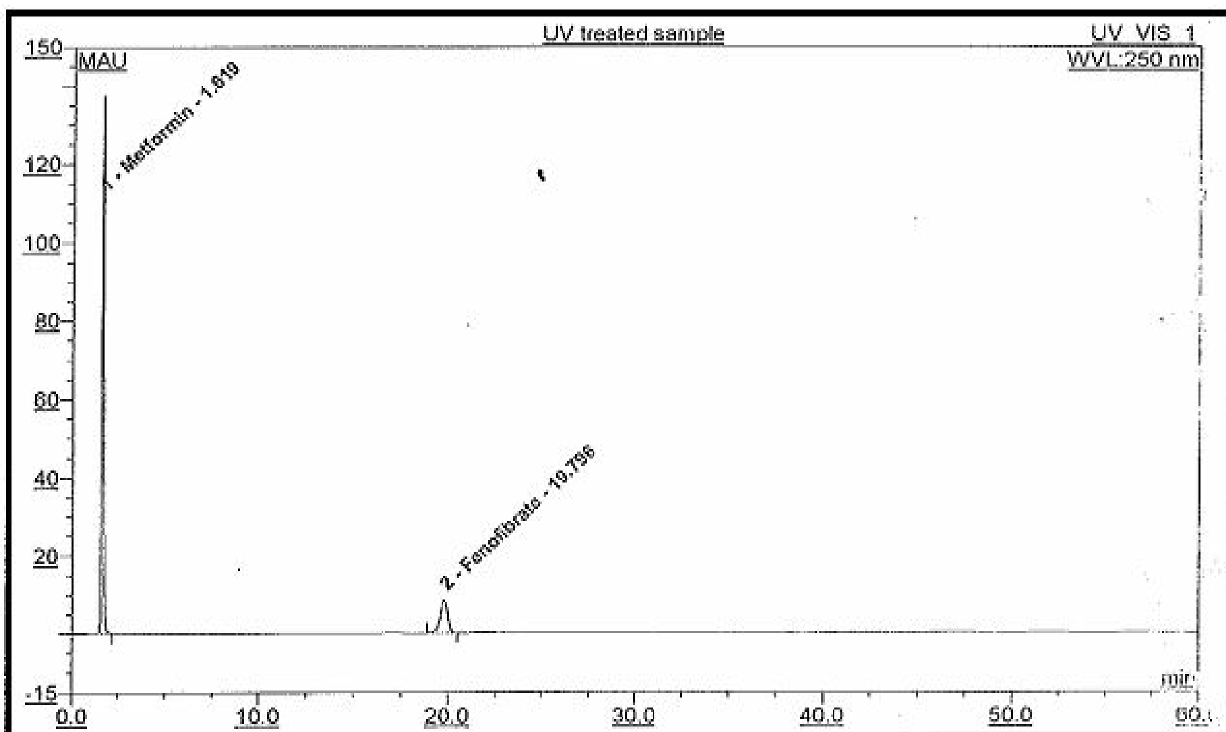

Fig 3e: UV treated sample (for 7 days)

Conclusion

For the determination of Metformin Hydrochloride and Fenofibrate, stability indicating HPLC method was found to be superior due to a much more selective detection and increased sensitivity. High percentage recovery showed that method was free from interference of excipients used in the formulations. The results of the study indicates that the proposed stability indicating HPLC method of analysis can be used in quality control departments with respect to routine analysis for the assay of the tablets containing Metformin Hydrochloride and Fenofibrate.

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