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A VALIDATION ANALYTICAL METHOD DEVELOPMENT FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND FENOFIBRATE IN PURE AND IN TABLET DOSAGE FORM

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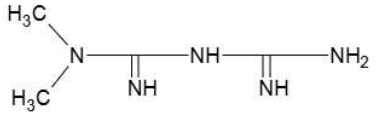
ABSTRACT
Two simple, sensitive, specific, accurate, UV-spectroscopic and RP-HPLC methods are developed for the estimation of Metformin hydrochloride and Fenofibrate in pure and in tablet dosage form. The UV-spectroscopic first method was a determination using the simultaneous equation method at 237nm and 258nm over the concentration range of 3-15µg/ml, 1-5µg/ml for Metformin hydrochloride and Fenofibrate respectively. The UV-spectroscopic second method was a determination using the derivative method at 221nm, 303nm over the concentration range of 9-45µg/ml and 3-15µg/ml for Metformin hydrochloride and Fenofibrate respectively. Calibration curve was linear with the correlation coefficient of 0.9999, 0.9998 and 0.9998, 0.9997 respectively. In the RP- HPLC method, separation of the drug in reverse phase mode using phenmenax Luna C_{18} column (150 mm x4.6 mm i.d. 5 µ). The mobile phase constituted of Methanol: Acetonitrile: 0.01M Ammonium acetate (60:30:10 % v/v/v) and flow rate 1.0 ml/min. Detection was performed at 256nm. The RT value of Metformin hydrochloride and Fenofibrate at 3.34 and 7.37 min. calibration curve was linear with correlation coefficient of 0.9994 and 0.9986 over a concentration range of 3-15 µg/ml and 1-5 µg/ml. the relative standard derivation (R.S.D)was found <2.0 % for both the methods. Both these methods have been successively applied to bulk and in tablet dosage form. The present methods were validated according to ICH guidelines.
Keywords: Metformin hydrochloride, Fenofibrate, UV-spectroscopic, RP-HPLC

INTRODUCTION

Metformin hydrochloride (MET) belongs to the class hypoglycemia. Chemical name N,N-dimethylimidodicarbonimidic diamide. Its main use is to reduce the blood sugar levels. Fenofibrate (FEN) is an HMG- COA reductase inhibitor shows major effect by reduction of LDL levels. Chemical name is propan -2- yl 2- [4-(4-chlorobenzoyl) phenoxy]-2- methyl propanoate.

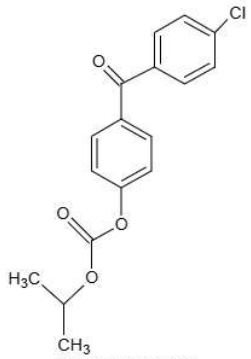
Literature survey revealed that few methods were available for estimation of MET and FEN individually as well as combination with other drugs. So far no method was available for estimation of MET and FEN simultaneously by UV-spectroscopic and RP-HPLC methods in pure and in tablet dosage form. Hence an attempt has been made to develop simple, precise, accurate UV and chromatographic methods for simultaneous estimation of Metformin hydrochloride and Fenofibrate in pure and in tablet dosage form.

Structure



.HCl

METFORMIN HYDROCHLORIDE



FENOFIBRATE

MATERIALS AND METHODS

Reagents

MET and FEN was procured as gift sample from Griffon Labortoires Pvt.Ltd., Mumbai. All chemical and reagents used were of HPLC grade and AR grade. Tablets were purchased from Indian market (sun pharmaceutical industitries). FIBMET^{R} ER containing (500mg of Metformin hydrochloride and 160mg Fenofibrate).

Shimadzu-1700 double beam UV-spectrophotometer with pair of 10 mm matched quartz cells, shimadzu HPLC system (LC-10 ATvp solvent deliver module,SPD-10 Avp UV-Visible detector) using phenomena luna C $_{18}$ column (150mm x 4.6 mm i.d. 5µ),was used for the analysis. The mobile phase constituted of Methanol: Acetonitrile: 0.01 m Ammonium acetate (60:30:10 % v/v/v) and flow rate was 1.0 ml/min. detection was performed 256nm.

UV-SPECTROSCOPIC METHOD

Preparation of standard stock solution of Metformin hydrochloride

Pure raw material of Metformin hydrochloride 25mg were accurately weighed and dissolved in methanol to produce 1000 µg/ml solution. From this 1.5ml of the solution was transferred into the 50ml volumetric flask and made up required volume with methanol to get concentration 30 µg/ml. It is used as a working standard.

Preparation of standard stock solution of Fenofibrate

Pure raw material of Fenofibrate 25mg were accurately weighed and dissolved in Methanol to produce 1000 µg/ml solution. From this 1ml of the solution was transferred into the 50ml volumetric flask and made up required volume with methanol to get concentration 20 µg/ml. It is used as a working standard.

Selection of Wavelengh

For the selection of wavelength for the estimation of Metformin hydrochloride and Fenofibrate a suitable standard solution to contain 10 µg/ml Metformin hydrochloride and Fenofibrate were prepared separately and scanned in the entire range from 200-400nm using Methanol as blank. From the spectra λ max of Metformin hydrochloride was found to be 237nm and for Fenofibrate 258nm was selected. From the IInd order derivative spectra of Metformin hydrochloride 221nm which is the zero crossing point for fenofibrate and from the Ist order derivative spectra of Fenofibrate 303nm which

is the zero crossing point for Metformin HCl was selected for the determination. The spectrum is shown in fig.1 &2.

Preparation of calibration graph

From the working standard stock solution of Metformin hydrochloride (30µg/ml) pipette out 1 to 5 ml into a series of five 10ml volumetric flask and made up to mark with methanol to get concentration range of 3-15 µg/ml and 9-45 µg/ml respectively . From the working standard stock solution of Fenofibrate (20 µg/ml) pipette out 0.5-2.5ml into a series of five 10ml volumetric flask and made up to mark with methanol to get the concentration range of 1-5 µg/ml and 3-15 µg/ml. The absorbances of these both solutions were measured at 237nm 258nm at zero order and 221nm at II^{nd} order, 303nm at I^{st} order and calibration curve was plotted using absorbance VS concentration. The optical characteristics of the method are listed in table.1

Quantification in formulation

Twenty tablets were weighed and average weight of each tablet was found and powered. The content of the drug equivalent to 50mg of Fenofibrate and 156.25mg of Metformin hydrochloride was transferred to a 50ml standard flask and the content of the flask was dissolved in methanol by sonication for 15 minutes and made up to the volume and filtered through whatmann filter paper (N0.41). The solution was diluted to get a concentration of 2µg/ml of Fenofibrate and 6.25µg/ml of Metformin hydrochloride in methanol. Absorbances of the diluted sample solution were measured at 237nm and 258nm for simultaneous estimation equation and 221nm & 303nm for derivative method.

Recovery studies

To the pre-analyzed sample solution ,a definite concentration was added and then its recovery was studied.1ml pre-analyzed formulation was taken in the separate 10ml volumetric flasks with these ,known concentration of pure drug (Metformin hydrochloride and Fenofibrate) at 80%,100% & 120% levels were added. The absorbances of resulting solution were measured at their corresponding wavelength and the percentage recovery was calculated.

RP-HPLC METHOD

Optimized Chromatographic conditions

The following optimized conditions were employed for analysis of Metformin hydrochloride and Fenofibrate by isocratic RP-HPLC method were Stationary phase : C_{18} column (150mm X4.6mm i.d. 5µ), Mobile phase : Acetonitrile: Methanol: 0.01 M Ammonium acetate (30: 60: 10% v/v), Detection wavelength : 256nm, Flow rate : 1.0ml/min, Sample load : 20 µl. The solution of Metformin hydrochloride and Fenofibrate was injected and the respective chromatograph was recorded. The chromatograph is shown in fig-3

Standard solution

Weighed accurately 25mg of Metformin hydrochloride and Fenofibrate transferred into a 25ml standard volumetric flask separately and dissolved with minimum quantity of methanol and the volume was made up to the mark with methanol. From the above solutions 1.5ml were transferred to a 50ml volumetric flask and diluted with methanol to get concentration of 30µg/ml for Metformin hydrochloride.

From the standard solution the of Fenofibrate pipette out 1ml and made up to the mark with methanol in 50ml volumetric flask to get the concentration of 20 µg/ml. Aliquots of working standard solution (1-5ml) into a serious of five 10ml volumetric flask and made up to the mark with mobile phase to obtain the concentration range from 3-15 µg/ml for MET and from the standard solution , pipette out 0.5-2.5ml into a serious of five 10ml volumetric flask and made up to the mark with mobile phase to obtain the concentration range from 1-5 µg/ml for Fenofibrate solution were injected and chromatograph was recorded. The calibration curve was plotted using peak area VS concentration. The correlation coefficient was found to be 0.9994 and 0.9986.

Assay of tablet formulation

Twenty tablets were weighed and average weight of each was found and powered. The content of the drug equivalent to 50mg of Fenofibrate and which also contains 156.25mg of Metformin hydrochloride was transferred to a 50ml volumetric flask and dissolved in methanol and sonicated for 15minutes.the final concentration was 1000 µg/ml. the above solution was filtered through whatmann filter paper (No.41) and the clear solution was collected 2.5mlwas pipette into a 10ml volumetric flask and made up to the mark with methanol. From this 2ml was pipette into 10ml volumetric flask and made up to mark with the mobile phase to produce 2 µg/ml solutions. The peak area measurements were done by injecting sample (20 µl) six times and the amount of Metformin hydrochloride and Fenofibrate were calculated from their respective calibration curve. The results are shown in table.4

METHOD VALIDATION

Linearity

The developed methods were validated as per ICH guidelines. The plot of absorbance against concentration is shown in fig 4&5 for UV-spectroscopy and RP-HPLC methods respectively. It can be seen that plot I linear over the concentration range of Metformin hydrochloride and Fenofibrate 3-15 µg/ml &1-5 µg/ml for UV-spectroscopy and RP-HPLC with a correlation coefficient (r^2)of 0.9999, 0.9998 and 0.9994, 0.9986 respectively.

Precision

Intraday and interday precision was determined by repeating assay for three times on the same day and on three different days. The relative standard deviation for replicates of sample solution was less than 2.0% which meet the acceptance criteria established for both the methods. The obtained results were present in table.5

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out at 80%, 100%, 120% of test concentration as per ICH guidelines and low relative standard deviation value show the accuracy of the UV-spectroscopy and RP-HPLC methods. The data were presented in table 3&6

LOD &LOQ

The LOD and LOQ were separately determined based on the standard deviation of intercept and the average value of slope.

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature for 24hrs.the relative standard deviation was found below 2.0%. It shows that standard and sample solution were stable up to 24 hrs at room temperature.

RESULTS AND CONCLUSION

In this study a simple ,precise, accurate and sensitive UV-spectroscopy and RP-HPLC methods were developed for the simultaneous estimation of Metformin hydrochloride and Fenofibrate in pure and in tablet dosage form. As these proposed methods have the lowest LOD valves and wider linearity range is more sensitive method. From the results obtained, we conclude that the suggested methods showed high sensitivity, accuracy, reproducibility and specificity. Moreover these methods were simple and in expensive and this can be employed for the routine quality control of MET & FEN in pure and in tablet dosage form.

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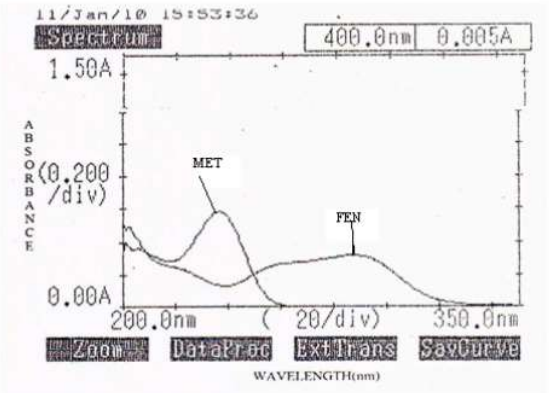


Figure 1: Overlaid Spectrum of Metformin hydrochloride and Fenofibrate

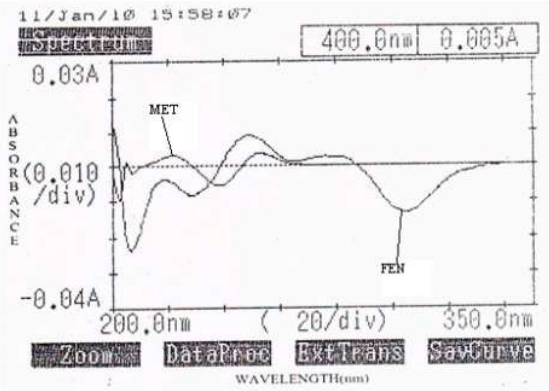


Figure 2: Overlaid derivative spectra of Metformin hydrochloride and Fenofibrate.

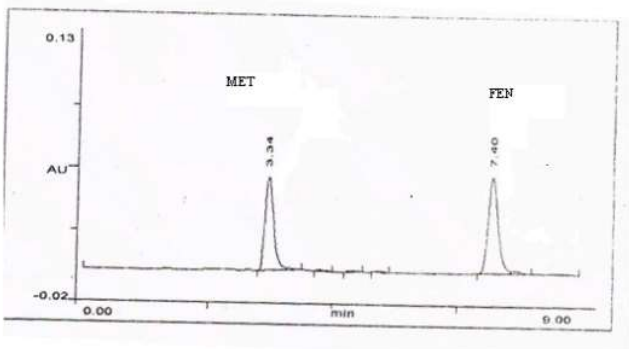


Figure 3: Chromatogram of Metformin hydrochloride and Fenofibrate.

TABLE.1: Optical characteristics of Metformin hydrochloride and Fenofibrate

Parameters	Metformin hydrochloride for simultaneous equation	Metformin hydrochloride for derivative method	Fenofibrate for simultaneous equation	Fenofibrate for derivative method.
λ max (nm)	237nm	221nm	258nm	303nm
Beer's law limit(µg/ml)	3-15	9-45	1-5	3-15
Sandell's sensitivity (µg/cm^2/0.001 A.U)				
Molar absorptivity (L/mol/cm)	0.010839	2.432890463	0.022559	.049044472
Correlation coefficient (r)				
Regression equation (Y=mX+C)	51875.0863	-	161013.2773	-
Slope(m)	0.9999	0.9998	0.9998	0.9997
Intercept (c)	Y=0.09225X+	Y=0.000405X	Y=0.04433X+	Y=0.002028CX+
Precision	0.00603	+0.0001451	0.00044	0.00020791
Inter day, intra day	0.09225	0.000405	0.04433	0.002028
Accuracy	0.00603	0.0001451	0.0004	0.000207
LOD (µg/ml)	0.09400	0.027891	0.01791	0.022923
LOQ (µg/ml)	0.28484	0.04863	0.03578	0.069466
Standard error	0.000663	0.000145	0.000155	0.000247

TABLE 2: Quantification of Metformin hydrochloride and Fenofibrate by simultaneous equation and derivative spectroscopic method

Drug		Percentage Obtained by*	S.D	R.S.D	S.E
MET	Simultaneous equation	100.5	1.1713	1.1654	0.4782
	Derivative spectroscopic method	100.64	0.97351	0.96252	0.39751
FEN	Simultaneous equation	101.01	1.37954	1.3660	0.5633
	Derivative spectroscopic method	100.14	0.66347	0..6626	0.2709

*Mean of six observations

TABLE 3: Recovery of Metformin hydrochloride and Fenofibrate by simultaneous equation and derivative spectroscopic method

Drug		Percentage	% recovered	S.D	% R.S.D	S.E
MET	Simultaneous equation	80 100 120	100.2 101.4 100.7	0.4262	0.4230	0.2460
	Derivative spectroscopic method	80 100 120	100.3 99.46 99.72	0.3041	0.3046	0.1755
FEN	Simultaneous equation	80 100 120	100.2 100.82 102.09	0.6812	0.6743	0.3933
	Derivative spectroscopic method	80 100 120	100.56 101.8 101.09	0.4399	0.4349	0.2540

*Mean of three observations

TABLE 4: Quantification of Metformin hydrochloride and Fenofibrate by RP-HPLC method.

Drug	Average (%)	S,D	R.S.D	S.E
MET	100.44	0.3793	0.37768	0.15487
FEN	99.75	0.3468	0.3476	0.1416

Table.5: Indra day and Inter day analysis of formulation (FIBMET^R ER) by simultaneous equation and derivative spectroscopic method.

Drug		Condition	% obtained	% R.S.D
MET	Simultaneous equation	Intraday Interday	100.01 99.8	0.7226 0.7319
	Derivative spectroscopic method	Intraday Interday	100.64 100.57	0.7226 0.7526
FEN	Simultaneous equation	Intraday Interday	100.72 102.1	0.5299 0.2256
	Derivative spectroscopic method	Intraday Interday	100.69 100.96	0.8074 0.3321

TABLE.6 Recovery studies of 50% pre analyzed formulation (FIBMET^R) by RP-HPLC.

Drug	Percentage (%)	% Recovery	S.D	% R.S.D	S.E
MET	80 100 120	99.47 100.1 100.71	0.4396	0.4392	0.2538
FEN	80 100 120	100.07 102.05 99.66	0.9037	0.8984	0.5217

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