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UV Spectrophotometric estimation of Ezetimibe and Fenofibrate in Bulk drug and Dosage form using Simultaneous Equation Method

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Abstract: A simple, accurate, sensitive, selective, precise and robust spectrophotometric method of analysis was suggested for determination of antilipidemic drugs; Fenofibrate and Ezetimibe. A spectrometric one depends interaction between {4-[(4-chlorophenyl)-carbonyl] phenoxy} - ring of Fenifibrate and 3-(4-fluorophenyl) - 3 (S) hydroxyl propyl ring of Ezetimibe. The estimation of drugs was carried out at λ_{max}; 286.6 nm and 232.6 nm for Fenofibrate and Ezetimibe respectively. The proposed method was found to be linear in the range of 2 – 20 µg/ml with mean recovery more than 98%, for both the drugs. The developed new method was validated according to ICH guidelines and it found to be accurate and precise. Thus the proposed method can be successfully applied for simultaneous determination of Fenofibrate and Ezetimibe in routine analysis work.
Keywords: Fenofibrate, Ezetimibe, Spectrophotometric.

INTRODUCTION

Ezetimibe (EZ), 1- (4-Fluorophenly) – 3 (R) - [3-(4-fluorophenyl) - 3 (S) hydroxyl propyl]-4 (S) – (4-hydroxy phenyl) – 2 azetidinones is a therapeutically beneficial drug that works by inhibiting the protein transporters on small intestinal brush border, which brings about this active transport of cholesterol. In addition, it also inhibits phytosterol absorption [3]. EZ has no inhibitory effect on absorption of lipid soluble vitamins[1-4, 14].

Fenofibrate is belongs to the fibrate class[5-8]. Fenofibrate is chemically propan-2-yl 2{4-[(4-chlorophenyl)-carbonyl]phenoxy}-2-methylpro panoate[10]. It is mainly used to reduce cholesterol levels in patients at risk of cardiovascular disease. It helps to reduces low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels, as well as reducing triglycerides (TG) level[7-13]. It is used alone or in combination with statins in the treatment of hypercholesterolemia and hypertri - glyceridemia[14-16].

The ezetimibe and fenofibrate combination was recently approved by the FDA for treatment of hyperlipidemia. This lipid-modifying therapy has the advantage of the different mechanisms of action of the two individual components[17]. Ezetimibe selectively inhibits intestinal absorption of dietary and biliary cholesterol, and exerts its effect mainly on the low-density lipoprotein cholesterol (LDL-C). Fenofibrate activates the PPAR-alpha, hence increases the tissue lipoprotein lipase activity and decomposition of triglycerides in VLDL[18]. The combination therapy of ezetimibe and fenofibrate has very good safety profile and represents another alternative in the clinical treatment of hyperlipidemia[19, 20].

Literature survey revealed that there is no UV method has been reported yet for the analysis of these two drugs in combination without preliminary separation that makes it worthwhile to pursue the present work.

EXPERIMENTAL

INSTRUMENTATION

The present work was carried out on JASCO spectrophotometer, model no. V-530 with 1 cm matched quartz cells was used for experiments. The absorption spectra of reference and test solution were carried out in a 1 cm quartz cell over the range of 200-400 nm.

REAGENTS AND CHEMICALS

A gift sample of Ezetimibe was obtained from Alkem Pharmaceutical Ltd, Mumbai and Fenofibrate was obtained from Smurthi Organics Ltd, Solapur. Methanol as solvent (HPLC grade) which was procured from Finar Chemicals Ltd.Ahmedabad, India.

EXPERIMENTAL CONDITION

According to the solubility characteristics of drugs, methanol was selected as solvent for analysis. From the scanning of both the drug by UV spectra wavelengths was selected for estimation of FENO 286.6nm and the estimation of EZ 232.6nm.

STANDARD STOCK AND SUB STOCK SOLUTION

UV analysis was done by using the standard stock solution of 100 g/ml of each FENO and EZ by dissolving 10mg of each standard drug separately in methanol. And aliquots of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 g/ml was prepared by diluting stock solution for calibration curve.

SAMPLE PREPARATION

Accurately weighed and powdered ten tablets of *"stanlip- EZ"* containing 10 mg of ezetimibe and 145 mg of fenofibrate. Then weigh powder equivalent to label claim of "*stanlip- EZ"* of drugs was weighed and transferred to a sintered glass crucible and drug was extracted thrice with 20 ml of methanol, and then final volume of the solution was made up to 100 ml with methanol to get a stock solution containing 100 µg/ml of EZ and 1450 µg/ml FENO, and further dilutions were made to get a concentration of 1µgm/ml of ezetimibe and 14.5 µg/ml of fenofibrate. The contents were mixed thoroughly and filtered through a 0.45 µ membrane filter.

WAVELENGTH SELECTION

The standard solution of ezetimibe and fenofibrate were separately scanned at different concentration in the range of 200-400 nm for determination of λ max. The overlain spectrum of both the drugs was also run.

Table-1: Result of UV analysis

Parameters	Ezetimibe	Fenofibrate
Detection Wavelength	232.6nm	286.6nm
Beers Law Limit	2-20µg/ml	2-20µg/ml
Molar Absorptivity	1.94×10^4 L/mol.cm	1.66×10^4 L/mol.cm
Regression Equation	y = mx + c	y = mx + c
Slope	0.049	0.043
Intercept	-0.017	0.027
Correlation Coefficient	0.997	0.999

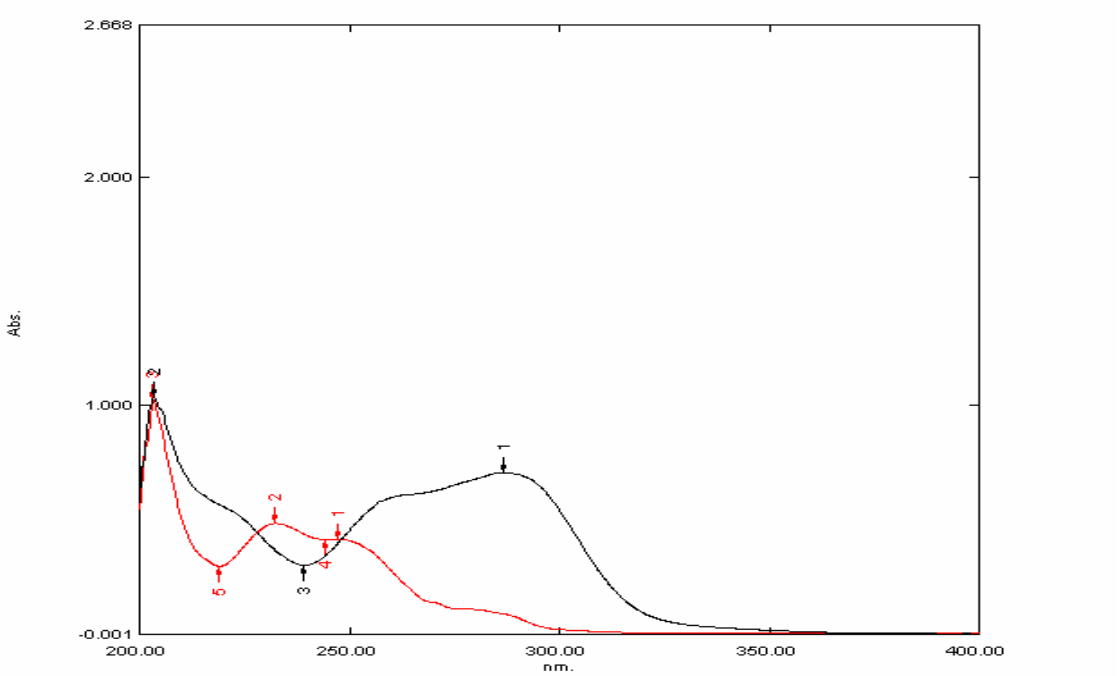

Figure 1: UV overlain spectra for Ezetimibe and Fenofibrate

Table-2: Result of UV analysis for marketed tablet formulation

Formulation	Drug	Label Claim (mg)	% Label Claim*, Mean ± S.D.	% R.S.D.
Tablet	Ezetimibe	10mg	100.2±0.00173	0.00172
	Fenofibrate	145mg	102±0.00152	0.00148

(S.D. - Standard Deviation, R.S.D.- Relative Standard Deviation, * Average of six determinations.)

Table-3: Result of recovery study

Drug	Level of Recovery (in %)	Amount present (in µg / ml)	Amount found (in µg / ml)	% Recovery	%RSD
Ezetimibe	80%	1.8	1.79	99.61%	0.00152
	100%	2	1.97	98.28%	0.00584
	120%	2.2	2.199	99.99%	0.00123
Fenofibrate	80%	26.1	26.7	102.49%	0.00168
	100%	29	29.7	102.41%	0.00097
	120%	31.9	32.6	102.42%	0.00195

Table no.4- Precision study of Ezetimibe:-

Conc. (µg/ml)	Intra-day Absorbance				± SD	%RSD	Inter-day Absorbance				± SD	%RSD
	Trial 1	Trial 2	Trial 3	Man absorbance			Day 1	Day 2	Day 3	Mean absorbance		
2	0.106	0.108	0.105	0.10633	0.001528	0.0144	0.106	0.106	0.110	0.1073	0.002309	0.0215
4	0.171	0.179	0.165	0.1716	0.007024	0.0409	0.171	0.175	0.176	0.174	0.002646	0.0152
6	0.265	0.267	0.265	0.2653	0.001155	0.0044	0.265	0.266	0.261	0.264	0.002646	0.01

Table no.5- Precision study of Fenofibrate:-

Conc. (µg/ml)	Intra-day Absorbance				± SD	%RSD	Inter-day Absorbance				± SD	%RSD
	Trial 1	Trial 2	Trial 3	Man absorbance			Day 1	Day 2	Day 3	Mean absorbance		
16	0.723	0.716	0.724	0.721	0.00082	0.006	0.723	0.721	0.723	0.7223	0.001155	0.0016
18	0.813	0.810	0.815	0.8126	0.00121	0.0031	0.813	0.812	0.818	0.8143	0.003215	0.0039
20	0.903	0.902	0.910	0.905	0.00306	0.048	0.903	0.903	0.901	0.9023	0.001155	0.0013

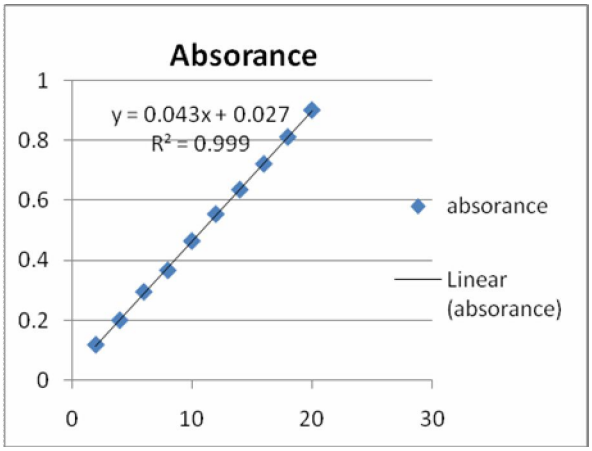

Figure 2: Calibration curve for fenofibrate

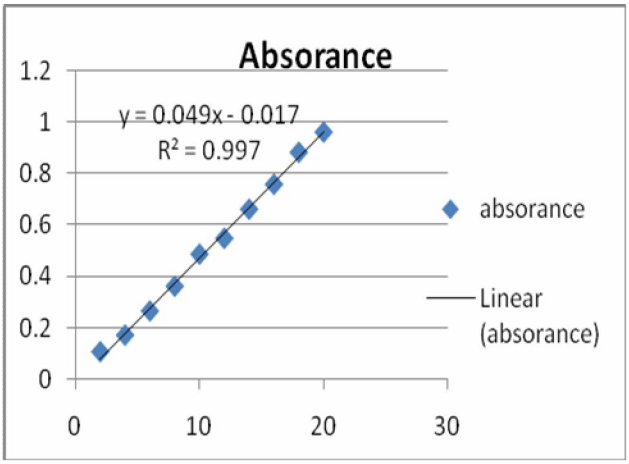

Figure 3: Calibration curve for Ezetimibe

METHOD VALIDATION

Accuracy was determined by recovery study. The recovery experiment was carried out by spiking the already analyzed sample of the tablets with their different known concentration of standard FENO and EZ. Precision for assay were determined by repeatability, inter-day, intra-day precision for both drugs (each in three replicate). Table no.5 &6

LINEARITY

The linearity for spectrophotometric method was established in the concentration of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20g/ml for both the drugs. (Fig no. 2&3)

RECOVERY

To evaluate the accuracy, precision and reproducibility of the method, known amount of pure drug was added to the analyzed sample of tablet powder and the mixture was analyzed for the drug content using the proposed method. The recovery experiments indicated the absence of interference from the commonly encountered pharmaceutical additives and excipients. Result of recovery study has been shown in **Table 3.**

RESULTS AND DISCUSSION

The proposed method for determination of EZ and FENO showed molar absorptivity 1.94×10^4L/mol.cm and 1.66×10^4L/mol.cm respectively .The calibration curve of EZ and FENO plotted at 232.6nm and 286.6nm respectively (represented in fig.2 &3) A linear relationship was obtained for both the drugs in the concentration range of 2-20µg/ml. Further the simultaneous estimation of marketed tablet formulation was carried out. The result of recovery study shown in Table-3 clearly indicates that the percentage recovery was found to be within range of 98.67-100.34% for both the drugs.

CONCLUSIONS

The proposed spectrophotometric method is accurate, precise and reliable for the simultaneous measurement of FENO and EZ in combined dosage form. The developed spectrophotometric method was validated for parameters like linearity, range, accuracy and precision. The % RSD for all parameters was found to be less than one, which revealed the validation of new method and assay results obtained by this method are in fair agreement. The developed new method can be used for routine quantitative simultaneous estimation of FENO and EZ in multi-component pharmaceutical preparation.

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