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Qualitative and Quantitative Analysis on Fibrates - A Spectroscopic Study

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In the present work, qualitative and quantitative analyses were made on the lipid lowering drugs namely gemfibrozil and fenofibrate through the means of infrared, Raman and UV-Visible spectroscopy. Some specific modes of vibration are identified and the absorbance values are noted under the different conditions of exposure. The ratios of absorbance among some specific modes of vibration are calculated, which represents the internal standards. The sets of internal standards of these drugs are compared with suitable storage conditions to check whether any change has taken place due to the exposure. Also a systematic approach has been adopted using UV-Visible spectroscopic method to study the light absorption activity under different storage conditions and the interaction of these drugs with some of the trace elemental constituents such as sodium, potassium, calcium, iron and lead ions. The study of interaction of drug with these ions reveals the nature of bonding, affinity of ion with drug molecules. The estimation of the active substance of a drug is an important quantitative analysis. The method of assay is experimented with these drugs using UV-Visible spectroscopic measurements.

Key Words: Spectroscopy, Genfibrozil, Fenofibrate.

INTRODUCTION

The word 'drug' is derived from the Fernch word drogue which means dry herb. In a general way, a drug may be defined as a substance used in the prevention, diagnosis, treatment or cure of disease in man or other animals. According to WHO, a drug may be defined as any substance or product which is used or intended to be used for modifying or exploring physiological systems or pathological states for the benefits of the recipient[1].

The process of discovery of a drug and its development is complex. It involves the collective contributions of many scientific specialists, such as organic, physical and analytical chemists, biochemists, bacteriologists, physiologists, pharmacologists, toxicologists, hematologists, immunologists, endocrinologists, pathologists, pharmaceutical scientists, clinical physicians and many others.

Pharmaceutical science deals with the identification, selection, presentation and standardization of various drugs. Quality assurance plays a vital role in determining the safety and efficacy of medicines. Modern spectroscopic techniques are very effective and sensitive tool for the qualitative and quantitative analysis of many compounds and the results are well employed in the quality control laboratories of pharmaceutical firm.

To investigate the structure and analysis of some pharmaceutical and biological active compounds spectroscopic techniques have been widely used in the recent past. Gunasekaran *et al.*[2-9] have done the qualitative analysis and structural conformation on some sample of pharmaceutical and biological importance using IR and UV-Visible spectral measurements. By keeping all these factors, in the present investigation a qualitative and quantitative analysis have been made on the lipid lowering drugs gemfibrozil and fenofibrate.

Though investigations on gemfibrozil and fenofibrate have been made by many but not much work is done on these said drugs spectroscopically. Hence the present study aims to make use of FTIR, FT Raman and UV-Visible spectroscopic methods in the qualitative and quantitative analysis of these drugs.

EXPERIMENTAL

The spectroscopic pure samples of gemfibrozil and fenofibrate were procured from Sigma Aldrich Company,USA and used as such. The FTIR and FT Raman spectra were measured using Bruker IFS 66V spectrophotometer over the region 4000-400 cm^{-1} and The FT Raman spectra were measured using 1064 nm line of Nd: YAG Laser operating at 200 mW on Bruker FRA 106/Bruker RSS 100 spectrometer in the region 4000-50 cm^{-1}, respectively at Sophisticated Analytical Instrumentation Facility (SAIF), IIT, Chennai, India. Transmitted light samples are prepared for FTIR and FT Raman spectra by mixing the sample powder with spectra grade KBr powder, grinding to mix and then pressing to form a semi-transparent disk of KBr containing the suspended sample powder. Both the spectra have been recorded at 303 K. The frequencies of all the sharp bands are accurate to ± 1 cm^{-1}. A spectral width of 4.29 cm^{-1} was used and spectra were measured with a scanning speed of 1.87 cm^{-1}/min. The UV-Visible spectral measurements are carried using ELICO SL-159 UV-Visible spectrophotometer of readability 0.1 nm, repeatability 0.2 nm and the drive speed of 900 nm/min. The UV-Visible spectra were recorded over the region 200-400 nm at spectrophysics research laboratory, Pachaiyappa's College, Chennai, India.

RESULTS AND DISCUSSION

The infrared spectrum of a compound is the superposition of the absorption bands of specific functional groups. As such, the infrared spectrum can be used as a finger print for identification of unknown in comparison with previously recorded reference spectra. By observing the position, shape and relative intensities of the vibrational bands in FTIR and FT Raman spectra of the drugs a satisfactory vibrational band assignments has been made. The molecular structures of gemfibrozil and fenofibrate are shown in Fig. 1. The vibrational band assignments of the drugs are summarized in Tables 1 and 2.

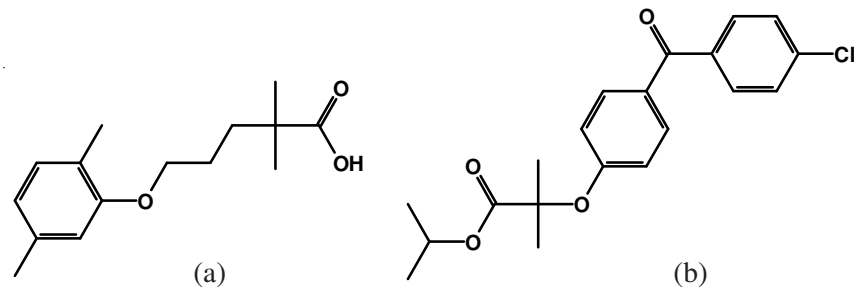

Fig. 1. Molecular structure of (a) Gemfibrozil and (b) Fenofibrate

Gemfibrozil and fenofibrate

Ring vibrations: Polynuclear, aromatic condensed-ring compounds absorb in the same general regions as benzene derivatives. Aromatic C-H stretching vibrations of heterocyclic aromatic compounds give rise to a band at 3100-3010 cm^{-1}. In the present study, the vibrational frequencies exhibited at 2944, 2919, 2877 and 2729 cm^{-1} in the FTIR spectrum and the bands 2917, 2880 and 2728 cm^{-1} in FT Raman spectrum are considered to be due to C-H stretching vibrations of gemfibrozil[10]. The vibrational frequencies exhibited at 2984 and 2936 cm^{-1} in the FTIR spectrum are considered to be due to C-H stretching vibration of fenofibrate.

Bands of variable intensity are observed in the regions 1300-1180 and 1100-1000 cm^{-1} due to C-H deformation vibrations. The sharp bands present at 1214, 1718, 1159, 1130, 1065, 1048, 996, 957, 941 and 904 cm^{-1} are suggested as C-H in plane deformations. The C-H out of plane deformations occur in the range 900-675 cm^{-1} and the bands exhibited at 833, 803, 749 and 721 cm^{-1} in the FTIR spectrum and the bands at 842, 811, 760 and 719 cm^{-1} in FT Raman spectrum are allotted as C-H out of plane deformations of gemfibrozil. The sharp peaks observed at 899, 859, 844, 819, 764 and 739 cm^{-1} in the FTIR spectrum are assigned to C-H out of plane deformation of fenofibrate.

TABLE-1
VIBRATIONAL SPECTRAL ASSIGNMENT OF GEMFIBROZIL

Frequency (cm^{-1})		Assignment
FTIR	FT Raman	
3300 w	–	O-H Stretching
2958 w	2965 vs	O-H Stretching
2944 s	–	CH_3/CH_2 Stretching
2919 s	2917 vvs	CH_3/CH_2 Stretching
2877 w	2880 s	CH_3/CH_2 Stretching
2729 w	2728 m	CH_3/CH_2 Stretching
1708 ms	–	C=O Stretching
1613 s	1612 s	C=O Stretching/C---C ring Stretching
1586 s	–	C---C ring Stretching
1510 vs	–	C---C ring Stretching
1473 s	–	C---C ring Stretching
1459 s	1451 s	C---C ring Stretching
1403 s	1414 w	C---C ring Stretching
1388 w	1393 w	CH_3 deformation
1377 w	1377 s	CH_3 deformation
1317 w	1309 s	OH deformation
1286 s	1293 w	OH deformation
1214 vs	1210 w	C-H in plane deformation
1718 s	1171 m	C-C Stretching/C-H in plane deformation
1159 s	1159 s	C-C Stretching/C-H in plane deformation
1130 s	1147 w	C-H in plane deformation
1065 vw	1074 m	C-H in plane deformation
1048 s	1039 m	C-H in plane deformation
996 s	1002 vw	C-H in plane deformation/C-O Stretching
957 m	–	C-H in plane deformation
941 s	934 s	C-H in plane deformation
904 w	899 w	C-H in plane deformation
833 m	842 vw	C-H out of plane deformation
803 s	811 w	C-H out of plane deformation
749 s	760 s	C-H out of plane deformation
721 m	719 m	C-H out of plane deformation
609 s	–	C-C in plane deformation
588 s	589 m	C-C in plane deformation
555 m	569 vw	C-C in plane deformation
531 w	540 w	C-C in plane deformation

Two bending vibrations can occur within a methyl group. The first of these, the symmetric bending vibrations involves in-plane bending of C-H bands. The second, the assymetrical bending vibration, involves out-of-plane bending of the C-H bands. The symmetrical bending vibration occurs

TABLE-2
VIBRATIONAL SPECTRAL ASSIGNMENT OF FENOFIBRATE

Frequency (cm^{-1})		Assignment
FTIR	FT Raman	
3067 w	3069 s	O-H stretching
2984 w	2985 s	$CH_3/CH_2/CH$ stretching
2936 w	2940 s	$CH_3/CH_2/CH$ stretching
1651 w	1648 m	C=O stretching
1599 w	1598 vs	C=O stretching
1588 m	1598 vvs	C=O stretching
1572 w	-	C=O stretching
1561 vs	-	C----O stretching
1504 m	1505 w	C----O stretching
1486 w	1480 m	C----O stretching
1466 s	1472 m	C----O stretching
1451 s	1442 m	CH_3 deformation
1419 s	1419 m	CH_3 deformation
1397 s	1390 m	CH_3 deformation
1385 s	-	CH_3 deformation
1377 vvs	-	CH_3 deformation
1368 m	1359 w	CH_3 deformation
1303 m	1310 m	O-H in plane deformation
1287 m	1286 w	O-H in plane deformation
1275 w	1286 m	O-H in plane deformation
1248 w	1248 vs	C-N stretching/C-O stretching
1205 m	1203 vw	C-N stretching/C-F stretching
1183 s	1180 w	C-C-H in plane deformation
1173 w	1180 m	C-C-H in plane deformation
1159 m	1145 s	C-C-H in plane deformation/O-H in plane deformation
1145 m	1145 m	C-C-H in plane deformation/O-H in plane deformation
1116 s	1105 vs	C-C-H in plane deformation
1101 s	1105 s	C-C-H in plane deformation
1013 s	1010 w	C-C-H in plane deformation
974 s	975 w	C-C-H in plane deformation
937 vs	943 vw	C-C-H in plane deformation
925 vs	920 vw	C-C-H in plane deformation
899 vs	–	C-H out of plane deformation
859 m	856 m	C-H out of plane deformation
844 m	856 m	C-H out of plane deformation
819 s	818 m	C-H out of plane deformation
764 vs	769 s	C-H out of plane deformation
739 vs	740 m	C-H out of plane deformation
656 m	657 m	O-H out of plane deformation
637 w	630 w	O-H out of plane deformation
626 s	630 vw	C-C-C deformation
597 m	600 m	C-C-C deformation
574 vs	578 m	C-C-C deformation
550 vw	550 vw	C-C-C deformation
520 w	515 w	C-C-C deformation
506 s	515 w	C-C-C deformation

near 1375 cm^{-1}, the asymmetric bending vibrations near 1450 cm^{-1}. The symmetric bending generally overlaps with the scissoring vibration of methylene groups. Two distinct bands are observed, however in compound such as diethyl ketone, in which the methylene scissoring band has been shifted to lower frequency, 1439-1399 cm^{-1} and the increased intensity because of its proximity to the carbonyl group. The absorption band at 1375 cm^{-1} arising from the symmetric bending of the methyl C-H bands, is very stable in position when the methyl group is attached to another carbon atom. Based on these factors, the bands observed at 1388 and 1377 cm^{-1} in the FTIR spectrum and the bands at 1393 and 1377 cm^{-1} in FT Raman spectrum are assigned to CH_3/CH_2 deformation vibrations of methyl group. The peaks at 1451, 1419, 1397, 1385, 1377 and 1368 cm^{-1} in the FTIR spectrum of fenofibrate are assigned to the deformation vibrations of methyl group and they are confirmed by FT Raman bands.

The bands between 1650 and 1400 cm^{-1} benzene derivatives are assigned to skeleton stretching of C-C vibrations[11,12]. Two double degenerate frequencies split into totally symmetric components under C_s symmetry. The bands observed at 1613, 1586, 1510, 1473, 1459 and 1403 cm^{-1} in the FTIR spectrum and the bands at 1612, 1451 and 1414 cm^{-1} in FT Raman spectrum are assigned as aromatic ring stretching vibrations which are present in both FTIR and FT Raman spectra. Present conclusions agree well with the literature values[13,14]. The bands observed at 1561, 1504, 1486 and 1466 cm^{-1} in the FTIR spectrum of fenofibrate are allotted to aromatic ring stretching vibrations which are present in FT Raman at 1505, 1480 and 1472 cm^{-1}.

Aromatic ring deformation vibrations occur below 700 cm^{-1} and normally in plane deformation vibration is at a higher frequency than out of plane deformations. With this note, the bands observed at 609, 588, 555 and 531 cm^{-1} in the FTIR spectrum and the bands at 589, 569 and 540 cm^{-1} in FT Raman spectrum are assigned as ring deformations of gemfibrozil and the bands observed at 626, 597, 574, 550 and 506 cm^{-1} in the FTIR spectrum are assigned as ring deformation.

O-H Vibrations: As a result of the presence of hydrogen bonding, carboxylic acids in liquid and solid phases exhibit a broad band at 3300-2500 cm^{-1} due to the OH stretching vibrations, which sometimes in the lower half of the frequency range has two or three weak bands superimposed on it[15]. The bands at 3300 and 2958 cm^{-1} in the FTIR spectrum and 2965 in the FT Raman spectrum are assigned to OH stretching vibrations. The bands at 1317 and 1286 cm^{-1} in the FTIR spectrum and the bands at 1309 and 1293 cm^{-1} in the FT Raman spectrum are assigned to OH deformation vibrations of gemfibrozil and the bands at 1310, 1286, 657 and 630 cm^{-1} are assigned to O-H deformation vibrations of fenofibrate.

C-O and C=O Vibrations: A strong absorption band due to C=O stretching occurs in the region 1850-1550 cm^{-1}. Because of high intensity and the relatively interference free region in which it occurs, this band is reasonably easy to recognize[16]. The sharp band present in the expected region being at 1708 and 1613 cm^{-1} in the FTIR spectrum is assigned to C=O stretching vibrations of gemfibrozil. Keeping this in mind the sharp band present in the expected region being at 1651, 1599, 1588 and 1572 cm^{-1} in the FTIR spectrum are allotted to C=O vibrations of fenofibrate. A band of medium strong intensity may be found in the region 1325-1115 cm^{-1} for aliphatic ketones. Hence the band at 996 cm^{-1} in FTIR spectrum and the bands at 1002 in FT Raman spectrum are allotted to C-O stretching vibration of gemfibrozil. The band at 1248 cm^{-1} in both FTIR and FT Raman spectrum is assigned to C-O stretching vibrations of fenofibrate.

C-N Vibrations: Silverstein *et al.*[17] assigned C-N stretching absorption in the region 1342-1266 cm^{-1}. In analogy with the previous work, the bands observed at 1248 and 1205 cm^{-1} in the FTIR spectrum and the band at 1248 and 1203 cm^{-1} in the FT Raman spectrum are assigned to C-N stretching vibrations of fenofibrate.

C-F Vibrations: In the vibrational spectra of related compounds, the band due to the C-F stretching vibration[18] may be found over a wide frequency range 1360-1000 cm^{-1}, since the vibration is easily affected by adjacent atoms or groups. Monofluorinated compounds have a strong band in the frequency range 1110-1000 cm^{-1} due to C-F stretching vibration. In the present work, the band observed at 1205 cm^{-1} in the FTIR spectrum and at 1203 cm^{-1} in the FT Raman spectrum is assigned to C-F stretching vibrations of fenofibrate.

Thus, a satisfactory vibrational band assignment has been made by observing the nature, shape and intensity of the vibrational bands both in IR and Raman spectra of all the chosen drugs and hence studied their quality.

Study of storage condition

Among the different quality assurance measures used in the control of manufacturing and formulation of drugs, two parameters are important which are the check on the shelf life of the drugs, stability under different storage conditions which has to be tested at every stage. Hence during the fabrication of the drugs, the various raw materials that are used in the fabrication of the drugs should undergo a rigourous qualitative test. Spectroscopic techniques, including mid-infrared and UV-Visible bands were made to study the quality of the drugs under different storage conditions.

Maintaining proper storage conditions for health commodities and essential medicines is vital to ensuring the quality. Product expiration dates are based on ideal storage conditions and protecting product quality until their expiration date is important for serving customers and conserving

resources. Medications last only as long as their storage conditions are favourable. Drugs can lose their potency long before the expiration date if exposed to oxygen, heat, light or humidity. In order to maintain their potency, medications should be stored in a place that is dry, cool and dark.

The drugs used in the present study should be stored in air tight containers[19]. Four sets of equal amount of gemfibrozil in the powder form (each 25 mg) have been taken for the investigation. One set of the drug was stored at room temperature in an air tight container, while another was stored at cold condition, the third set of the drug was exposed to infrared radiation and yet another exposed to sunlight continuously for a stipulated period of 4 h to make an internal standard calculation on the compound gemfibrozil. The FTIR spectra are recorded in the absorbance mode and normalized. The same procedure is adopted for fenofibrate. The overlaid FTIR spectra of gemfibrozil and fenofibrate at different storage conditions are presented in Figs. 2 and 3. The vibrational frequencies for the specific modes of vibration chosen for internal standard calculation are presented in Tables 3 and 4. Internal standards at specific modes of vibration are found and these sets of internal standards of the drugs stored under different storage conditions are compared with that of the drugs stored under suitable condition (air tight container) to check whether any change in the light absorption characteristics of the drug has taken place. From the tables, it is observed that the internal standard calculation for the various storage conditions showed significant change with the drug stored in the air tight container.

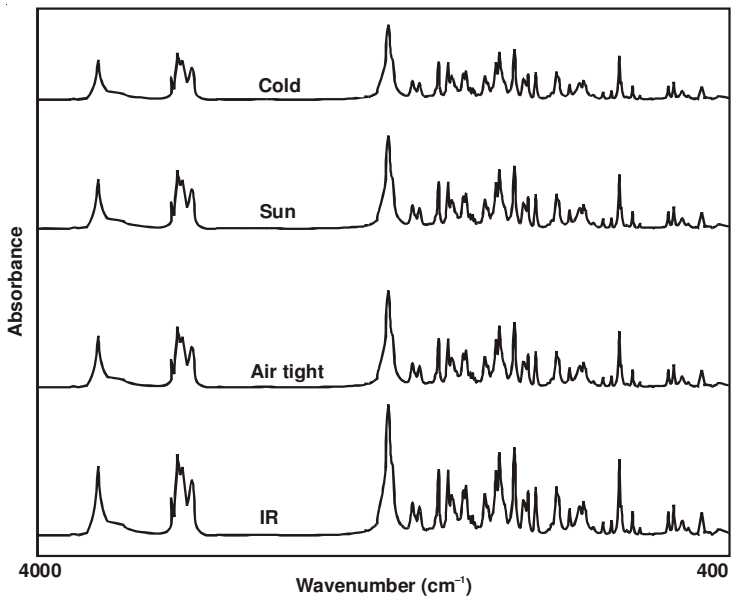

Fig. 2. An overlaid FTIR spectra of gemfibrozil at different storage conditions

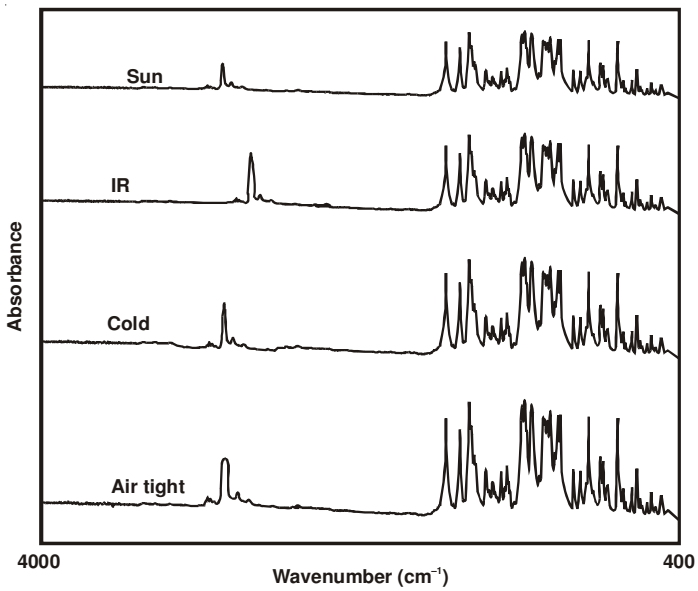

Fig. 3. An overlaid FTIR spectra of fenofibrate at different storage conditions

TABLE-3
INTERNAL STANDARD CALCULATION OF GEMFIBROZIL UNDER DIFFERENT STORAGE CONDITION

Storage conditions	Internal standard ratio for the specific modes of vibrations						
	A_{3300} / A_{2972}	A_{2972} / A_{1708}	A_{2972} / A_{1473}	A_{2972} / A_{1329}	A_{1473} / A_{1329}	A_{1708} / A_{803}	A_{803} / A_{555}
Airtight	0.4309	0.3209	0.9027	1.2617	1.3979	2.3320	3.1125
Sunlight exposure	0.5302	0.3918	1.4008	1.3714	1.4442	2.5715	3.4096
Cold condition	0.5417	0.4083	1.6715	1.3897	1.4709	2.6888	3.5215
IR Radiation	0.5179	0.3814	1.3259	1.3479	1.4256	2.4110	3.2714

TABLE-4
INTERNAL STANDARD CALCULATION OF FENOFIBRATE UNDER DIFFERENT STORAGE CONDITION

Storage conditions	Internal standard ratio for the specific modes of vibrations						
	A_{2984} / A_{1651}	A_{2984} / A_{1561}	A_{2984} / A_{1419}	A_{2984} / A_{1303}	A_{1419} / A_{1303}	A_{3315} / A_{1159}	A_{1101} / A_{819}
Airtight	1.405	0.934	0.825	1.721	2.085	3.554	0.392
Sunlight exposure	1.321	0.997	0.828	1.636	1.976	3.426	0.448
Cold condition	1.162	0.749	0.640	1.319	2.060	1.531	0.312
IR Radiation	1.045	0.790	0.583	1.295	2.221	1.782	0.355

Storage condition of the drugs can also be analyzed by using UV-Visible spectroscopic method. Many molecules absorb ultraviolet or visible light. Absorbance is directly proportional to the path length 'l' and the concentration 'c', of the absorbing species. Beer's law states that $A = \varepsilon cl$, where ε is a constant of proportionality, called the absorbtivity. Different molecules absorb radiation of different wavelengths. An UV-Visible absorption spectrum will show a number of absorption bands corresponding to structural groups within the molecule.

The UV-Visible spectral measurement are carried out on gemfibrozil and by checking the sample obeying Beer's law, the absorption peaks are identified. The sample is dissolved in methanol and a stock solution is prepared. From the stock solution, a solution of 0.07 mg/mL concentration is made as for this concentration onwards only a smooth spectrum had been obtained. The experiment is repeated for other concentrations. As expected theoretically the absorption level of the peaks decreases as the concentration is decreased, thus verifying Beer's law. The UV-Visible spectra of gemfibrozil show the wavelength maxima λ_{max} at 227 and 275 nm. The same procedure is adopted for fenofibrate. Fenofibrate shows the wavelength maximum at 232, 260 and 288 nm.

UV-Visible spectral investigation has been carried out to study the variations in the absorbance of λ_{max} in gemfibrozil at different storage conditions. The samples of gemfibrozil are exposed to different storage conditions *viz.*, air tight container, cold condition, sunlight and IR radiation. An overlaid UV-Visible spectra of gemfibrozil is shown in Fig. 4. The variation in the absorbance of λ_{max} at different storage conditions are presented in Table-5. It is observed from the table, that the absorbance of drug kept under IR radiation and cold condition show changes in absorbance to a larger extent when compared to the absorbance of the drug kept in air tight container. Hence, it can be concluded that the drug under study is to be store in air tight container to retain its pharmaceutical properties. Similarly, the experiment was performed for fenofibrate. The overlaid UV-Visible spectra of gemfibrozil and fenofibrate at different storage conditions are presented in Figs. 4 and 5, respectively. The variation in the absorbance of λ_{max} at different storage conditions for gemfibrozil and fenofibrate are presented in Tables 5 and 6. The internal standard ratio among the absorbance of wavelength maxima are calculated and the sets of internal standards of these drugs stored under different storage conditions are compared with that of the drugs stored under suitable condition (air tight container) to check whether any change in the light absorption characteristics of the drugs has taken place. A graph of concentration *vs.* absorbance (linearity graph) was also drawn (Fig. 6) and the slope values in different conditions *i.e.*, air tight container, cold condition, exposed to sun and IR radiation of gemfibrozil were

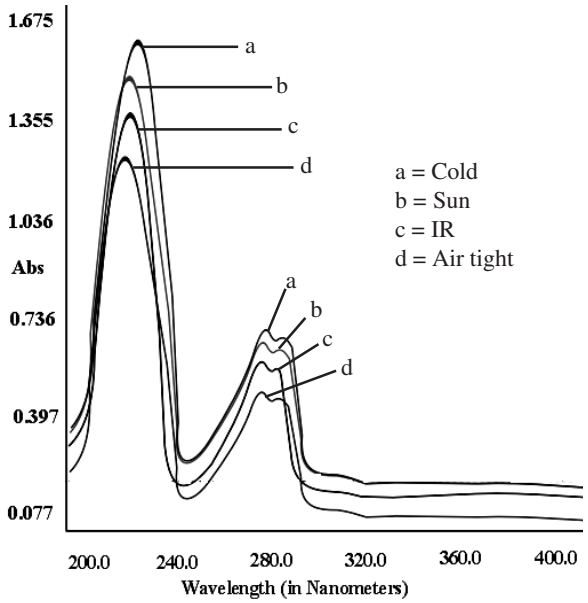

Fig. 4. An overlay UV-Visible spectra of gemfibrozil at different storage conditions

TABLE-5
VARIATION OF ABSORBANCE UNDER DIFFERENT STORAGE CONDITIONS OF GEMFIBROZIL

Conc. (mg/dl)	Absorbance (A)							
	λ = 227 nm				λ = 275 nm			
	Air tight	Cold	Sun	IR	Air tight	Cold	Sun	IR
0.03	1.150	1.099	1.150	1.382	0.479	0.459	0.582	0.621
0.04	1.286	1.288	1.296	1.533	0.517	0.507	0.660	0.707
0.05	1.317	1.315	1.317	1.742	0.547	0.547	0.681	0.823
0.06	1.548	1.405	1.548	1.949	0.638	0.638	0.703	0.979
0.07	1.977	1.879	1.977	2.128	0.818	0.769	0.769	1.013

TABLE-6
VARIATION OF ABSORBANCE UNDER DIFFERENT STORAGE CONDITIONS OF FENOFIBRATE

Conc. (mg/dl)	Absorbance (A)											
	λ = 232 nm				λ = 260 nm				λ = 288 nm			
	Sun	IR	Cold	ATC	Sun	IR	Cold	ATC	Sun	IR	Cold	ATC
0.03	0.131	0.135	0.149	0.162	0.221	0.230	0.242	0.287	0.269	0.274	0.278	0.252
0.04	0.285	0.310	0.296	0.352	0.480	0.491	0.506	0.638	0.557	0.562	0.566	0.533
0.05	0.420	0.422	0.436	0.501	0.710	0.724	0.736	0.865	0.828	0.829	0.821	0.762
0.06	0.564	0.580	0.592	0.648	0.960	0.962	0.965	1.145	1.110	1.113	1.115	0.995
0.07	0.702	0.739	0.789	0.812	1.193	1.195	1.999	1.425	1.371	1.383	1.389	1.271

ATC = Air tight container.

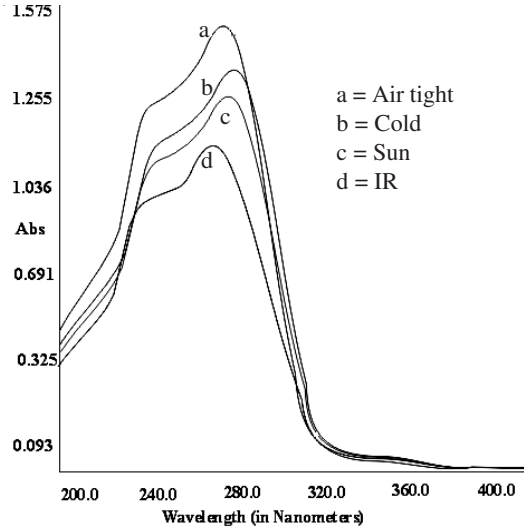

Fig. 5. An overlay UV-Visible spectra of fenofibrate at different storage conditions

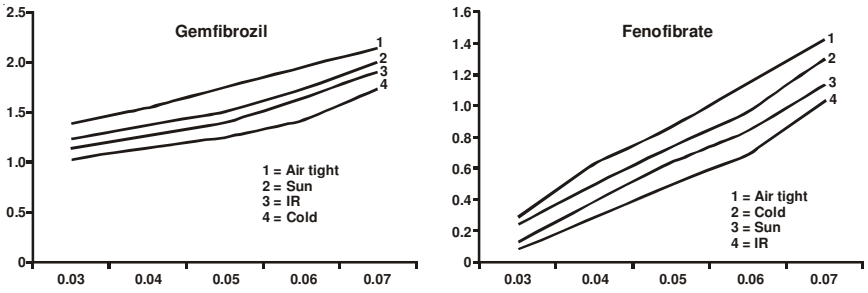

Fig. 6. Linearity graph to find the slope values of gemfibrozil and fenofibrate at different storage condition

found to be 0.052, 0.055, 0.051 and 0.053, respectively. In fenofibrate, the slope values are found to be 0.036, 0.038, 0.043 and 0.040, respectively. It is observed that the drug activity changes more significantly due to improper storage of the drugs. Hence the spectral data obtained from UV-Visible spectroscopy of the chosen drugs, kept under different storage condition are in accordance with the results obtained by the IR spectroscopy.

Drug-Trace element interaction

Trace elements are also known as micronutrients and are found only in minute quantities in the body yet they are vitally important. In nutrition, minerals are those elements for which the body's requirement is at least

100 mg/d and trace minerals are those elements that are needed in smaller amounts. Dietary minerals are derived from the earth's crust. Plants extract the minerals from the soil and humans and animals, in their turn, consume the plants. There are seven major minerals. Calcium occurs mainly in the teeth and bones, but a small amount is found in blood plasma and other body fluids, where it influences nerve transmission, blood clotting and muscle contraction. Dairy products and green leafy vegetables are dietary sources of calcium and an adequate intake of vitamin D is required for calcium absorption. Phosphorus, also found in dairy products, is closely allied to calcium in bone and tooth formation and its association with vitamin D. It is present in every cell in compounds such as nucleic acids and adenosine triphosphate. Magnesium, also present in every cell, is necessary for carbohydrate and protein metabolism, cell reproduction and smooth muscle action. Dietary sources include nuts, soy beans and cocoa. Sodium is in the skeleton and extracellular fluids and is necessary for fluid and acid-base balance, cell permeability and muscle function[20]. It occurs in table salt (sodium chloride, the main source) and such foods as milk and spinach. Potassium, which is found in intra- and extracellular fluid, plays a major role in fluid and electrolyte balance and in heart muscle activity and is also required for carbohydrate metabolism and protein synthesis. Its sources include legumes, whole grains and bananas. Chlorine is found in extracellular fluid, where it helps maintain normal fluid-electrolyte and acid-base balance and in the stomach, where it helps provide the acidic environment necessary for digestion. Table salt is its main dietary source. Sulfur, which is important to the structure of proteins, is also necessary for energy metabolism, enzyme function and detoxification[21]. Sulfur is obtained from protein foods, such as meat, eggs and legumes. Some trace minerals are considered "essential" in human nutrition. The essential trace minerals include iron, which is a constituent of hemoglobin; iodine, which is necessary for thyroxine synthesis; and cobalt, which is a component of vitamin B_{12}. Other essential trace minerals are chromium, copper, fluorine, manganese, molybdenum, selenium and zinc.

Many trace elements are better absorbed in humans and animals if they are in ionic form magnesium, for example, from a variety of more expensive organic salts (acetate, citrate, lactate) and less expensive organic salts (carbonate, chloride, oxide, phosphate and sulfate) have been shown to be equally absorbed from green leafy vegetables (organic magnesium) is equivalent to the absorption of magnesium from magnesium chloride (inorganic magnesium). These finds and many others, question the assumption that 'organic' minerals are somehow superior to 'inorganic' minerals. For the body to function normally, the level of each ion must be kept in

balance within a very narrow range; any significant deviation can result in symptoms.

Recent research indicates that trace minerals may play a significant role against a variety of degenerative diseases and processes. They may also prevent and reduce injury from environmental pollutants and enhance the ability to work and learn. They can also protect the body from the effects of toxic minerals. New studies suggest that numerous trace minerals, when in proper balance with one another, may be performing important non-classical biochemical functions especially important to age-related health problems.

When a drug is administrated to a living system, it is important to know the manner in which the drug is absorbed. Every drug is distributed throughout the body in a characteristic manner depending upon its physio-chemical properties. Individual differs both in degree and the character of the response and therefore the optimum dose of a drug which produces the desired therapeutic effect varies from person to person. The important factors which influence the effect of the drug are body weight, age, sex, diet and environment, route of administration, emotional factors, genetic factors, presence of disease, cumulation, additive effect, synergism, antagonism, drug tolerance, drug dependence *etc.*[22]. In the case of chemical anatagonism, the biological activity of a drug can be reduced or abolished by a chemical reaction with another agent[23]. Metal ions, as well as proteins, lipids, carbohydrates and vitamins are an essential part in the life processes. In this connection, while the relavant role played in biological systems, by some metal ions has been relatively well established, for other elements the scenario is almost totally obscure.

In the present investigation, an attempt has been made to study the interaction of the chosen lipid lowering drugs with the metal ions *viz.*, iron, zinc, potassium and lead by UV-Visible spectroscopic method.

The salt solution (ferrous sulphate) is prepared in the laboratory by dissolving 10 mg of ferrous sulphate in 10 mL of distilled water. Then it is diluted to obtain by dissolving 1, 2, 3, 4 and 5 ppm with gemfibrozil solution. The absorbance values corresponding to different concentrations of the trace elements with gemfibrozil are presented in Table-7. The same procedure is adopted for zinc, potassium and lead ions. The same procedure is performed for fenofibrate and the absorbance values corresponding to the various concentrations of these drugs are summarized in Table-8. It is observed from the study that, the light absorption characteristics of the drug varies with the variying concentrations of the trace elemental constituents of the blood such as Fe, Zn, K and Pb.

TABLE-7
INTERACTION OF GEMFIBROZIL WITH THE TRACE ELEMENTS AT DIFFERENT CONCENTRATIONS

Conc. (ppm)	Absorbance (A)							
	λ = 227 nm				λ = 275 nm			
	Fe	Zn	K	Pb	Fe	Zn	K	Pb
10	0.173	0.097	0.11	0.169	0.042	0.043	0.048	0.033
20	0.122	1.008	0.125	0.174	0.061	0.056	0.059	0.051
30	0.179	1.027	0.128	0.191	0.075	0.063	0.069	0.073
40	0.199	1.046	0.143	0.204	0.081	0.079	0.084	0.094
50	0.207	1.079	0.272	0.217	0.094	0.098	0.101	1.002

TABLE-8
INTERACTION OF FENOFIBRATE WITH THE TRACE ELEMENTS AT DIFFERENT CONCENTRATIONS

Conc. (ppm)	Absorbance (A)											
	λ = 232 nm				λ = 260 nm				λ = 288 nm			
	Fe	Zn	K	Pb	Fe	Zn	K	Pb	Fe	Zn	K	Pb
10	0.173	0.167	0.165	0.188	0.293	0.254	0.247	0.299	0.261	0.249	0.241	0.253
20	0.189	0.179	0.175	0.201	0.301	0.271	0.259	0.314	0.279	0.267	0.258	0.271
30	0.194	0.186	0.188	0.215	0.331	0.283	0.263	0.346	0.283	0.271	0.271	0.289
40	0.207	0.189	0.194	0.228	0.347	0.299	0.274	0.361	0.295	0.284	0.28	0.303
50	0.222	0.201	0.217	0.249	0.358	0.307	0.288	0.374	0.309	0.295	0.295	0.317

Assay of drugs

Tablets remain more popular dosage form because of the advantages offered both to the manufacturer in terms of simplicity and economy of preparation, stability and convenience in packaging, transporting and dispensing and to the patient for accuracy of dosage, compactness, portability, blandness of taste and ease of administration[24]. In the present work, medicines of gemfibrozil and fenofibrate in the form of tablets are subjected for the quantitative estimation of the drug substance in the tablet. According to Indian Pharmacopoeia the method of assay is by different methods in each of the above tablets. As a model experiment to understand how UV-Visible spectroscopic technique is employed in the assay, this method is applied in gemfibrozil and fenofibrate to estimate the active substance in the tablet.

The tablets containing gemfibrozil as the active ingredient is obtained from a leading pharmaceutical company labelled as lopid 300 mg. In the case of fenofibrate a tablet having strength 10 mg (fibator 10 mg) is used

for the analysis. The UV-Visible spectra are recoded for all the samples in the pure form and for the tablets. By comparing the absorbance in the pure and tablet form of the sample, the quantitative estimation of the drug can be estimated. Quantitative spectrometry is an extension of calorimetry and many pharmacopoeial substances are assayed spectrophotometrically[25]. A solution of the test substance is made at a known concentration in a suitable solvent. The absorbance is noted at a selected wavelength which is preferably that of wavelength maxima having a fairly broad, flat-topped peak. Then by making a parallel determination of a solution prepared from a pure reference sample for the same concentration, the amount of active ingredient in the test substance is calculated.

All the measurements are based on the fundamental law of spectrophotometry (*i.e.*) Beer-Lambert's law which is stated as $A = \log (I/I_0) = abc$ where, I_0 is the intensity of the incident monochromatic beam which emerges with intensity I through a solution of path length of 'b' having concentration 'c' and A is the absorbance. In drug analysis, the determination of the drug content is carried out by preparing a stock solution of the test sample and the solution is diluted to the same concentration as that of the standard sample and the absorbance of the resulting solution is measured[26]. The drug content of the tablet is calculated from Beer's law as

$$\text{Drug content of the tablet or assay} = \frac{\text{Test absorption}}{\text{Standard absorption}} \times \frac{\text{Standard weight}}{\text{Test weight}} \times \text{Average wt. of one tablet}$$

In the present work, a single component system is chosen for the assay. From the spectroscopic point of view, a single component system is the one for which, at the wavelength selected for the measurement, the determination of the analyte is not influenced either by another substance or by background absorption.

Gemfibrozil: With the UV-Visible spectral measurements of the tablet containing gemfibrozil as active substance and with the pure sample of gemfibrozil, the estimation is done. The UV-Visible spectrum of the sample exhibits wavelength maximum at 276 nm. The average weight of one tablet is found to be 453.7 mg. From the powdered tablet 32 mg of the sample (equivalent to 25 mg of active substance gemfibrozil) is weighed and the stock solution is done to make the same concentration as above for pure sample. The UV-Visible spectrum is recorded for all the sample solutions and the absorbance of wavelength maximum is noted in each case. Fig. 7 presents the UV-Visible spectrum of gemfibrozil. Table-9 summarizes the estimation of active substance lopid in 300 mg.

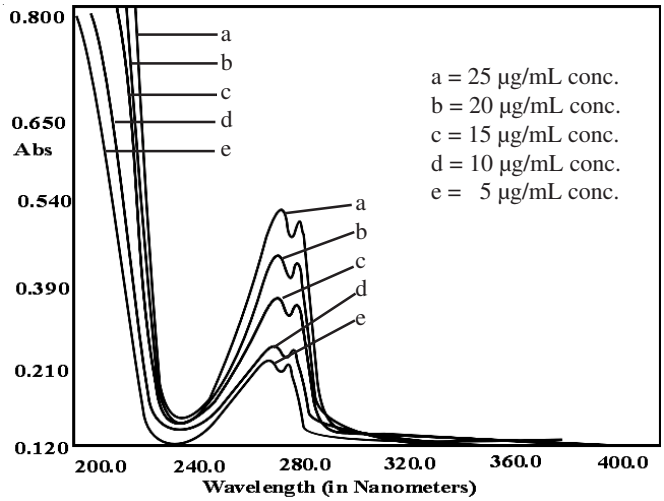

Fig. 7. An overlaid UV-Visible spectra of lopid for various concentrations

TABLE-9
ESTIMATION OF ASSAY IN LOPID (300 mg)

Wavelength (nm)	Conc. (µg/mL)	Average absorbance of wavelength maximum		Estimation of assay Lopid
		Pure	Lopid	
276	5	0.117	0.116	300.400
276	10	0.213	0.212	301.567
276	15	0.321	0.319	301.103
276	20	0.424	0.421	300.846
276	25	0.535	0.532	301.290

Fenofibrate: Fibator is the combination of atorvastatin and fenofibrate. From the stock solution of concentration, dilute solutions are prepared at concentrations 25, 20, 15, 10 and 5 µg/mL for which the spectral recordings are carried out. For the estimation of assay fenofibrate, fibator 10 mg is used. As the average weight of one tablet is 437.1, 82 mg of the powder of tablet (equivalent to 25 µg of pure substance) is dissolved in distilled water. The spectral recordings are carried out for the same concentrations of the pure sample. Fig. 8 presents the UV-Visible spectrum of fenofibrate which exhibits a wavelength maximum at 286 nm. Table-10 compress the absorbance by wavelength maxima at 286 nm for pure and tablet, from which the assay is estimated to be around 20.369 mg.

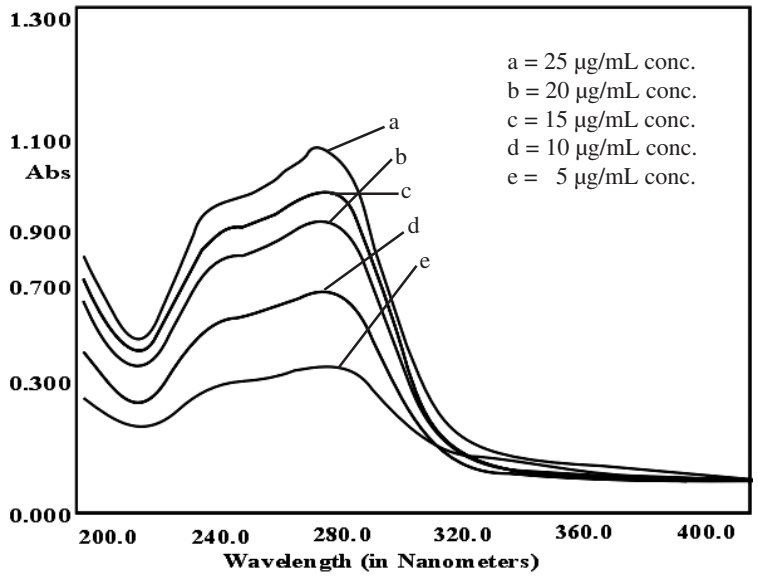

Fig. 8. An overlaid UV-Visible spectra of fibator for various concentrations

TABLE-10
ESTIMATION OF ASSAY IN FIBATOR (20 mg)

Wavelength (nm)	Conc. (µg/mL)	Average absorbance of wavelength maximum		Estimation of assay Fibator
		Pure	Fibator	
286	5	0.278	0.216	20.374
286	10	0.566	0.399	20.358
286	15	0.831	0.586	20.389
286	20	1.115	0.798	20.384
286	25	1.389	0.984	20.377

Conclusion

Thus the qualitative analysis of the drugs gemfibrozil and fenofibrate have been analyzed using FTIR, FT Raman and UV-Visible spectral measurements. The complete vibrational band assignments on the basis of their relative intensity, characterization, position, correlation and vibrational bands of related compounds have been made available using FTIR and FT Raman spectra for these compounds. The internal standard calculation has been made at different specific modes of vibration for the drugs exposed to different environmental conditions. From the internal standard calculation,

it is observed that the best storage condition for the drugs *viz.*, gemfibrozil and fenofibrate in air tight container to retain its pharmaceutical properties. Also a systematic approach has been employed using UV-Visible spectroscopic method to study light absorption activity under different storage conditions and the interaction of the drugs with the trace elemental constituents such as iron, zinc, potassium and lead ions. The study of interaction of drug with various metal ions reveals the nature of bonding/affinity of the metal ion with drug molecules site. As the concentration of the metal ion is increased the absorption of the drug decreases revealing higher of the metal ion interaction with the drug. The estimation of the substance of a drug is one of the monograph specified by pharmacopoeia, which has to be checked from time to time. The method had been employed in two tablets, lopid and fibator. Lopid 300 mg is used for the assay estimation in gemfibrozil and the experimental value is found to be 300.329. In fibrator 20 mg, the active substance present is calculated as 20.369 mg.

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