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Qualitative Analysis of Anti-protozoan Drug Tinidazole by Vibrational and UV-Visible Spectroscopy

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Modern spectroscopic techniques serve as indispensable tools for the qualitative analysis of pharmaceutical products. In this work, a qualitative analysis of a commonly used anti-protozoan drug, tinidazole has been carried out using FTIR, FT-Raman and UV-visible spectroscopic techniques. A satisfactory vibrational band assignment of the drug has been made based on the position, shape and relative intensity of the recorded spectra, in correlation with bands assigned in structurally related molecules. The quality of the drug has been studied by storing it in different conditions and analyzing their spectra by internal standard ratio method. The stress degradation behaviour of the drug has been studied by employing FTIR and UV-Visible spectral techniques.

Key Words: Tinidazole, Quality, Storage, FTIR spectrum, UV-Visible spectrum.

INTRODUCTION

Parasitic protozoa are responsible for a significant portion of the global burden of illness due to infectious agents. The disease burden is greatest in lower socioeconomic groups of under developed areas of the world[1]. Tinidazole and metronidazole are some of the important drugs used commonly to treat such protozoan infections. Because of the important role these two drugs play in numerous pathological processes, their stress degradation behaviour, thermodynamic properties, pharmacokinetics, *in vitro* activities have all been widely studied in recent years[2,3]. In recent times, FTIR, Raman and UV-Visible spectroscopy are used as powerful tools to analyze the quality of drugs in terms of stability under different storage conditions and interaction with trace elements[4,5]. The current work presents the quality analysis on the drug, tinidazole by employing such tools. The vibrational analysis of the compounds ascertains the presence of the basic functional groups of the compounds. The change in the quality of drugs when exposed to different environmental conditions has been analyzed by internal standard calculations done, based on the FTIR and UV-visible spectral data.

Tinidazole is structurally derived from metronidazole, with a 2-(ethyl sulphonyl) ethyl moiety replacing a 2-(hydroxyl) ethyl group in the 5-nitroimidazole backbone[1]. Chemically, tinidazole is 1-(2(ethyl sulphonyl)ethyl)-2-methyl-5-nitroimidazole, with the molecular formula, $C_8H_{13}N_3O_4S$ and molecular weight 247.28 g/mol.

It forms pale yellow crystals or crystalline powder having a slight characteristic odour. The Indian Pharmacopoeia recommends that it should be stored in well-closed, light-resistant containers[6]. It is sparingly soluble in water and slightly soluble in ethanol, chloroform and ether. The structure of the azole drug tinidazole is given in Fig. 1.

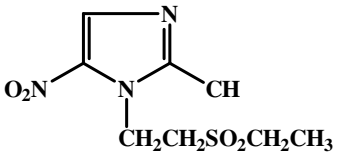

Fig. 1. Structure of tinidazole

EXPERIMENTAL

High-grade pure sample of tinidazole was procured from reputed pharmaceutical firms in Chennai, India and used for spectral recording as such without further purification. The FTIR spectra of the samples were recorded in the region 4000-400 cm^{-1}, using Bruker IFS 66V spectrophotometer, in the solid state by KBr pellet method, at Sophisticated Analytical Instrumentation Facility, IIT, Chennai. The Laser Raman spectra were recorded using Bruker FRA 106 FT-Raman spectrophotometer at CECRI, Karaikudi, India. The Indian Pharmacoepia recommends storing the tinidazole in well-closed light resistant containers (LRC)[6]. The FTIR spectra of the samples have been recorded for the pure drug stored in (i) well-sealed light resistant containers (ii) at ice-point (iii) exposed to sunlight for a period of 5 h. All the spectral recordings were carried out in absorbance mode at room temperature on the same day.

The UV-visible spectral measurements of the drugs were carried out at Dr. CEEAL Analytical Lab, Chennai, using Shimadzu-160A spectrophotometer. For the purpose, the linearity range in which the drugs obey Beer-Lambert's law has been figured out by analyzing the sample at various concentrations. For regression analysis, the stock solution of tinidazole of concentration 1000 µg/mL was prepared by dissolving 99.7 mg of pure sample in 100 mL of 0.1 M HCl. The solution was then sonicated to ensure thorough mixing of the contents. The working standard solution of concentrations 10, 20, 30, 40, 50 µg/mL was prepared by further dilution using the same solvent and then scanned in the wavelength range 200-400 nm. The absorbance values of the compound at 278 nm for various concentrations in the range 10-50 µg/mL are used to plot the linearity curve.

RESULTS AND DISCUSSION

The functional groups present in the compound, tinidazole was identified and a satisfactory vibrational band assignment of the drug was made by comparing the spectra of similar compounds and also by observing the nature, shape and intensity

of the vibrational bands in the spectra[7,8]. The vibrational band assignments of the compound are presented in Table-1.

TABLE-1
VIBRATIONAL FREQUENCY ASSIGNMENT FOR TINIDAZOLE

Frequency (cm^{-1})		Vibrational band assignment
FTIR	FT-Raman	
2962(w)	-	Aliphatic CH_3 stretching
2957(w)	2956(w)	Aliphatic CH_3 stretching
2950(w)	-	Aliphatic CH_2 stretching
2875(w)	-	Aliphatic CH_3 stretching
2847(w)	-	Aliphatic CH_2 stretching
1535(s)	1523(w)	Aromatic C=C stretching (imidazole I band)
1486(vs)	-	Methyl bending
1473(vs)	1477(m)	Aromatic C=C / C=N stretching
-	1420(vw)	Aromatic C=C / C=N stretching
1369(vs)	1362(s)	SO_2 stretching
1265(m)	1262(m)	C-S-C deformation
1159(m)	-	SO_2 stretching
-	1147(vw)	Aromatic C-N stretching
1187(s)	-	Aromatic C-N stretching
1047(m)	-	Aromatic C-N stretching / C-CH_3 stretching
969(vw)	-	N-CH_2 stretching
825(w)	830(w)	C-N-C bending
743(vw)	742(vw)	Methylene rocking vibration
675(vw)	-	Alkyl C-S stretching
650(vw)	655(vw)	Alkyl C-S stretching
605(vw)	-	Scissor vibration of SO_2 group
559(vw)	565(vw)	SO_2 in-plane deformation
503(vw)	-	SO_2 wagging
-	466(vw)	N-C=C deformation
450(vw)	-	N-C=C deformation

Sulphone group vibrations: The presence of two strong bands in the region 1415-1300 and 1200-1120 cm^{-1} are attributed to asymmetric and symmetric stretching vibrations respectively of the SO_2 group in sulphones, sulphonamides, sulphonates and organic sulphates. The position of these bands provide a reliable method for the detection of sulphones and sulphonic acid derivatives which contain O=S=O groups. All sulphones also show a medium to strong band at 610-545 cm^{-1} due to the scissor vibration of the -SO_2 group[9]. Gunasekaran *et al.*[10] have reported that the IR band at 1342 and 1158 cm^{-1} as due to SO_2 asymmetric and symmetric vibrations in the antidiabetic drug glibenclamide. In the compounds chlorothiazide and diazoxide, this band occur at around 1300 and 1200 cm^{-1} and the bending vibrations occur at frequencies in the range[11] 1500 and 1159 cm^{-1}. Based on these factors, in the present study, the infrared bands of strong intensity present at 1369 and 1159

cm^{-1} in tinidazole has been assigned to asymmetric and symmetric sulphone vibrations, respectively. The counterpart of these bands in the FT-Raman spectrum is present at 1362 and 1147 cm^{-1}, respectively. Also, the scissoring and wagging of the same group results in band near 600 cm^{-1} region in both IR and Raman.

Ring vibrations: In aromatic heterocyclic compounds having nitrogen, the coupled C=C and C=N stretching vibrations give rise to several characteristic bands in the general region[9] between 1600 and 1300 cm^{-1}. These are due to the stretching and contraction of all the bonds present in the ring and also the interaction between them. The intensity of the C=N stretch is usually more than C=C stretch. Imidazoles have several bands of variable intensity in the range 1660-1450 cm^{-1} due to C=N and C=C stretching vibrations, named as the imidazole-I bands. Studies indicate that 1,4,5-trisubstituted imidazoles have a medium to strong absorption at 660-650 cm^{-1} and a weak to medium band at 420-390 cm^{-1} due to ring deformation vibrations, in addition to imidazole-I bands[12,13].

In some imidazole substituted indoles the (C=N) and (C=C) stretching vibrations are observed to occur in the range[12] 1600-1500 cm^{-1}. The vibrations seem to occur in the same region in nicotinaldehyde and a few pyrazole derivatives also[14,15]. Following these, for the compound under study, the (C=N) and (C=C) vibrations are assigned to frequencies observed at 1522, 1455 and 1366 cm^{-1}. The corresponding lines in the FT-Raman spectrum are observed at 1523 and 1477 cm^{-1}. The C-N stretching vibrations occur in the range 1200-1000 cm^{-1} for the compound under investigation. The bending vibrations are generally found at lower wavenumber region of the spectra. The bands observed at frequencies 503, 466 and 450 cm^{-1} are assigned to C=C-N and N-C=N ring deformations, respectively.

C-H vibrations: Willard *et al.*[16] suggest that the shape of an absorption band around 3000 cm^{-1} gives an idea of the CH group present. While alkyl groups have their CH stretching frequencies lower than 3000 cm^{-1}, alkenes and aromatics have them slightly higher than 3000 cm^{-1}. In tinidazole, the asymmetric methyl vibrations occur at the frequencies 2962 cm^{-1} and 2957 cm^{-1}, while it's symmetric counterpart is observed at 2875 cm^{-1}. The methylene group vibrations of tinidazole occur at 2950 and 2847 cm^{-1}. This assignment agrees well with the values reported by Green and Harrison, in their work on mono-substituted nitrobenzenes[17].

The bending vibrations of CH bands of methyl and methylene groups occur at frequencies around 1465 and 1380 cm^{-1} as medium intense band. The band at 1380 cm^{-1} is due to methyl group and is sensitive to the electronegativity of the substituents attached to it. The CH_2 wagging occurring in the frequency region 1340-1190 cm^{-1} can be clearly seen in the solid phase spectra of long straight chain compounds such as acids and soaps. The CH_2 twisting vibrations are quite weak and appear at a little lower frequency than the CH_2 wagging frequency. Earlier workers have assigned the CH_3 deformation vibrations in the range[11,18] 1475-1357 cm^{-1}. In the present case, the methyl bending vibrations are located at 1490 cm^{-1}.

Other vibrations: The C-S stretching mode does not give rise to strong bands in the infrared spectra.The most characteristic bands are those of the CH_2 or CH_3 groups attached to the sulphur. The C-S symmetric and asymmetric stretching vibrations of thiazolidenones, compounds possessing antitubercular and antibacterial activity are traced[19] at 680 and 678 cm^{-1}. Horrocks and Cotton have assigned the bands occurring at 689 and 672 cm^{-1} in DMSO to asymmetric and symmetric C-S stretching frequencies, respectively, on the basis of Raman polarization data[20]. Based on these factors, in the current investigation, the weak bands found at 675 and 650 cm^{-1} in the IR spectra of tinidazole are assigned to C-S stretching vibrations.

The C-N vibration due to the methyl group attached to nitrogen atom in the side chain is observed at around 1150 cm^{-1} in N-methyl pyridinium ion[21]. The vibrations occur at 973 cm^{-1} in caffeine and at 980 cm^{-1} in theophylline as strong bands[22]. In tinidazole, the N-CH_2 vibrations are assigned to frequencies 969 cm^{-1}. The C-CH_3 stretching vibrations have been identified in the 1160-950 cm^{-1} region. The C-CH_3 symmetric stretching vibration in chloroxylenol is reported to occur at 1052 cm^{-1} and the asymmetric stretching[23] at 1191 cm^{-1}. In analogy with this, the C-CH_3 stretching vibration is assigned to the frequency 1047 cm^{-1} in tinidazole.

Quality analysis on the drug by FTIR spectroscopy: Vibrational spectroscopy is employed widely in pharmaceutical laboratories to analyze the quality of drug formulations. The degradation behaviour of the drug was analyzed by comparing the sets of internal standards that were arrived at from absorbance ratios of the samples kept at suitable storage condition at specific modes of vibration, with those of the samples exposed to sunlight and stored at ice-point. The intensity ratios of these selected modes of vibration with respect to other modes under various environmental conditions to which the drug was exposed are given in Table-2. The analysis indicates that storing the drug in any way other than that prescribed in pharmacopeias will certainly lead to degradation of the drug.

Quality analysis by UV-Vis spectroscopy: UV-Vis spectroscopy has been widely employed by many researchers for the quality analysis of pharmaceutical compounds[24,25]. The maximum absorbance for tinidazole, obtained at 278 nm arise due to the $n \rightarrow \pi^*$ transitions of the nitro group chromophore present in the heteroaromatic ring of the compounds and is called the R (radical-like) band. The UV-Visible spectrum of tinidazole is presented in Fig. 2. The linearity equation obtained was $Y = 0.02601X + 0.00439$ and the regression coefficient was 0.9997. These values show the accuracy of the method adopted for the analysis. Table-3 clearly indicates the change in absorption characteristics of the drug with change in storage condition. These results support the internal standard evaluations done using FTIR spectra of the drug, confirming the fact that that drugs change their behaviour when they are stored in altered conditions. These results lead us to conclude that in order to retain the good quality of the drugs they must be stored in the prescribed conditions.

TABLE-2
INTERNAL STANDARD EVALUATION FOR TINIDAZOLE

Conditions of exposure	Internal standard of specific modes of vibration at 2957 cm^{-1}						
	2957 / 2957	1535 / 2957	1473 / 2957	1369 / 2957	1265 / 2957	1187 / 2957	1159 / 2957
Labeled condition	1.0000	2.2941	3.0588	2.9176	1.9059	2.5882	1.6471
Exposed to sunlight	1.0000	4.1364	6.5455	5.9091	5.8909	5.9995	2.9541
At ice-point	1.0000	4.4001	6.0034	5.6021	5.8202	6.0035	3.0213
	Internal standard of specific modes of vibration at 1535 cm^{-1}						
	2957 / 1535	1535 / 1535	1473 / 1535	1369 / 1535	1265 / 1535	1187 / 1535	1159 / 1535
Labeled condition	0.4357	1.0000	1.3333	1.2718	0.8308	1.1282	0.7179
Exposed to sunlight	0.2418	1.0000	1.5824	1.4286	1.4242	1.4388	0.7143
At ice-point	0.2273	1.0000	1.3636	1.2727	1.3182	1.3576	0.6818
	Internal standard of specific modes of vibration at 1473 cm^{-1}						
	2957 / 1473	1535 / 1473	1473 / 1473	1369 / 1473	1265 / 1473	1187 / 1473	1159 / 1473
Labeled condition	0.3269	0.7500	1.0000	0.9538	0.6231	0.8462	0.5385
Exposed to sunlight	0.1528	0.6319	1.0000	0.9028	0.9000	0.9028	0.4514
At ice-point	0.1667	0.7333	1.0000	0.9334	0.9667	1.0312	0.5055
	Internal standard of specific modes of vibration at 1369 cm^{-1}						
	2957 / 1369	1535 / 1369	1473 / 1369	1369 / 1369	1265 / 1369	1187 / 1369	1159 / 1369
Labeled condition	0.3427	0.7863	1.0484	1.0000	0.6532	0.8871	0.5645
Exposed to sunlight	0.1692	0.7054	1.1077	1.0000	0.9969	1.0231	0.5022
At ice-point	0.1786	0.7857	1.0714	1.0000	1.0357	1.0714	0.5357
	Internal standard of specific modes of vibration at 1265 cm^{-1}						
	2957 / 1265	1535 / 1265	1473 / 1265	1369 / 1265	1265 / 1265	1187 / 1265	1159 / 1265
Labeled condition	0.5247	1.2037	1.6049	1.5309	1.0000	1.3580	0.8642
Exposed to sunlight	0.1698	0.7022	1.1111	1.0031	1.0000	1.0045	0.5015
At ice-point	0.1724	0.7856	1.0345	0.9655	1.0000	1.0445	0.5172
	Internal standard of specific modes of vibration at 1187 cm^{-1}						
	2957 / 1187	1535 / 1187	1473 / 1187	1369 / 1187	1265 / 1187	1187 / 1187	1159 / 1187
Labeled condition	0.3864	0.8864	1.1818	1.1273	0.7364	1.0000	0.6364
Exposed to sunlight	0.1698	0.7089	1.1077	1.0074	0.9969	1.0000	0.5017
At Ice point	0.1667	0.7333	1.0214	0.9334	0.9667	1.0000	0.5006
	Internal standard of specific modes of vibration at 1159 cm^{-1}						
	2957 / 1159	1535 / 1159	1473 / 1159	1369 / 1159	1265 / 1159	1187 / 1159	1159 / 1159
Labeled condition	0.6071	1.3929	1.8571	1.7714	1.1571	1.5714	1.0000
Exposed to sunlight	0.3385	1.4023	2.2154	2.0045	1.9938	2.0314	1.0000
At ice-point	0.3333	1.4667	2.0003	1.8667	1.9333	2.0091	1.0000

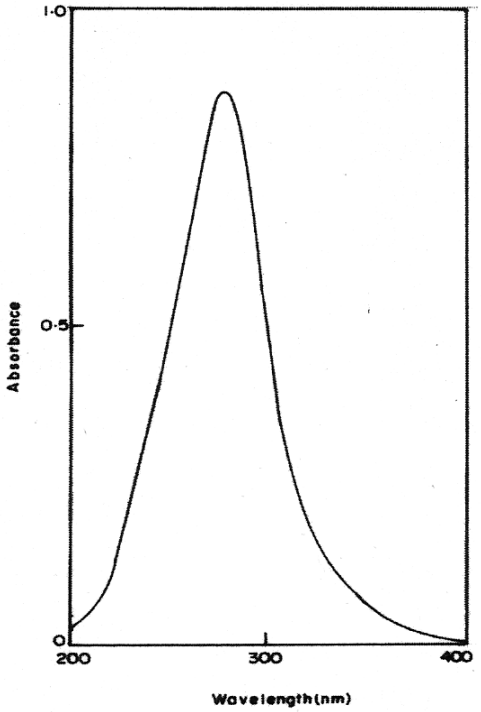

Fig. 2. UV-Visible spectrum of tinidazole

TABLE-3
VARIATION OF ABSORBANCE OF THE DRUG UNDER DIFFERENT STORAGE CONDITIONS

Drug	Absorbance (A) under different storage conditions		
	Labelled condition	Ice-point	Exposed to sunlight
Tinidazole	0.7782	0.9457	0.6538

Conclusion

Spectroscopic techniques have been employed for a qualitative analysis of the nitroimidazole anti-protozoan agent-tinidazole. A calculation of the intensity ratio among the specific modes of vibration, using the FTIR spectra of the sample, clearly shows that absorbance of most vibrational bands are altered when exposed to sunlight or placed at icepoint. The analysis by employing UV-Visible spectrum also supports the result of vibrational spectroscopic studies. These results clearly indicate that the quality of the drug gets altered due to storage at different condition other than the prescribed one, thus stressing the necessity of storing the drug only in the recommended condition.

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