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Vibrational Spectroscopic Investigation of Ornidazole - An Antiprotozoan Agent

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In this work, a qualitative analysis of an anti-protozoan drug ornidazole, which belongs to the potent nitroimidazole category of drugs, has been carried out using FTIR and FT Raman spectroscopic techniques. A satisfactory vibrational band assignment of the drug has been made based on the postion, shape and relative intensity of the recorded spectra, in correlation with bands assigned in structurally related molecules. The stress degradation behaviour of the drug has been studied by employing FTIR spectroscopy.

Key Words: Qualitative analysis, Nitroimidazole, FTIR spectroscopy, Internal standard calculation.

INTRODUCTION

Medicinal chemists, pharmacologists, analytical chemists and medical professionals have jointly paved the way to combat and reduce the sufferings of human beings. In this integrated effort, the role of an analyst in checking the chemical purity of pharmaceutical substances and drugs made using them has become very vital. Quality testing of drugs, their stress degradation behaviour, chemical and biological assay, can all be considered as different aspects of product safety research and development, since they give the analyst an idea of final dosage form that is available for direct patient's usage. Literature survey reveals that exhaustive work has been done on using spectroscopic techniques like FTIR, FT Raman, UV-Visible, NMR and spectrometry for qualitative and quantitative analysis of pharmaceutical products[1-3].

In present work, vibrational spectroscopy has been used for qualitative and quantitative analysis of nitroimidazole antiprotozoan drug, ornidazole. The constant interest in nitroimidazoles was originally caused by the discovery of the naturally occurring antibiotic azomycin (2-nitromidazole). Concerning the activity of nitroimidazoles against pathogenic microorganisms, several investigations towards structure-activity relationships of this substance class were accomplished and led to 1-alkylated, 4- and 5-nitroimidazoles, which showed the 5-nitro compounds were

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more effective than their 4-nitro isomers. Ornidazole (Fig. 1) belongs to this potent nitroimidazole category of drugs. The latest developments in this field of research show that the nitroimidazole moiety is used as a substructure in pharmacologically relevant compounds *i.e. bis*(nitroimidazolyl) alkane-carboxamides as hypoxia-selective antitumour agents or as ligands in organometallic compounds *i.e.* in osmium(III) complexes $[Os^{3+}(L)_3]$ as antiparasitic agents[4]. Ornidazole has a nitroimidazole-based nucleus with a 2-hydroxy-3-chloro-propyl group, in position 1 and a methyl group in position 2. It has the IUPAC name 1-chloro-3-(2-methyl-5-nitroimidazole-1-yl) propan-2-ol and a molecular weight of 219.625 g/mol[5]. It is more effective than metronidazole and tinidazole against amoebiasis and equally as effective as metronidazole in relation to trichomoniasis[6].

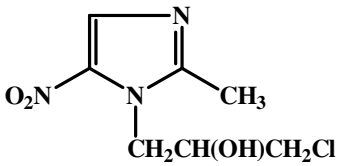


Fig. 1. Structure of ornidazole

EXPERIMENTAL

High-grade pure samples of ornidazole were procured from Dr. Ceeal Analytical Lab, Chennai and were used 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 frequencies of all the sharp vibrational bands are accurate to ± 1 cm^{-1}.

RESULTS AND DISCUSSION

Qualitative analysis: The vibrational spectrum of a compound is the superposition of the absorption bands of specific functional groups. Raman and infrared spectroscopes are complementary techniques, but differ such that each is capable of providing information not easily obtainable from the other. The functional groups present in ornidazole were identified and a satisfactory vibrational band assignment of the drugs were made by comparing the spectra of similar compounds and also by observing the nature, shape and intensity of the vibrational bands in the spectra. The vibrational band assignment of the compound is presented in Table-1.

Chlorine vibrations: Halogen containing compounds absorb strongly over a wide range of 1400-500 cm^{-1} due to C-X stretching vibrations. The more massive the halogen, the lower is the frequency of absorption. Survey of literature reveals that in niclosamide, the C-Cl stretching vibrations have resulted in a medium strong band at 744 cm^{-1}, whereas, in the antifungal drug flucytosine[7], the halogen involved

TABLE-1
VIBRATIONAL FREQUENCY ASSIGNMENT FOR ORNIDAZOLE

Frequency (cm^{-1})		Vibrational band assignment
FTIR	FTRaman	
3130(w)	3127(w)	OH stretching
2956(w)	2966(w)	Aliphatic CH_2 stretching
2913(w)	-	Aliphatic CH_2 stretching
1522(s)	1531(w)	Aromatic C=C/C=N stretching
-	1486(m)	Aromatic C=C/C=N stretching/aliphatic CH_2 in-plane deformation
1455(vs)	-	Aromatic C=C/C=N stretching/aliphatic CH_2 in-plane deformation
-	1421(w)	Aromatic C=C/C=N stretching/aliphatic CH_2 in-plane deformation
-	1385(m)	C=N stretching
1366(vs)	1364(ms)	C=N stretching
1301(vs)	-	NO_2 stretching
1265(vs)	1270(m)	CH_2 wagging
1191(vs)	1189(vs)	CH_2 wagging/C-OH stretching
-	1149(w)	Aromatic C-N stretching
1137()	-	Aromatic C-N stretching
1123(vs)	-	Aromatic C-N stretching
1046(w)	-	Aromatic C-N stretching
947(vw)	-	N-CH_2 stretching
-	859(vw)	Aromatic C-N-C deformation
829(m)	828(w)	Aromatic C-N-C deformation
786(m)	793(w)	C-Cl stretching
743(w)	-	NO_2 in-plane deformation
680()	689(vw)	OH out-of-plane deformation
-	669(vw)	OH out-of-plane deformation
-	568(vw)	C-Cl bending
550(vw)	-	C-Cl bending
517(w)	523(vw)	C-N=C deformation/ NO_2 out-of-plane deformation
463(w)	-	N-C=C deformation

is fluorine, causing the C-X stretching vibration to occur at a much higher frequency of 1123 cm^{-1}. In general, when several chlorine atoms are attached to one carbon atom, the bond is usually more intense and at the higher frequency end of the assigned limits. Bands occuring at 711 cm^{-1} in chloroxylenol and at 632 cm^{-1} in clonidine have been reported to occur due to C-Cl stretching vibration by Gunasekaran and his co-workers[8]. Similar bands in thiadiazoles occur at 777 cm^{-1} and at 764 cm^{-1}, 768 cm^{-1} in thiazolidines[9]. Using the above as reference, band of medium intensity occurring at 786 cm^{-1} in the FTIR spectrum and at 793 cm^{-1} in the FTIR spectrum of ornidazole has been assigned to C-Cl stretching mode of vibration and its first overtone is traced at 1625 cm^{-1}.

Hydroxy group vibrations: The value of OH stretching is a measure of the strength of a hydrogen bond. The stronger the hydrogen bond, the longer is the OH bond, the lower the vibration frequency and broader and more intense will be the

absorption band[10]. In their analysis of some compounds of pharmaceutical interest, Gunasekaran and his co-workers[11] have assigned OH group vibrations at 3250, 3072 and 3218 cm^{-1}. Along the same line, in the current work, the weak band appearing at 3434 cm^{-1} in ornidazole is attributed to OH vibrations. The appearance of the band on the lower frequency end of the range and also its broad nature suggests the presence of intermolecular hydrogen bonding in the compound. Alcohols and phenols in the liquid state display broad OH out-of-plane bending vibrations of the bonded OH group in the region[12] from 770 to 650 cm^{-1}. The OH out-of-plane bending vibrations of the compound under study are traced at 680 cm^{-1} in the FTIR spectrum and at 689 cm^{-1} in FT Raman spectrum.

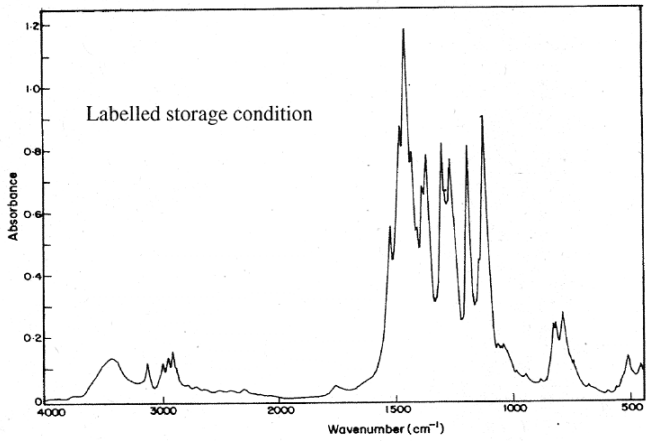
Ring vibrations: Thiazoles, iminocarbonates show C=N stretching vibrations in the region 1689-1471 cm^{-1}. While pyridine shows 4 bands in this region, furans and pyrroles display 2 to 4 bands in the same region[13]. Bands of variable intensity that occur in the range 1660-1450 cm^{-1} in imidazoles are due to C=N and C=C stretching vibrations and are named as imidazole-I bands. 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. In mercaptopurine[13], a drug used in the treatment of leukemia, the C=N stretching vibrations of the imidazole group has been traced at 1520 cm^{-1} and C-N vibrations at 1120 and 1224 cm^{-1}. Following this, in ornidazole, the C=N/C=C vibrations are assigned to bands occurring at 1522, 1455 and 1366 cm^{-1}. The corresponding lines in the FT Raman spectrum are observed at 1523 and 1477 cm^{-1} and at 1531 and 1364 cm^{-1}. The bands observed at frequencies 463 and 507 cm^{-1} in ornidazole are assigned to C=C-N and N-C=N ring deformations, respectively.

CH vibrations: The bending vibrations of CH bands of methyl and methylene groups occur at frequencies around 1465 and 1380 cm^{-1} as a medium intense band. 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[12]. In ornidazole the methylene bending vibrations are traced at 1455 cm^{-1}. The CH_2 wagging mode in compounds with a CH_2X group gives rise to a strong band whose frequency depends on X, X being a halogen atom. When X is replaced by CH_2Cl, the range in which the bands are observed[14] is 1300-1250 cm^{-1}. In the present study, the CH_2 wagging mode of vibration is assigned to the frequencies 1265 and 1191 cm^{-1}.

Other vibrations: Bands of strong intensity in the region 1260-1000 cm^{-1} of the spectrum occur due to the C-O stretching vibrations. These bands are sensitive to the nature of the substituents bonded to the carbinol carbon. Consequently, the bands can be used to obtain information on the nature of the hydroxy compound. In ornidazole, where a secondary alcohol functional group is present, the C-OH stretching vibration manifests itself as a very strong band at the frequency 1191 cm^{-1}. This is in agreement with values assigned for the same vibration by earlier workers on

similar compounds[15]. In caffeine and theophylline, the C-N vibrations due to the methyl group attached to nitrogen atom in the side chain is observed in the range 900 cm^{-1}, while in N-methyl pyridinium ion it is present[16] at around 1100 cm^{-1}. In ornidazole the N-CH_2 vibrations are assigned to frequency 947 cm^{-1}.

Quantitative analysis: The aim of the current investigation is to study the degradation behaviour of the compound ornidazole when exposed to different environmental conditions. The British pharmacopoeia recommends the drug be stored in well-closed light resistant container[17]. In the present investigation, the behaviour of the drug that was stored under the prescribed storage condition, with those exposed to altered conditions has been compared. The FTIR spectra of the samples have been recorded for the pure drugs stored in (i) well-sealed light resistant container (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 and are given in Fig. 2. The degradation behaviour of the drug was analyzed by comparing



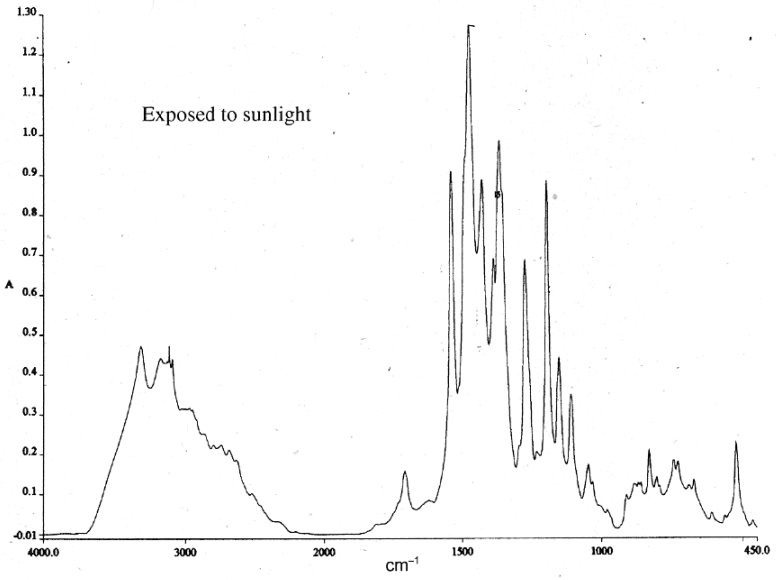


Fig. 2. FTIR spectra of ornidazole under different storage conditions

the sets of internal standards that were arrived at from absorbance ratios of the sample kept at suitable storage condition at specific modes of vibration, with those of the samples exposed to different environmental conditions. Table-2 provides the set of vibrational modes chosen for internal standard evaluation and their corresponding absorbance values. The intensity ratios of these selected modes of vibration with respect to other modes under various environmental conditions to which the drug was exposed to are given in Table-3. The analysis leads us to conclude that exposing the drug to extreme environmental conditions, rather than storing it as recommended by pharmacopeias will lead to a change in the quality of the drug.

TABLE-2
ABSORBANCE FOR CERTAIN MODES OF VIBRATION UNDER DIFFERENT CONDITIONS OF STORAGE FOR ORNIDAZOLE

Frequency (cm^{-1})	Absorbance			Assignments
	Labelled condition	Exposed to sunlight	At ice point	
3130	0.145	0.458	0.415	OH stretching
1522	0.556	0.913	0.905	C=C/C=N stretch
1425	0.775	0.875	0.850	C=C/C=N stretch
1191	0.805	0.865	0.875	CH_2 wag/C-OH stretch
1046	0.195	0.164	0.175	CN stretching
829	0.241	0.198	0.200	CNC deformation
743	0.153	0.175	0.162	NO_2 in-plane deformation

TABLE-3
INTERNAL STANDARD EVALUATION FOR ORNIDAZOLE

Conditions of exposure	Internal standard of specific modes of vibration at 3130 cm^{-1}						
	3130/ 3130	1522/ 3130	1425/ 3130	1191/ 3130	1046/ 3130	829/ 3130	743/ 3130
Labelled condition	1.0000	3.7931	5.3448	5.5172	1.3448	1.6552	1.0345
Exposed to sunlight	1.0000	2.0121	1.9444	1.9222	0.3556	0.4445	0.3889
At ice point	1.0000	2.0111	1.8889	1.9444	0.3889	0.4444	0.3556
	Internal standard of specific modes of vibration at 1522 cm^{-1}						
	3130/ 1522	1522/ 1522	1425/ 1522	1191/ 1522	1046/ 1522	829/ 1522	743/ 1522
Labelled condition	0.2636	1.0000	1.4091	1.4545	0.3545	0.4364	0.2727
Exposed to sunlight	0.5442	1.0000	0.9722	0.9611	0.1778	0.2234	0.1944
At ice point	0.4972	1.0000	0.9392	0.9669	0.1934	0.2210	0.1768
	Internal standard of specific modes of vibration at 1425 cm^{-1}						
	3130/ 1425	1522/ 1425	1425/ 1425	1191/ 1425	1046/ 1425	829/ 1425	743/ 1425
Labelled condition	0.1971	0.7097	1.0000	1.0323	0.2516	0.3097	0.1935
Exposed to sunlight	0.5143	1.0286	1.0000	0.9886	0.1829	0.2263	0.2045
At ice point	0.5294	1.0647	1.0000	1.0294	0.2059	0.2353	0.1882
	Internal standard of specific modes of vibration at 1191 cm^{-1}						
	3130/ 1191	1522/ 1191	1425/ 1191	1191/ 1191	1046/ 1191	829/ 1191	743/ 1191
Labelled condition	0.1813	0.6875	0.9688	1.0000	0.2438	0.3009	0.1875
Exposed to sunlight	0.5202	1.0405	1.0116	1.0000	0.1850	0.2289	0.2023
At ice point	0.5143	1.0343	0.9714	1.0000	0.2286	0.2207	0.1829
	Internal standard of specific modes of vibration at 1046 cm^{-1}						
	3130/ 1046	1522/ 1046	1425/ 1046	1191/ 1046	1046/ 1046	829/ 1046	743/ 1046
Labelled condition	0.7436	2.8205	3.9744	4.1026	1.0000	1.2308	0.7692
Exposed to sunlight	2.8125	5.6250	5.4688	5.4063	1.0000	1.2375	1.0938
At ice point	2.5714	5.1714	4.5871	5.0325	1.0000	1.1429	0.9143
	Internal standard of specific modes of vibration at 829 cm^{-1}						
	3130/ 829	1522/ 829	1425/ 829	1191/ 829	1046/ 829	829/ 829	743/ 829
Labelled condition	0.6042	2.2917	3.2292	3.3333	0.8125	1.0000	0.6250
Exposed to sunlight	2.2727	4.5455	4.4192	4.3687	0.8081	1.0000	0.8838
At ice point	2.2500	4.5250	4.2500	4.3750	0.875	1.0000	0.8090
	Internal standard of specific modes of vibration at 743 cm^{-1}						
	3130/ 743	1522/ 743	1425/ 743	1191/ 743	1046/ 743	829/ 743	743/ 743
Labelled condition	0.9667	3.6667	5.1667	5.3333	1.3083	1.6098	1.0000
Exposed to sunlight	2.5714	5.1429	5.0666	4.9429	0.9143	1.1314	1.0000
At ice point	2.8125	5.6563	5.3125	5.4688	1.0938	1.2544	1.0000

Conclusion

FTIR spectroscopic technique has been employed for a qualitative analysis of the nitroimidazole anti-protozoan agents- ornidazole. The various functional groups present in the compound were identified and a vibrational band assignment for the compound was made by comparing the vibrational spectra of similar compounds. For quality analysis of the chosen drugs, the behaviour of the drugs there were stored under the prescribed storage condition, with those exposed to altered conditions has been compared. The FTIR spectra of the samples have been recorded for the pure drugs stored as recommended by the manufacturer and the drugs exposed to sunlight and placed at ice point. Internal standard evaluations done clearly indicate that most vibrational bands are altered when exposed to sunlight or placed at ice point. This is an indication of the change in the quality of the drug due to change in the storage condition. The results are an indication of the need to store the drug as prescribed, failing which its quality may be altered, resulting in loss of potency of the drug.

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