SPECTRAL ANALYSIS OF HYDROCORTISONE

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ABSTRACT

In the present investigation, FTIR, FTRaman and UV-Vis spectroscopic methods have been employed successfully on Hydrocortisone. It is a corticosteroid. This medicine is used for the treatment of inflamed areas of the body, organ transplantation, brain swelling, ulcerative colitis, spinal cord injuries. The structural analysis and internal standard calculation of the drug have been studied using FTIR and FTRaman methods. Also the quality analysis of the drug has been analyzed using UV-Visible spectroscopic method and the results are discussed.

Key words: Hydrocortisone, FTIR, FTRaman, UV-Visible spectroscopy

1. INTRODUCTION

Spectroscopy is mainly concerned with the interaction of radiation and matter may result in intensity of electromagnetic radiation with frequency (or wavelength). Infrared and Raman spectroscopic methods are being extensively used to identify the structural groups present in a compound. UV- Visible spectroscopic methods have been employed to investigate the samples of biological interest. In the recent past an extensive work has been carried out in the analysis on the samples of pharmaceutical importance (Gunasekaran, 2003; Gunasekaran and Abitha, 2003; Gunaskearan and Kanjanadevi, 2004; Krishnakumar et al, 1997; Gunasekaran et al., 1993). In the present work, FTIR, FTRaman and UV-Vis spectroscopic methods have been employed successfully on Hydrocortisone and the results obtained are discussed. Thus the spectroscopic methods have been used in the analysis of drugs and hence it has been established that the sophisticated spectrophotometers are used as powerful tools for quality control in pharmaceutical laboratories.

2. PHARMACEUTICAL DATA AND IMPORTANCE OF HYDROCORTISONE

Hydrocortisone is one of the steroid hormones produced by the adrenal gland (adrenal cortex) which plays a complex role in regulating body functions. Hydrocortisone Tablets are taken as a replacement for the natural hormone where this is deficient either because there is a primary failure (or insufficiency) of Hydrocortisone production by the adrenal cortex gland (Addison's disease) or adrenal failure. Hydrocortisone puts down the body's reduces swelling, redness, itching and other symptoms of allergy. It also reduces the body's ability to fight infection. Hydrocortisone is 11â, 17á, 21-trihydroxypregn-4-ene-3, 20-dione.The molecular formula is $C_{21}H_{30}O_5$. It is white to practically white, crystalline powder. It is sparingly soluble in ethanol and in acetone; slightly soluble in chloroform, very slightly soluble in ether. It should be stored in well-closed light resistant containers. Orally, in the treatment of adrenocortical insufficiency, 20 to 30 ng daily, in divided doses; by intramuscular injection or by slow intravenous injection or infusion. 100 to 500 mg 3 to 4 times in 24 hours or as required (Pharmacopoeia, 1996).

3. EXPERIMENTAL

The pure sample of Hydrocortisone in powder form was obtained from Sigma Aldrich Chemical Company, USA and used as such without further purification. FTIR spectrum of the sample was recorded on Perkin Elmer model spectrophotometer in the region 4000 – 400 cm^{-1} using KB pellet and the FT-Raman spectrum was recorded in the region 4000–100 cm^{-1} using Bruker IFS 66V model spectrophotometer at the SAIF, I.I.T., Chennai. UV-Visible spectral measurements have been made Using an Elico SL 164 - UV-Visible double spectrophotometer, Department of chemistry, St.Joseph's College of Arts and Science, Cuddalore-1. The molecular structure of Hydrocortisone is shown in Fig.1. The vibrational spectra of Hydrocortisone are presented in Fig.2

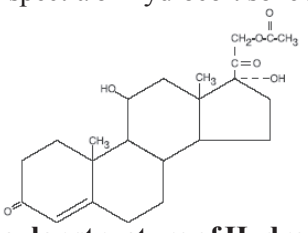

Fig.1. Molecular structure of Hydrocortisone

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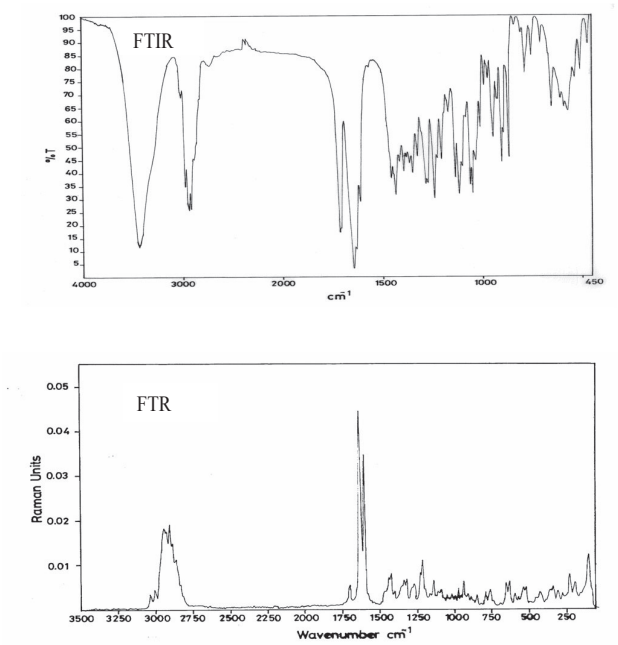

Fig.2. Vibrational spectra of Hydrocortisone

4. VIBRATIONAL ANALYSIS

From the FTIR spectra, vibrational band assignments have been made in terms of fundamentals, overtones and combinations on the molecular structure of the pharmaceutical compounds under study. A qualitative study on the vibrational band assignments of the drugs is given below. The vibrational assignments are presented in Table 1.

C-H vibrations

The substituted benzene gives rise to C-H stretching C-H in-plane bending and C-H out-of-plane bending vibrations. The aromatic compounds commonly exhibit multiple weak bands in the region 3100-3000 cm^{-1} due to aromatic C-H stretching vibrations. The most prominent and most informative bands in the spectra of aromatic compounds occur in the low-frequency range between 900-675 cm^{-1}. These strong absorption bands result from the out-of-plane bending of the ring C-H bonds. In-plane bending bands appear in the 1300-1000 cm^{-1} region (Robert et al., 1997). The vibrational bands presented at 2825, 2954, 3001 and 3027 cm^{-1} in the FTIR spectra of Xanthine derivatives have been identified by (Gunasekaran et al., 2005). (Krishnakumar and Balachandran, 2004) have been reported the C-H in-plane bending vibrations at 1210,1070,1026,1000 and 935 cm^{-1} and C-H out-of-plane bending vibrations at 926,880,810,765 and 744 cm^{-1} for 2-hydroxy-3-methoxybenzaldehyde thiosemicarbozone compound. Hence in the present investigation, the FTIR band observed at 3018 cm^{-1} has been assigned to C-H stretching vibration. The bands at 966,942,918,900,864,831,781 and 700 cm^{-1} and bands observed at 1133, 1058 and 1047 cm^{-1} have been assigned to C-H out-of-plane bending and C-H in-plane bending vibrations respectively in the present work.

Table 1 Vibrational band assignments of Hydrocortisone

Frequency (cm^{-1})		Assignment
FTIR	FTRaman	
463 (vw)	-	C-C-O in plane bending
500 (w)	-	C-C-C out plane bending
527 (w)	-	C-C-C out of plane bending
566 (w)	551 (vw)	C-C-C out plane bending
648 (w)	647 (vw)	C-C-C in plane bending
700 (vw)	-	C-H out of plane bending
746 (vw)	-	C-O-H out plane bending
781 (vw)	778 (vw)	C-H out plane bending
831 (vw)	-	C-H out of plane bending
864 (m)	757 (w)	C-H out of plane bending
900 (m)	-	C-H out of plane bending
918 (w)	-	C-H out of plane bending
942 (w)	-	C-H out of plane bending
966 (w)	955 (vw)	C-H out of plane bending
985 (w)	-	CH_3 twisting
1006 (m)	-	"Ring breathing"
1031 (m)	-	C-C-C in plane bending
1047 (s)	-	C-H in plane bending
1058 (s)	-	C-H in plane bending
1133 (s)	-	C-H in plane bending
1166 (w)	1156 (vw)	CH_3 rocking
1202 (m)	-	CH_2 wagging
1237 (s)	1230 (vw)	C-C stretching
1281 (s)	1286 (vw)	C-O stretching
1322 (m)	-	C-C stretching
1348 (m)	1337 (vw)	CH_3 sym.bending
1391 (m)	-	C-O-H in plane bending
1432 (s)	1439 (vw)	CH_3 asym.bending
1453 (s)	-	CH_2 symmetric bending
1570 (w)	-	aromatic C=C stretching
1610 (s)	1615 (s)	aromatic C=C stretching
1642 (vs)	1647 (vs)	aromatic C=C stretching
1713 (vs)	1714 (vw)	C=O stretching
2894 (m)	-	CH_2 sym.stretching
2912 (s)	2918 (w)	CH_3 sym.stretching
2934 (s)	-	CH_2 asym.stretching
2970 (s)	2956 (w)	CH_3 asym.streching
3018 (w)	-	aromatic C-H stretching
3437 (vs)	-	O-H stretching

vs- very strong, s- strong, m-medium, w- weak , vw- very weak

Ring Carbon vibrations (skeletal vibrations)

Skeletal vibrations, involving carbon to carbon stretching with in the ring, absorb in the 1600-1585 cm^{-1} and in the 1500-1400 cm^{-1} region. The skeletal bands frequently appear as doublets, depending upon the nature of the ring substituents (Robert et al., 1997). The C-C ring breathing mode a_{1g} (995 cm^{-1}) and C-C-C in-plane bending non-degenerate b_{1u} (1010 cm^{-1}) vibrations of benzene give rise to combined modified modes under C_{2v} symmetry. In substituted benzene, one mode is observed at about 800 cm^{-1} and the other appears around 1000 cm^{-1} and the breathing mode is sensitive to the substitution. The carbon out-of-plane bending vibrations are derived from the non-degenerate b_{2g} and degenerate e_{2u} modes of benzene. Based on these assignments, in the present investigation, the FTIR bands observed at 1570, 1610 and 1642 cm^{-1} have been assigned to aromatic C=C stretching vibrations. The band appeared at 1006 cm^{-1} and band at 1031 cm^{-1} have been designated to "ring breathing" and C-C-C in-plane bending vibrations respectively. The FTIR bands at 500,527 and 566 cm^{-1} are assigned to C-C-C out-of-plane bending vibrations.

C=O vibrations

A great deal of structural information of a molecule can be derived from the exact position of the carbonyl stretching absorption peaks. The interaction of the carbonyl group with a hydrogen donor group does not produce such a drastic change in the frequency of C=O stretch as does by the interaction of N-H stretch. If a compound contains a carbonyl group, the absorption caused by C=O stretching is generally among the strongest present. The carbonyl group at the fourth position of cyclohexanone ring may interact with the other groups of the molecule and give rise to a complex pattern of spectrum. In general, the characteristic C=O stretching vibrations of cyclic ketones are found over a relatively wide rand depending on the ring size. (Chithambarathanu, 2002) identified the carbonyl stretching vibrations at 1707 cm^{-1} in 2,6-diphenyl-3-methyl piperidone . In the vibrational analysis of 2, 3,5-tri-iodobenzoic acid (Mohd Chaman and Verma, 2003) assigned the C=O stretching vibration at 1711 cm^{-1}. Consideration of these factors led to assign the IR band appeared at1713 cm^{-1} and the corresponding Raman band at 1714 cm^{-1} C=O stretching vibrations in the present investigation.

O-H and C-O vibrations

The characteristic bands observed in the spectra of alcohols and phenols result from O-H stretching and C-O stretching. These vibrations are sensitive to hydrogen bonding. The C-O stretching and O-H bending modes are not independent vibrational modes because they couple with the vibrations of adjacent groups. The unbonded or 'free' hydroxyl group f alcohols and phenols absorbs strongly in the 3650-3584 cm^{-1} region. Sharp, 'free' hydroxyl bands are observed only in the vapour phase or in very dilute solution in non-polar solvents. Intermolecular hydrogen bonding increases as the concentration of the solution increases, and additional bands start to appear at lower frequencies, 3550-3200 cm^{-1}, at the expense of 'free' hydroxyl band. In the present investigation the FTIR band observed at 3437 has been assigned to O-H stretching vibration.
The C-O stretching vibrations in alcohols and phenols produce a strong band in the 1260-1000 cm^{-1} region of the spectrum. The C-O stretching mode is coupled with the adjacent C-C stretching vibration; thus in primary alcohols the vibration might better be described as an asymmetric C-C-O stretching vibration. Two bands arising from C-O stretching and O-H bending appear in the spectra of carboxylic acids near 1320-1210 cm^{-1} and near 1440-1395 cm^{-1} respectively. Both of these bands involve some interaction between C-O stretching and in-plane C-O-H bending. The more intense band, near 1315-1280 cm^{-1} for dimmers, is generally referred to as the C-O stretching band and usually appears as a doublet in the spectra of long-chain fatty acids. The C-O-H bending band near 1440-1395 cm^{-1} is of moderate intensity and occurs in the same region as the CH_2 scissoring vibration of the CH_2 group adjacent to the carbonyl (Robert et al., 1997). Hence in the present work, the FTIR band appeared at 1281 cm^{-1} and FTRaman band at 1286 cm^{-1} and the IR band observed at 1391 cm^{-1} have been assigned to C-O stretching and C-O-H in-plane bending vibrations respectively. Similarly the other vibrational bands are assigned in the characteristic range.

5. STORAGE CONDITION

Maintaining proper storage conditions for health commodities is vital to ensuring their quality. As the infrared spectrum of a compound is the superposition of absorption bands of specific functional groups, some fundamental modes of vibration are identified in the

spectrum of Hydrocortisone. The respective absorbance values are noted in the four different conditions of exposure viz. normal (Light Resistance Container- LRC), Ice point, Sun light and Infrared. The various modes are calculated for all the four conditions, which represents the internal standards of the drug for that particular position of absorption. The sets of internal standards of the drug under the different storage conditions are compared with that of the light resistance container to check whether any change has taken place due to different storage condition. The FTIR spectra of Hydrocortisone exposed to Sunlight and IR radiations are presented in Fig.3. The Vibrational frequencies for the specific modes of vibration chosen for internal standard calculation are listed in Table 2. The Internal standard calculation at different storage conditions for the drug Hydrocortisone were calculated from the FTIR spectral data and are listed in Tables 3. From the tables, we observed that the internal standard calculation for the various storage conditions showed the significant change with the drug stored in the light resistance container.

Table 2: Specific modes of vibration of Hydrocortisone

Frequency cm^{-1}	Vibrational Assignment	Absorbance For			
		LRC	ICE	SUN	IR
864	C-H out of plane bending	0.2108	0.2197	0.1512	0.2235
899	C-H out of plane bending	0.2298	0.2367	0.1659	0.2259
1132	C-H in plane bending	0.4328	0.2698	0.3029	0.4292
1237	C-C stretching	0.4363	0.4587	0.3158	0.4309
1271	C-O stretching	0.3994	0.4275	0.2986	0.3827
1320	C-C stretching	0.3854	0.2316	0.2395	0.3078
1432	CH_3 asym.bending	0.4781	0.4918	0.3487	0.4719
1643	aromatic C=C stretching	1.5000	1.509	1.1570	1.4680
1714	C=O stretching	0.6177	0.6095	0.4086	0.5867
3434	O-H stretching	1.1320	1.4780	1.4890	1.5060

Table 3: Internal standard calculation of Hydrocortisone under different storage Conditions

Storage condition	Internal standard calculation for the specific modes of vibrations					
	864/899	899/1132	1432/1643	1643/1714	1714/3434	1432/3434
Stored at LRC	0.9173	0.5310	0.3183	2.4284	0.5457	0.4223
Stored at ICE Point	0.9281	0.8773	0.3259	2.4758	0.4124	0.3327
Exposed to Sun	0.9114	0.5477	0.3014	2.8316	0.2744	0.2342
Exposed to IR radiation	0.9894	0.5263	0.3215	2.5021	0.3896	0.3133

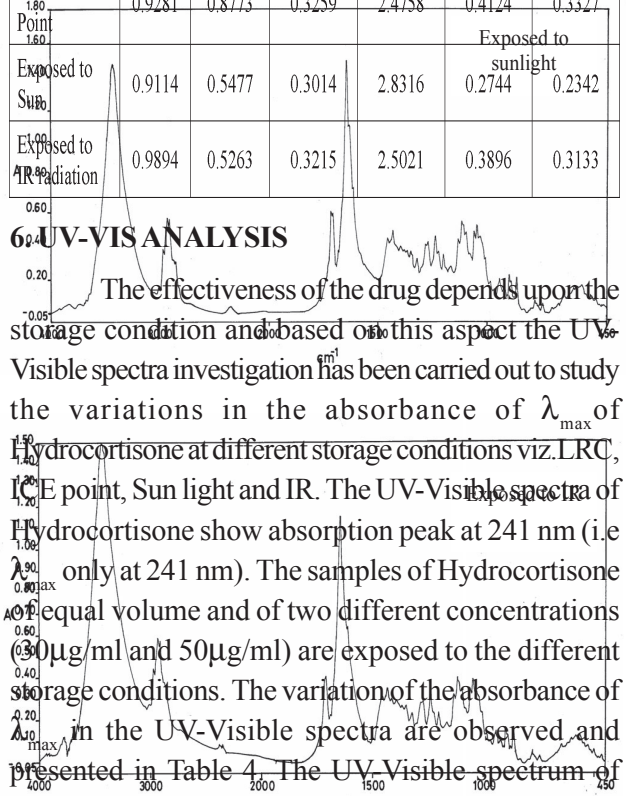

Fig.3. FTIR spectra of Hydrocortisone Exposed to Sun light & IR

6. UV-VIS ANALYSIS

The effectiveness of the drug depends upon the storage condition and based on this aspect the UV-Visible spectra investigation has been carried out to study the variations in the absorbance of λ_{max} of Hydrocortisone at different storage conditions viz.LRC, ICE point, Sun light and IR. The UV-Visible spectra of Hydrocortisone show absorption peak at 241 nm (i.e λ_{max} only at 241 nm). The samples of Hydrocortisone of equal volume and of two different concentrations (30µg/ml and 50µg/ml) are exposed to the different storage conditions. The variation of the absorbance of λ_{max} in the UV-Visible spectra are observed and presented in Table 4. The UV-Visible spectrum of Hydrocortisone stored at ICE Point is presented in Fig 4.

Table 4 Variation of absorbance under different storage

Concentration (ppm)	Storage Condition	Absorbance (λ_{max} = 241 nm)
30	Light Resistance Container	1.530
	Ice Point	1.490
	Sun Light	1.340
	Infrared Radiation	1.517
50	Light Resistance Container	2.297
	Ice Point	2.399
	Sun Light	2.542
	Infrared Radiation	2.660

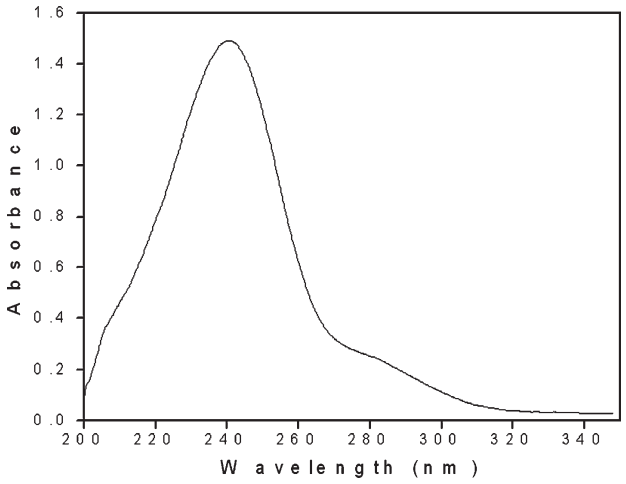

Fig.4 UV-Visible spectrum of Hydrocortisone stored at ICE Point

It is observed, that the absorbance of the drug kept under sunlight, IR radiation and Ice point shows changes to a maximum extent when compared to the absorbance at light resistance container. Hence it can be concluded that the drug under study is to be stored at LRC to retain its pharmaceutical properties.

7. CONCLUSION

The vibrational band assignments have been made on the basis of magnitude and relative intensities of the observed bands in FTIR, FTRaman spectra of Hydrocortisone. The effectiveness of the drugs depends upon the storage condition and based on this aspect, FTIR and UV-Visible spectral investigations have been carried out to study the variations in the effectiveness of the drug with different storage conditions. The best storage condition for the chosen drug is light resistance container and hence it retains its pharmaceutical properties.

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