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Research Paper

Spectroscopic Study of Characterisation of Commercial Drug and its Mixture

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The study of characterization of commercial drug was made through spectroscopic technique. Chloramphenicol is the commercial drug used as broad-spectrum antibiotic while Riboflavin is used as micronutrient with a key role in maintaining health. Many researches have been made on structural characterization of these commercial drugs. But few studies have been carried out to study drug interaction. UV-visible study shows the shift in λ_{max} of Riboflavin was observed due to interaction of chloramphenicol. An increase in absorbance is recorded at 275 nm and 199 nm treated to different proportion (1:2 and 2:1). Infrared study shows characteristics skeletal stretching modes of the semi unsaturated carbon-carbon bond leads to the appearance of a group of four bonds in the region of 1650-1450 cm^{-1}. Further shift in frequencies are observed in the interaction of Riboflavin in various combinations with Chloramphenicol. The influence of Riboflavin was more pronounced than the latter. UV and FTIR data were used for discriminant analysis which shows distinct variation existing among the drug sample as evidenced by spectroscopic technique.

Key Words: FTIR; UV Study; Commercial Drug; Characterization; Discriminant Analysis

1. Introduction

Pharmacology can be defined as the branch of science that includes history, physicochemical properties, dosage forms, methods of administration, absorption, distribution, mechanism of action, physiological and biochemical changes produced within the body, biotransformation and excretion, clinical uses, and adverse effects of the drugs. There is some structure activity relationship within the drug. The activity of drug is intimately related to its chemical structure. Knowledge about the chemical structure of a drug is useful for synthesis of new compounds with more specific actions and fewer adverse reactions. It also helps in understanding the mechanism of drug action [1]. The main objective of pharmaceutical drug analysis is to offer not only a ready reference, but also an intermediate level for the convenient analysis of pure pharmaceutical substances and their respective dosage forms wherever applicable.

Spectroscopy has recently emerged as a novel bio-medical technique that can potentially reveal a wealth of qualitative and quantitative information about a given pharmaceutical sample. The increasing use of FTIR spectroscopy demonstrates that this technique is a valuable tool owing to its high sensitivity in detecting changes in functional groups of tissue components, such as membranes, proteins, as well as for complex drug material. When infrared light is passed through a sample of an organic compound some of the frequencies are absorbed while others

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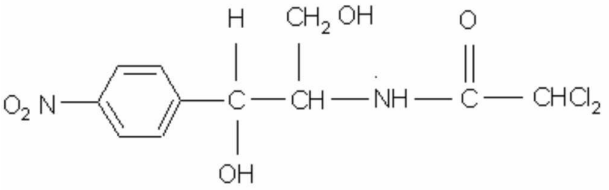
are transmitted. The variation of infrared absorbance against frequency gives the infrared spectrum. The infrared spectrum of a compound is essentially the superposition of absorption bands of specific functional groups. For qualitative analysis, the absorptions in specific frequency regions can be correlated with specific stretching and bending motions of these groups. Thus by interpreting the spectrum it is possible to state whether certain functional groups are present in a given sample. Many researches have been carried out to study the structural characterization of drugs using spectroscopic study [2-4]. The use of spectroscopic data in chemometrics is widely used nowdays because it provides an easier and more efficient way to perform the vast calculation from large data sets. Discriminant analysis (DA) is a technique for classifying a set of observations into predefined classes. The main purpose of discriminant function analysis is to predict group membership based on a interval variable. The procedure begins with a set of observations where both group membership and the values of the interval variables are known. The end result of the procedure is a model that allows prediction of group membership when variables alone are known. The second purpose of discriminant function analysis is to understand the data set and to find relationship between group membership and the variables used to predict group membership. It is typically used for one or more purposes; (i) to explore patterns of relationship in data (ii) to track properties of materials on a continuous basis. Also Chloramphenicol is a broad-spectrum antibiotic generally administrated for common diseases such as typhoid and Riboflavin a multivitamin administrated along with antibiotics for the speedy recovery of the patients. Hence keeping in view of these facts an attempt has been made to study, (i) to find the absorption position in UV-Visible spectrum, and to find any interaction existing among the drugs, (ii) the structural conformation and to assign the frequencies of different functional group of commercial tablet Chloramphenicol and Riboflavin using FTIR technique and (iii) significant changes in the sample using DA.

2. Materials and Methods

Description of Chloramphenicol Commercial Drug

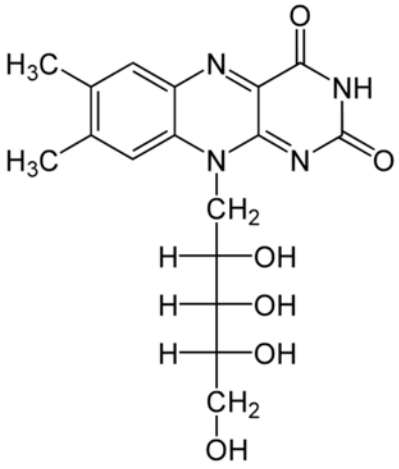
The molecular formula of Chloramphenicol is $C_{11}H_{12}Cl_2N_2O_5$ with the structure as



Chloramphenicol is considered as a broad-spectrum antibiotic and it is cheap and easy to manufacture. Chloramphenicol was primarily used in the treatment of typhoid and it is also used as universal antibiotic.

Description of Riboflavin Commercial Drug

The molecular formula of Riboflavin is $C_{27}H_{20}N_40_6$ with structure as



Riboflavin, also known as vitamin B_2 is an easily absorbed micronutrient with a key role in maintaining health. Many clinicians recommend administration of multivitamin preparations containing riboflavin in patients with vitamin deficiencies, since poor dietary habits often result in concurrent deficiencies [5].

Sample Preparation

The samples were made into fine powder. The drug powdered drug samples and KBr (all solid dry state) were kept in a oven at 25ºC in order to remove most bound water that might interfere with the measurement. Approximately 5 mg of the sample was mixed with 100 mg of dried KBr and then pressed into a clear pellet of 13 mm diameter and 1 mm thickness [6]. Absorbance spectra were recorded using Nicolet Avatar –360 FTIR spectrometer installed at Sophisticated Analytical Instrument Facility, I.I.T, Chennai. For each spectrum 100 scans were co-added, at a spectral resolution of 4 cm^{-1}. For interaction study different w/w ratio (1:1, 1:2 and 2:1) Riboflavin and Chloramphenicol were dissolved in water and then the dried sample was used for analysis. The statistical analysis was made using SPSS 16 software.

3. Results and Discussion

UV Spectral Measurements of Commercial Drugs and its Mixtures

The UV spectral measurements were carried out on Chloramphenicol and Riboflavin (Figs. 1-3). The sample was dissolved in methanol and a solution of 2% concentration was prepared and a smooth spectrum obtained. The spectral recording shows a peak at 278 nm for chloramphenicol. In the case of Riboflavin three absorbance was measured at 446 nm, 270 nm and 222 nm with the absorbance maximum of 0.16 0.43 and 0.39. In the case of Chloramphenicol the absorbance had increased to 0.615 which is measured at 278 nm.

To investigate the interaction among the drugs, spectra were recorded at different mixtures of concentration and listed in Table 1. In the case of 1:1 mixtures Riboflavin and Chloramphenicol, there is a shift in λ_{max} and the increase in absorbance is also recorded (275 nm and 199 nm), showing the influence of one drug on another (Fig. 1). But to find the exact nature of drug which influences one over the other, interaction among the drug in the ratio of 1:2 and 2:1 was studied. It was found that there is decrease in absorption in the case of 1:2 ratios but in the case of

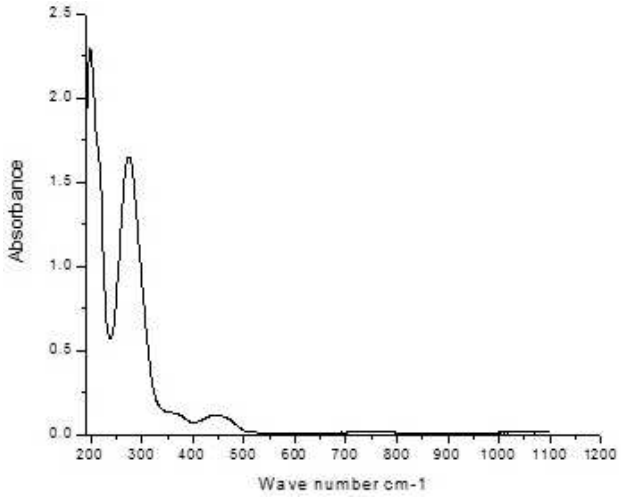


Fig. 1: UV-Visible spectra of commercial drug Riboflavin and Chloramphenicol-1:1 mixtures

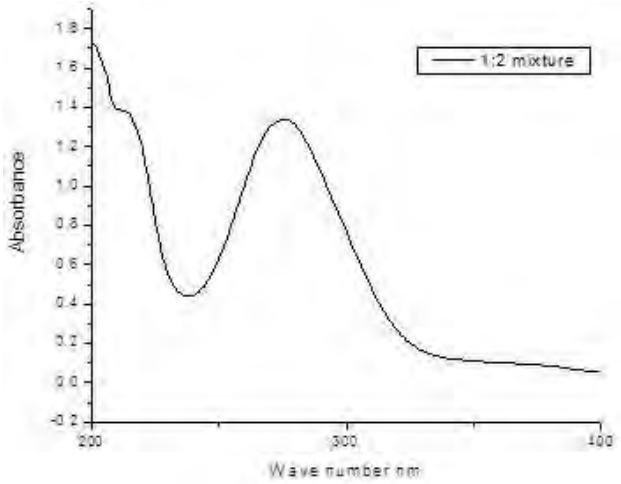


Fig. 2: UV-Visible Spectra of commercial drug Riboflavin and Chloramphenicol -2:1 mixture

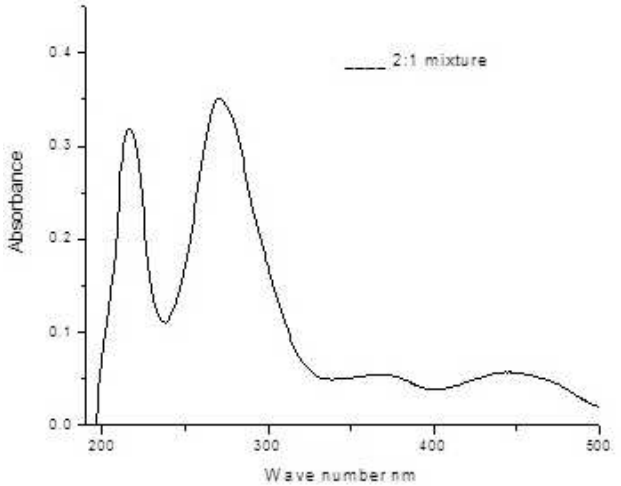


Fig. 3: UV-Visible Spectra of commercial drug Riboflavin Chloramphenicol- 1:2 mixture

Table 1: Variation of absorbance of λ_{max} of Chlorophenicol, Riboflavin and its mixtures

Drugs	λ_{max}	Absorbance	λ_{max}	Absorbance	λ_{max}	Absorbance
Chloramphenicol	-	-	278	0.615	-	-
Riboflavin	222	0.393	270	0.431	446	0.167
1:1 mixture of Riboflavin and Chloramphenicol	199	2.246	275	1.657	444	0.143
2:1 mixture of Riboflavin and Chloramphenicol	-	-	275	1.372	-	-
1:2 mixture of Riboflavin and Chloramphenicol	216	0.326	270	0.352	445	0.053

2:1 ratio the absorption corresponding to 275 nm alone was observed (Figs. 2 & 3). This corresponds to the characteristic nature of Chloramphenicol, indicating absence of characteristic nature of Riboflavin. This may be due reduction of Riboflavin while interacting with the Chloramphenicol, resulting in formation of complex structure.

Infrared Spectral Analysis of Commercial Drug-Chloramphenicol and Riboflavin

The FTIR spectra of commercial drug Chloramphenicol and Riboflavin are given in Table 2 & 3. The spectra recorded for commercial drug and their mixtures at different proportions are presented in Figs. 4&5. By observing the position, shape and relative intensities of the vibrational bands in FTIR spectra of the drug Chloramphenicol/Riboflavin a satisfactory vibrational band assignment has been made. As solid or liquid, a broad band of high intensity was observed in the region ~3400 cm^{-1} exhibiting the presence of OH group.

C-N Stretching/C-O and C=O Vibration

The C-O stretching absorption of primary alcohol is strong and occurs in the region 1071-1065cm^{-1}. For aromatic and unsaturated amines, two bands are observed at 1360-1250 cm^{-1}. The spectra of benzene and derivative substituted compound exhibit a band in the region 1220-1210 cm^{-1}. The band region 1222 cm^{-1} in IR and 1226 cm^{-1} has been assigned to C-N symmetry stretching of the compound [7].

Saturated aliphatic ketone absorbs strongly in the range 1700-1680 cm^{-1} and this band shifted from its expected position by a number of parameters due to the adjacent position. The bands due to C-O stretching vibrations are strong and occur in the region 1260 cm^{-1}. In fluorouracil the bands are observed at 1621 cm^{-1} in IR. A strong absorption band due to C=O stretching occurs in the region ~1371 cm^{-1}. Because of high intensity and the relatively interference free region in which it occurs, this band is reasonably easy to recognize. A band of medium to strong intensity may be found in the region 1325-115 cm^{-1} for aliphatic ketones [8]. Hence the bands at ~1065 cm^{-1} and 990 cm^{-1} in FTIR spectrum are allotted to C-O stretching vibration in secondary and primary alcohol.

Ring Vibration

For aromatic six member rings, there are two or three bands in this region due to skeletal vibrations, the strongest usually being about 1500 cm^{-1}. In acetaminophen the bands of strong intensity at ~1641 cm^{-1} are assigned to asymmetric and symmetric vibrations of the ring C-N stretching vibrations. The ring symmetry and asymmetry bending vibrations result in bands ~808 cm^{-1} in FT-IR [9]. The symmetrical deformation of the hydrogen atoms of a methyl group results in an absorption band in the range 1385-1370 cm^{-1} which is stable in position [10]. In the present case the band observed at ~1388 cm^{-1} in the FTIR spectrum allotted is due to CH_3/CH_2 deformation.

The fundamental study in benzene vibrations having the characteristics of skeletal stretching

Table 2: Tentative frequency assignment of commercial drug Chloramphenicol mixed at different ratios with Riboflavin

Pure drug	1:1 ratio	1:2 ratio	2:1 ratio	Frequency assignment
3401(s)	3352(s)	3356(s)	3372(s)	OH stretching
2927(m)	2925(m)	2910(s)	2925(m)	Aromatic C-H stretching
1732(s)	-	-	-	C = stretch amide II
1647(vs)	1686(vs)	1686(s)	1685(s)	Amide I
1550(s)	1561(s)	1561(s)	1559(s)	N-0 stretch ($ArNo_2$)
1071(s)	1066(s)	1064(s)	1065(s)	C-O stretch (Primary alcohol)
876(m)	842(m)	817(s)	818(w)	C-N stretch (Out of plane NH-bending)
1346(s)	1348(s)	1348(s)	1348(m)	C=O stretching mode

Table 3: Tentative frequency assignments of commercial drug Riboflavin mixed at different ratios with Chloramphenicol

Pure drug	1:1 ratio	1:2	2:1	Frquency assginment
3400(s)	3352(s)	3356(s)	3372(s)	N-H and O-H stretching, and possibly intra molecular hydrogen bonded –OH groups
1650(s)	1686(vs)	1686(m)	1685(m)	C=O Diene, triens; C=N-
1562(s)	1561(s)	1561(s)	1559(s)	Aryl H- vibration frequencies
1450(s)	1454(m)	1412(s)	1414(s)	-C-H deformations
1388(s)	-	-	-	-CH3 symmetrical deformations
1240(m)	1244(s)	1243(s)	1245(m)	-O-H bending
1065(s)	1066(s)	1064(s)	1065(s)	C-O stretching (2^o alcohol)
990(m)	-	-	-	C-O stretching (1^o alcohol)
817(m)	842(m)	817(s)	818(w)	Meta di-substituted aromatic ring ortho-di-substiuted aromatic ring due to –H, moving out of plane of the benzene ring

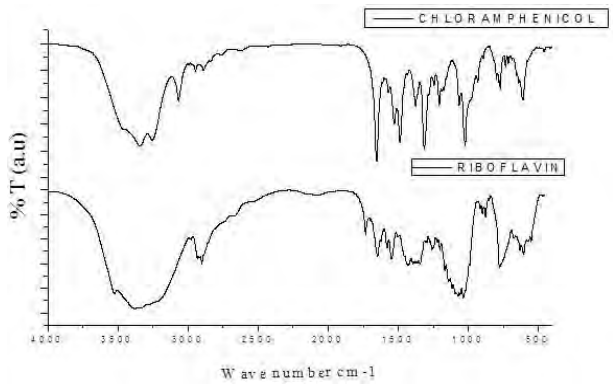


Fig. 4: FTIR spectrum of commercial drug Chloramphenicol and Riboflovin

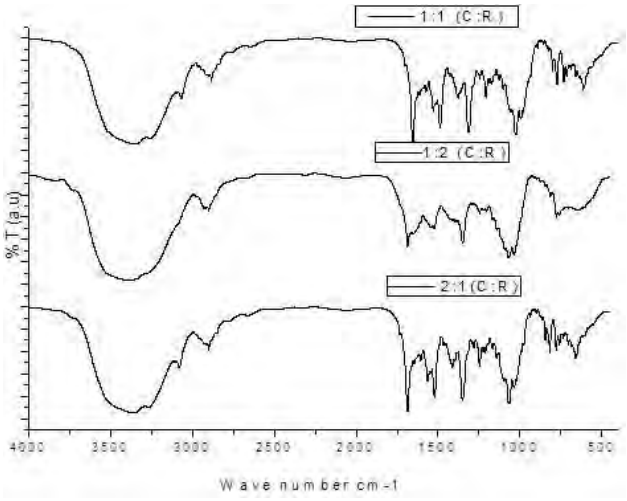


Fig. 5: FTIR spectrum of commercial drug Chloramphenicol and riboflavin drug treated at different proportion

appears in the range of 1650-1450 cm^{-1}. The bands observed at ~1562 cm^{-1} and ~1550 cm^{-1} are assigned to aromatic ring stretching vibrations [11]. Aromatic ring deformation vibrations occur below 700 cm^{-1} and normally the plane deformation vibrations are at a higher frequency than the out of plane deformations. Thus a satisfactory vibrational band assignment has been made available for acetaminophen through infrared spectroscopy. The remaining bands observed in the spectra may be due to overtones and combinations of fundamental vibrations. Further shift in frequencies and change in intensities are observed in the interaction of Riboflavin at various combinations with Chloramphenicol. The result shows that the influence of riboflavin is more pronounced than chloramphenicol. It was evident from the study that in the case of 2:1 ratio of Riboflavin and chloramphenicol, disappearance of frequency at ~1388 cm^{-1}, corresponds to the symmetrical deformation – CH_3, which may interact with chloramphenicol resulting in formation CH_2Cl_2. Further the band corresponds to ~990 cm^{-1} (primary alcohol-CH_2OH) of Riboflavin and may interact with the ~1732 cm^{-1} (CN stretching/NH bending) of Chloramphenicol resulting in elimination of water molecule and the possibility of forming complex structure. These results are further confirmed by the UV-Visible study where the absence λ_{max} corresponding to 275 nm in the 2:1 ratio of Riboflavin and Chloramphenicol was observed.

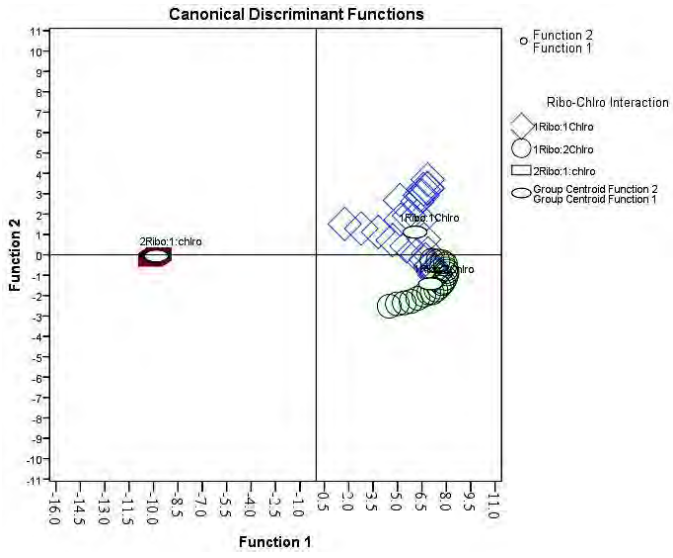


Fig. 6: Canonical Discrimination function of UV data of commercial drug at different ratio

4. Chemometric-Discriminant Analysis

UV Spectral Data

A data matrix was constructed with a column representing drug samples (9 objects) and rows corresponding to wave number (78 variables). These variables belonging to the same group were analyzed. These groups were then termed a 'category'. The three samples (each with three replicates) were used to populate the input matrix on the study. The charts present scatter plots showing the discriminant scores of nine samples. Fig. 6 shows that DA resulted in better classification of drug samples according to their interaction. In the replication experiments, each drug sample was plotted at almost exactly the same point, to demonstrate that the drugs of different trademark can reproducibly be separated and clearly classified. To investigate the potential of chemical components for the drug classification, table of equality of group means (Table 4) was generated by selected univariate ANOVAs. This indicates whether there is a statistically significant difference among the dependent variable means (group) for each independent variable. The Wilks' Lambda was used as a statistical criterion to add or remove variables from the analysis. In the ANOVA, the smaller the Wilks's Lamba, the more important is the independent variable to the discriminant function. As a result we found that absorption of UV spectra around 260-280 nm and 440-450 nm made the drug sample separable into different areas of the scatter plot as shown in Fig. 6.

FTIR Spectral Data

The 810 inputs were used to populate the input matrix of the study. The Fig. 7 shows the scatter plots

Table 4: Test of quality of group means for UV data

Sample	Wilks' Lambda	F	Sig.
1Ribo:1Chlro	.067	522.601	.000
1Ribo:2Chlro	.054	653.371	.004
2Ribo:1Chlro	.175	176.300	.007

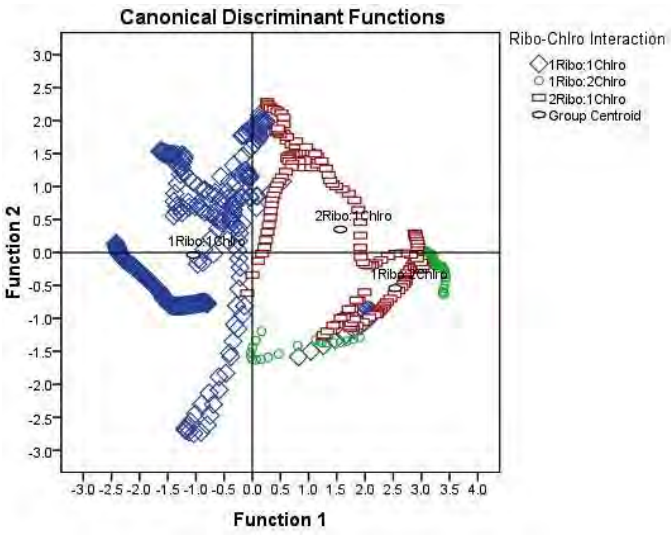


Fig. 7: Canonical discrimination function of FTIR data of commercial drug at different ratio

Table 5: Test of quality of group means for FTIR data

Sample	Wilks' Lambda	F	Sig.
1Ribo:1Chlro	.812	70.232	.000
2Chlro:1Ribo	.709	124.585	.006
2Ribo:1Chlro	.709	124.537	.002

showing the discriminant scores of three cases used in the classification. It shows better classification among the drug samples. About 80.8% of cross validated cases were correctly classified for the drug sample. The Wilk's Lambda was used as statistical criteria for the variables from the analysis. The smaller the Wilk's Lambda, the more important the independent variable to the discriminant function. The Wilk's Lambda was significant by the F test (Table 5) and the important variables indicated by the higher component are closely related to the FTIR spectrum.

5. Conclusion

A rapid and simple UV and FTIR procedure has been used in the analysis of the drug sample. The chemometric analysis is used to distinguish between very similar chemical components of spectral data. The difference in the drug sample was studied from the difference in absorption/ transmission in UV and FTIR data. The discriminant analyses demonstrate that the interaction of the drug sample can be effectively distinguished by spectroscopic method. In conclusion the spectroscopic studies using FTIR and UV-Visible can be effectively used for qualitative analysis of commercial drug to a certain limit and further investigation with other spectroscopic techniques provides complete characterization of the drug and it plays a vital role in the field of pharmacology.

Acknowledgement

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