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A Review On: Quantitative Analysis of Doxorubicin Hydrochloride by using IR Spectroscopy

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ABSTRACT:

Environment-friendly fast and accurate mid-infrared spectroscopic method have been developed for the quantitative analysis of doxorubicin hydrochloride (DOX) in bulk and marketed formulations. Both transmittance and reflectance modes have been used for the analysis and a comparison has been drawn for better accuracy. The analytical methods were validated in accordance with International Council for Harmonization (ICH) guidelines.The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation.

Keywords: Spectroscopy, Qualitative, Instruments, Pharmaceutical, Doxorubicin Hydrochloride, Quantitative.

INTRODUCTION:

Analysis is a detailed examination of anything complex in order to understand its nature or to determine its essential thorough study doing a careful analysis of the problem. A statement of such an examination. Separation of a whole into its component parts. Quantitative analysis is a chemical analysis designed to determine the amounts or proportions of the components of a substance. In recent years, Fourier transform infrared spectroscopy (FTIR) has been widely explored for the quantitative analysis , quality control, and supervision of manufacturing process of pharmaceutical products .Doxorubicin hydrochloride (DOX) is a naturally occur-ring anthracycline derivative and is used for the treatment of a number of malignancies including solid tumors, transplantable leukemia's and lymphomas .[1]

Infrared (IR) spectroscopy is one of the most common spectroscopic techniques used by organic and inorganic chemists. Simply, it is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. The main goal of IR spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of IR radiation.[1]

2.1 Principle of Infrared (IR) Spectroscopy:

- Infrared Spectroscopy is the analysis of infrared light interacting with a molecule.
- Portion of the infrared region most useful for analysis of organic compounds have a wavelength range from 2,500 to 16,000 nm, with a corresponding frequency range from 1.9*1013 to 1.2*1014 Hz.
- Photon energies associated with this part of the infrared (from 1 to 15 kcal/mole) are not large enough to excite electrons, but may induce vibrational excitation of covalently bonded atoms and groups.
- It is known that in addition to the facile rotation of groups about single bonds, molecules experience a wide variety of vibrational motions, characteristic of their component atoms.
- Consequently, virtually all organic compounds will absorb infrared radiation that corresponds in energy to these vibrations.[3-4]

2.2 Molecular vibration:

- **Stretching vibrations:**

  The stretching vibrations are those in which two bonded atoms oscillate continuously, without altering bond axis or bond angles.

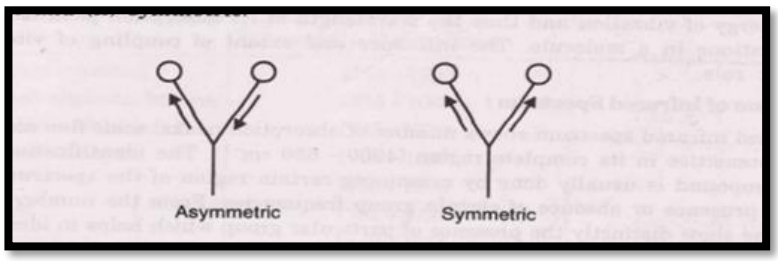


Fig.No.1(a) Molecular Vibration

- **Bending (or deformed) vibrations**:

  These are characterised by continuously changing bond angle and axis with common atom. These are of various types as (+ve indicates movement out of plane above and -ve indicates movement back of plane)[2-4]

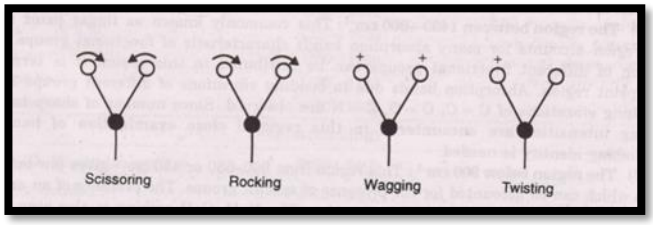


Fig No 1(b). Molecular Vibration

2.3 Instrumentation of Infrared Spectroscopy:

1. Light source 2.Monochromators and optical material
3.Sample holder 4.A radiation Detector

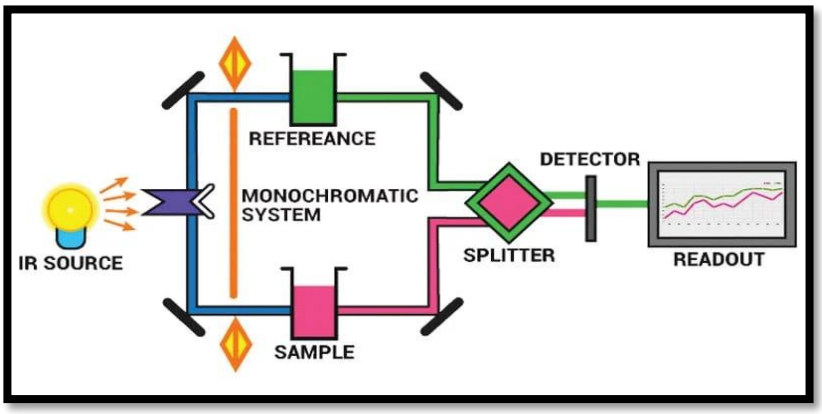


Fig No 2. Instrumentation Of IR

2.3.1 Light source:

Sources for different IR regions are-

- Near IR region: Tungsten filament, Nichrome wire, Rhodium wire.
- Mid IR region: Nernst glower and Global Sources two are most commonly used IR Sources.
- Far IR region: Mercury arc lamp and Carbon dioxide laser.

2.3.2Monochromators:

The radiation source emits radiations of Variable frequencies. But the sample in The IR spectroscopy absorbs only at certain frequencies. It is, therefore. Necessary to select desired frequencies from the radiation source and reject the others. The selection has been achieved by means of monochromators.

I. Prism:

The prism disperse the light radiation into its individual wavelength .The Resolution depends on the size and Refractive index of the prism.

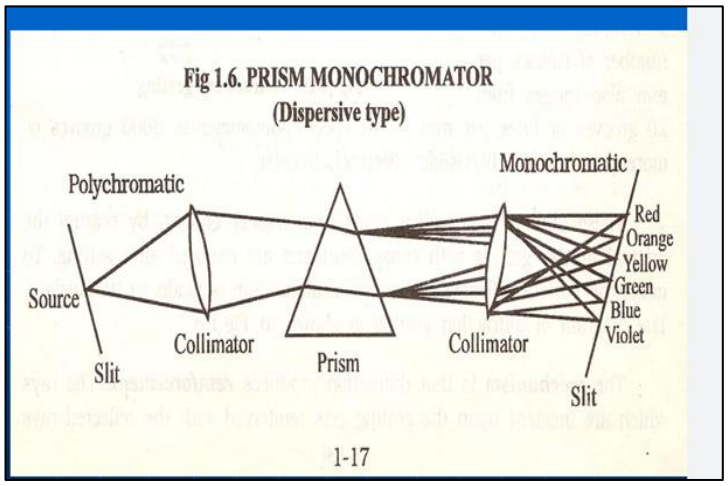


Fig No 3PrismMonochromatic

2.3.3 Sample handling: As IR spectroscopy has to use for characterization of solid, liquid or gas samples, it is evident that samples different phases are have to be handled and to be treated differently

2.3.4 Detectors:

- Near-infrared : Lead Sulphide photoconductive
- Mid-infrared: Thermopile, Thermistor or Pyro electric
- Far –infrared: Golly, pyro electric at the shorter- wavelength end , below about 1.2 microns, the preferred detection methods are the same as those used for visible and U V radiation.

➢ **Thermal Detector** –

Generally thermal detectors are used in IR spectroscopy

a. Bolometer
b. Golly call (an Expansion of a solid or fluid)
c. Thermistor (electrical resistance)
d. Thermocouple (Voltage induced at the junction of dissimilar material)

e. Pyro electrical Detector(Electronic Polarisation) [5-8]

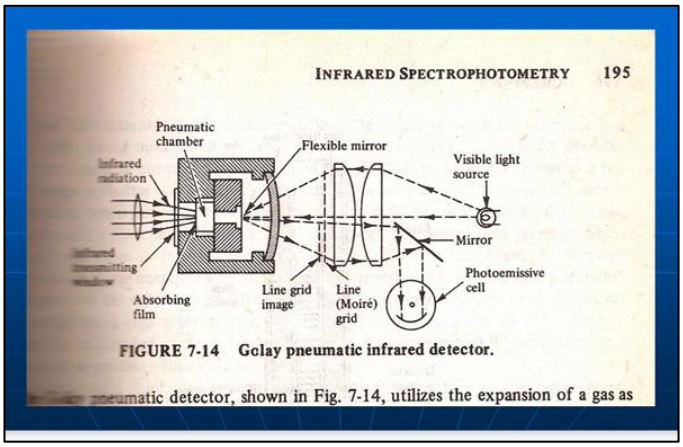


Fig No 4. Golly Cell

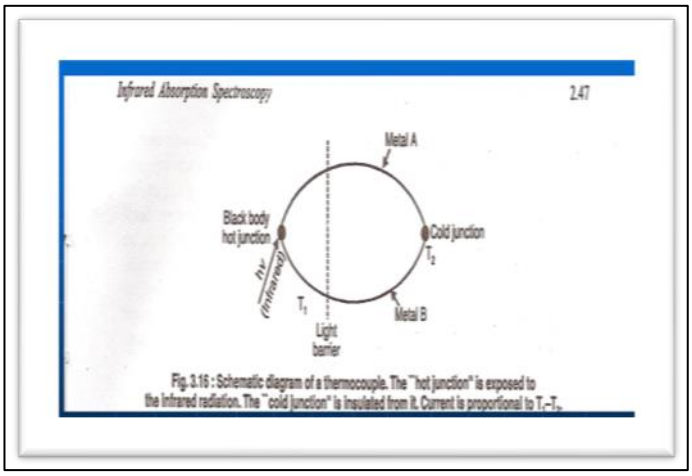


Fig No 5. Thermocouple

- **Functional Groups and IR Tables**

IR Absorptions of Common Functional Groups

Functional Group	*Absorption Location (cm^{-1})*	*Absorption Intensity*
Alkane (C–H)	2,850–2,975	Medium to strong
Alcohol (O–H)	3,400–3,700	Strong, broad
Alkene (C=C)	1,640–1,680	Weak to medium
(C=C–H)	3,020–3,100	Medium
Alkyne		
(C≡C)	2,100–2,250	Medium
(C≡C–H)	3,300	Strong
Nitrile (C≡N)	2,200–2,250	Medium
Aromatics	1,650–2,000	Weak
Amines (N–H)	3,300–3,350	Medium
Carbonyls (C=O)		Strong
Aldehyde (CHO)	1,720–1,740	
Ketone (RCOR)	1,715	
Ester (RCOOR)	1,735–1,750	
Acid (RCOOH)	1,700–1,725	

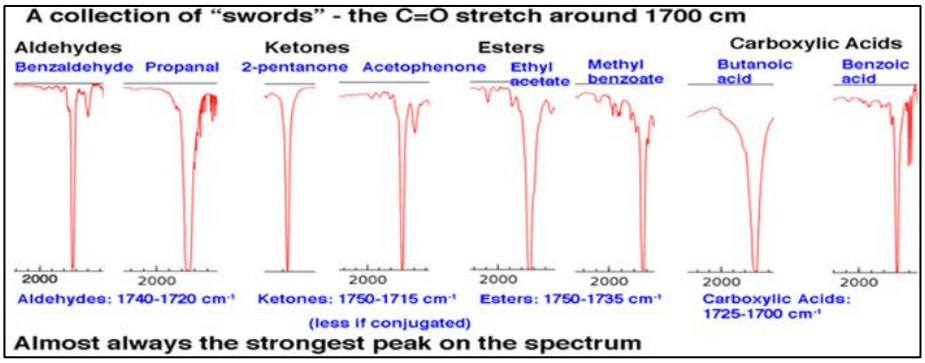


Fig.No 6. Spectrum[6]

2.4 Application of IR:

I) Qualitative Analysis (Compound Identification)

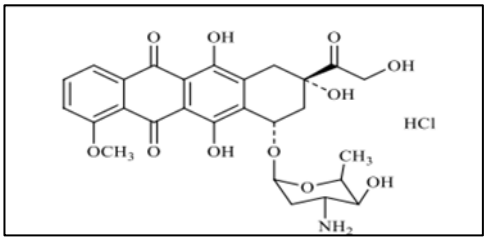
Main application use of IR, with NMR and MS, in late 1950's revolutionized organic chemistry decreased the time to confirm compound identification (10-1) examine what functional groups are present by looking at group frequency region- 3600 cm-1 to 120.

II) Quantitative Analysis:

Not as good as UV/Vis in terms of accuracy and precision more complex spectra narrower bands (Beer's Law deviation) limitations of IR instruments (lower light throughput, weaker detectors) high background IR. Difficult match reference and sample cells changes in e (A=ebc) common potential advantage is good selectivity, since so many compounds have different IR spectraone common application is determination of air contaminants.[14]

4. Drug Profile

- **Structure**



- **IUPAC NAME:** 7S,9S)-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione;hydrochloride
- **Mechanism of action:**
  Doxorubicin has antimitotic and cytotoxic activity through a number of proposed mechanisms of action: Doxorubicin forms complexes with DNA by intercalation between base pairs, and it inhibits topoisomerase II activity by stabilizing the DNA-topoisomerase II complex, preventing the relegation portion of the ligation-relegation reaction that topoisomerase II catalyzes.
- **Method of Synthesis :**

i. In the first step, Adriamycin one converts into dioxolone derivative.

ii. It is then condensed with N-O-ditriflouroacetyl-alpha-daunosaminyl chloride.
iii. The formed compound is then treated with methanol to remove the O-triflouroacetyl group.
iv. First alkaline and then an acid treatment is given to the compound to give doxorubicin.

➢ **Structural Activity Relationship:**

I. Substitution at 2nd position decreases the biological activity of drug. Presence of any substituent at R2 position also decreases the biological activity of drug.
II. Biological activity can be increased by substitution at 3rd,8th position has direct relationship with the biological activity of drug and thus,
III. Substitution at 8th position can increase the biological activity of drug. Substitution at 1st and 7th position will have negative impact on the biological activity of the drug.

➢ **Uses:**

I. Doxorubicin is used in combination with other medications to treat certain types of bladder, breast, lung, stomach, and ovarian cancer;
II. Hodgkin's lymphoma (Hodgkin's disease) and non-Hodgkin's lymphoma (cancer that begins in the cells of the immune system); and certain types of leukemia (cancer of the white blood cells.[13-14]

4. Material and Methodology –

➢ Requirement –
➢ Chemical – Potassium pellet, Potassium powder, Doxorubicin HCL
➢ Glass wear – Cover slip, Mortar pestle
➢ Instrument – FTIR

4.1 Materials and methods:

Fig No. 6 FTIR Instrumentation

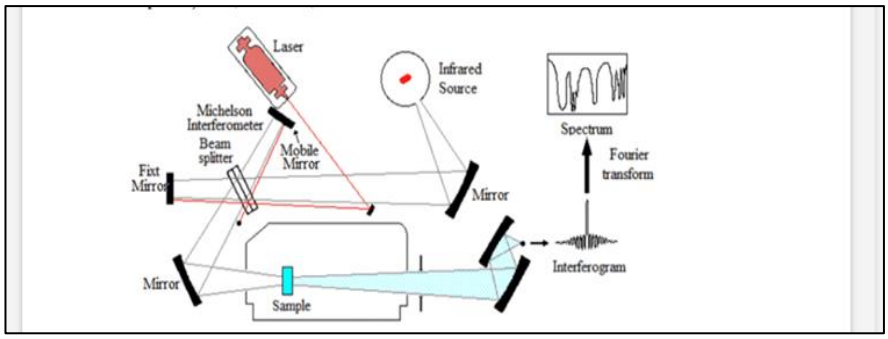


Fig No 7 FTIR SPECTROMETER

➢ **Method A-transmission mode Sample preparation**:

I. Five different concentrations (% w/w, 0.6, 0.8, 1.0, 1.2 and 1.4 in case of doxorubicin) were prepared by homogeneously mixing.

II. Add required amount of the requisite drug in KBr powder to make total weight of 100 mg.

III. weigh 65 mg of sample mixture for pellet preparation.

IV. The spectra for each concentration were recorded in transmittance mode.
Standard curve was obtained by plotting peak area versus concentration.

➢ **Method B-reflectance mode Sample preparation:**

I. Five different concentrations (% w/w) of doxorubicin and were prepared in a similar way as mentioned in method A

II. weigh70 mg of the sample mixture in a macro cup for analysis.

III. A cover slip was then dragged across the top of the cup to remove excess powder and smooth the sample surface.

IV. The spectra obtained in reflectance mode were transformed to Kubelka Munk (KM) mathematical function.[13-15]

➢ **Assay Validation :**

I. **Linearity**: Linearity of the proposed methods was established by analysing five different samples of the drugs (0.6%, 0.8%, 1.0%, 1.2%, 1.4% w/w of DOX) the standard plot was linear over the concentration range 0.6-1.4%w/w

II. Accuracy:

i. To take three different concentration of 0.6%,1.0%,1.4%w/w. of DOX Accuracy was assessed as the percentage relative standard deviation (RSD) and mean percentage recovery.

ii. Specific amount of pure drug were added to known previously analysed concentration and the total concentration was determined using the method

iii. Standard addition method was performed to give additional support to recovery study analysis.

The percent recovery of the added pure drug was calculated as

$$\% \text{ recovery} = [(Cv - Cu)/Ca] \times 100, (10\text{-}11)$$

➢ **Precision:**

i. Repeatability of the method was determined by preparing and analysing three different concentrations levels of Drug concentrations 0.6%, 1.0%, 1.4% w/w of DOX for intra and inter day precision.

ii. Prepared each concentration and analysed in same day at three different times in a day for intraday precision study.[n=27]

➢ **Limit of detection (LOD) and limit of quantitation (LOQ):**

i. LOD and LOQ were calculated as 3.3σ/S and 10σ/S, Respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation.

[LOD=3Sa/b, LOQ=10Sa/b]

➢ **Robustness:**

i. Robustness of the proposed method was checked by deliberately varying the weight of the DOX-KBr and -KBr mixture by ± 2 mg for estimation.

ii. Robustness was determined as percentage relative standard Deviation.

➢ **Estimation in formulations**:

- Marketed lyophilized formulation 'Duxocin®' for doxorubicin and tablet of'Synriam®' for estimation of were used. For transmission analysis, the drug (5 mg for dox and 15 mg for) was weighed and mixed with KBr (95 mg for dox and 85 mg for) in pestle mortar to obtain a homogenous fine powder. A pellet of 65 mg was prepared and analysed using transmission method.[15-17]

5. Results and discussion:

I. Doxorubicin methods such as LC-MS, reverse phase HPLC and UV-Visible spectrometry have been reported and validated for quantitative analysis of doxorubicin hydrochloride in bulk and pharmaceutical dosage forms [12].

II. All these methods require use of solvents, however due to unstable nature of DOX at high pH, in solvents and at temperature above 8 °C, there is a need of an alternative analytical technique [15-18].

III. A fast, green and specific analytical method which do not require toxic solvents and can be performed without any sample preparation is the need of the hour.

Table 1(a) IR spectral analysis of Doxorubicin hydrochloride

Wave number (cm-1)	Functional group
3331	N-H stretch
3525	O-H stretch
2935,2897	C-H stretch
1729	C=O stretch
1617,1582,1414	C=C ring stretch
1115,1073	C-O-C stretch
805,688	C=H bend=C ring bend

Parameter	Transmittance Mode	Reflectance
LOD	**0.11**	**0.15**
LOQ	**0.30**	**0.48**

Table No1(b) Result of LOD and LOQ

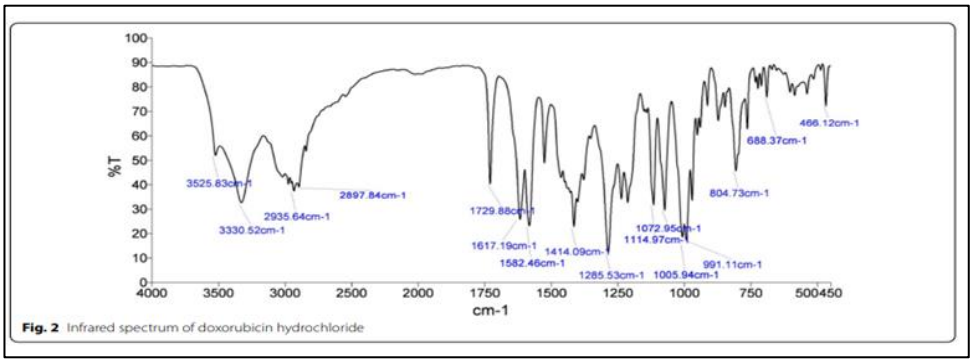


Fig No. 8Infrared Spectrum of DOX

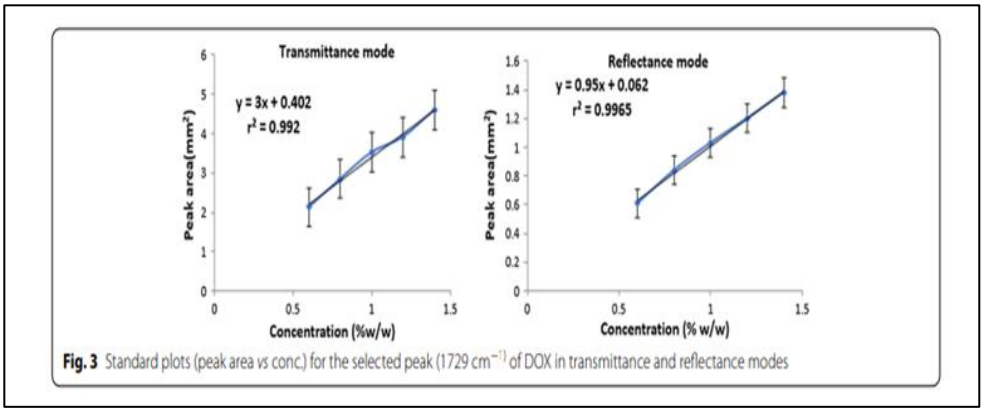


Fig No. 9 Standard Graph of DOX

6. Conclusion:

The proposed methods have been successfully developed and validated for the quantification of Doxorubicin in solid bulk and dosageform . The developed methods offer a simple and dependable alternative for the routine quantitative analysis of drug .The developed methods are accurate, precise, robust, and easy to the time consuming chromatographic methods reported in literature which also requires high quantities of solvent .FTIR methods although are quite sensitive but accuracy may be affected due to manual errors which could be minimized by practice and expertise.[18]

7. Reference:

1. Fanelli S, Zimmermann A, Totóli EG, Salgado HRN (2018) FTIR spectro-photometry as a green tool for quantitative analysis of drugs: practical application to amoxicillin. J Chem. 2018:1–8
2. Etzion Y, Linker R, Cogan U, Shmulevich I (2004) Determination of protein concentration in raw milk by mid-infrared Fourier transform infrared/attenuated total refectance spectroscopy. J Dairy Sci 87:2779–2788
3. Kawamura S, Natsuga M, Takekura K (2001) Development of an automatic rice-quality inspection system. IFAC Proc 34:71–76
4. Silverstein RM, Bassler GC (2001) Infrared spectroscopy. Spectrometric identifcation of organic compounds, 6th edn. Wiley, New York, pp 66–84
5. Robinson JW (1991) Infrared spectroscopy. Practical handbook of spec-troscopy, 4th edn. CRC Press, Boca Raton, pp 34–56
6. Smith BC (1996) Introduction to infrared spectroscopy. Fundamentals of Fourier transform infrared spectroscopy, 2nd edn. CRC Press, Boca Raton, pp 43–57
7. Blanco M, Coello J, Iturriaga H, Maspoch S, Pezuela C (1998) Near-Infrared spectroscopy in the pharmaceutical industry. Analyst 123:135–50
8. Tripathi S, Mishra HN (2009) A rapid FT-NIR method for estimation of afatoxin B 1 in red chili powder. Food Control 20:840–846
9. Bansal R, Guleria A, Acharya PA (2013) FT-IR method developmentand validation for quantitative estimation of zidovudine in bulk and tablet dosage form. Drug Res 63:165–170
10. Tacar O, Sria2.
11. mornsak P, Dass CR (2013) Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol 65:157–170
12. Octavia Y, Tocchetti CG, Gabrielson KL (2012) Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. J Mol Cell Cardiol. 52:1213–25
13. Rodrigues AS, Lopes RA, Couceiro A, Ribeiro BA (2009) Development of an analytical methodology for simultaneous determination of vincristine and doxorubicin in pharmaceutical preparations for oncology by HPLC-UV. J Chromatogrhttps://medlineplus.go
14. https://www.researchgate.net
15. https://medlineplus.gov
16. https://images.app.goo.gl/ZTWvV8K789HiwQEn6
17. https://microbenotes.com/infrared-ir-spectroscopy/
18. https://pubchem.ncbi.nlm.nih.gov/compound/Doxorubicin#:~:text=Doxorubicin%2C%20isolated%20from%20the%20bacterium,and%20ultimately%20inhibiting%20protein%20synthesis