



QUANTITATIVE ANALYSIS OF EUGENOL IN DIFFERENT PARTS OF CLOVE

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ABSTRACT

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Eugenol is the key active constituent of clove. The aim of this study was to analyze the concentration of Eugenol in different parts of dried Clove (tail, mid body and flower) by IR Spectroscopy and graphical comparison of the concentration. In this experiment the different part of dried clove was used to perform the quantitative analysis. Two methods were followed for the IR Spectroscopy, Attenuated Total Reflectance (ATR) sampling method and KBr Pellet method. KBr Pellets were prepared at 1:99 (Clove part: KBr) and IR spectrum drawn between IR region (650 to 4000). Total 5 samples were analysed [Sample 01- Clove flower, Sample 02- just below of flower, Sample 03- mid body, Sample 4 -mid Body 2nd and Sample 05 -tail part]. Two wavelengths were target 1513 cm⁻¹ and 1431 cm⁻¹. The quantitative analysis was performed for % transmittance and % absorbance. Plotted Spectra was compared with the Spectral Database for Organic Compounds (SDOC) and spectra-base at Wiley. Sample 03 mid body shows higher area comparatively remaining four samples at both peaks (1513 cm⁻¹ as well as 1431 cm⁻¹). Results shows that the concentration of Eugenol is higher at Ovules than tail and flower.

Contribution/Originality: Several literatures stating that Clove is a rich source of Eugenol but not specific study saying that which part of clove is having highest concentration of Eugenol. In the present study we have evaluated each part of the clove and confirm the part which contains highest % of eugenol.

1. INTRODUCTION

Clove (*Syzygium aromaticum*) is a precious spice, a member of the *Myrtaceae* family that has been employed for centuries (around 2000 years) as a food preservative and medicine due to its antimicrobial and antioxidant properties and native to Indonesia (Agrawal et al., 2014; Hussain, Rahman, Mushtaq, & Belaskri, 2017; Mbaveng & Kuete, 2017). *Eugenia caryophyllata* Thunb is a tree and Clove is obtained from the dried flower bud of this tree (Zheng, Kenney, & Lam, 1992). There have been many investigations and reports by many researchers that a good quality or bad clove contains eugenol (80-85%), volatile oil 12-15% and some other constituents such as acetyl eugenol, gallic, methyl furfural, gum, resin and some other components (Chaieb et al., 2007; Gaydou & Randriamiharisoa, 1987; Gopalakrishnan & Narayanan, 1988; Gopalakrishnan, Narayanan, & Mathew, 1988;

Mittal, Gupta, Parashar, Mehra, & Khatri, 2014; Pino, Marbot, Agüero, & Fuentes, 2001). The exact identity of clove's active compounds has not yet been established, but the medicinal effect is believed to be mainly due to eugenol (Smiley & Miles, 2002). Eugenol - C₁₀H₁₂O₂; Figure 1 a volatile phenolic constituent (Smiley & Miles, 2002), 2-methoxy-4-(2-propenyl) phenol, is an allyl chain substituted guaiacol, it is slightly soluble in water and soluble in organic solvents (Rapp, 2007). Sample preparation is an important first step in herbicide analysis, as it is necessary to extract desired chemical constituents from herbal material for further separation and characterization example lack of consistency, safety, and efficacy. Same as sample preparation, the operation of Spectrophotometry is very crucial too. IR Spectrophotometry has been applied to determine eugenol in different parts of the clove which is much accurate comparatively TLC method. Thin-layer chromatography (TLC) depends on the separation principle and IR spectroscopy deals with the infrared region of the electromagnetic spectrum, it generally refers to the analysis of the interaction of a molecule with infrared light.

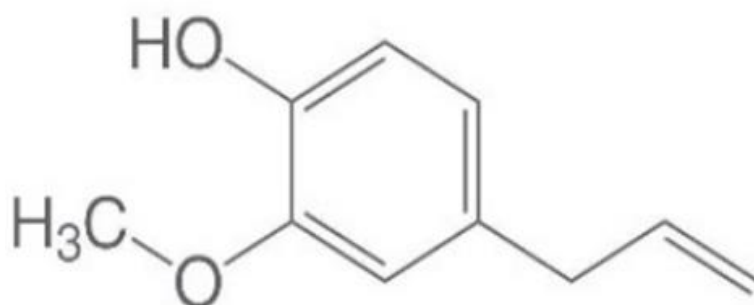


Figure 1. Chemical structure of eugenol (C₁₀H₁₂O₂) (Nejad, Özgüneş, & Başaran, 2017).

2. MATERIALS AND METHODS

Plant Materials: Dried Cloves were purchased from a local supermarket (Reliance Super, Gaurav Path, RIICO Industrial Area, Bhiwadi, Rajasthan 301019. However, the samples originated from Indonesia. Samples were maintained in the dark and cool place at 25°C.

2.1. Chemical Reagent

2.1.1. Potassium Bromide for IR Spectroscopy was Procured from Merck

2.1.1.1. Sample Preparation –

A single Clove bud was divided into five parts and named as Sample 01- Clove flower, Sample 02- just below of flower, Sample 03 -mid body, Sample 4 -mid body 2nd and Sample 05 -tail part Figure 2 representing clove and sample area). Individual part was grinded using stone pestle and mortar and then sifted through USA standard test sieve No.# 20 Mesh (ASTM specification, with an 850 μm pore-size) to maximize surface area. ASTM stand for American Society for Testing and Materials, which is a nonprofit organization that develops and publishes technical standards.

Two methods were followed for IR Spectroscopy - Attenuated Total Reflectance (ATR) sampling method and KBr (Potassium Bromide) Pellet method.

- (a) KBr Pellet Method - Ground clove sample (200 mg) and KBr (1.8 gm) were taken into the stone mortar and pestle and mixed well. Approx. 1 gm of this sample was taken in the KBr Pellet die and a pressure of 10 ton was applied. The prepared pellet was carefully removed from the die and placed in the KBr pellets cage of the IR spectrometer. Determination was performed for five separate pellets of each section of the sample, and each sample was analyzed within the same parameters.
- (b) Attenuated Total Reflectance is a method based on internal reflection, and the length of the sample path depends on the depth of penetration of infrared energy into the sample (Clagg, Hold, Kumar, Koenig, &

Angelaud, 2019; Dunn et al., 2019; Wang, Gérardy, Gauron, Damblon, & Monbaliu, 2019). A 50-100 mg and ~10 μm thick solid sample was placed on the ATR unit and the infrared spectrum was recorded.

2.2. Chromatographic Conditions

IR Spectroscopy was performed using an FTIR Spectrometer, Model Spectrum one, Sr No 69566 (Manufactured by Perkin Elmer Precisely) at $450\text{-}4000\text{ cm}^{-1}$ for IR and $650\text{ - }4000\text{cm}^{-1}$ for AT. Eugenol was eluted within a min and quantification was carried out using a calibration curve and peak area measurements.

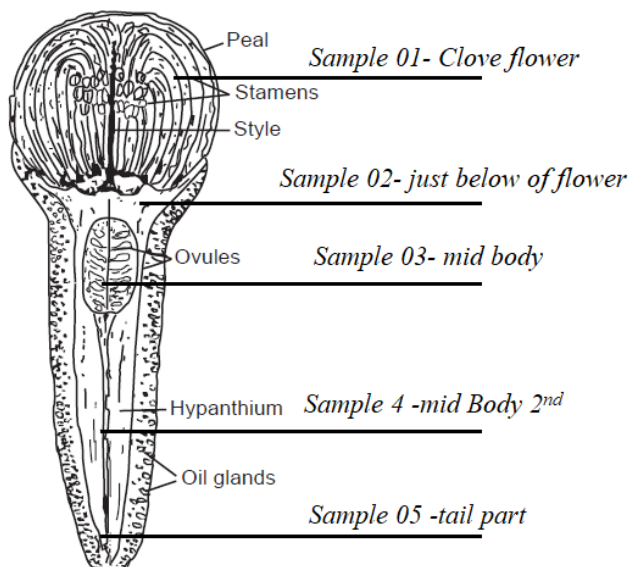


Figure 2. Representation of samples collected from a Clove.

2.3. System Suitability

The system suitability parameters with respect to KBr Pellets, Samples, wavelength between eugenol peak and other constituent peaks were defined and compared with the reference peak.

3. RESULTS AND DISCUSSION

In order to obtain the IR Spectra with better resolution of adjacent peaks within a short time, the Sample, KBr Pellets and detecting wavelength were investigated. Before each pellet preparation the pastel-mortar, spatula, die and sample reader were cleaned with IPA (Iso Propyl Alcohol) and dried thoroughly. As per the reference IR spectra of eugenol $1600\text{-}1420\text{ wavenumber/cm}^{-1}$, all samples were analyzed for (a) % absorbance Vs wavelength and (b) % transmittance wavenumber/ cm^{-1} . Reference spectra (Figure 3 and 4) were compared to the spectra of each sample and it was identically same peak in the both way % absorbance as well as %transmittance, which confirmed the presence of eugenol in all the samples. In comparison of all fives samples with respect to the total transmittance area, each sample has different unit of area, which conclude the concertation of eugenol is not same at each section of the clove. At 1513 cm^{-1} mid part of the clove has highest % transmittance (736.72) and flower have the lowest (489.89) area and same at 1431cm^{-1} mid part of the clove has highest % transmittance (133.35) and flower have the lowest (58.22) area.

Based on total area at two different peaks it can be proved that the middle part of the clove has the highest concentration of the clove.

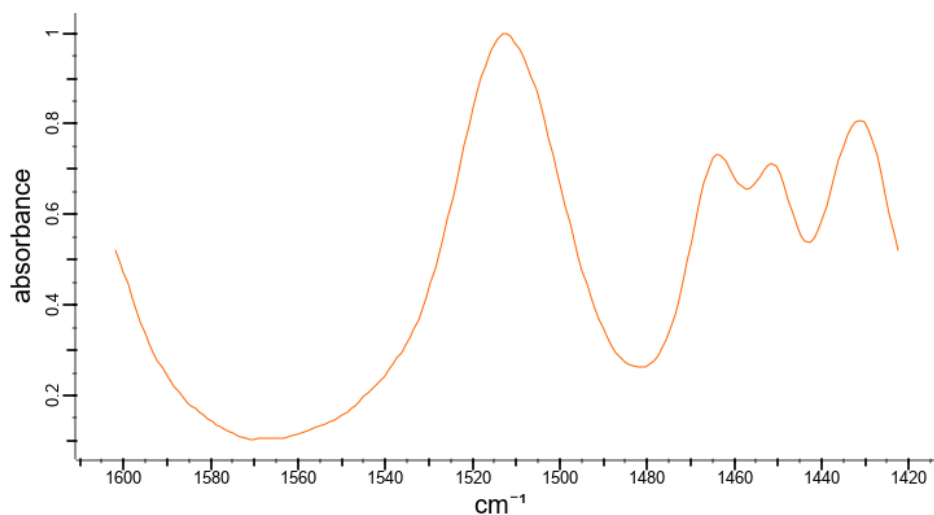


Figure 3. Reference infrared (IR) Spectrum of Eugenol; %absorbance vs wavenumber (Spectral Database for Organic Compounds (SDBS), 2022).

4. CONCLUSIONS

According to ayurveda, clove is a best home remedy for treating several ailments and disorders. The established analytical method was applied to quantitatively analyse eugenol within 10 samples collected from different location of a single clove. The content of eugenol in different locations of a clove is represented in the total area of transmittance in Figure 5 and 6. Results shows that the content of eugenol in Ovules (Mid of the clove) was significantly different in comparative of other location of clove. Therefore, this quantitative analysis can provide a scientific and technical data as a prove that Ovules is the richest source of Eugenol.

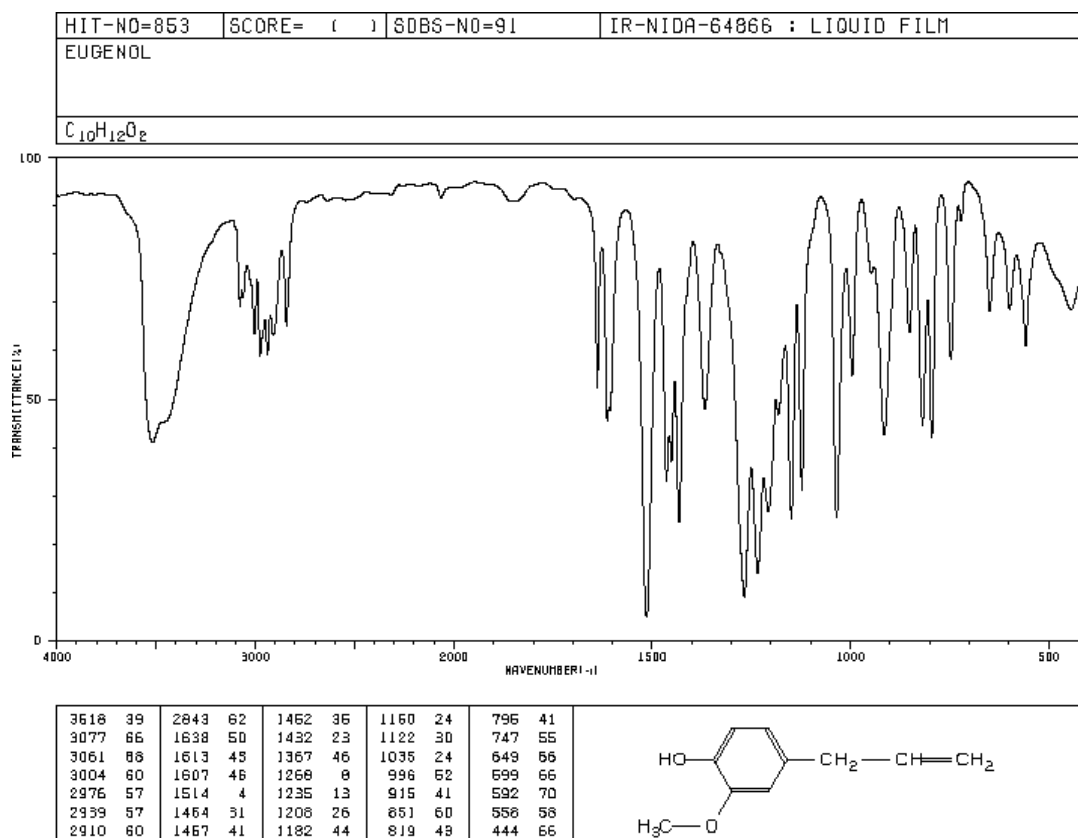


Figure 4. Reference IR Spectra of Eugenol; % transmittance vs wavenumber 20.

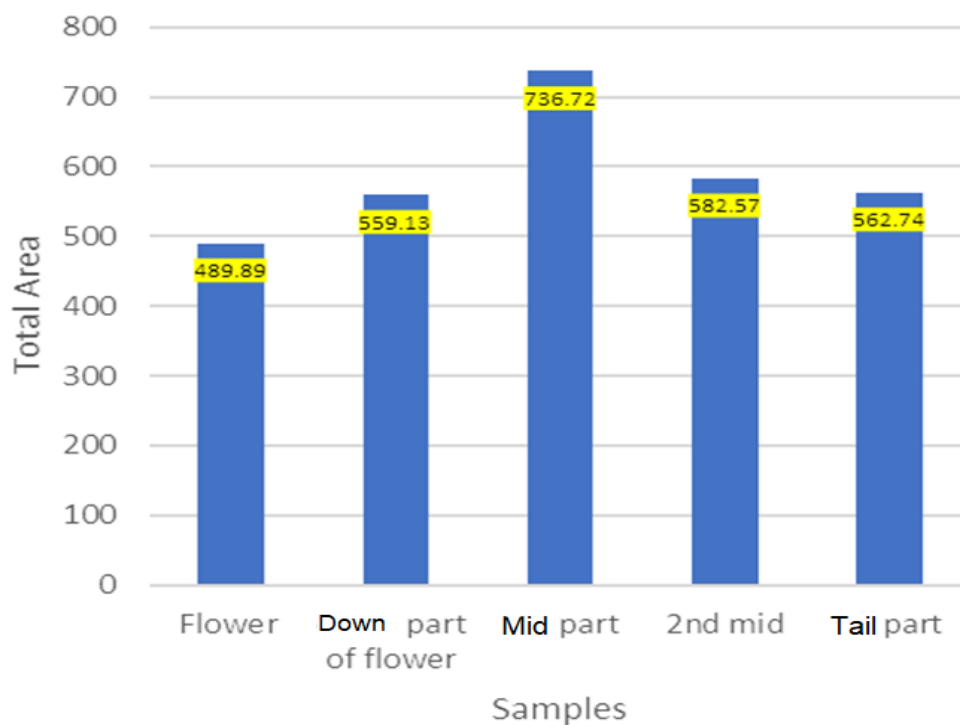


Figure 5. Graph representing the total area of IR absorbance at 1513cm^{-1} .

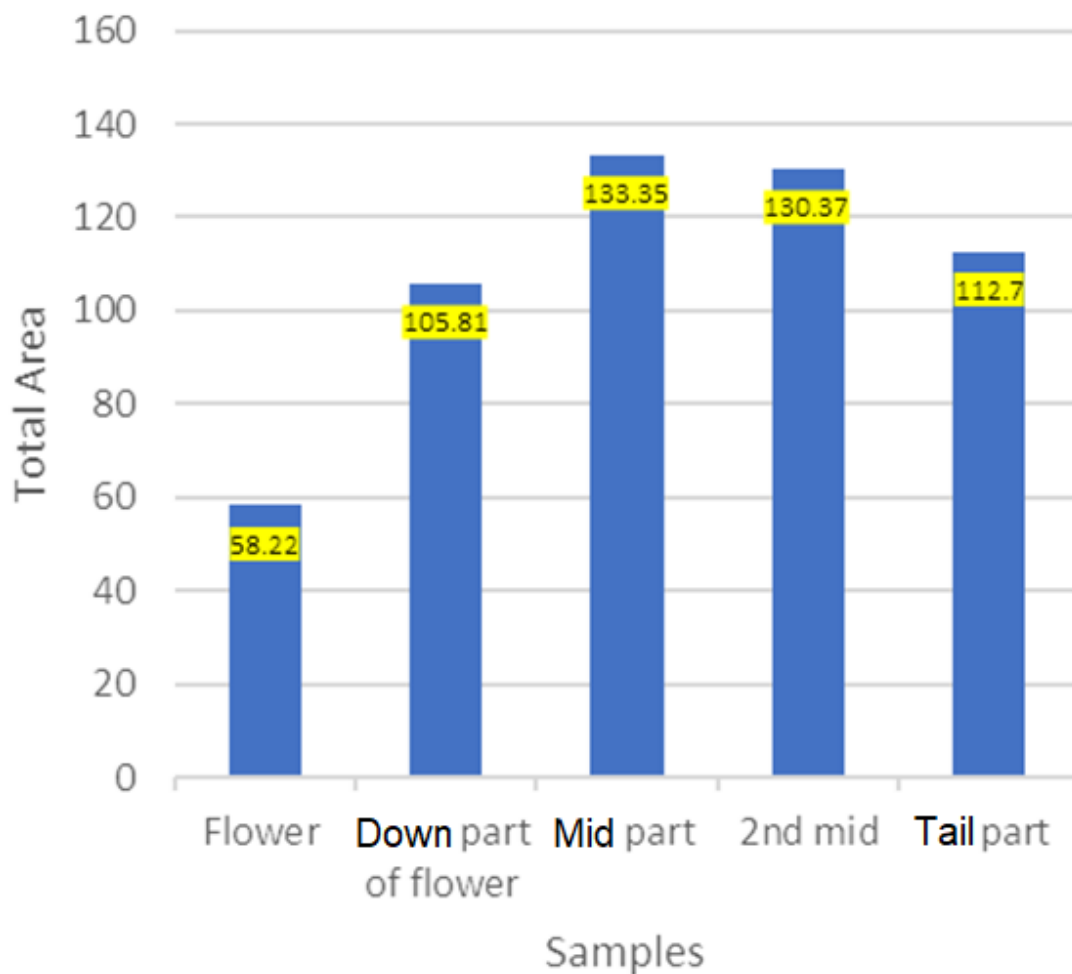


Figure 6. Graph representing the total area of IR absorbance at 1431cm^{-1} .

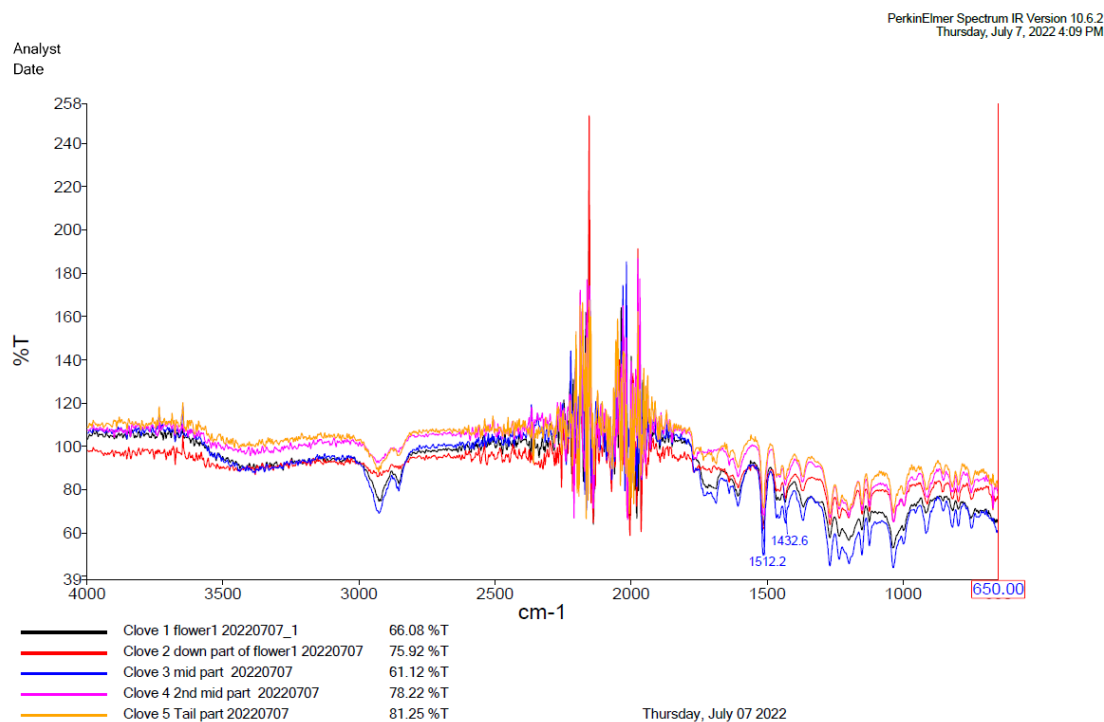


Figure 7. IR spectra of all five Samples (overlay)-% Transmittance VS wavenumber.

Figure 7 presents IR spectra of individual part of clove. The graph is between % transmittance ad wavelength. Each graph starts at same %transmittance so called overlay.

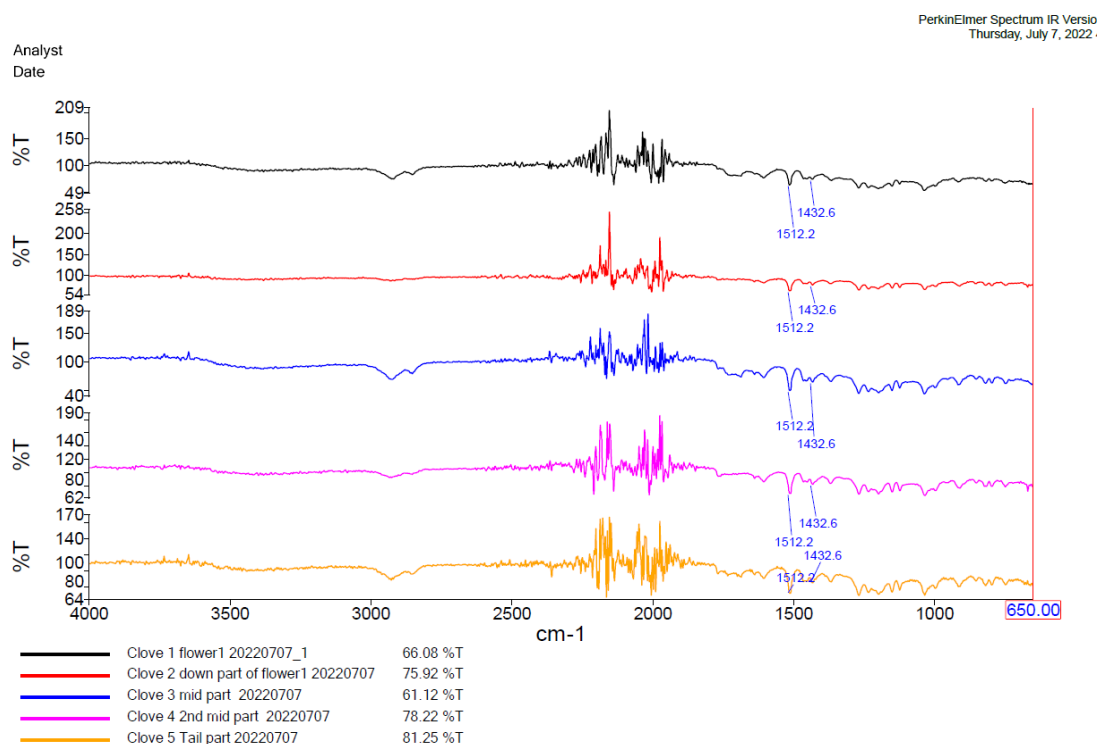


Figure 8. IF Spectra of all five Sample (Split)-% Transmittance VS wavenumber.

Figure 8 presents IR spectra of individual part of clove. The graph is between % transmittance ad wavelength. The curve is spited into individual parts.

Figure 9 illustrates IR Spectra of Sample 01(Clove flower)- % Transmittance VS wavenumber.

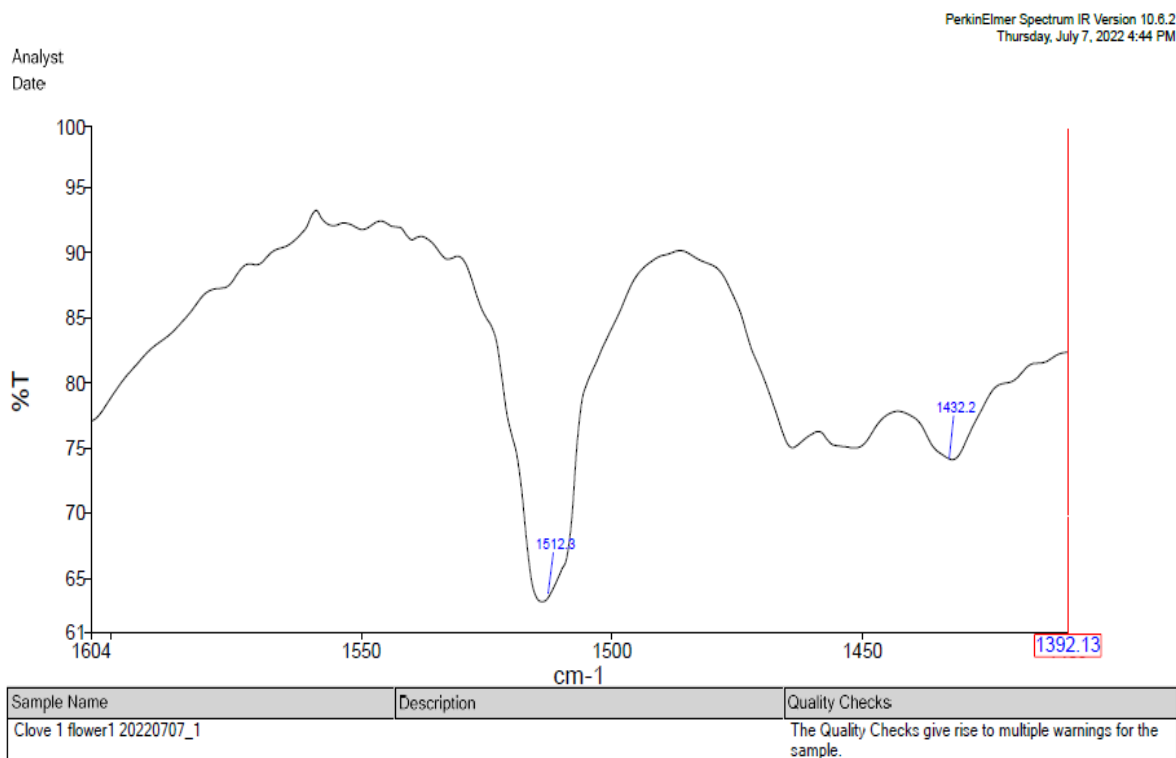


Figure 9. IR Spectra of Sample 01(Clove flower)- % Transmittance VS wavenumber.

Figure 10 illustrate IR Spectra of Sample 01 (Clove flower)- % Absorbance VS wavenumber.

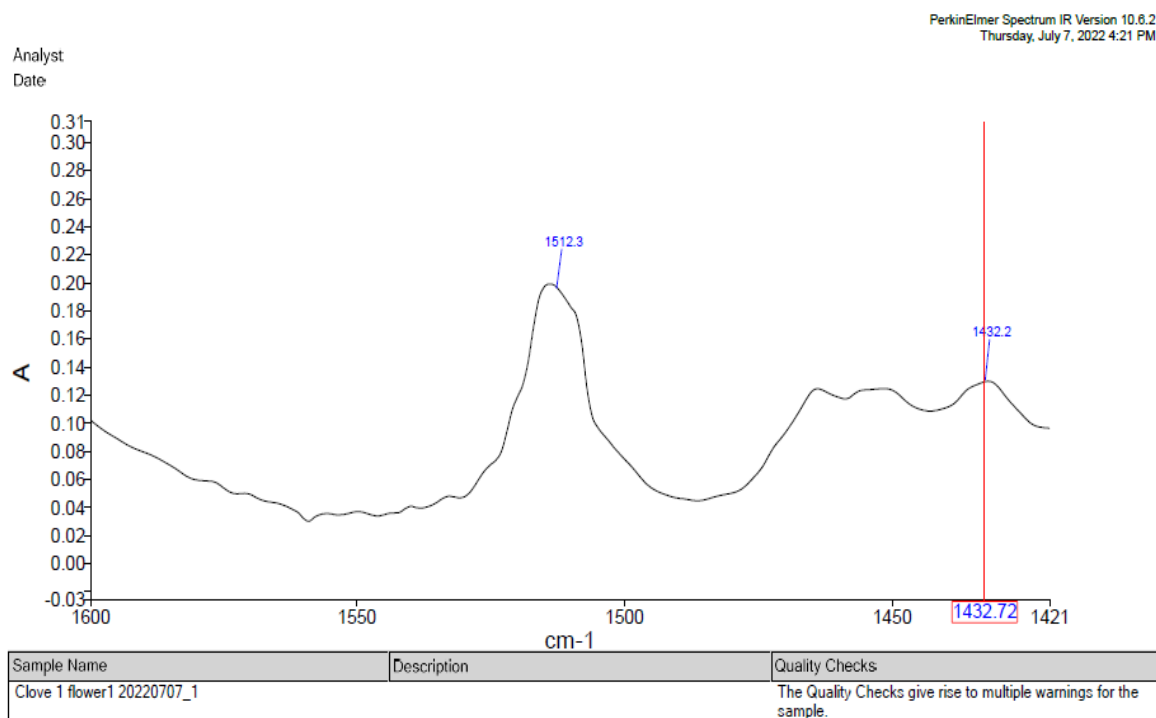


Figure 10. IR Spectra of Sample 01 (Clove flower)- % Absorbance VS wavenumber.

Figure 11 illustrates IR Spectra of Sample 02 (down part of flower)- % Transmittance VS wavenumber.

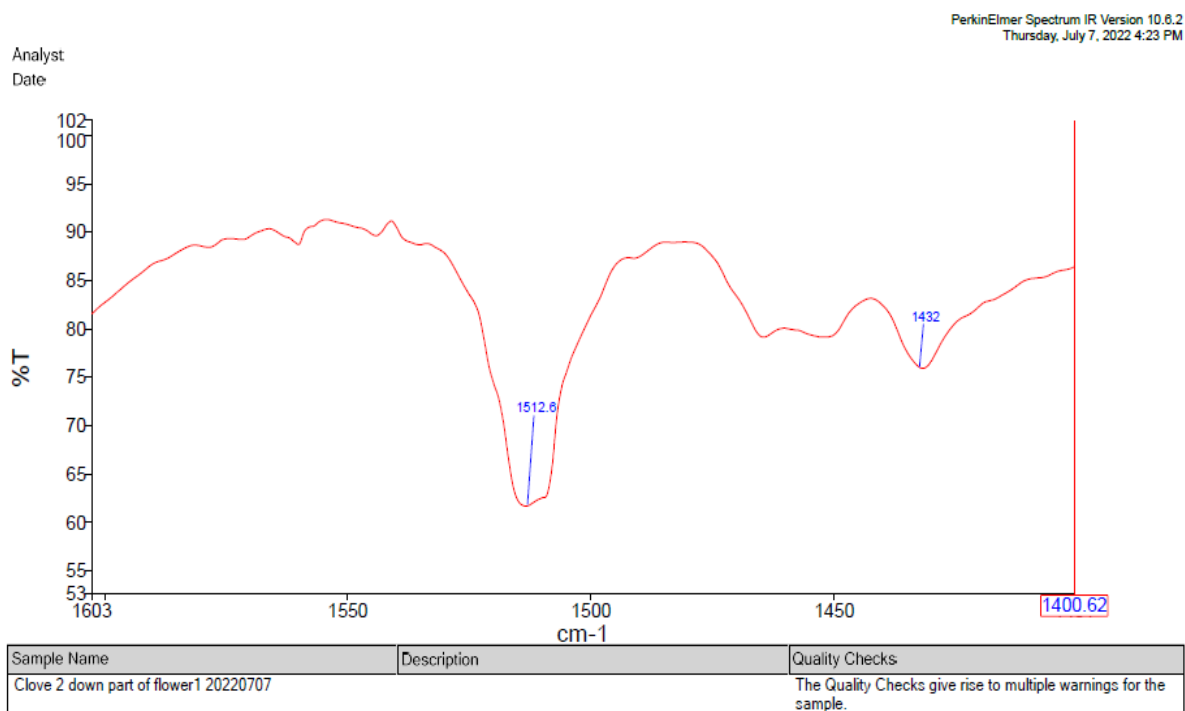


Figure 11. IR Spectra of Sample 02 (down part of flower)- % Transmittance VS wavenumber.

Figure 12 illustrate IR Spectra of Sample 02 (down part of flower)- % Absorbance VS wavenumber.

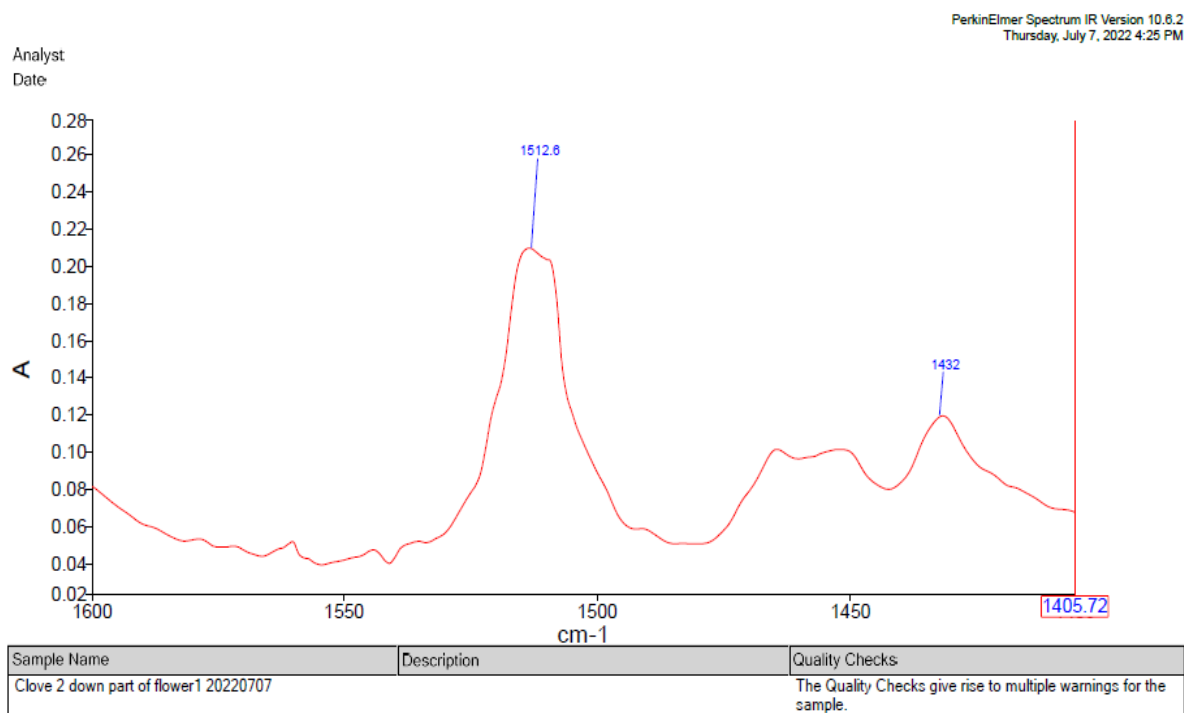


Figure 12. IR Spectra of Sample 02 (down part of flower)- % Absorbance VS wavenumber.

Figure 13 Illustrates IR Spectra of Sample 03 (mid part)- % Transmittance VS wavenumber.

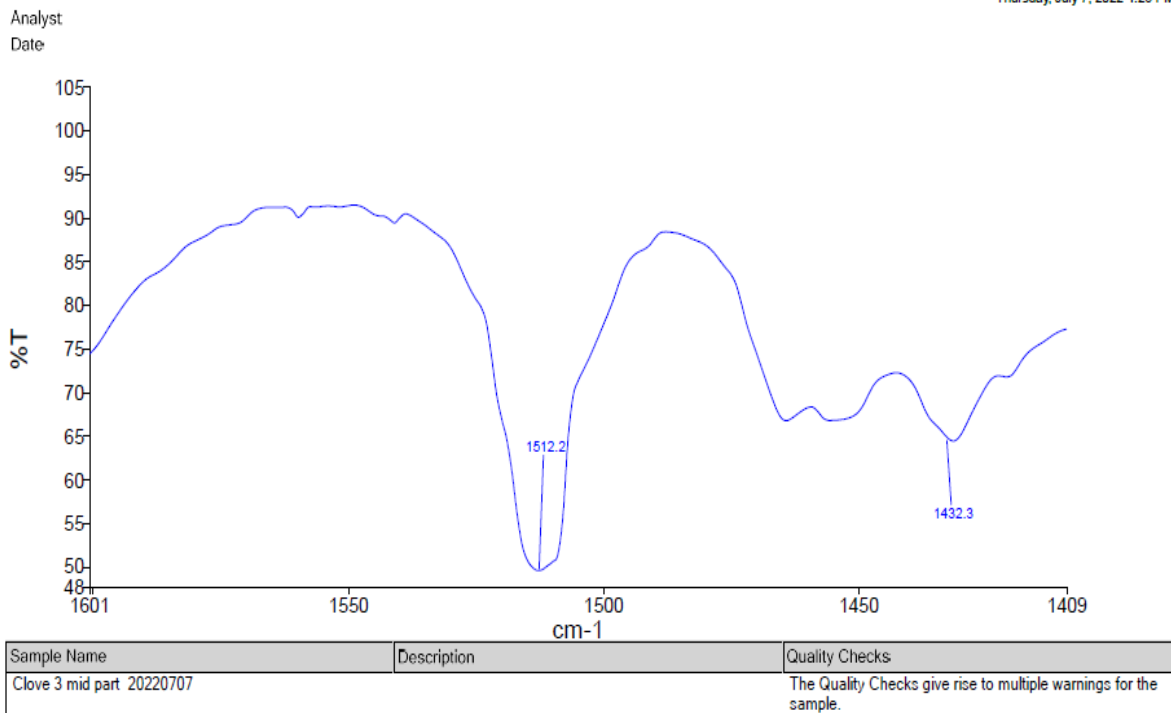


Figure 13. IR Spectra of Sample 03 (mid part)- % Transmittance VS wavenumber.

Figure 14 illustrate IR Spectra of Sample 03 (mid part)- % Absorbance VS wavenumber.

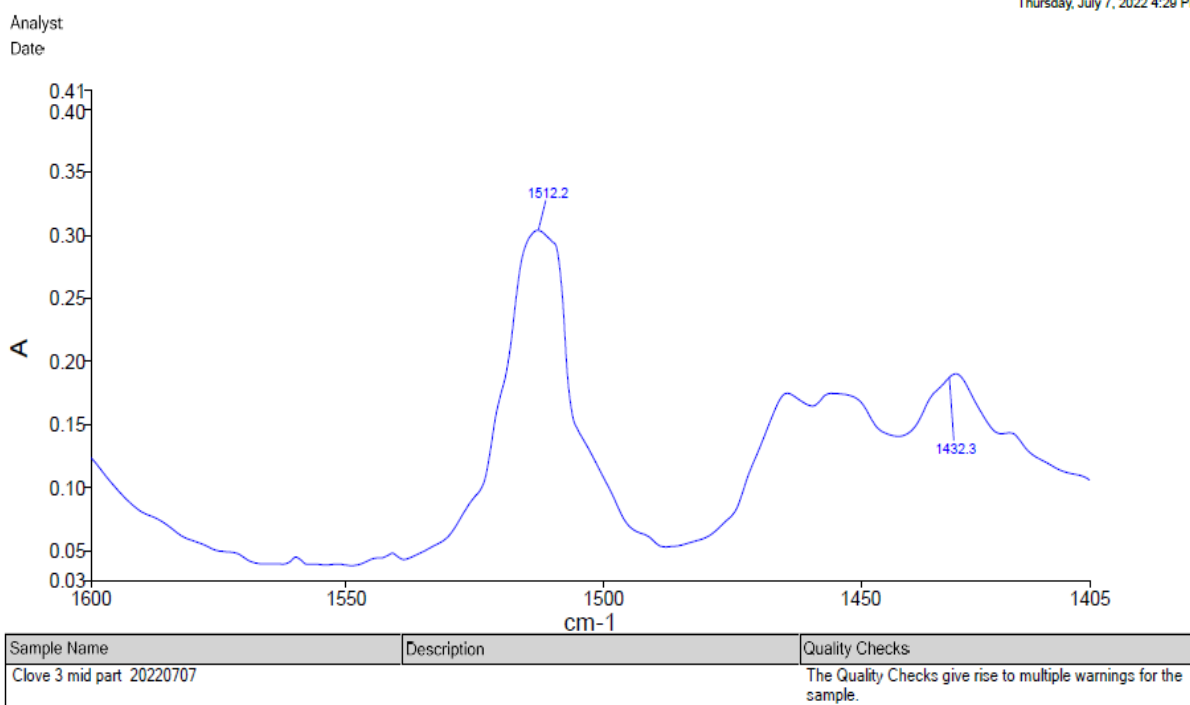


Figure 14. IR Spectra of Sample 03 (mid part)- % Absorbance VS wavenumber.

Figure 15 Illustrates IR Spectra of Sample 04 (2nd mid part)- % Transmittance VS wavenumber.

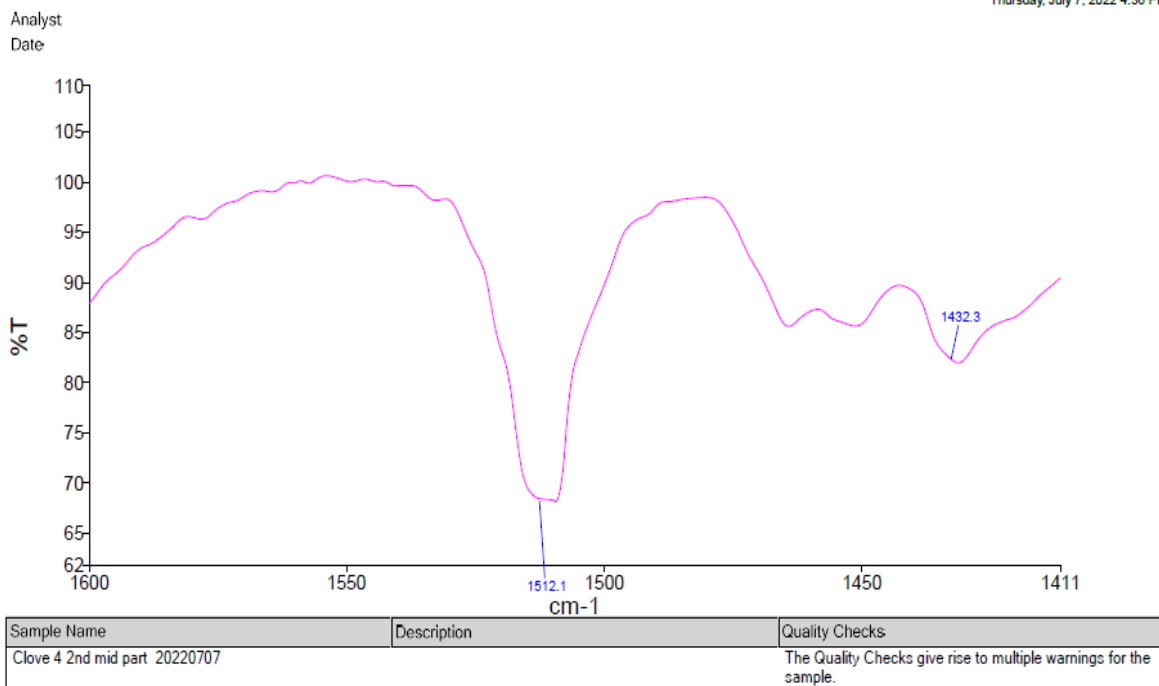


Figure 15. IR Spectra of Sample 04 (2nd mid part)- % Transmittance VS wavenumber.

Figure 16 Illustrates IR Spectra of sample 04 (2nd mid part)- % Absorbance VS wavenumber.

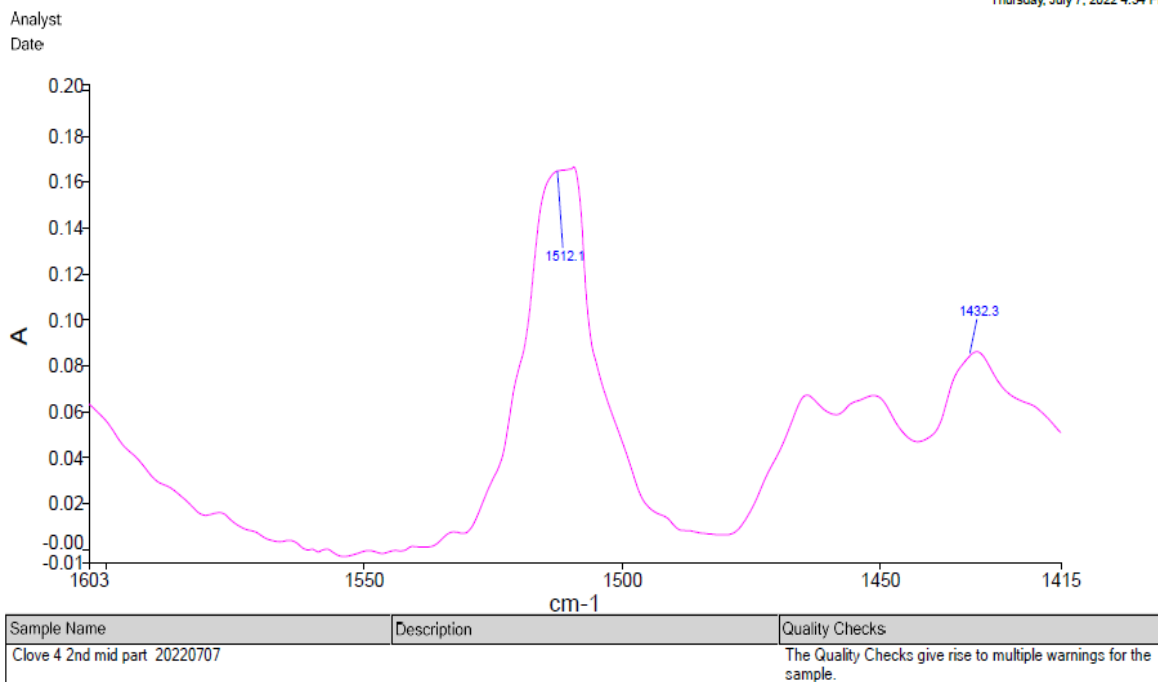


Figure 16. IR Spectra of sample 04 (2nd mid part)- % Absorbance VS wavenumber.

Figure 17 Illustrates IR spectra of sample 05 (tail part)- % Transmittance VS wavenumber.

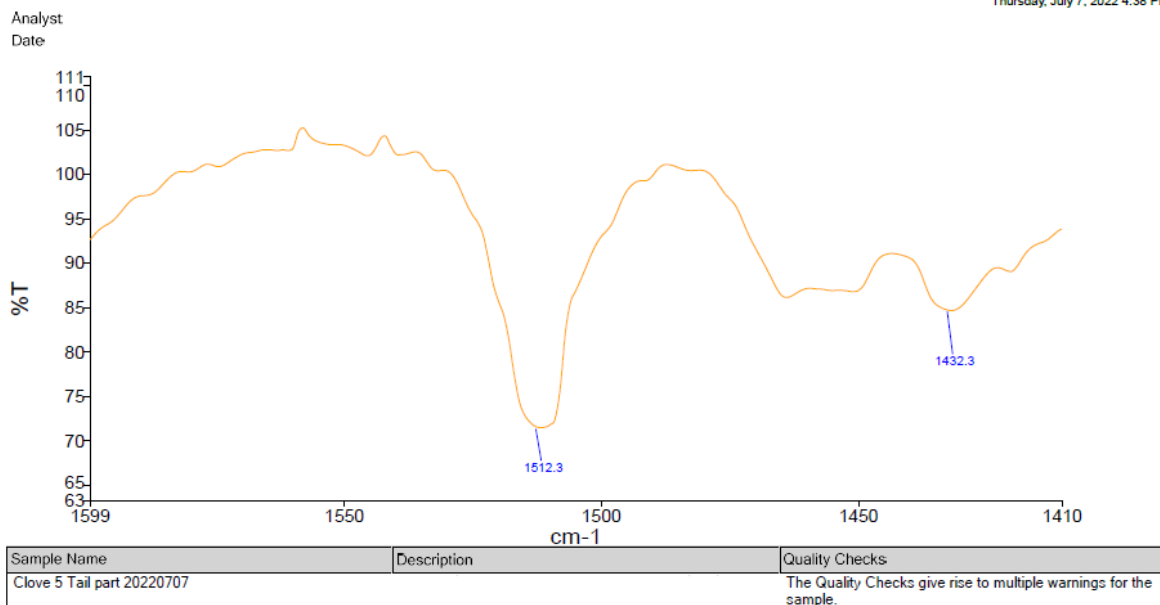


Figure 17. IR spectra of sample 05 (tail part)- % Transmittance VS wavenumber.

Figure 18 illustrates ASTM International, founded as the American Society for Testing and Materials, is a nonprofit organization that develops and publishes approximately 12,000 technical standards IR Spectra of Sample 05 (tail part)- % Absorbance VS wavenumber.

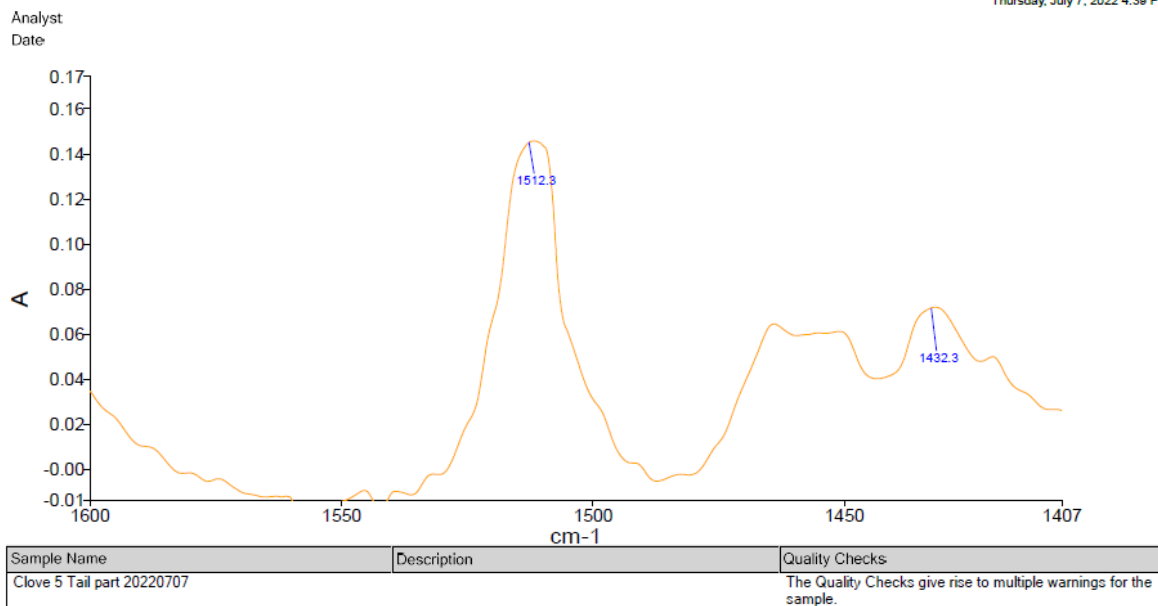


Figure 18. IR Spectra of Sample 05 (tail part)- % Absorbance VS wavenumber.

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