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Characterization and Quantitative Assessment of Antibiotic Cefixime Drug Using Raman and Time-domain Terahertz Spectroscopy

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This paper reports the use of Raman and time-domain terahertz spectroscopy to ascertain the potential of antibiotic cefixime drug which is widely used for curing bacterial infections and obtained from different pharmaceuticals companies. The indigenously designed terahertz spectrometer between 0.1−2.8 THz range was employed for recording of time domain spectra of drug molecules. The refractive index, absorption coefficients and absorbance strength of this drug in THz domain were also ascertained. THz-Raman spectra simultaneously helps to characterize the crystalline nature of cefixime.s and its chemical composition and effect and concentration of its principal ingredients. The Raman spectra of these drugs molecules was recorded for cross verification of previous experimental findings. Finally, we have identified the absorbance strength of two important ingredients, i. e., sulpha pyridine and sulpha thiazoles and specific absorbance of these ingredients was ascertained in THz domain to quantify the concentration per milligram level between 0.5 and 0.8 THz range. The obtained results reveal that concentration of these two ingredients varies from sample to sample. It help to determine the efficacy and side effects of the drugs produced from different manufacturers.

Keywords: Raman, THz spectroscopy, sulpha, drug, absorption, refractive index, concentration.

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1. Introduction

Terahertz (THz) or sub millimeter/far-infrared waves refer to electromagnetic radiation in between 0.1 to 10 THz frequency ranges. THz spectroscopy is a nondestructive, non-contact, and real-time technique that requires very little sample preparation, it can be utilized for improving quality and uniformity of pharmaceutical products, such as identifying polymorphic forms, measuring tablet coating thickness, and 3D chemical mapping. These properties contribute greatly to drug dissolution and bioavailability [1–5]. Moreover, it covers the fingerprint region of vibrational.rotational lines of most of the organic, inorganic and bio molecules. Also, terahertz radiation has low energy and deep penetration ability from leather, cloths, and PTFE (Teflon) and packing material [6].

In the present study, we have employed indigenously designed THz spectrometer tunable between 0.1−3.0 THz range. In present system LT GasAs based photoconductive antenna and ZnTe crystals were used as a source and detector. A femtoseconds laser source of 800 nm wavelength of 140 fs pulse duration obtained from Ti:sapphire oscillator at 80 MHz repetition rate [7]. The study was mainly focused on the Cefixime tablets collected from five major pharmaceuticals companies and sold in different names and designated as FK027, FRI7027 and CL284635, and is an orally administered cephalosporin with a broad spectrum of antibacterial activity in vitro. Cefixime has 3-hour elimination half-life that is taken twice daily by patients suffering from bacterial infections. In many instances, it is also administered once daily. Comparative trials, by various pharmaceutical companies indicate that the clinical and bacteriological efficiency of cefixime in doses of 200 to 400 mg daily which is administered as a single dose or in 2 divided doses over the duration of a day is comparable with that of multiple daily doses of co-trimoxazole (trimethoprim + sulphamethoxazole) or amoxicillin in acute uncomplicated urinary tract infections. Several comparative trials in children with acute obits media demonstrate the similar effectiveness of cefixime 8 mg/kg daily (in 2 divided doses or as a single daily dose) [8]. The most frequently reported adverse effects of consuming cefixime tablet are diarrhoea and stool changes which have been observed in various patients. These symptoms are usually mild to moderate in severity and are transient in nature which mostly occurs in the first few days of treatment. Thus, cefixime is an effective orally active cephalosporin with a relatively long elimination half-life that permits a simplified treatment procedure. It is a suitable alternative to therapeutic trials comparing the clinical and bacteriological efficacy of cefixime with that of other orally active antibacterial drugs in adults.

THz radiation interacts with molecules and generates various resonances associated with vibrations and weak phonons modes in optical and acoustic domains. Whereas Raman spectroscopy also provides similar information in forms of Stokes and antiStokes lines which are associated with vibrational states of the entire molecule but can.t detect as many resonant states as can be detected by THz due

to higher sensitivity [9–11]. The quantification of principal ingradient of drug molecules is one of the important tasks in THz domain which can be very useful for curing the patients. This can be achieved by using absorption and effective mass measurement data [12]. To the best of our knowledge it is the first report on the quantification of principal ingredient in cefixine antibiotic drug.

2. Experimental Details

The experiment was carried out in three parts. In first step, we have prepared the medicine pallets in PTFE (Teflon) matrix. The medicine tablets were first crushed and grinded using a mortar-pestle. Now 200 mg of test sample was mixed with equal weight of PTFE (Teflon) powder using 20 ml of CH_3OH solvent. The mixture was dried for 20 minutes and finally grinded with mortar to convert it into fine homogenous powder. The powder mixture was further divided into two halves and transferred to press mills for making pellets. The thickness and diameter of pellet were 2 and 12 mm, respectively. The THz wavelength matches with the size of the sample present in the pallets and are responsible for the inhomogeneous broadening of the spectral peaks due to scattering effect. Moreover, scattering in THz spectra caused by refractive index mismatch between the sample medicine and the surrounding pellet medium (PTFE in this case) is also observed. The experimental setup used for the experiment is similar to our previous work where PC antenna source was replaced by the ZnTe crystal [7].

Ti: Sapphire femtosecond oscillator (Coherent Chameleon Ultra II) was used as a source. It delivers P-polarized laser pulses of 140 fs pulse duration with 80 MHz repetition rate. The laser output wavelength was selected at 800 nm for THz generation. The variable attenuator (from Eskpla Company) was employed to attenuate the average output power. The incident average power was allowed to be incident on the ¡110¿ oriented ZnTe crystal of 2.0 mm thickness for THz generation. The crystal was housed in a Teflon holder and placed in an optical rotator for vertical rotation. The incident femtoseconds laser pulses were loosely focused onto the crystal by means of a plane-convex lens of focal length 10 cm. The spot diameter of laser pulse on ZnTe crystal was ~ 234 microns. The generated THz radiation was collected using two half axis parabolic mirrors. The diameter (D) and an effective focal length (f_e) of parabolic mirrors (MPD508762-90-M01) were ~ 50 and ~ 150 mm, respectively. The residual transmitted laser pulses from the source were separated from THz radiation using a high resistivity float zone (HRFZ) silicon plate (diameter 50 mm, thickness 2 mm) filter. The photocurrent generated by PC antenna detector was amplified with the preamplifier and the fed to the lock-in amplifier. The output signal from lock-in amplifier was sent to personal computer for the recording of time and frequency domain signals using an indigenously designed data acquisition program made in Lab view. In the third step, these samples were subjected to 780 nm wavelength for the recording of different bands using Raman spectroscopy.

Table 1 shows the details of the medicine samples used along with other physical characteristics of the sample used.

The Raman Spectra of drugs molecules and their corresponding vibrational bands which are decoded are represented in Table 2. Since Raman spectroscopy is treated as a valuable technique for the investigation of solid-state drugs in molecular level, because it provides the rich intra-molecular structural information and also inter-molecular (e. g., hydrogen bonding effect) and interactions between the molecules. Raman spectra of the cefixime tablet (their physical mixture), is recorded in the wave-number range of $200-3000\,\mathrm{cm}^{-1}$ as shown in Fig. 2

3. Results and Discussion

This section is divided into five subsections. The first subsection deals with the time domain THz spectroscopy of drugs molecules while subsection 2 with Raman spectra and identification of some important vibartional modes and their corresponding band assignments. Subsection 3 deals with comparison between THz and Raman spectroscopy and fingerprint spectra in terms of structural and chemical configurations. Subsection 4 highlights the THz refractive index, absorption coefficients of drugs molecules and identification of their crystalline nature and presence of two important ingredients of sulpha groups. Final subsection 5 is quantitative analysis of some important chemical groups in THz range.

3.1. Time domain THz Spectroscopy

Figure 1 shows the temporal and spectral profiles of air, PTFE (Teflon) and medicine samples in THz domain. It is observed that THz pulse show different delay time after passing through different samples of cefixime. It is attributed to the change in optical properties and path length of the transmitted radiation which varies from sample to sample. It is attributes to change in the refractive index of the molecules. The spectral peaks clearly show the variation in the intensity and shift in the absorption peaks of medicine samples. The experiment was performed in ambient conditions at room temperature.

Figure 1 depicts the temporal and frequency domain spectrum of the samples between $0.1-2.4$ THz range. The dynamic range of the amplitude of spectral lines lies between 10^{-4} to 10^{-7} ranges on log scale which helps us in recording of the weak absorption peaks of the medicine samples [13].

3.2. Raman Spectroscopy

The drugs samples were subjected to Raman Spectrometer and spectra were recorded at 780 nm wavelength. We have used WITEC-alpha 300 micro Raman spectrometer

Table 1. The details of the medicine samples used along with other physical characteristics of the sample used

Sample Nomber	Sample Name	Manufacturing Company	Major Component	Mass, mg	MolecularWeight, g/mol	Chemical Formula
1.	Cefixime TabletIP	Cipla	Cefixime	200	453.444	$C_{16}H_{21}N_5O_{10}S_2.H_2O$
2.	Cefixime Tablet IP	Sun Pharmaceutical Ind. Ltd	Cefixime	200	453.444	$C_{16}H_{21}N_5O_{10}S_2.H_2O$
3.	Cefixime-AZ	German Remedies	Cefixime	200	453.452	$C_{16}H_{21}N_5O_{10}S_2.H_2O$
4.	Taxim-O	ALKEM Health Science	Anhydrous Cefixime	200	453.444	$C_{16}H_{21}N_5O_{10}S_2$
5.	Milixim	Glenmark	Anhydrous Cefixime	200	453.444	$C_{16}H_{21}N_5O_{10}S_2$

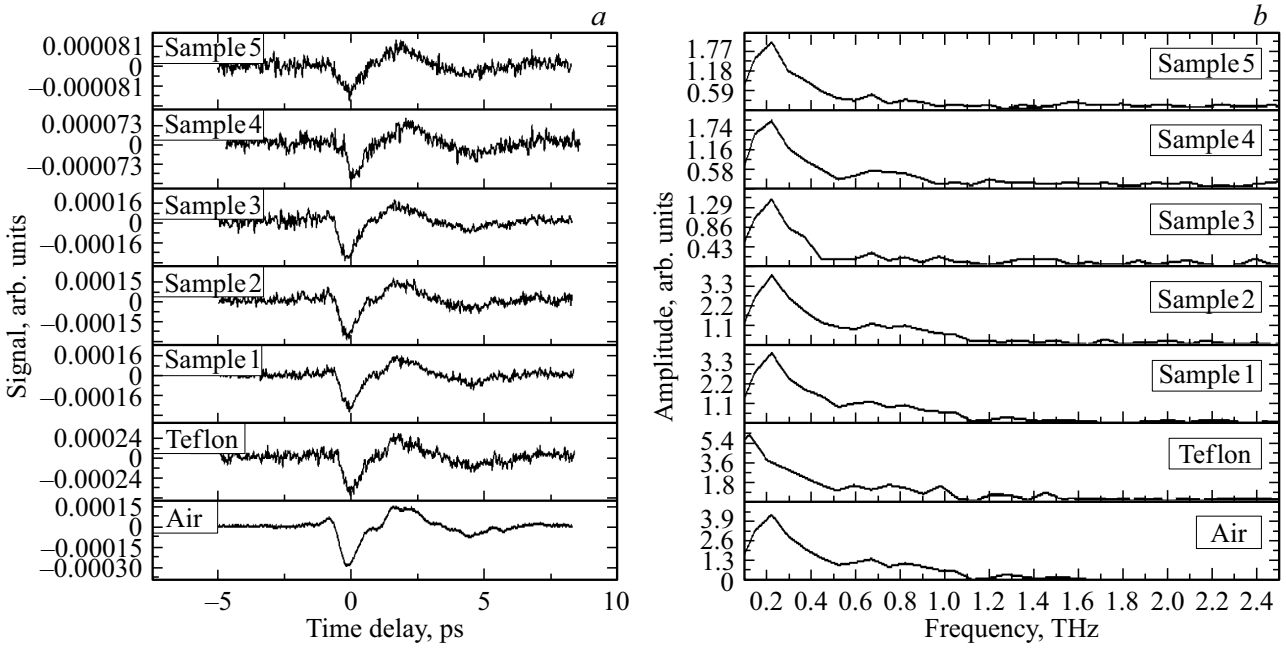

Figure 1. The schematic THz spectrum: (*a*) temporal profile of THz-pulse, (*b*) frequency domain specta of different drug samples.

(Germany) for the recording of the spectra. The software was used for the scan-control spectroscopy and controlling the stage movement. Figure 3 shows the experimental results of all the samples (1–5) and share a common peak related to *D* band at 1612.97 cm^{-1}. The presence of D band in all samples show the similarity among all tablets from different sources; cefixime is sold by different manufacturers in the market but the most important point to be taken in consideration is that apart from this peak, there is no other peak/band found common among these drug samples which should ideally have been the case if all the samples had the same composition. It is a well known fact that the intensities and vibrational modes of Raman spectra are associated with the change in polarization of specific chemical bonds within molecules. The peaks observed and their corresponding band location is represented in Table 2. Raman spectra are based on the band intensity values from the full biologically relevant spectral region, i.e., from 600–1800 cm^{-1} and from 2800–3100 cm^{-1} (this approach is also known as FRDA). D and G bands are presented in all the samples but aliphatic chain shows strongest intensity in case of S_4 and are missed in S_5 while S_1 shows moderate intensity. However, S_2 and S_3 show weak peaks of aliphatic chain.

Since D and G notations in the Raman spectroscopy are used for the ordered carbon structure but it can also be used for other types of organic samples. For example we have used the role of D and G bands information of carbon in detection of TNT explosive molecules [14]. Since antibiotics molecules also belong to organic group. Therefore, we have used the idea of D and G bands in case of antibiotics samples. The experimental results obtained from Raman spectra clearly show that all the samples (1–5) share a common peak at 1612.97 cm^{-1} which corresponds to D band. This helps us to conclude that there is some similarity among all the drugs samples containing organic molecules which contain ordered carbon structures. It is also evident from the chemical formula and structures of different polymorphs of cefixime antibiotic samples as given in the Table 1. We can clearly see that they too contain ordered carbon structures.

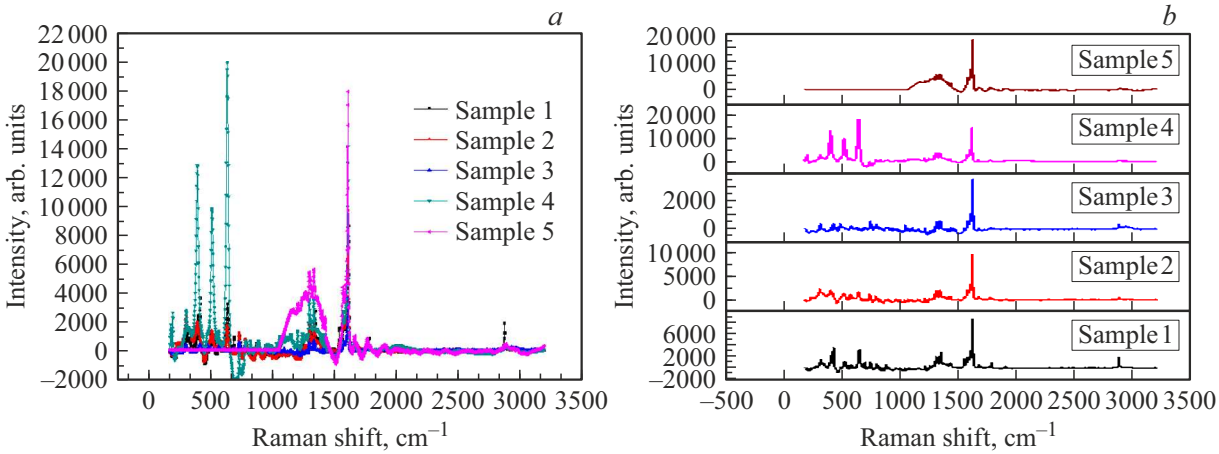

Figure 2. Raman spectroscopy of medicine samples (Sample 1– Sample 5).

Table 2. The correspondence of various peaks obtained in the Raman spectra to its corresponding bond assignments

Common properties			Aliphatic Chain	Aliphatic Chain	D	D	D	D	G	G
S_1	Intensity (arb.units)	373.578	173.164	506.643	598.360	401.4031	16.0139	47.5579	560.931	3570.124
	Raman shift (cm^{-1})	302.764	302.764	302.764	302.764	302.764	302.764	302.764	302.764	302.764
S_2	Intensity (arb.units)	1409.44	3597.306	1934.9	2068.56	2721.211	–	1530.94	1870.402	8602.194
	Raman shift (cm^{-1})	304.946	413.442	1300.65	1336.19	1345.9073	–	1541.01	1570.094	1612.62
S_3	Intensity (arb.units)	–	–	5400.71	5589.86	–	2699.18	–	4383.901	17870.18
	Raman shift (cm^{-1})	–	–	1330.26	1336.64	–	1403.37	–	1570.8	1612.939
S_4	Intensity (arb.units)	2270.27	1450.89	1858.74	2000.78	–	–	–	2088.358	9654.499
	Raman shift (cm^{-1})	305.223	413.4	1300.83	1336.00	–	–	–	1569.29	1612.967
S_5	Intensity (arb.units)	2726.21	–	3502.19	3549.17	–	–	–	2687.5	14135.15
	Raman shift (cm^{-1})	306.88	–	1300.415	1336.08	–	–	–	1569.93	1613.809

3.3. Comparison between THz Spectroscopy and Raman Spectroscopy

Generally, Raman spectroscopy is associated with molecular vibrations in the 200–4000 cm^{-1} spectral range .Within this range, the region between 500 and 1500 cm^{-1} is usually known as the „fingerprint“ of the molecules. But in case of molecules having very low vibrational modes it is very difficult to analyse using Raman spectroscopy. Whereas THz spectroscopy can be used for identification of weaker vibrational modes which also falls in the FIR or sub-millimetre (100 GHz to 3.0 THz or 3–100 cm^{-1}) range. Therefore, peaks which were missed in the Raman spectroscopy due to low phonon absorption and low vibrational frequencies could easily be detected in THz time domain spectra. The range of THz generated for the medicine samples is 0.1–1.6 THz as shown in Fig. 1, *b* which corresponds to 3.3356–53.3702 cm^{-1} range and shown in Fig. 2. We can clearly see that such low vibrational frequency between 3.3356–53.3702 cm^{-1} is not covered in the Raman spectroscopy range but it becomes possible in case of THz spectroscopy. This is also shown in Table 3.

Table 3. Comparison between Raman and THz spectroscopy

Parameter (Experimental)	THz spectroscopy, THz	Corresponding Raman spectroscopy, cm^{-1}
Initial frequency obtained	0.1	3.3356
Final frequency obtained	1.6	53.3702

The following schematic frequency scale diagram (Fig. 3) explains the common region between Raman Spectroscopy and time-domain THz spectroscopy [15–17].

Since the Stokes lines of Raman spectra is extended up to the far IR spectral range. Therefore, extension of THz spectral range may overlap the considerable part of Raman spectra. These features are attributed to molecular vibrations or phonon modes of the material of interest; however, unlike

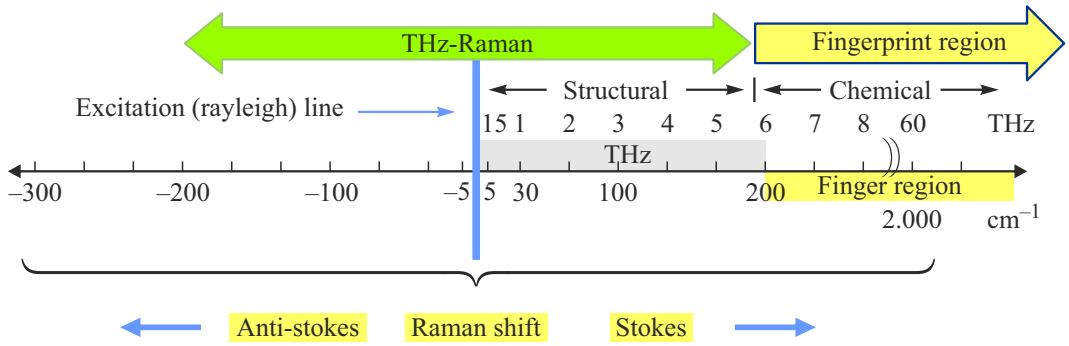

Figure 3. Schematic spectroscopic scale of Raman and THz spectroscopy.

IR spectroscopy, these features are sampled in a form of inelastic scattering, because they deal with the measurement of the transfer of energy between the molecule and the photons of the excitation laser.

Therefore, the difference between conventional Raman spectroscopy and THz-Raman not only covers the different spectral range, but also complimentary to each other and support to provide the set of structural information of the given organic sample In this study we have only covered THz spectroscopy between 0.1–2.4 THz range which only covers with the structural aspects of the drugs sample whereas given range of Raman spectra covers the chemical information in terms of D and G bands along with aliphatic chain.

3.4. Absorption Coefficient and Refractive Index

The time domain terahertz signal received after passing through the samples was converted into frequency domain spectra using FFT. The value of absorption coefficient $\alpha(\omega)$ was calculated form FFT spectrum. The transmitted field T is given by equation

$$T = \frac{E_{\text{sample}}}{E_{\text{reference}}}. \quad (1)$$

Here E_{sample} and $E_{\text{reference}}$ are the amplitudes of THz radiation after passing through material and reference samples, respectively. We can calculate the effective thickness l of the sample distributed in PTFE (Teflon) matrix using formula

$$l = \frac{m}{\rho}\frac{4}{\Pi D^2}. \quad (2)$$

Here, m — mass of the sample (100 mg), D — diameter of sample (12 mm), ρ — density of the sample. Since the pellets contains mixture of two sample absorption coefficient α can be ascertained using formula [18].

$$\alpha == \frac{1}{l}\ln\frac{T_m}{T_s}. \quad (3)$$

Here l is the effective thickness of the sample and T_m, T_s are the transmitted intensity from material sample and reference, respectively. The refractive index of the sample can be calculated using equation

$$n = 1 + \frac{\Delta\phi c}{2\pi\nu d}. \quad (4)$$

Where $\Delta\phi$ — the phase difference between reference and sample, ν is the frequency, d is thickness of pellet and c — the velocity of light. The complex refractive index may be presented using Kramer's–Kronig model:

$$\mathbf{n} = n + i\kappa. \quad (5)$$

Where n is the real part of the refractive index and κ is extinction coefficient which is related to the power absorption coefficient α.

The absorption peak is attributed to the intermolecular vibrational modes in terahertz region. The corresponding terahertz absorption peaks for samples 1–5 are located at 0.5, 1.5, 2.00 and 2.5 cm^{-1} range, respectively (Fig. 4). The peaks which were missing in the Raman spectroscopy due to low phonon absorption could only be detected in THz domain. The absorption coefficients of the sample 1 to sample 5 follow the following order. At 0.5 THz: $S_5 > S_2 > S_3 > S_4 > S_1$, at 1 THz: $S_4 > S_5 > S_2 > S_1 > S_3$, at 1.5 THz: $S_4 > S_2 > S_3 > S_5 > S_1$, at 2.0 THz: $S_5 > S_4 > S_1 > S_2 = S_3$ and at 2.5 THz: $S_2 > S_4 > S_5 > S_3 > S_1$. The refractive index constantly decreases up to 1.5 THz for all samples and then it becomes constant. The refractive index of S_4 is highest and has the value 1.75 followed by S_5, S_2, S_3 and S_1. Absorption peaks located at 0.5 and 2.6 THz range show highest value and assigned to sulpha pyridine and sulpha thiazole, respectively. But intensity of 0.5 THz band for S_1 is the lowest and S_3 show the highest values. In addition, the value of refractive index drops rapidly from 0.25 to 1.0 THz range. It also confirms the crystalline nature between these ranges. But S_1 shows crystalline nature between 0.1–0.5 THz range.

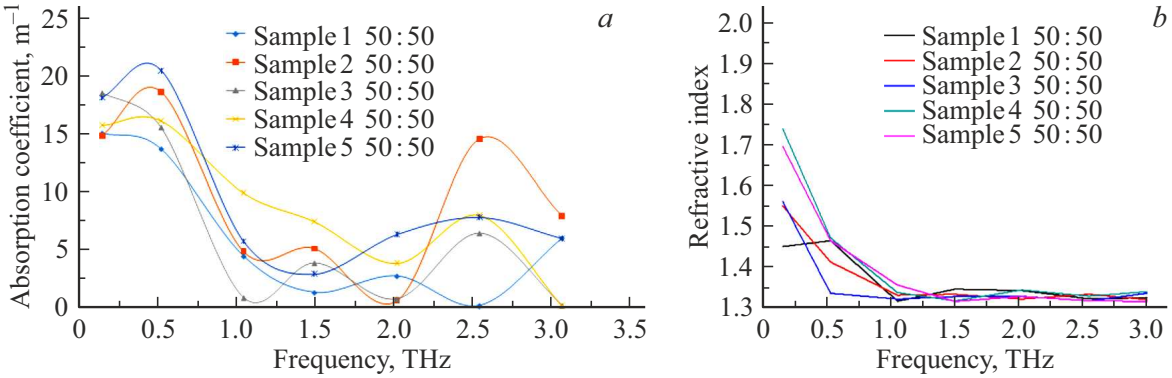

Figure 4. The absorption coefficient and the refractive index of the medicine samples 1–5.

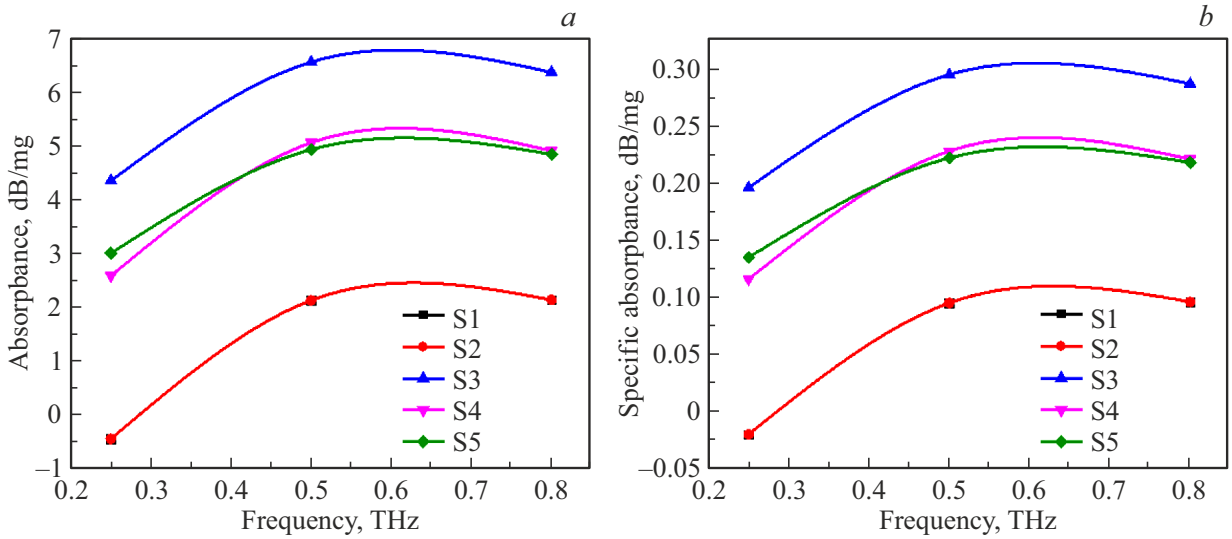

Figure 5. The absorbance strength and the specific absorbance of the samples.

3.5. Absorbance Strength

Once the spectral data of a sample were obtained, the absorbance $A(\nu)$ may be calculated (in dB) as

$$A(\nu) = -10 \log \frac{E_{\text{sample}}(\nu)}{E_{\text{reference}}(\nu)},$$

where $E(\nu)$ represents the strength of the THz peak [16]. In order to calculate the specific absorbance strength, the effective mass M_{eff} is required to be calculated. Thus,

$$M_{\text{eff}} = M_{\text{sample}} \frac{\text{cross-section of THz beam}}{\text{cross-section of sample pellet}}.$$

Hence, specific absorbance $A_s(\nu)$ is given as,

$$A_s(\nu) = \frac{A(\nu)}{M_{\text{eff}}}.$$

The specific absorbance is calculated at 0.5 THz. The results are provided in Table 4.

Table 4. Absorbance strength and specific absorbance at 0.5 THz

Sample	Absorbance strength, $A(\nu)$	Specific absorbance, $A_s(\nu)$
S_1	2.13	0.095
S_2	2.13	0.095
S_3	6.57	0.295
S_4	5.07	0.228
S_5	4.94	0.222

The following graphs (Fig. 5) represent the above data at three frequencies: 0.25, 0.5 and 0.8 THz.

The most important part of present work is quantitative analysis by means of absorbance strength and specific absorbance. The effective mass Meff is calculated to ascertain the actual quantity of drug participated in transmission/absorption process. This also indicates the strength of particular functional group and efficacy of drug and most importantly to distinguish actual and fake medicines.

4. Conclusions

We have successfully recorded the time domain THz and Raman spectra of cefixine drug of different manufacturers. In addition, we have ascertained their refractive indices and absorption coefficients in THz domain and carried out quantitative analysis using absorbance strength. The Raman spectra help to identify the aliphatic chain along with D and G bands .Whereas THz spectroscopy identify the crystalline nature and quantify the sulphha pyridine and thiazole groups which are the principal ingredients of the drugs molecules. The higher or lower percentage of these groups can help to define the efficacy and side effects of the same drugs produced by different manufacturers and help to open a new channel research.

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