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**Review Article**

# In Situ Monitoring of Ritonavir Protective and Therapeutic Influence as a Potent Drug on *Coronavirus* Disease–2019 (*COVID–19*) Infection by Attenuated Total Reflectance–Fourier Transform Infrared (ATR–FTIR Fingerprint) Biospectroscopy

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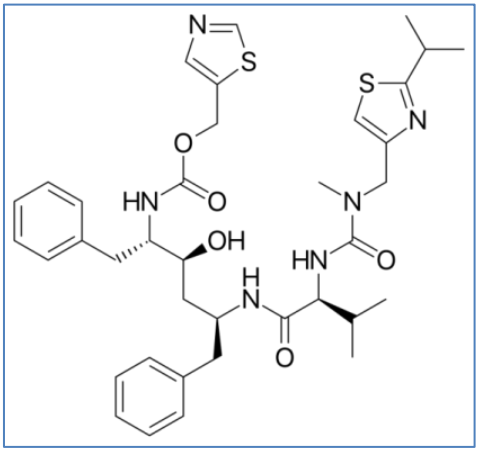
## Abstract

Ritonavir is an antiretroviral of the protease inhibitor class. It is used against HIV infections as a fixed–dose combination with another protease inhibitor, ritonavir (lopinavir/ritonavir). In the current research, the stimulated ATR–FTIR biospectroscopy of liquid sample of Ritonavir was investigated. The stimulated ATR–FTIR diffractions emitted through focusing the second harmonic laser beam Nd:YAG into the sample were recorded by Echelle spectrometer and ICCD detector. Increasing the energy of laser beam from 2.6 (mJ) to 16 (mJ) was led to increase in stimulated ATR–FTIR signal but after breakdown threshold of liquid sample, more increasing of energy was led to decrease in stimulate ATR–FTIR signals and for energies higher than 20 (mJ), they were disappeared.




Skeletal formula of ritonavir (original trade name Norvir)—an HIV protease inhibitor and a pharmacokinetic booster. Orientation made to match to show the structural similarity between ritonavir and cobicistat. Created with ChemDoodle 8.0.0.b1 and Adobe Illustrator CC 2015.

**Keywords:** ATR–FTIR Biospectroscopy, Stimulated ATR–FTIR Biospectroscopy, Ritonavir, Breakdown, Coronavirus Disease–2019, COVID–19, Infection, Protective and Therapeutic Effect, Potent Drug.



## INTRODUCTION

ATR–FTIR biospectroscopy is a vibration biospectroscopy based on the influence of ATR–FTIR [1–47]. The influence of ATR–FTIR is elastically diffracting the electromagnetic ray due to rotational and vibrational transitions in molecules and its characteristic is changing the energy of diffracted beam photons compared to incident beam [48-95]. The difference between wavelength of incident beam light

and diffracted light is related to molecular vibrations and is considered as exclusive "chemical finger print" of sample and can be used in identification of molecular compounds on a surface, into a liquid or into the air [96–142].

The stimulated ATR–FTIR diffraction is a non–linear effect [143-189]. If the pumping intensity exceeds the threshold of this effect, it observes [190–237]. The pumping threshold limit for stimulated ATR–FTIR depends on ATR–FTIR active material [238–285]. Regarding the spectral characteristics, stimulated ATR–FTIR can be distinguished from normal ATR–FTIR [286–333]. While the intensity of ATR–FTIR bands are several times smaller than pumping laser intensity in normal ATR–FTIR, the intensity of ATR–FTIR bands in stimulated ATR–FTIR can be similar to laser intensity and for most materials, only strongest ATR–FTIR bands of material are intensified and are dominant in the recorded spectrum of material [334–377].

In the current research, the stimulated ATR–FTIR spectrum are obtained through pumping the second harmonic beam laser Nd:YAG and it is performed by a spectrometer and detector. The resulted spectra and their characteristics are investigated here.

**Experimental Arrangement**

The experimental arrangement used in the current study is schematically shown in Figure (1). The first harmonic bicolor mirror reflects 1064 (nm) but passes the second harmonic one. As a result, the first harmonic removes from laser beam. The second harmonic laser Nd: YAG with wavelength of 532 (nm) and pulse width of 8 (ns) interacts with the sample after passing through bicolor mirror and lens with focal length of 3.5 (cm). The resulted emissions from this interaction filters by an optical system consisting some lens and optical fiber conducts to Eschelle spectrometer. The necessary time range for collecting spectra and its start time in ICCD detector controls by delayer device. Optical emissions of sample collects and intensifies from the striking moment of laser to sample until 5 (ms) after that moment. Test was repeated five times for each energy level for laser energy from 2.4 (mJ) to 29 (mJ).

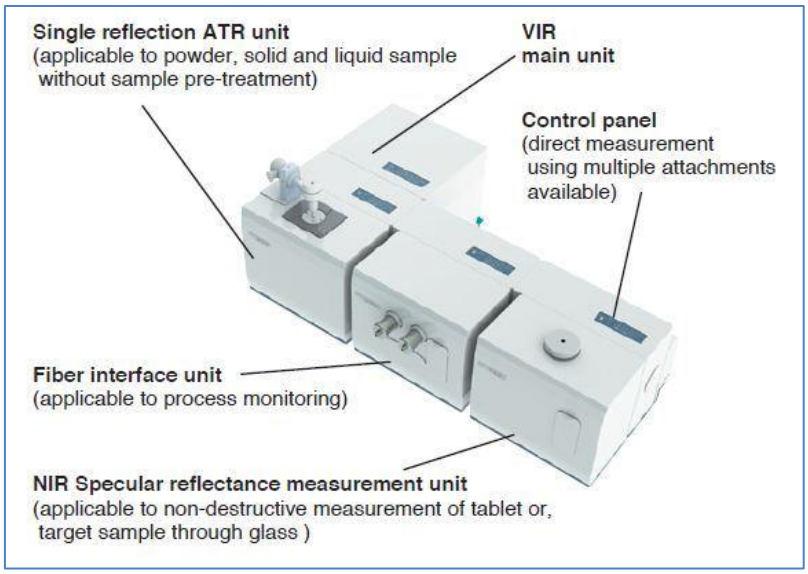



**Fig-1: Schematic of stimulated ATR–FTIR biospectroscopy test arrangement**

## RESULTS AND DISCUSSION

Figure (2) shows the normal and stimulated ATR–FTIR spectra. Normal ATR–FTIR spectrum can be obtained when laser beam is not focused on the sample. When laser beam focuses on sample using a lens, non–linear effects stimulate and stronger bands of ATR–FTIR spectrum intensify up to some levels of laser intensity.

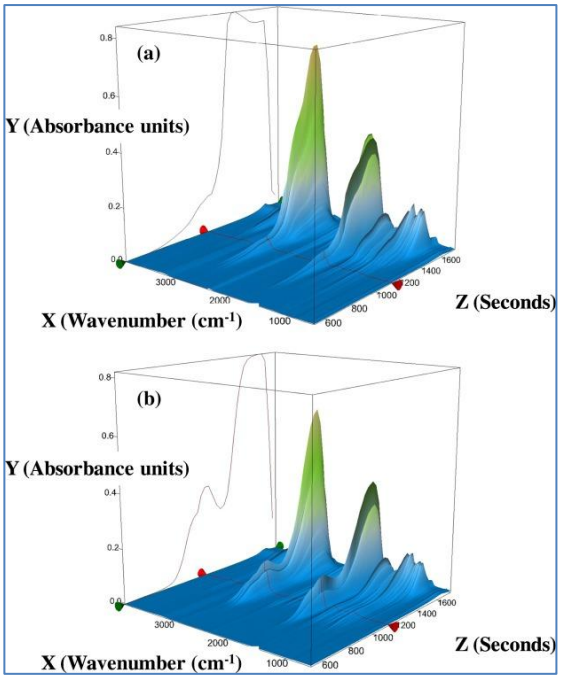



**Fig-2: (a) Normal and (b) stimulated ATR–FTIR spectra for Ritonavir**

By increasing the energy of laser beam, the intensity of main bands of 3333 ($cm^{-1}$) and 3563 ($cm^{-1}$) also are increased and for energy levels higher than 8 (mJ), anti–Stokes ATR–FTIR band corresponding to 3333 ($cm^{-1}$) intensifies in the spectrum and can be observed at left hand side of laser line in ATR–FTIR shift of –3333 ($cm^{-1}$). Recording the anti–Stokes band necessitates the occupation of corresponding vibration level through diffraction of Stokes ATR–FTIR (Table 1).

By more increasing the energy level higher than 16 (mJ), all four graphs of Figure (3) shows reduction in intensity. The reason for this reduction is creation of spark in the Ritonavir liquid due to increase in energy of laser more than the breakdown threshold of liquid. As a result of this spark, which creates in the center of liquid, laser beam absorbs by liquid and some part of it diffracts and only this part plays a role in creation of stimulated ATR–FTIR. By increasing the energy, beam has higher contribution in making the spark and the diffracted emission which reaches to detector decreases.

**Table-1: ATR–FTIR modes for Ritonavir**

| | ATR–FTIR Shift ($cm^{-1}$) | ATR–FTIR Mode |
|---|---|---|
| **1** | 1085 ($cm^{-1}$) | C–H Stretch |
| **2** | 1593 ($cm^{-1}$) | $CH_2$ Rocking |
| **4** | 1927 ($cm^{-1}$) | $CH_2$ Wagging |
| **5** | 3333 ($cm^{-1}$) | $CH_2$ Symmetric Stretch |
| **7** | 3563 ($cm^{-1}$) | C–H Asymmetric Stretch |

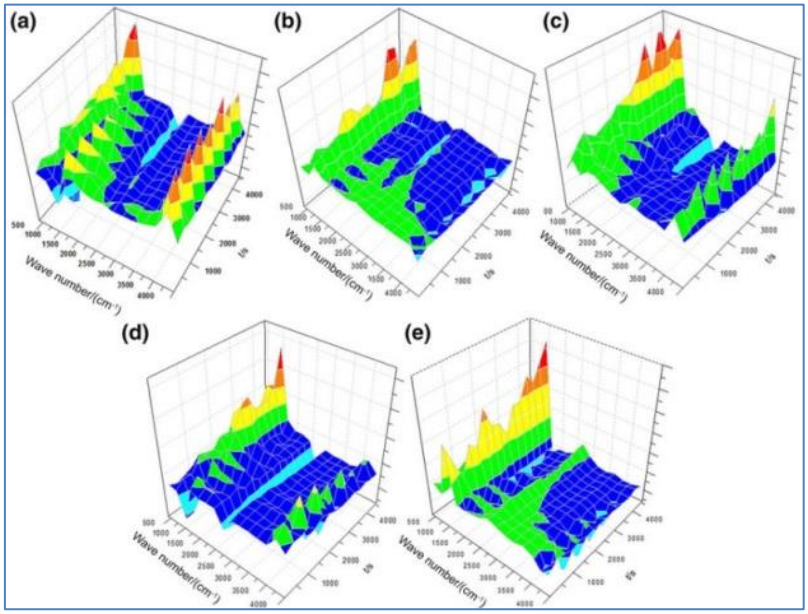



**Fig-3: Peak intensity (a) band 1593 ($cm^{-1}$), (b) 1927 ($cm^{-1}$), (c) band 3333 ($cm^{-1}$), (d) band 3563 ($cm^{-1}$) and (e) band –3333 ($cm^{-1}$) based on increase in energy level of beam focused on the liquid**

## CONCLUSIONS AND SUMMARY

The stimulated ATR–FTIR biospectroscopy test was performed for liquid sample of Ritonavir. The main band at 3333 ($cm^{-1}$) shows an intensity level comparable to pumping laser intensity. The intensity of stimulated ATR–FTIR spectrum at 16 (mJ) energy level is the highest intensity in this test and more increasing the energy level reduces the intensity of spectrum. The reason for this reduction is creation of spark in the Ritonavir liquid due to increase in energy of laser more than the breakdown threshold of Ritonavir.

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