

## Structural Characterization and Isotopic Abundance Ratio Analysis of the Consciousness Energy Healing Treated Ofloxacin

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### Abstract

Ofloxacin is an antibiotic, useful against the number of bacterial infections. This scientific investigation was performed to identify the impact of the Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment on the structural properties and the isotopic abundance ratio of ofloxacin using sophisticated analytical techniques. Ofloxacin sample was divided into control and treated parts. Only the treated ofloxacin received the Consciousness Energy Healing Treatment remotely by a well-known Biofield Energy Healer, Mr. Mahendra Kumar Trivedi. The LC-MS spectra of both the samples of ofloxacin at retention time 3 minutes exhibited the mass of the protonated molecular ion peak at  $m/z$  362.17  $[M+H]^+$ . The chromatographic peak area% of the treated ofloxacin (52.4%) was increased by 2.03% compared to the control sample (51.36%). The LC-MS based isotopic abundance ratio of  $P_{M+1}/P_M$  in the Biofield Treated ofloxacin was significantly increased by 22.43% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of  $P_{M+1}/P_M$  in the Biofield Treated ofloxacin was significantly increased by 19.24% compared with the control sample. The LC-MS and GC-MS based isotopic abundance ratio of  $P_{M+1}/P_M$  ( $^2H/^1H$  or  $^{15}N/^{14}N$  or  $^{13}C/^{12}C$  or  $^{17}O/^{16}O$ ) was significantly increased in the Biofield Treated ofloxacin as compared to the control sample. Thus,  $^2H$ ,  $^{15}N$ ,  $^{13}C$ , and  $^{17}O$  contributions from  $(C_{18}H_{21}FN_3O_4)^+$  to  $m/z$  363.17 in the treated ofloxacin were significantly increased compared with the control sample. The increased isotopic abundance ratio of the Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treated ofloxacin may increase the intra-atomic bond strength and increase its physical stability. The new form of treated ofloxacin would be more stable, better soluble, and bioavailable compared to the control sample. It would be more useful to design efficacious pharmaceutical formulations that might offer better therapeutic response against infections in the urethra, urinary tract, gonorrhoea, pneumonia, infectious diarrhoea, bronchitis, cellulitis, bacterial infection of the eye and ear, multidrug-resistant tuberculosis, prostatitis, otitis media, plague, etc.

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## Introduction

Ofloxacin is a class of antibiotics, useful against the number of bacterial infections caused by *Escherichia coli*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Proteus*, *Salmonella* and *Shigella species*, *Neisseriaceae*, *Yersinia enterocolitica*, *Haemophilus influenza*, etc. [1]. Ofloxacin act by means of inhibiting bacterium's DNA [2]. It is useful in the treatment of infections of the urethra, cervix, and urinary tract, infectious diarrhoea, cellulitis, chronic bronchitis, prostatitis, tuberculosis, pneumonia, otitis media, plague, etc. [1, 3, 4]. The side effects associated with the use of ofloxacin are diarrhoea, tendon rupture, numbness, headache, skin rash, vomiting, seizures, psychosis, etc. [1]. It may increase the drug concentration in the blood i.e., theophylline, warfarin, cyclosporine, etc., and also increase the anticoagulant, cardiotoxicity and arrhythmias activity of acenocoumarol, barbiturate, etc. like drugs [4, 5]. The major problems associated with ofloxacin has short biological half-life, and its bioavailability is more dependent upon the physiological condition of the GIT. It is soluble in acidic media but precipitates in alkaline media, which lead to loss of its solubility [4]. Dissolution, absorption, and bioavailability of the pharmaceutical compound depend on the physical and chemical properties it [6]. The Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment also called the Biofield Energy Healing Treatment has been significantly improved the physicochemical properties, isotopic abundance ratios, and bioavailability of pharmaceutical and nutraceutical compounds [7-9]. The Trivedi Effect<sup>®</sup> is a natural and lone scientifically proven phenomenon, which involves that an expert individual can harness this inherently intelligent energy from the "Universe" and transfer to any object(s) on the planet *via* the possible mediation of neutrinos [10]. The "Biofield Energy" in an electromagnetic energy field which exists surrounding all the living beings, generated by the continuous movement of the electrically charged particles, i.e., ions, cells, etc. inside the body. The process of the harness of energy and transmit the energy into any living and non-living object(s) is called Biofield Energy Healing Treatment, which has significant outcomes against various disease [11-14]. Such Biofield Energy is recommended by the National Institutes of Health/

National Center for Complementary and Alternative Medicine (NIH/NCCAM) under the category of Complementary and Alternative Medicine (CAM) along with other therapies, medicines, and practices such as Ayurvedic medicine, homeopathy, traditional Chinese herbs and medicines, massage, acupressure, acupuncture, yoga, meditation, Reiki, hypnotherapy, Tai Chi, Qi Gong, aromatherapy, chiropractic/osteopathic manipulation, movement therapy, cranial-sacral therapy, applied prayer, etc. The CAM therapies have huge acceptance by the USA people with a beneficial effect in the treatment of various disease conditions [15, 16]. Various scientific experimental studies reported the astounding capability of the Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment for its significant effect on the different objects in the field of materials science, agriculture science, microbiology, medical science [17-24], etc.

The Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment could be an economical approach for the alteration of the physicochemical and thermal properties of ofloxacin for the designing of the better pharmaceuticals formulations. To understand the isotope effects, the stable isotope ratio analysis has various applications in different scientific fields [25, 26]. Isotope ratio analysis can be performed by using the mass spectrometry techniques such as gas chromatography - mass spectrometry (GC-MS) and liquid chromatography - mass spectrometry (LC-MS) in low micromolar concentration with sufficient precision [25, 27]. Thus, the LC-MS and GC-MS were used to evaluate the impact of the Trivedi Effect<sup>®</sup> - Consciousness Energy Healing Treatment on the structural properties and isotopic abundance ratio analysis of  $P_{M+1}/P_M$  ( $^2\text{H}/^1\text{H}$  or  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  or  $^{17}\text{O}/^{16}\text{O}$ ) and  $P_{M+2}/P_M$  ( $^{18}\text{O}/^{16}\text{O}$ ) in treated ofloxacin compared to the control sample.

## Materials and Methods

### Chemicals and Reagents

The ofloxacin powder sample was purchased from Sigma Aldrich, USA and remaining reagents used during the experiments were purchased in India.

### Consciousness Energy Healing Treatment Strategies

The ofloxacin powder sample was equally divided into two parts and termed as the control and Biofield Energy Treated sample. The treated ofloxacin

was received the Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 minutes by the popular Biofield Energy Healer, Mahendra Kumar Trivedi, USA. The Biofield Energy Treatment was provided through the healer's unique energy transmission process. However, the control ofloxacin received treatment from a "sham" healer who did not have any knowledge about the Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment. After the treatment both the samples were kept in sealed conditions and analyzed using sophisticated analytical techniques.

#### Characterization

##### Liquid Chromatography-Mass Spectrometry (LC-MS)

##### Analysis and Calculation of Isotopic Abundance Ratio

The LC-MS analysis of the ofloxacin samples was carried out with the help of LC-MS/MS ThermoFisher Scientific (USA), fitted out with an ion trap detector connected with a triple-stage quadrupole mass spectrometer. A reversed-phase Thermo Scientific Synchronis C18 (Length-250 mm X ID 4.6 mm X 5 micron) column was used maintained at 25°C. 10 µL of ofloxacin solution in methanol was injected. The analyte was eluted in gradient phase using 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B) pumped at a constant flow rate of 0.6 mL/min for 10 min. Peaks were monitored at 254 nm using the PDA detector. The Electrospray ionization (+ve) technique was used in the mass spectrometric analysis. The total ion chromatogram and mass spectrum of the individual peak (appeared in LC-MS) were recorded. The natural abundance of each isotope (C, O, H, and N) can be predicted from the peak [26, 28-30].

##### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS of the ofloxacin samples were analyzed with the help of Perkin Elmer Gas chromatograph equipped with a PE-5MS (30M x 250 micros x 0.250 microns) capillary column and coupled to a single quadrupole mass detector was operated with a positive electron impact (EI) ionization mode. The Oven temperature was programmed from 75°C (5 min hold) to 280°C (14 min hold) @ 10°C /min (total run time 40 min). The diluent for the sample preparation was acetonitrile in water.

The % change in the LC-MS and GC-MS based

isotopic abundance ratios ( $P_{M+1}/P_M$  and  $P_{M+2}/P_M$ ) for the control and Biofield Energy Treated ofloxacin was calculated.

% Change in isotopic abundance ratio =  $[(IAR_{Treated} - IAR_{Control}) / IAR_{Control}] \times 100$

Where  $IAR_{Treated}$  = isotopic abundance ratio in the treated ofloxacin and  $IAR_{Control}$  = isotopic abundance ratio in the control ofloxacin.

## Results and Discussion

### Liquid Chromatography-Mass Spectrometry (LC-MS)

The chromatograms of both the ofloxacin samples showed the single major chromatographic peak at the retention time ( $R_t$ ) 3 minutes (Figure 1). The  $R_t$  indicated that the polarity of the Biofield Energy Treated ofloxacin (3.04 minutes) was slightly increased compared to the control sample (3.02 minutes). The peak area% of the treated ofloxacin (52.4%) was increased by 2.03% compared to the control sample (51.36%). Which indicated that the Biofield Energy Treated ofloxacin would be more soluble compared to the control sample. The result was supported by one of the studies that the particle size of the physicochemical properties of Biofield Energy Treated ofloxacin was significantly decreased and the surface area was increased compared to the control sample [7]. The increased surface area of the Biofield Energy Treated ofloxacin might be the cause of increased solubility Ofloxacin shows the molecular ion peak  $[M+H]^+$  at  $m/z$  362 in positive ion mode [31]. The mass spectra of both the samples of ofloxacin (Figure 2) exhibited the protonated molecular ion peak at  $m/z$  362.17  $[M+H]^+$  (calculated for  $C_{18}H_{21}FN_3O_4^+$ , 362.15), along with the fragment ion peaks near  $m/z$  318.08, 261.08, and 214 corresponded to the molecular formula  $C_{17}H_{21}FN_3O_2^+$ ,  $C_{13}H_9FNO_4^+$ , and  $C_9H_5NO_2^+$ , respectively (Figure 3).

The mass spectra of both the samples showed the mass of the molecular ion peak  $[M+H]^+$  at  $m/z$  362.17 (calculated for  $C_{18}H_{21}FN_3O_4^+$ , 362.15) with relative intensity of 100%. The theoretical calculation of  $P_{M+1}$  for ofloxacin was presented as below:  
 $P(^{13}C) = [(18 \times 1.1\%) \times 100\% \text{ (the actual size of the } M^+ \text{ peak)}] / 100\% = 18.8\%$   
 $P(^2H) = [(21 \times 0.015\%) \times 100\%] / 100\% = 0.315\%$   
 $P(^{15}N) = [(3 \times 0.4\%) \times 100\%] / 100\% = 1.2\%$   
 $P(^{17}O) = [(4 \times 0.04\%) \times 100\%] / 100\% = 0.16\%$   
 $P_{M+1}$ , i.e.  $^2H$ ,  $^{15}N$ ,  $^{13}C$ , and  $^{17}O$  contributions from

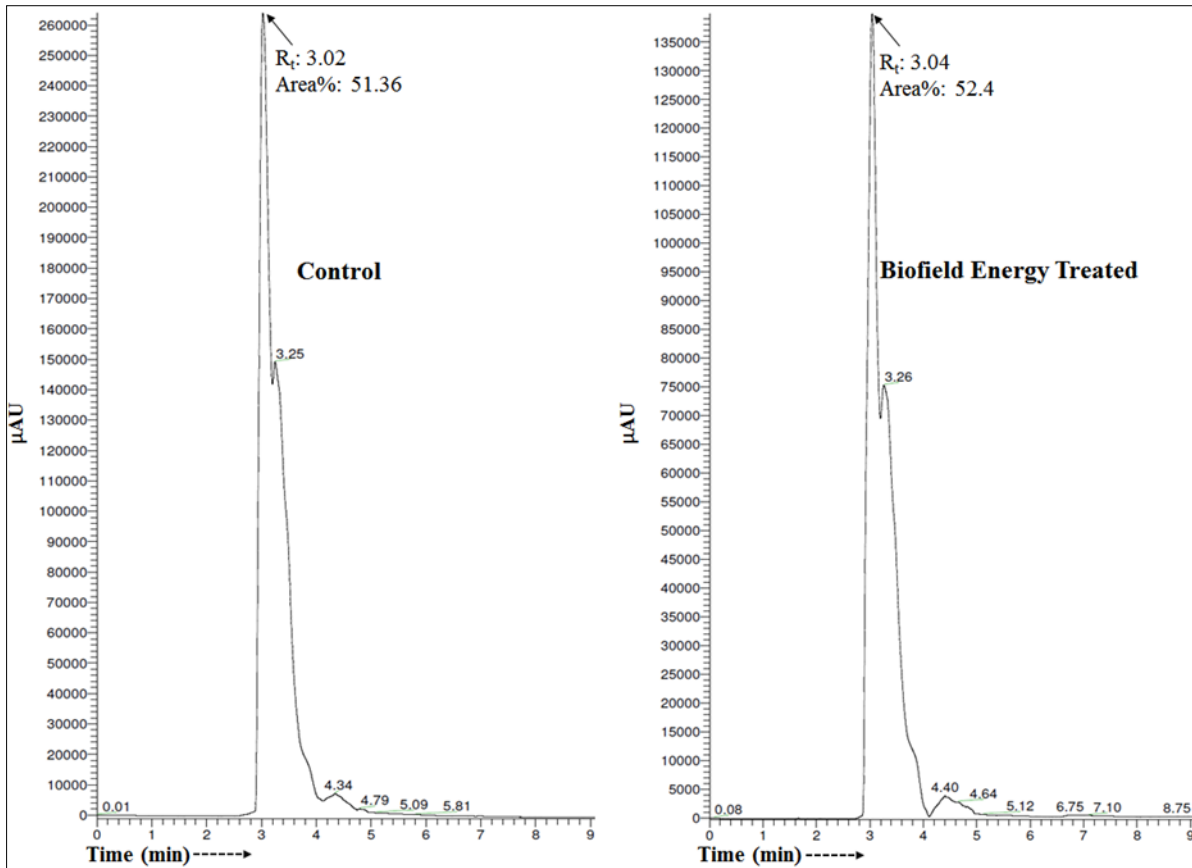


Figure 1. Liquid chromatograms of the control and treated ofloxacin.

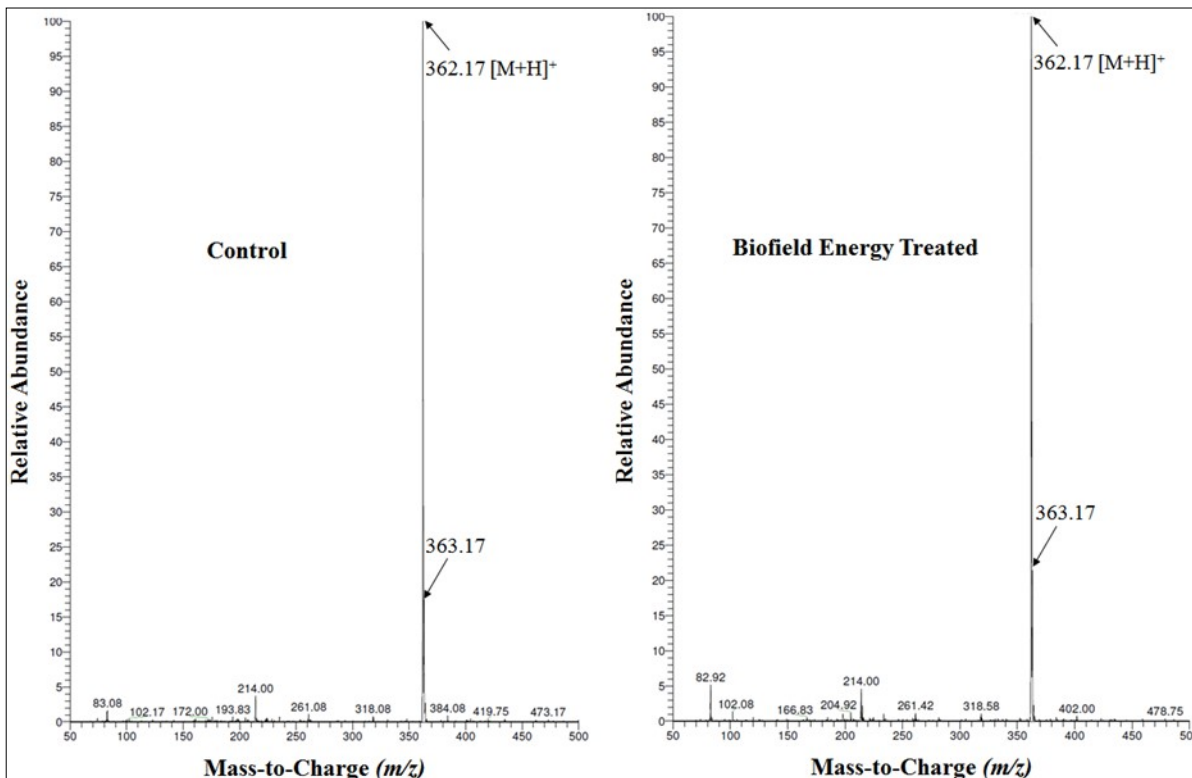


Figure 2. Mass spectra of the control and Biofield Energy Treated ofloxacin at  $R_t$  3 minutes.

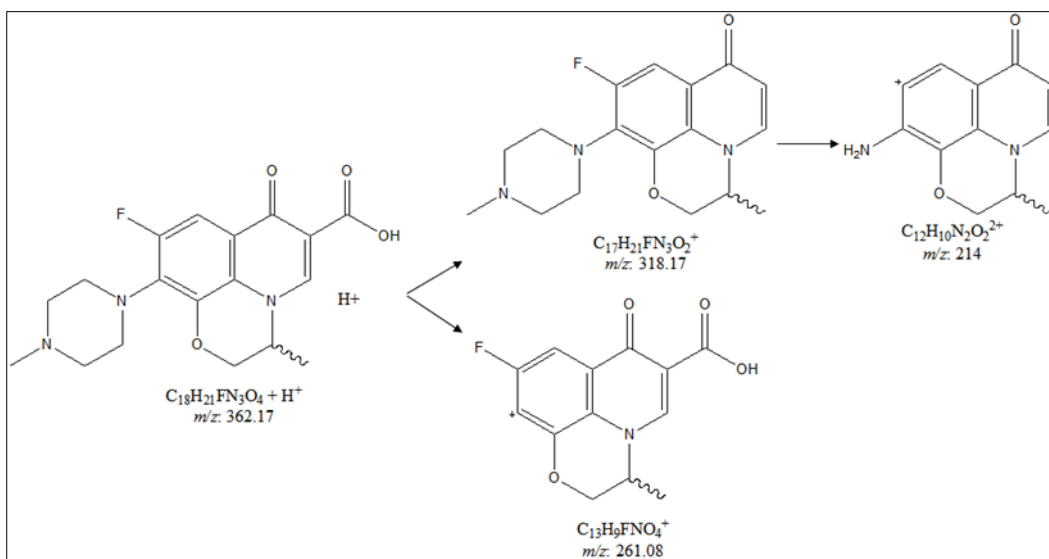


Figure 3. Proposed fragmentation pattern of ofloxacin with respect to the mass spectra.

$(C_{18}H_{21}FN_3O_4)^+$  to  $m/z$  363.17 = 20.28%

From the above calculation, it has been found that  $^{13}C$  and  $^{15}N$  have major contribution to  $m/z$  363.17. The calculated isotopic abundance is close to the experimental observed value (Table 1).

The LC-MS based isotopic abundance ratio of  $P_{M+1}/P_M$  in the Biofield Energy Treated ofloxacin was significantly increased by 22.43% compared with the control sample (Table 1). Thus,  $^{13}C$ ,  $^2H$ ,  $^{15}N$ , and  $^{17}O$  contributions from  $(C_{18}H_{21}FN_3O_4)^+$  to  $m/z$  363.17 in the treated ofloxacin were significantly increased compared with the control sample.

#### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS chromatograms showed that the  $R_t$  of the Biofield Energy Treated ofloxacin (23.9 minutes) was altered compared to the control sample (22.93 minutes). The peak area% of treated ofloxacin (86.41%) was increased by 1.72% compared to the control sample (84.95%). The dehydrated molecular ion peak at  $m/z$  317 (calculated for  $C_{17}H_{21}FN_3O_2^+$ , 317.15) was observed in the control (Figure 4) and treated (Figure 5) ofloxacin mass spectra. The theoretical calculation of  $P_{M+1}$  for ofloxacin was presented as below:

$$P(^{13}C) = [(17 \times 1.1\%) \times 25.32\% \text{ (the actual size of the } M^+ \text{ peak)}] / 100\% = 4.73\%$$

$$P(^2H) = [(21 \times 0.015\%) \times 25.32\%] / 100\% = 0.08\%$$

$$P(^{15}N) = [(3 \times 0.4\%) \times 25.32\%] / 100\% = 0.3\%$$

$$P(^{17}O) = [(2 \times 0.04\%) \times 25.32\%] / 100\% = 0.02\%$$

$P_{M+1}$ , i.e.  $^2H$ ,  $^{15}N$ ,  $^{13}C$ , and  $^{17}O$  contributions from  $(C_{17}H_{21}FN_3O_2)^+$  to  $m/z$  318 = 5.13%

From the above calculation, it has been found that  $^{13}C$  and  $^{15}N$  have a major contribution towards the  $m/z$  318.

Similarly, the theoretical calculation of  $P_{M+2}$  for ofloxacin was presented as below:

$$P(^{18}O) = [(2 \times 0.2\%) \times 25.32\%] / 100\% = 0.1\%$$

$P_{M+2}$ , i.e.  $^{18}O$  contributions from  $(C_{18}H_{21}FN_3O_4)^+$  to  $m/z$  319 = 0.1%

From the above calculation, it has been found that  $^{18}O$  have major contribution to  $m/z$  319. The calculated isotopic abundance is close to the experimental observed value (Table 2).

The GC-MS based isotopic abundance ratio analysis of the control and treated ofloxacin samples were calculated for its dehydrated molecular mass at  $m/z$  317 (calculated for  $C_{17}H_{21}FN_3O_2^+$ , 317.15). The  $P_M$ ,  $P_{M+1}$ , and  $P_{M+2}$  for both the ofloxacin near  $m/z$  317 [ $M^+$ ], 318 [ $(M+1)^+$ ], and 319 [ $(M+2)^+$ ], respectively (Table 2). The isotopic abundance ratio of  $P_{M+1}/P_M$  in the treated ofloxacin was significantly increased by 19.24% compared with the control sample (Table 2). But, the isotopic abundance ratio of  $P_{M+2}/P_M$  in the treated ofloxacin was similar compared with the control sample (Table 2). This indicated that the  $^{13}C$ ,  $^2H$ ,  $^{15}N$ , and  $^{17}O$  contributions from  $(C_{18}H_{21}FN_3O_4)^+$  to  $m/z$  318 in the treated ofloxacin were significantly increased compared with the control sample.

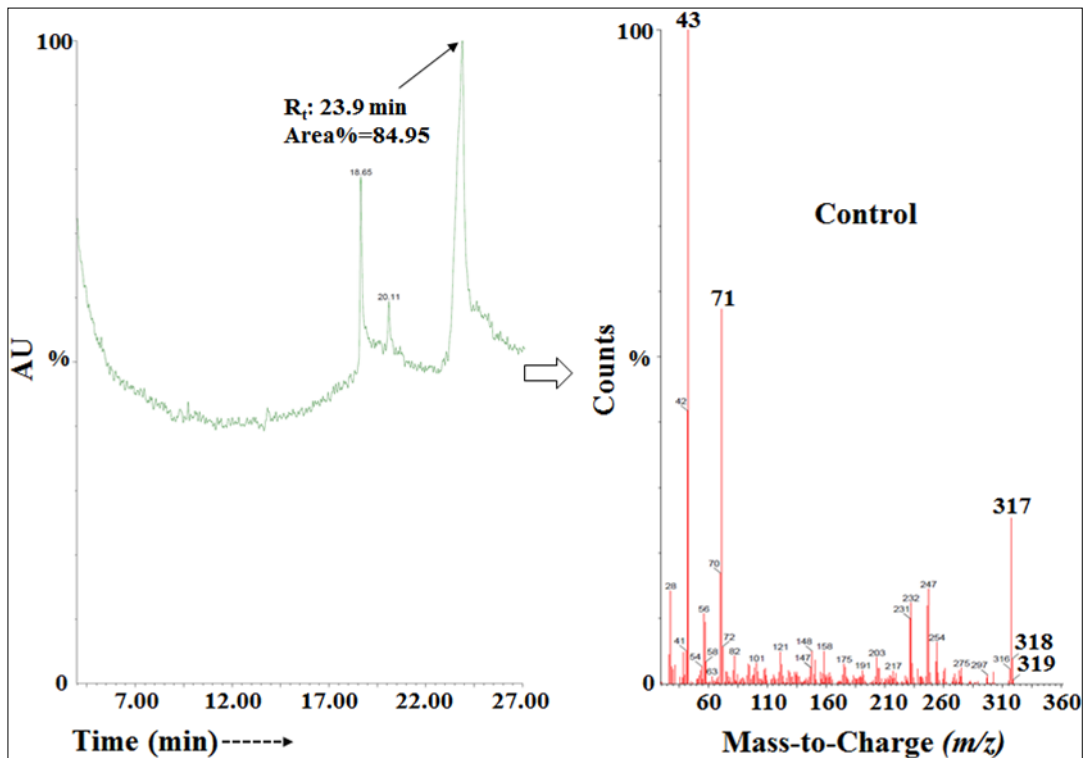


Figure 4. The GC-MS chromatogram and mass spectra of the control ofloxacin.

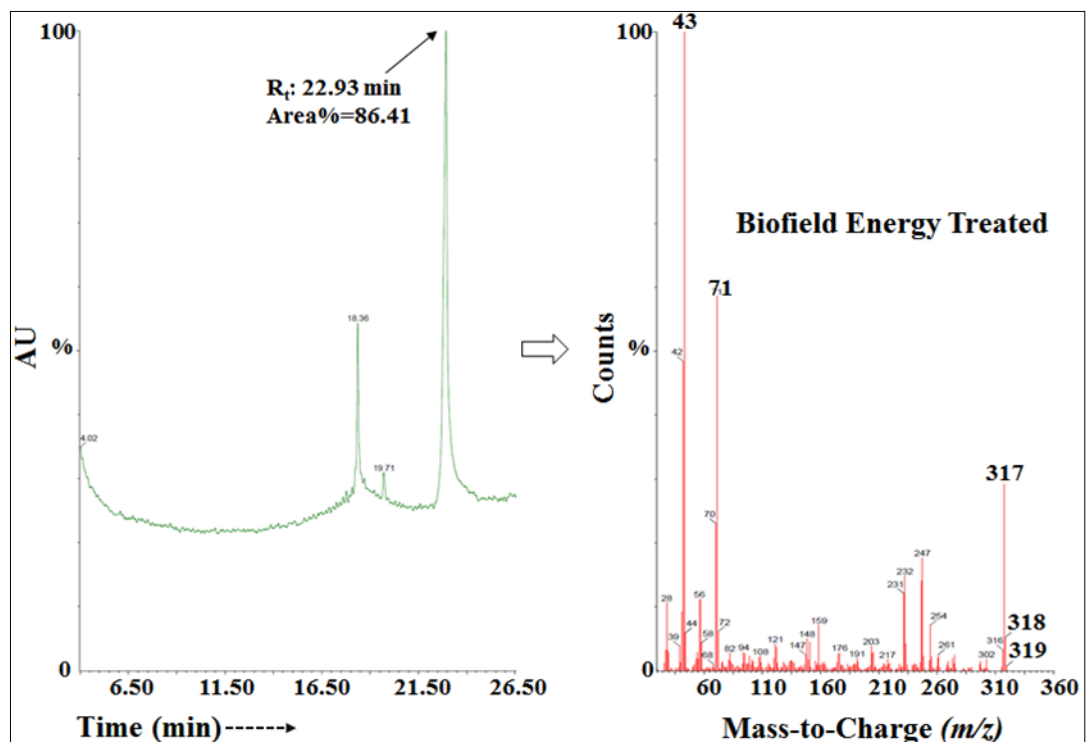


Figure 5. The GC-MS chromatogram and mass spectra of the Biofield Energy Treated ofloxacin.

Table 1. Comparative LC-MS based isotopic abundance results analysis of the Biofield Energy Treated ofloxacin vs the control sample.

Parameter	Control Sample	Biofield Energy Treated Sample
$P_M$ at $m/z$ 362.17 (%)	100	100
$P_{M+1}$ at $m/z$ 363.17 (%)	17.48	21.4
$P_{M+1}/P_M$	0.17	0.21
% Change of isotopic abundance ratio ( $P_{M+1}/P_M$ ) with respect to the control sample		22.43

$P_M$ : the relative peak intensity of the parent ofloxacin ion  $[M^+]$ ;  $P_{M+1}$ : the relative peak intensity of the isotopic ofloxacin ion  $[(M+1)^+]$ , M: mass of the parent molecule.

Table 2. Comparative GC-MS based isotopic abundance results analysis of the Biofield Energy Treated ofloxacin vs the control sample.

Parameter	Control Sample	Biofield Energy Treated Sample
$P_M$ at $m/z$ 317 (%)	25.32	29.19
$P_{M+1}$ at $m/z$ 318 (%)	3.79	5.21
$P_{M+1}/P_M$	0.15	0.18
% Change of isotopic abundance ratio ( $P_{M+1}/P_M$ ) with respect to the control sample		19.24
$P_{M+1}$ at $m/z$ 319 (%)	0.60	0.69
$P_{M+2}/P_M$	0.02	0.02
% Change of isotopic abundance ratio ( $P_{M+2}/P_M$ ) with respect to the control sample		0.00

$P_M$ : the relative peak intensity of the parent ofloxacin ion  $[M^+]$ ;  $P_{M+1}$ : the relative peak intensity of the isotopic ofloxacin ion  $[(M+1)^+]$ ;  $P_{M+2}$ : the relative peak intensity of the isotopic ofloxacin ion  $[(M+2)^+]$ , M: mass of the parent molecule.

The LC-MS and GC-MS based isotopic abundance ratio of  $P_{M+1}/P_M$  ( $^2\text{H}/^1\text{H}$  or  $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$  or  $^{17}\text{O}/^{16}\text{O}$ ) was significantly increased compared to the control sample. The increased in isotopic abundance could be due to changes in neutron to proton ratio in the nucleus possibly through the interference of neutrino particles *via* the Trivedi Effect<sup>®</sup> [9, 10]. The neutrino is electrically neutral and the mass is so small. It interacts protons and neutrons in the nucleus *via* the weak subatomic force and gravity, which indicated a close relation between neutrino and the isotope formation [10, 28, 29]. According to Ben Xu *et al.* 2018, reported that varying the isotopic ratios of  $^{13}\text{C} : ^{12}\text{C}$  can change the relative positions and intensities of infrared-active vibrational modes of atoms in a non-linear and mode-dependent fashion [32]. In this experiment, the Biofield Energy Treated ofloxacin has increased the isotopic ratios by 22.43% as compared to the untreated ofloxacin. Based on the findings, authors assumed that the Biofield Treated ofloxacin could be influence the atomic bond vibration. As per literature reported that, accumulation and/or concentrate of heavier isotope that trends to strongest bond [33]. Based on the findings, authors assumed that the Biofield Energy Treated ofloxacin may increase the intra-atomic bond strength, and that leads to increase its physical stability. The new form of treated ofloxacin would be more stable, better soluble, and bioavailable compared to the control sample, which was also supported by the recently published article [7]. It would be more useful to design efficacious pharmaceutical formulations that might offer better therapeutic response against infections in the urethra, urinary tract, gonorrhoea, pneumonia, infectious diarrhoea, bronchitis, cellulitis, bacterial infection of the eye and ear, multidrug-resistant tuberculosis, prostatitis, otitis media, plague, *etc.*

### Conclusions

The Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment showed a significant impact on the peak area and isotopic abundance ratios of the ofloxacin. The LC-MS spectra of both the samples of ofloxacin at  $R_t$  3 minutes exhibited the mass of the protonated molecular ion peak at  $m/z$  362.17  $[\text{M}+\text{H}]^+$ . The chromatographic peak area% of the Biofield Energy Treated ofloxacin was increased by 2.03% compared with the control sample. The LC-MS based isotopic

abundance ratio of  $P_{M+1}/P_M$  in the Biofield Energy Treated ofloxacin was significantly increased by 22.43% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of  $P_{M+1}/P_M$  in the Biofield Energy Treated ofloxacin was significantly increased by 19.24% compared with the control sample. The LC-MS and GC-MS based isotopic abundance ratio of  $P_{M+1}/P_M$  ( $^2\text{H}/^1\text{H}$  or  $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$  or  $^{17}\text{O}/^{16}\text{O}$ ) was significantly increased compared to the control sample. Thus,  $^{13}\text{C}$ ,  $^2\text{H}$ ,  $^{15}\text{N}$ , and  $^{17}\text{O}$  contributions from  $(\text{C}_{18}\text{H}_{21}\text{FN}_3\text{O}_4)^+$  to  $m/z$  363.17 in the treated ofloxacin were significantly increased compared with the control sample. The increased isotopic abundance ratio of the Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treated ofloxacin may increase the intra-atomic bond strength, increase its physical stability. The new form of Biofield Energy Treated ofloxacin would be more stable, better soluble, and bioavailable compared to the control sample. It would be more useful to design efficacious pharmaceutical formulations that might offer better therapeutic response against infections in the urethra, urinary tract, gonorrhoea, pneumonia, infectious diarrhoea, bronchitis, cellulitis, bacterial infection of the eye and ear, multidrug-resistant tuberculosis, prostatitis, otitis media, plague, *etc.*

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