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Research Article

Evaluation of the Impact of Consciousness Energy Healing Treatment on the Isotopic Abundance Ratios (P_{M+1}/P_M and P_{M+2}/P_M) of Ofloxacin

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Abstract

Ofloxacin is a class of fluorinated quinolone antibiotics, useful against most of the Gram-positive and Gram-negative bacterial infections. This study was designed to investigate the impact of the Trivedi Effect®-Consciousness Energy Healing Treatment on the structural properties and the isotopic abundance ratio of ofloxacin using LC-MS and GC-MS spectroscopy. The ofloxacin sample was divided into control and treated parts. The control ofloxacin did not receive the Consciousness Energy Healing Treatment, while the treated ofloxacin received the Consciousness Energy Healing Treatment remotely by a renowned Biofield Energy Healer, Dahryn Trivedi. The LC-ESI-MS spectra of both the samples of ofloxacin at the retention time 3.05 minutes exhibited the mass of the protonated molecular ion peak at *m/z* 362.17 $(M+H)^+$ (calculated for $C_{18}H_{21}FN_3O_4^+$, 362.15). The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated ofloxacin was significantly increased by 56.57% compared with 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 considerably increased compared with the control sample. The GC-MS based isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in the treated ofloxacin were significantly increased by 9.53% and 12.94%, respectively compared with the control sample. Hence, ^2H, ^{15}N, ^{13}C, ^{17}O, and ^{18}O contributions from $(C_{18}H_{21}FN_3O_4)^+$ to *m/z* 318 and 319 in the treated ofloxacin were significantly increased compared with the control sample. The LC-MS and GC-MS based isotopic abundance ratios of P_{M+1}/P_M ($^2H/^1H$ or $^{15}N/^{14}N$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$), and P_{M+2}/P_M ($^{18}O/^{16}O$) in the treated ofloxacin were considerably improved compared to the control sample. The increased isotopic abundance ratio of the treated ofloxacin would increase the chemical bond strength and increase the stability in the body. The new form of treated ofloxacin would be more stable compared to the control sample and would be very useful to design improved pharmaceutical formulations that might offer better therapeutic response

against infections of the urethra and cervix, infectious diarrhoea, urinary tract infections, cellulitis, chronic bronchitis, pneumonia, prostatitis, multidrug-resistant tuberculosis, plague, otitis media, *etc.*

Keywords:
Ofloxacin; The Trivedi Effect®; Biofield Energy; Consciousness Energy Healing Treatment; LC-MS; GC-MS

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Introduction

Ofloxacin is a class of fluorinated quinolone antibiotics, useful against most of the Gram-positive and Gram-negative (*Escherichia coli, Klebsiella, Citrobacter, Enterobacter, Proteus, Salmonella and Shigella species, Neisseriaceae, Yersinia enterocolitica, Haemophilus influenza, etc.*) bacterial infections [1]. Ofloxacin restricts the bacteria cell division restricted by means of inhibiting DNA gyrase, which separates the replicated DNA [2]. It is used for the treatment of diseases like infections of the urethra and cervix, infectious diarrhoea, urinary tract infections, cellulitis, chronic bronchitis, pneumonia, prostatitis, multidrug-resistant tuberculosis, plague, otitis media, *etc.* [1], [3], [4]. Some of the common side effects are headache, vomiting, tendon rupture, diarrhoea, numbness, skin rash, psychosis, seizures, *etc*. [1]. It may increase the blood levels of other drugs such as theophylline, cyclosporine, warfarin, *etc.* by inhibiting the drug metabolizing enzymes. It shows to increase the anticoagulant, cardiotoxicity, and arrhythmias effects of the drugs such as acenocoumarol, barbiturate, *etc*. [4], [5].

Ofloxacin has a short biological half-life, and its bioavailability is dependent upon the physiological condition of the gastrointestinal tract (GIT). It is soluble in acidic media and precipitates in alkaline media; therefore, it loses its solubility [4]. The physical and chemical properties of a pharmaceutical sample play a crucial role in its dissolution, absorption, and bioavailability in the body [6]. In this scenario, the Consciousness Energy Healing Treatment (Trivedi Effect®; Biofield Energy Healing Treatment) has been scientifically proven with a significant impact on the physicochemical properties, isotopic abundance ratios, and bioavailability of pharmaceutical and nutraceutical compounds [7], [8], [9]. The Consciousness Energy Healing Treatment is a natural and lone scientifically proven phenomenon in which an individual can harness this inherently intelligent energy and transfer it anywhere on the planet through the possible mediation of neutrinos (bio-photons) [10]. The "Biofield Energy" in the electromagnetic energy field, which exists surrounding the living beings, generated by the continuous movement of the electrically charged particles (ions, cells, etc.) inside the body. The Biofield Energy Healing experts have the ability to transmit the energy into any living and non-living object(s). This process is called Biofield Energy Healing Treatment [11], [12], [13]. The Biofield based Energy Therapies have been reported with significant outcomes against various diseases [14]. The National

Center of Complementary and Integrative Health has recognized and accepted Biofield Energy Therapy as a Complementary and Alternative Medicine health care approach in addition to other therapies, medicines, and practices such as Qi Gong, Reiki, Tai Chi, yoga, hypnotherapy, etc. [15], [16].

The Trivedi Effect® had been scientifically proved with beneficial outcomes in different fields, *i.e.*, materials science [17], [18], agriculture science [19], [20], microbiology [21], [22], medical science [23], [24], etc. The Consciousness Energy Healing Treatment could be an economical approach and solution for the practical challenges of ofloxacin in designing better pharmaceutical formulations. The isotopic composition of the molecule the stable isotope ratio analysis has various applications in different scientific fields for understanding the isotope effects [25], [26]. Isotope ratio analysis can be performed by using the conventional 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]. Therefore, LC-MS and GC-MS were used in this study to characterize the structural properties and evaluate the isotopic abundance ratio analysis of P_{M+1}/P_M ($^2H/^1H$ or $^{17}O/^{16}O$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$) and P_{M+2}/P_M ($^{18}O/^{16}O$) in the Trivedi Effect® - Consciousness Energy Healing Treated ofloxacin compared to the control sample.

Materials and Methods

Chemicals and Reagents

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

Consciousness Energy Healing Treatment Strategies

The test sample ofloxacin powder was divided into two parts and categorised as the control and Biofield Energy Treated ofloxacin. The control ofloxacin did not receive the Biofield Energy Treatment but was treated by a "sham" healer who did not have any knowledge about the Biofield Energy Treatment. However, the Biofield Energy Treated ofloxacin received the Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 minutes by the renowned Biofield Energy Healer, Dahryn Trivedi, USA. The Biofield Energy Treatment was provided through the healer's unique energy transmission process. After the treatment, both the ofloxacin samples were kept in sealed conditions and analyzed using LC-MS and GC-MS analytical techniques.

Characterization

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis and Calculation of Isotopic Abundance Ratio

The LC-MS analysis of the control and Biofield Energy Treated ofloxacin was carried out with the help of LC-MS/MS ThermoFisher Scientific, the USA equipped with an ion trap detector connected with a triple-stage quadrupole mass spectrometer. The column used here was a reversed phase Thermo Scientific Synchronis C_{18} (Length-250 mm X ID 4.6 mm X 5 micron), maintained at 25˚C. 10 µL of ofloxacin solution in methanol was injected, and the analyte was eluted 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. Chromatographic separation was achieved using gradient conditions, and the total run time was 10 min. Peaks were monitored at 254 nm using the PDA detector. The mass spectrometric analysis was performed under +ve ESI mode. 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], [29], [30].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS of the control and Biofield Energy Treated ofloxacin 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 electron impact (EI) ionization in positive 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

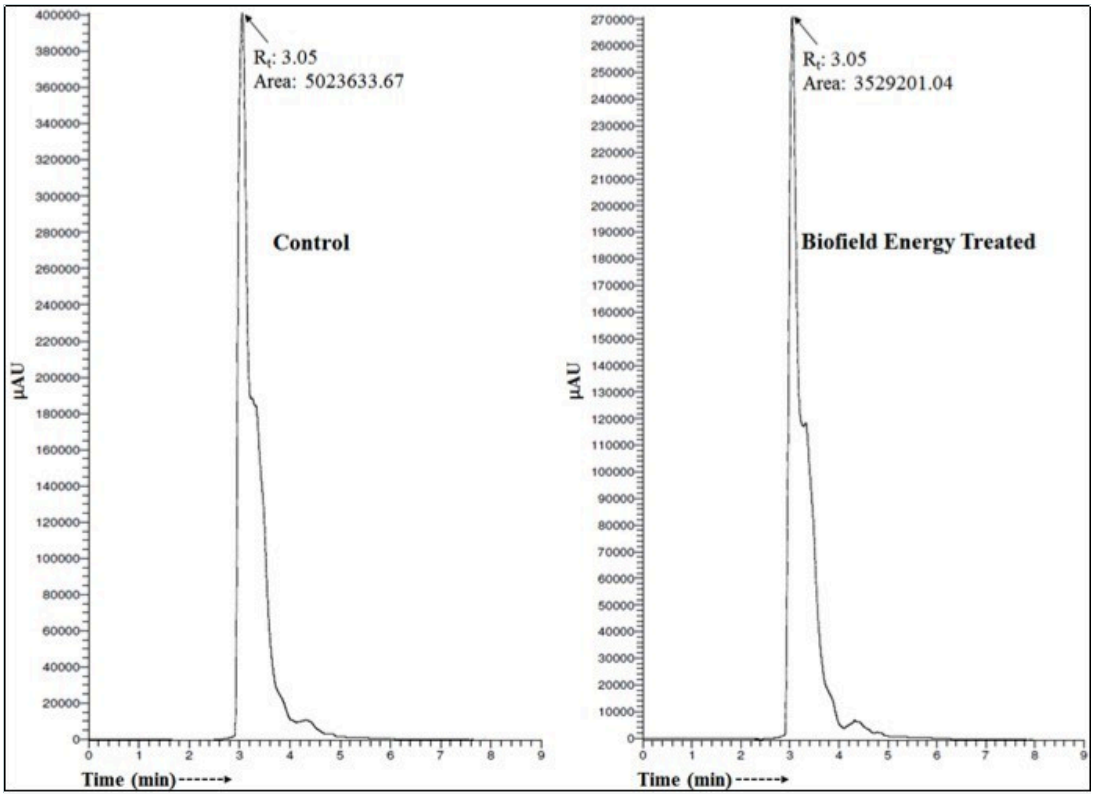


Figure 1. Liquid chromatograms of the control and treated ofloxacin.

acetonitrile in water. Mass spectra were scanned from *m/z* 40 to 400. The identification of the analyte was made by GC retention times and by a comparison of the mass spectra of samples.

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.

Percentage (%) 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 LC chromatograms of both the ofloxacin samples showed the single major chromatographic peak at the retention time (R_t) 3.05 minutes (Figure 1). This R_t indicated that the polarity of both the control and Biofield Energy Treated ofloxacin remained the same. The peak area of the control sample was more than the treated ofloxacin. This indicated that the Biofield Energy Treated ofloxacin would be more stable compared to the control sample. This was proved by the study of the physicochemical properties of Biofield Energy Treated ofloxacin compared to the control sample [7].

Ofloxacin was detected with the molecular mass peak $(M+H)^+$ at *m/z* 362 in the LC-MS spectrum in positive ion mode as per the literature [31]. The mass spectra of both the samples of ofloxacin (Figure 2) at the retention time 3.05 minutes exhibited the mass of 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* 318.17, 261.08, and 213.92 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 LC-ESI-MS spectra of both the control and Biofield Energy Treated ofloxacin 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 x 1.1%) x 100% (the actual size of the M^+ peak)] / 100% = 18.8%

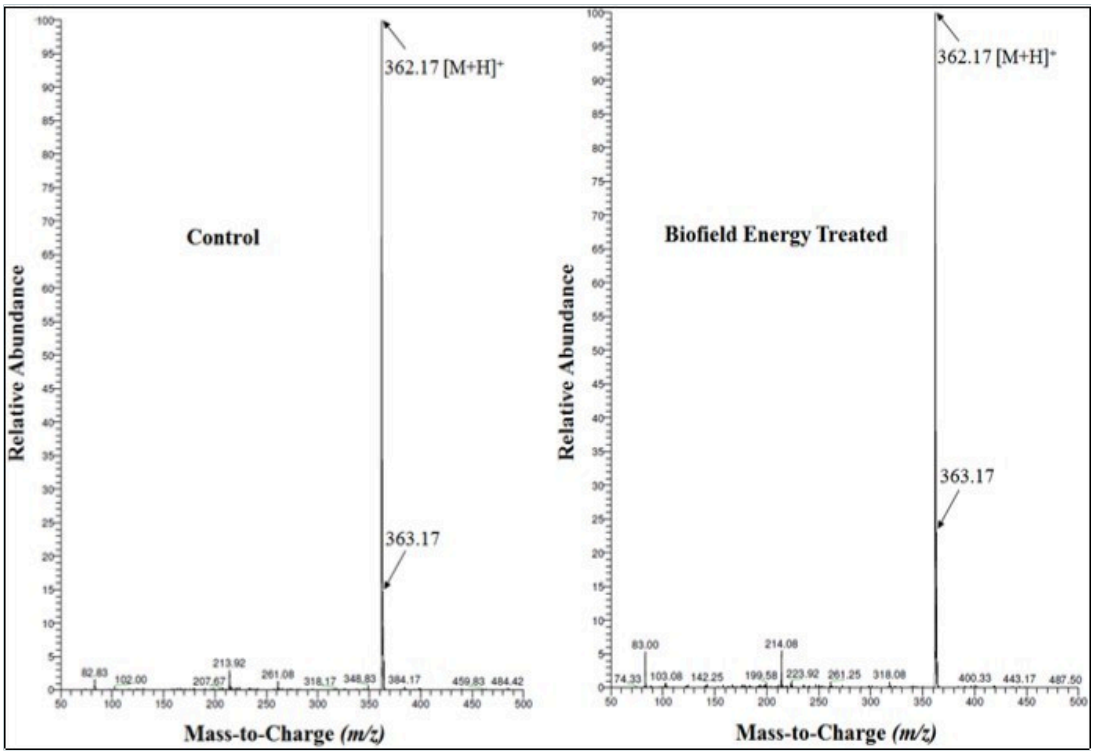


Figure 2. Mass spectra of the control and Biofield Energy Treated ofloxacin at R_t 3.05 minutes.

P (^{2}H) = [(21 x 0.015%) x 100%] / 100%= 0.315%

P (^{15}N) = [(3 x 0.4%) x 100%] / 100% = 1.2%

P (^{17}O) = [(4 x 0.04%) x 100%] / 100% = 0.16%

P_{M+1}, *i.e.* ^{2}H, ^{15}N, ^{13}C, and ^{17}O contributions from $(C_{18}H_{21}FN_3O_4)^+$ to *m/z* 363.17 = 20.28%

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

The LC-MS based isotopic abundance ratio of ofloxacin in control and Biofield Energy Treated ofloxacin samples was calculated for its molecular mass at *m/z* 362.17. The P_M and P_{M+1} for ofloxacin

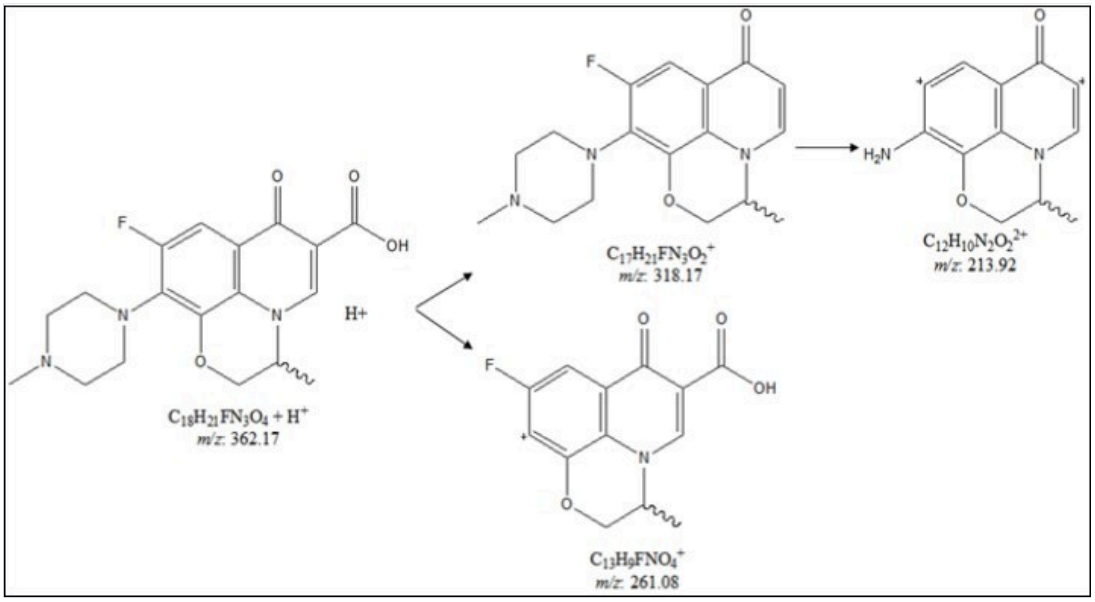


Figure 3. Proposed fragmentation pattern of ofloxacin with respect to the mass spectra.

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 (%)	14.69	23
P_{M+1}/P_M	0.15	0.23
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample	56.57	

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.

were near *m/z* 362.17 and 363.17, respectively for both the samples, which were obtained from the observed relative peak intensities of (M^+) and $((M+1)^+)$ peaks, respectively (Table 1). The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated ofloxacin was significantly increased by 56.57% compared with the control sample (Table 1). Hence, ^{13}C, ^{2}H, ^{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 retention time of the Biofield Energy Treated ofloxacin (23.31 minute) was close to that of the control sample (22.99 minutes). The peak area% of treated ofloxacin (85.45%) was almost closer compared to the control sample (86.54%). The molecular fragment peak at *m/z* 317 (calculated for $C_{17}H_{21}FN_3O_2^+$, 317.15) and *m/z* 261 (calculated for $C_{12}H_{10}N_2O_2^{2+}$, 261.04) was observed in control and Biofield Energy Treated ofloxacin (Figure 4 and Figure 5) was proposed from both the mass spectra.

The mass peak intensities influence the isotopic abundance ratio, which was well supported by the LC-MS based isotopic abundance ratio analysis. The GC-MS spectra of both the control and Biofield Energy

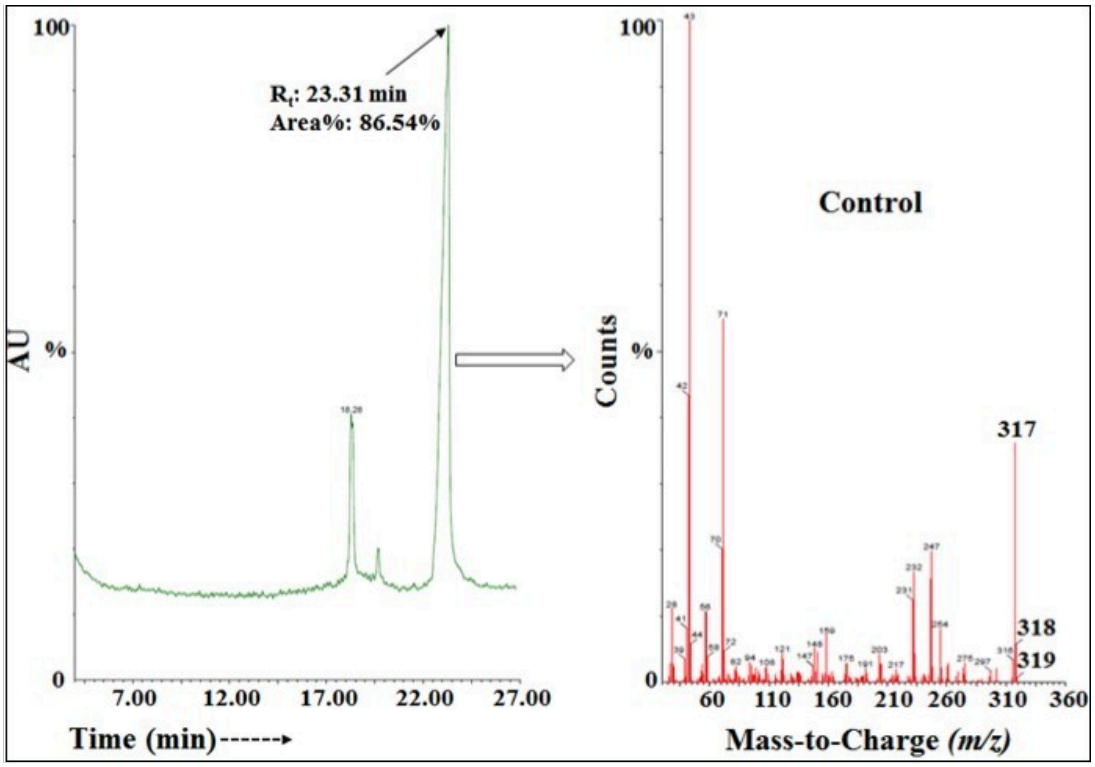


Figure 4. The GC-MS chromatogram and mass spectra of the control ofloxacin.

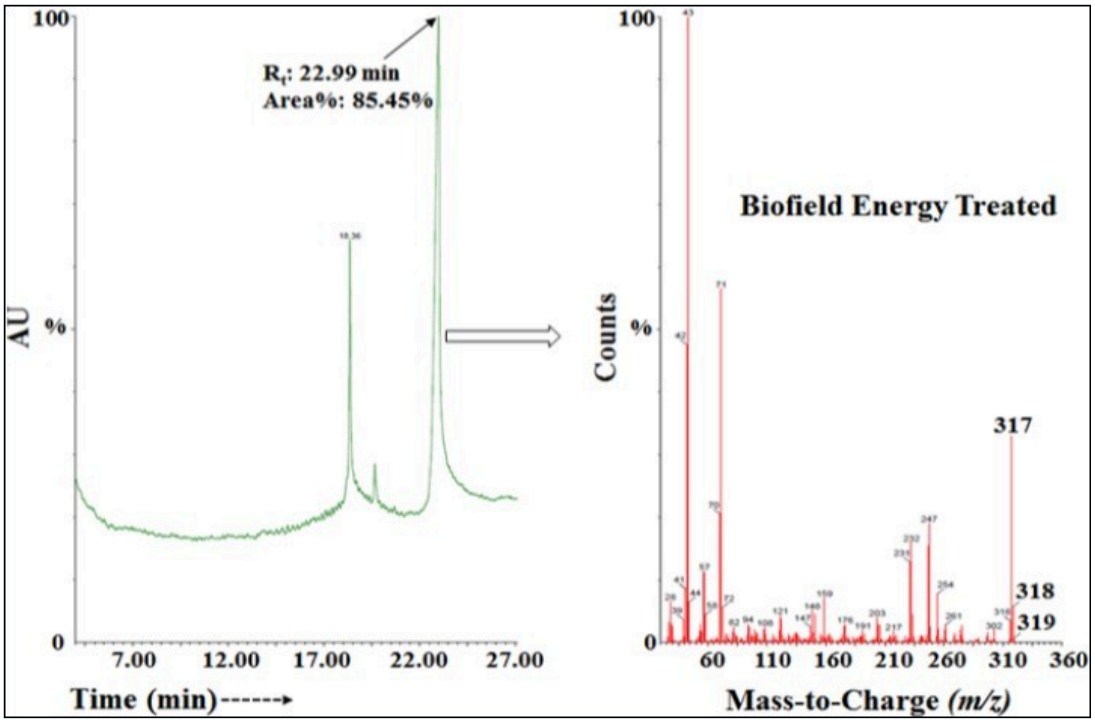


Figure 5. The GC-MS chromatogram and mass spectra of the Biofield Energy Treated ofloxacin.

Treated ofloxacin showed the mass of the fragmented molecular ion peak $[M]^+$ at *m/z* 317 (calculated for $C_{17}H_{21}FN_3O_2^+$, 317.15). The theoretical calculation of P_{M+1} for ofloxacin was presented as below:

P (^{13}C) = [(17 x 1.1%) x 36.13% (the actual size of the M^+ peak)] / 100% = 6.76%

P (^{2}H) = [(21 x 0.015%) x 36.13%] / 100%= 0.11%

P (^{15}N) = [(3 x 0.4%) x 36.13%] / 100% = 0.43%

P (^{17}O) = [(2 x 0.04%) x 36.13%] / 100% = 0.03%

P_{M+1}, *i.e.* ^{2}H, ^{15}N, ^{13}C, and ^{17}O contributions from $(C_{17}H_{21}FN_3O_2)^+$ to *m/z* 318 = 7.33%

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 x 0.20%) x 36.13%) / 100% = 0.14%

P_{M+2}, *i.e.* ^{18}O contributions from $(C_{18}H_{21}FN_3O_4)^+$ to *m/z* 319 = 0.14%

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

The GC-MS based isotopic abundance ratio analysis of ofloxacin in the control and treated ofloxacin samples was calculated for its fragmented 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 ofloxacin near *m/z* 317 M^+, 318 ($(M+1)^+$), and 319 ($(M+2)^+$), respectively of both the samples in the ESI-MS spectra (Table 2). The isotopic abundance ratio of P_{M+1}/P_M in the treated ofloxacin was significantly increased by 9.53% compared with the control sample (Table 2). This indicated that the ^{13}C, ^{2}H, ^{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. Similarly, the isotopic abundance ratio of P_{M+2}/P_M in the treated ofloxacin was increased by 12.94% compared with the control sample (Table 2). Hence, ^{18}O contributions from $(C_{18}H_{21}FN_3O_4)^+$ to *m/z* 319 in the treated ofloxacin were significantly increased compared with the control sample.

The LC-MS and GC-MS based isotopic abundance ratios of P_{M+1}/P_M ($^{2}H/^{1}H$ or $^{15}N/^{14}N$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) in the treated ofloxacin were significantly altered compared to the con-

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 (%)	36.13	32.93
P_{M+1} at *m/z* 318 (%)	5.70	5.69
P_{M+1}/P_M	0.158	0.173
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		9.53
P_{M+1} at *m/z* 319 (%)	0.68	0.70
P_{M+2}/P_M	0.019	0.021
% Change of isotopic abundance ratio (P_{M+2}/P_M) with respect to the control sample		12.94

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.

trol sample. Modern physics explains that neutrinos change identities. It is only possible if the neutrinos possess mass and have the ability to interchange from one phase to another internally. The neutrinos have the ability to interact with protons and neutrons in the nucleus, which indicated a close relation between neutrino and the isotope formation [10], [28], [29]. The alteration in the isotopic composition in the Consciousness Energy Healing Treated ofloxacin might be the cause of the alteration in neutron to proton ratio in the nucleus. The improvement in isotopic abundance could be due to changes in nuclei possibly through the interference of neutrino particles *via* the Trivedi Effect®. The increased isotopic abundance ratio of the Consciousness Energy Healing Treated ofloxacin would increase the chemical bond strength and increase the stability in the body.

The new form of Biofield Energy Treated ofloxacin would be more stable compared to the control sample [32]. It would be very useful to design better pharmaceutical formulations that might offer better therapeutic response against infections of the urethra, urinary tract infections, gonorrhea, infectious diarrhoea, pneumonia, chronic bronchitis, cellulitis, bacterial infection of the eye and ear, multidrug-resistant tuberculosis, prostatitis, plague, otitis media, *etc*.

Conclusions

The Trivedi Effect®-Consciousness Energy Healing Treatment showed a significant impact on the isotopic abundance ratios and mass peak intensities of ofloxacin. The LC-ESI-MS spectra of both the samples of ofloxacin at the retention time 3.05 minutes exhibited the mass of the protonated molecular ion peak at *m/z* 362.17 $(M+H)^+$. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the Consciousness Energy Healing Treated ofloxacin was significantly increased by 56.57% compared with 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 Consciousness Energy Healing Treated ofloxacin were significantly increased compared with the control sample. The GC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated ofloxacin was significantly increased by 9.53% compared with the control sample. Hence, ^2H, ^{15}N, ^{13}C, and ^{17}O contributions from $(C_{18}H_{21}FN_3O_4)^+$ to *m/z* 318 in the Consciousness Energy Healing Treated ofloxacin were significantly increased compared with the control sample. Similarly, the isotopic abundance ratio of P_{M+2}/P_M in the Consciousness Energy Healing Treated ofloxacin was increased by 12.94% compared with the control sample. Therefore, ^{18}O contributions from $(C_{18}H_{21}FN_3O_4)^+$ to *m/z* 319 in the Consciousness Energy Healing Treated ofloxacin were considerably increased compared with the control sample. The LC-MS and GC-MS based isotopic abundance ratios of P_{M+1}/P_M ($^2H/^1H$ or $^{15}N/^{14}N$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) in the treated ofloxacin were considerably improved compared to the control sample. The increased isotopic abundance ratio of the treated ofloxacin would increase the chemical bond strength and increase the stability in the body. The new form of Consciousness Energy Healing Treated ofloxacin would be more stable compared to the control sample and would be very

useful to design better pharmaceutical formulations that might offer better therapeutic response against infections of the urethra and cervix, infectious diarrhoea, urinary tract infections, cellulitis, chronic bronchitis, pneumonia, prostatitis, multidrug-resistant tuberculosis, plague, otitis media, *etc.*

Competing Interests

The authors have declared that no competing interests exist.

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