Journal of Antibiotics Research
Volume 4 | Issue 1
ISSN: 2574-5980

Research Article Open Access

The Consciousness Energy Healing Treatment and Its Impact on the Structural Properties and the Isotopic Abundance Ratio of Cefazolin Sodium

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Citation: Trivedi D, Trivedi MK, Branton A, Nayak G, Jana S (2020) The Consciousness Energy Healing Treatment and Its Impact on the Structural Properties and the Isotopic Abundance Ratio of Cefazolin Sodium. J Antibioti Res 4(1): 101

Received Date: January 25, 2020 **Accepted Date:** August 24, 2020 **Published Date:** August 27, 2020

Abstract

Cefazolin sodium is a broad-spectrum antibiotic useful for the treatment of many Gram-positive and some Gram-negative bacterial infections. This study was designed to investigate the impact of the Trivedi Effect® on the structural properties and the isotopic abundance ratio of cefazolin sodium using LC-MS and GC-MS spectroscopy. Cefazolin sodium sample was divided into two parts, one part of cefazolin sodium was considered as control, while the second part was treated with the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a famous Biofield Energy Healer, Alice Branton and termed as a treated sample. The LC-MS spectra of both the samples of cefazolin at the retention time (R_t) 4.6 minutes exhibited the mass of the protonated molecular ion peak at *m/z* 455 [M+H]+ (calculated for $C_{14}H_{15}N_8O_4S_3^+$, 455.05). The LC-MS based isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$ or $^{33}S/^{32}S$) in the treated cefazolin was significantly increased by 18.90% compared with the control sample. Thus, ^{13}C, ^2H, ^{15}N, ^{17}O, and ^{33}S contributions from ($C_{14}H_{15}N_8O_4S_3$) + to m/z 456 in the treated sample were significantly increased compared with the control sample. The GC-MS mass peak intensities of the control sample at m/z 56, 132, 219, and 264 were significantly decreased by 33.51%, 44.74%, 66.26%, and 73.77%, respectively compared to the treated sample. Hence, ^{13}C, ^2H, ^{15}N, ^{17}O, and ^{33}S contributions from ($C_{14}H_{15}N_8O_4S_3$) + to m/z 456 in the treated sample were significantly altered compared with the control sample. The isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$ or $^{33}S/^{32}S$) and peak intensities in the treated cefazolin was significantly altered compared to the control sample. The new form of treated cefazolin sodium would be better designing novel pharmaceutical formulations that might offer better therapeutic response against cellulitis, respiratory tract infections, urinary tract infections (UTI), genital infections, joint infection, biliary tract infections, pneumonia, endocarditis, blood infections, and also prevent group B streptococcal disease at the time of delivery and before surgery, etc.

Keywords: Consciousness Energy Healing Treatment; Cefazolin Sodium; The Trivedi Effect®; Isotopic Abundance

Introduction

Cefazolin is a broad-spectrum antibiotic. It is useful for the treatment of a number of both Gram-positive (i.e., Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus pneumonia, Streptococcus agalactiae, and other strains of streptococci) and Gram-negative (i.e., Escherichia coli, Proteus mirabilis, etc.) bacterial infections [1,2]. Cefazolin act as a bactericidal (kills the bacteria) by inhibiting the bacterial cell wall biosynthesis by binding penicillin binding proteins (bacterial proteins) and stops peptidoglycan synthesis, which is needed to maintain the cell wall [3]. It is used for the treatment of urinary tract infections (UTI), cellulitis, pneumonia, endocarditis, blood infections (sepsis), respiratory tract infections, joint infection, genital infections, biliary tract infections, and also prevent group B streptococcal disease around the time of delivery and before surgery, etc. [1-3]. General safety needs to follow while using cefazolin during pregnancy and breastfeeding as a small amount of cefazolin enters the breast milk [2,4]. Very common side effects associated with the cefazolin are diarrhoea, stomach pain or stomach upset, vomiting, rash, blood dyscrasias, allergic skin reaction, etc. [2,3]. Chemical structure of cefazolin contains an N-methylthiodiazole (NMTD) side-chain releases free NMTD in the body, which can cause hypoprothrombinemia [5]. Cefazolin sodium is the sodium salt of cefazolin available in various dosage forms, i.e., injectable, eye drop, powder for injection, etc. [6]. Physicochemical properties aspects, it is white or near white crystalline powder, freely soluble in water, slightly soluble in ethanol and methanol, and practically insoluble in acetone, chloroform, dichloromethane, ethyl acetate, and isopropanol [7].

Since the physicochemical properties of a pharmaceutical compound play a crucial role in its dissolution, absorption, and bioavailability profile in the body [8], therefore, many research activities are carrying out throughout the world by the researchers for improving the physicochemical properties of the pharmaceuticals or nutraceuticals compounds. In this scenario, it was observed that the Trivedi Effect®-Biofield Energy Healing Treatment has a significant impact on various properties such as particle size, surface area, and isotopic abundance ratios of pharmaceutical and nutraceutical compounds [9-11]. The Trivedi Effect® is a natural and only scientifically proven phenomenon in which a person can harness this inherently intelligent energy and transmit it anywhere on the planet through the possible mediation of neutrinos [12]. "Biofield Energy" the electromagnetic energy field which exists surrounding the living beings, which can transmit electromagnetic energy in the form of bio-photons generated by the continuous movement of the electrically charged particles (ions, cells, etc.) inside the body. Biofield Energy Healing specialists have the ability to harness the energy from the environment or the "Universal Energy Field" and can transmit into any living and non-living object(s). This process is called Biofield Energy Healing Treatment [13-15]. Biofield based Energy Therapies have been reported with significant outcomes against various diseases [16]. National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Energy Healing Treatment as a Complementary and Alternative Medicine (CAM) health care approach in addition to other therapies, medicines, and practices such as yoga, Qi Gong, Tai Chi, hypnotherapy, Reiki, etc. [17,18]. These therapies have been accepted by most of the USA people with several advantages [18]. Similarly, the Trivedi Effect®-Biofield Energy Healing Treatment had been proved with outstanding scientific data in the fields of materials science [19,20], agricultural science [21,22], microbiology [23,24], cancer research [25,26], pharmaceuticals and nutraceuticals [27,28], etc. The Trivedi Effect®-Biofield Energy Healing Treatment could be an economical approach for the practical challenges faced by cefazolin sodium with respect to the physicochemical properties for designing better pharmaceuticals formulations. The stable isotope ratio analysis has various applications in different scientific fields for understanding the isotope effects resulting from the variation of the isotopic composition of the molecule [29,30]. Isotope ratio analysis can be performed by using the conventional mass spectrometry (MS) techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) in low micromolar concentration with sufficient precision [29,31]. 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 $^{13}C/^{12}C$ or $^{17}O/^{16}O$ or $^{15}N/^{14}N$ or $^{33}S/^{32}S$) in the Trivedi Effect® - Consciousness Energy Healing Treated cefazolin sodium compared to the control sample.

Materials and Methods

Chemicals and Reagents

The test sample cefazolin sodium was purchased from Tokyo Chemical Industry Co., Ltd., Japan. The solvents like acetonitrile and formic acid were of analytical grade purchased from Merck, India.

Consciousness Energy Healing Treatment Strategies

The test sample cefazolin sodium powder was divided into two parts. One part of cefazolin sodium powder sample was considered as a control sample, which did not receive the Biofield Energy Treatment by the Healer. However, the other part of cefazolin sodium was received the Trivedi Effect®- Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 minutes by the famous Biofield Energy Healer, Alice Branton, USA, and known as the Biofield Energy Treated cefazolin sodium sample. Further, the control sample was treated with a "sham" healer who did not have any knowledge about the Biofield Energy Treatment. After the treatment, both the samples were kept in sealed conditions and characterized 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 cefazolin sodium 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 C18 (Length-250 mm X ID 4.6 mm X 5 micron), maintained at 25 °C. 5 µL of cefazolin sodium solution in acetonitrile was injected, and the analyte was eluted using 0.1% formic acid in water (mobile phase A; 15%), and acetonitrile (mobile phase B; 85%) pumped at a constant flow rate of 0.6 mL/min (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, peak area% and mass spectrum of the individual peak which was appeared in LC along with the full scan were recorded.

The natural abundance of each isotope (C, O, H, N, and S) can be predicted from the comparison of the height of the isotope peak with respect to the base peak. The values of the natural isotopic abundance of the common elements are obtained from the literature [30,33-37]. The change in the LC-MS based isotopic abundance ratios (P_{M+1}/P_M) for the Biofield Energy Treated cefazolin sodium was calculated compared to the control sample using equation 1.

% Change in isotopic abundance ratio = [(Treated – Control)/ Control)] x 100 (1)

Where IARTreated = isotopic abundance ratio in the treated sample and IARControl = isotopic abundance ratio in the control sample.

Gas chromatography-mass spectrometry (GC-MS) analysis: The cefazolin sodium was 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. Oven temperature was programmed from 75 ºC (5 min hold) to 280 °C (14 min hold) @ 10 ºC /min (total run time 40 min). The sample was prepared taking 20 mg of the cefazolin sodium is in 4.0 ml Acetone: Water (1:1) as a diluent. The identification of analyte was done by GC retention times and by a comparison of the mass spectra of samples.

The mass peak intensities of the Biofield Energy Treated cefazolin sodium was calculated compared to the control sample using equation 1.

Results and Discussion

Liquid chromatography-mass spectrometry (LC-MS)

The chromatograms and mass spectra of both the samples of cefazolin are shown in Figures 1 and 2, respectively. The chromatograms of cefazolin showed the single major chromatographic peak at the retention time (R_t) of 4.6 minutes in both the case (Figure 1). These results indicated that the polarity of both the control and Biofield Energy Treated cefazolin remained the same.

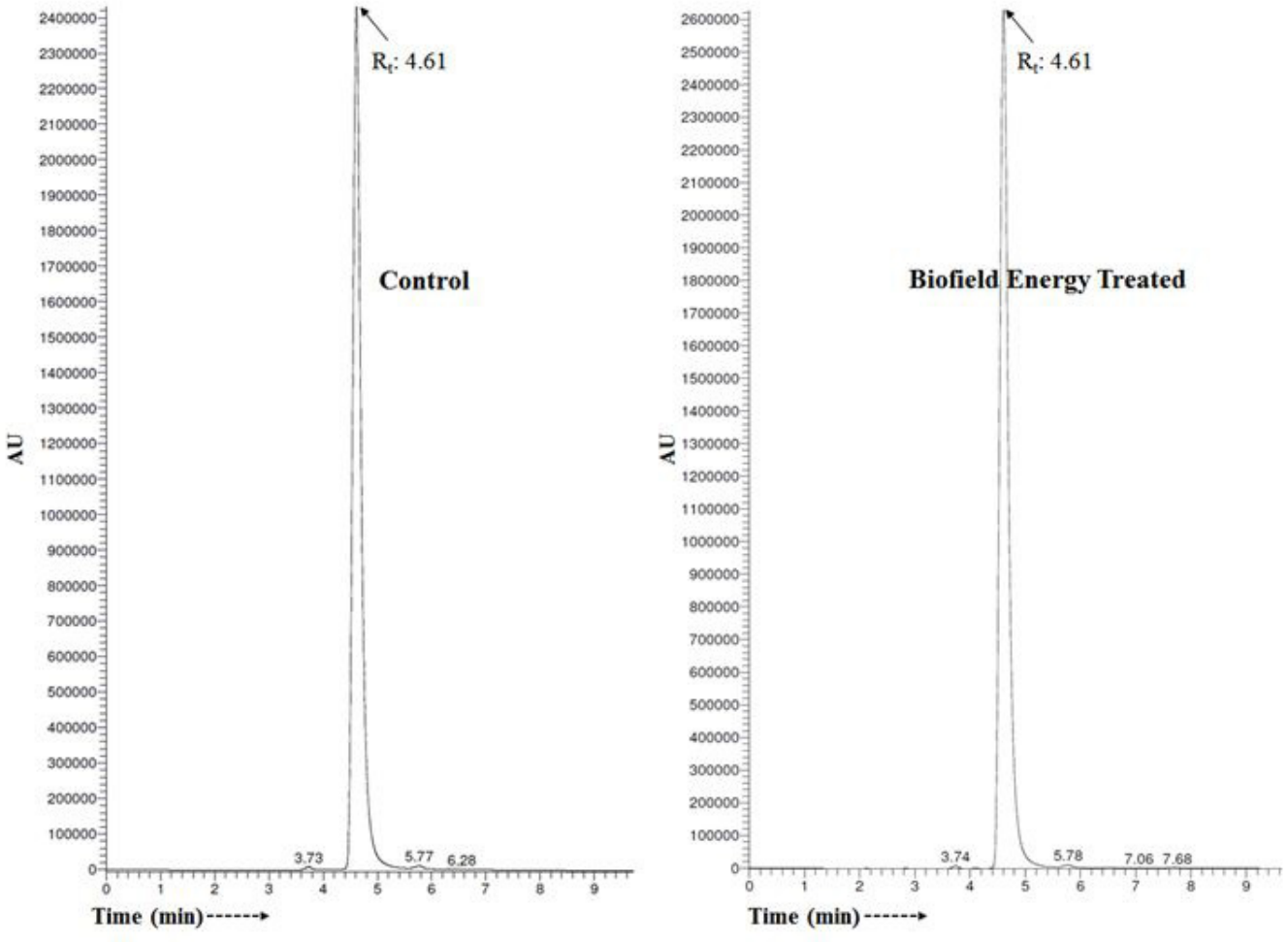

Figure 1: Liquid chromatograms of the control and Biofield Energy Treated cefazolin

As per the literature, cefazolin was detected with the molecular mass peak $[M+H]^+$ at m/z 455 LC-MS spectrum in positive ion mode [32]. The LC-MS spectra of both the samples of cefazolin (Figure 2) at the retention time 4.6 minutes exhibited the mass of the protonated molecular ion peak at m/z 455 $[M+H]^+$ (calculated for $C_{14}H_{15}N_8O_4S_3^+$, 455.05) in the control sample and Biofield Energy Treated sample, along with the fragment ion peaks near *m/z* 322.9 and 213.9 which were corresponded to the molecular formula $C_{11}H_{11}N_6O_4S^+$ and $C_8H_{10}N_2O_3S^+$, respectively in both the samples (Figure 3).

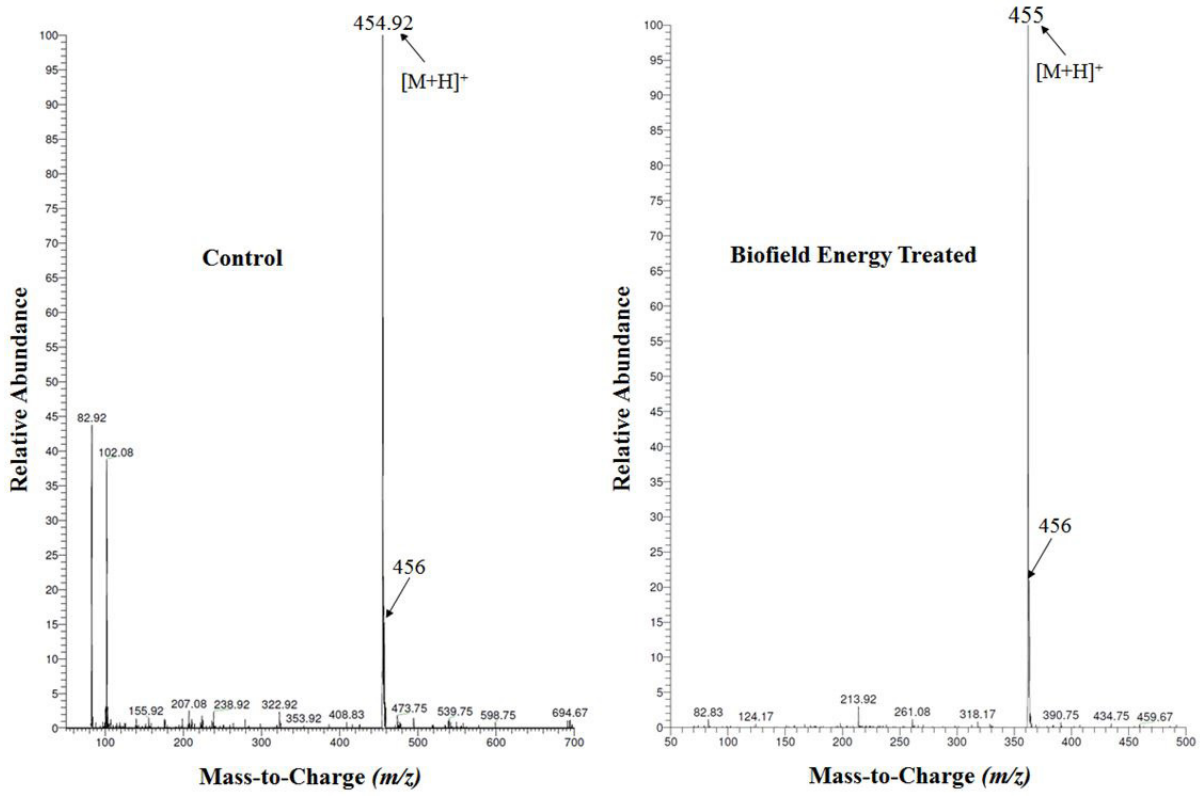

Figure 2: Mass spectra of the control and Biofield Energy Treated cefazolin at Rt 4.61 minutes

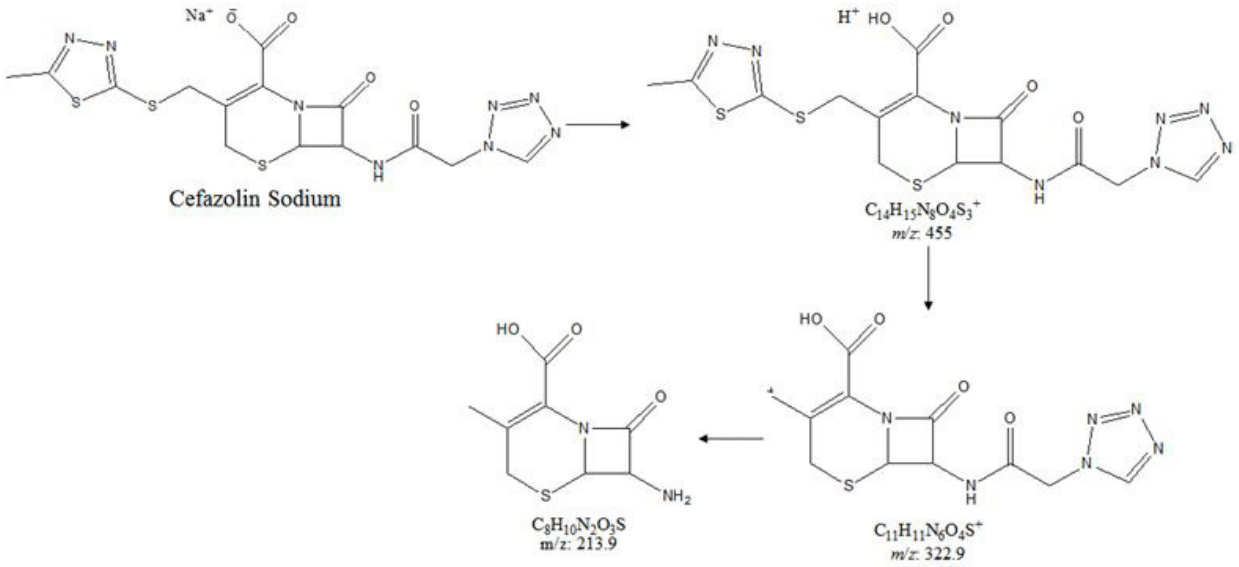

Figure 3: Proposed fragmentation pattern of cefazolin

The LC-ESI-MS spectra of both the control and Biofield Energy Treated cefazolin showed the mass of the molecular ion peak [M+H]+ at m/z 455 (calculated for $C_{14}H_{15}N_8O_4S_3$+, 455.05) with relative intensity of 100%. The theoretical calculation of P_{M+1} for cefazolin was presented as below:

P (^{13}C) = [(14 x 1.1%) x 100% (the actual size of the M+ peak)] / 100% = 15.4%

P (^{13}C) = [(14 x 1.1%) x 100% (the actual size of the M+ peak)] / 100% = 15.4%
P (^{2}H) = [(15 x 0.015%) x 100%] / 100%= 0.225%
P (^{15}N) = [(8 x 0.4%) x 100%] / 100% = 3.2%
P (^{17}O) = [(4 x 0.04%) x 100%] / 100% = 0.16%
P (^{33}S) = [(4 x 0.08%) x 100%] / 100% = 0.32%

P_{M+1}, *i.e.* ^{13}C, ^{2}H, ^{15}N, ^{17}O, and ^{33}S contributions from $(C_{14}H_{15}N_8O_4S_3)^+$ to m/z 456 = 19.31%

The calculated isotope abundance (19.31%) was close to the experimental value 21.9% (Table 1). From the above calculation, it has been found that ^{13}C and ^{15}N have major contribution to m/z 456.

The LC-MS based isotopic abundance ratio analysis of cefazolin in both the samples was calculated for its molecular mass at m/z 455. PM and PM+1 for cefazolin near m/z 455 and 456, respectively of the control and Biofield Energy Treated samples, which were obtained from the observed relative peak intensities of [M+] and $[(M+1)^+]$ peaks, respectively in the ESI-MS spectra and are presented in Table 1. The percentage change of the isotopic abundance ratio (P_{M+1}/P_M) in the Biofield Energy Treated cefazolin compared with the control sample are shown in Table 1. The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated cefazolin was significantly increased by 18.90% compared with the control sample (Table 1). Hence, ^{13}C, ^{2}H, ^{15}N, ^{17}O, and ^{33}S contributions from $(C_{14}H_{15}N_8O_4S_3)^+$ to m/z 456 in the Biofield Energy Treated sample were significantly increased compared with the control sample.

Parameter	Control sample	Biofield Energy Treated sample
P_M at m/z 455 (%)	100	100
P_{M+1} at m/z 456 (%)	21.9	26.04
P_{M+1}/P_M	0.22	0.26
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		18.90

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

Table 1: LC-MS based isotopic abundance analysis results of cefazolin in Biofield Energy Treated sample compared to the control sample

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC chromatograms of the control and Biofield Energy Treated samples showed the presence of a sharp, intense chromatographic peak (Figures 4 and 5). The retention time of the Biofield Energy Treated sample (14.25 minute) was close to those of the control sample (14.24 minutes). The mass spectra did not show the parent molecular ion peak of cefazolin in any of the mass spectra of control and Biofield Energy Treated samples. The fragment peaks at m/z 264, 219, 132, and 56 of the control (Figure 4) and Biofield Energy Treated (Figure 5) cefazolin were observed in both the mass spectra. The mass peak intensities of the control sample were significantly altered compared to the Biofield Energy Treated sample. The mass peak intensities of the control sample at m/z 56, 132, 219, and 264 were significantly decreased by 33.51%, 44.74%, 66.26%, and 73.77%, respectively compared to the Biofield Energy Treated sample (Table 2).

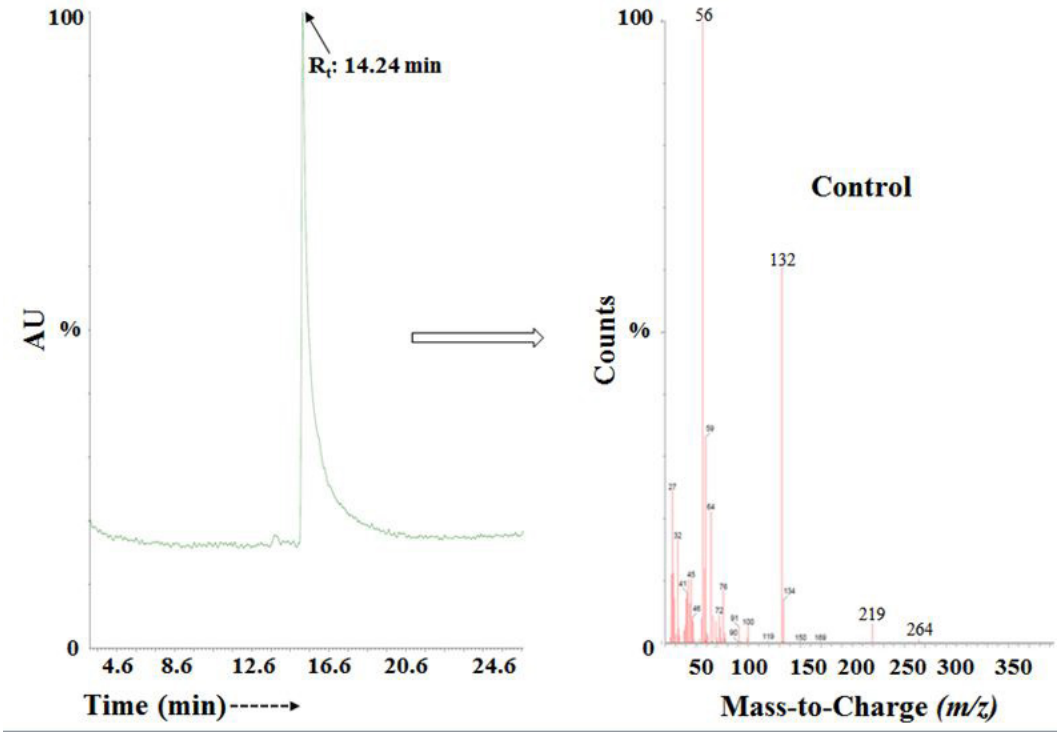

Figure 4: The GC-MS chromatogram and mass spectra of the control cefazolin

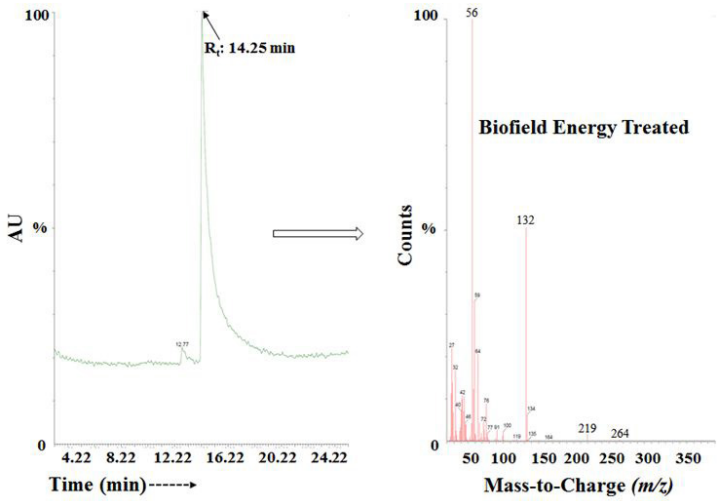

Figure 5: The GC-MS chromatogram and mass spectra of the Biofield Energy Treated cefazolin

m/z	Control	Biofield Energy Treated	% Change
56	1.88e^8	1.25e^8	-33.51
132	1.14e^8	0.63e^8	-44.74
219	5.78e^6	1.95e^6	-66.26
264	1.22e^6	0.32e^6	-73.77

Table 2: GC-MS peak intensities of the control and Biofield Energy Treated cefazolin

The LC-MS and GC-MS study confirmed the structure of cefazolin. The LC-MS based isotopic abundance ratio of PM+1/PM in the Biofield Energy Treated cefazolin was significantly altered compared to the control sample. The improved isotopic composition of the Consciousness Energy Healing Treated cefazolin might be due to the alteration in neutron to proton ratio in the nucleus via the Trivedi Effect®-Consciousness Energy Healing Treatment. 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 [12,33,34]. The isotopic abundance ratio ^{2}H/^{1}H or ^{13}C/^{12}C or ^{15}N/^{14}N or ^{17}O/^{16}O or ^{33}S/^{32}S would highly influence the atomic bond vibration and strength of the Biofield Energy Treated cefazolin [35]. The increased isotopic abundance ratio (P_{M+1}/P_M) of the Consciousness Energy Healing Treated cefazolin may significantly increase the chemical bond strength, improve its stability, solubility, and bioavailability in the body. The new form of the Biofield Energy Treated cefazolin would be better to design better pharmaceutical formulations that might offer better therapeutic response against cellulitis, respiratory tract infections, urinary tract infections (UTI), genital infections, joint infection, biliary tract infections, pneumonia, endocarditis, blood infections (sepsis), and also prevent group B streptococcal disease at the time of delivery and before surgery, etc. [1-3,36,37].

Conclusions

The experimental results revealed that the Trivedi Effect®-Consciousness Energy Healing Treatment showed a significant impact on the isotopic abundance ratios and mass peak intensities of cefazolin. The LC-MS spectra of both the control and Biofield Energy Treated samples of cefazolin at the retention time (R_t) 4.6 minutes exhibited the mass of the protonated molecular ion peak at m/z 455 [M+H]+. The LC-MS based isotopic abundance ratio of PM+1/PM in the Biofield Energy Treated cefazolin was significantly increased by 18.90% compared with the control sample. Thus, ^{13}C, ^{2}H, ^{15}N, ^{17}O and ^{33}S contributions from $(C_{14}H_{15}N_8O_4S_3)^+$ to *m/z* 456 in the Biofield Energy Treated sample were significantly increased compared with the control sample. The GC-MS mass peak intensities of the control sample at m/z 56, 132, 219, and 264 were significantly decreased by 33.51%, 44.74%, 66.26%, and 73.77%, respectively compared to the Biofield Energy Treated sample. Hence, ^{13}C, ^{2}H, ^{15}N, ^{17}O, and ^{33}S contributions from $(C_{14}H_{15}N_8O_4S_3)^+$ to *m/z* 456 in the Biofield Energy Treated sample were significantly altered compared with the control sample. The isotopic abundance ratio of P_{M+1}/P_M (^{2}H/^{1}H or ^{13}C/^{12}C or ^{15}N/^{14}N or ^{17}O/^{16}O or ^{33}S/^{32}S) and peak intensities in the Biofield Energy Treated cefazolin were significantly altered compared to the control sample. The increased isotopic abundance ratio (P_{M+1}/P_M) of the Consciousness Energy Healing Treated cefazolin may significantly increase the chemical bond strength, improve its stability, solubility, and bioavailability in the body. The new form of Biofield Energy Treated cefazolin would be better designing novel pharmaceutical formulations that might offer better therapeutic response against cellulitis, respiratory tract infections, UTI, genital infections, joint infection, biliary tract infections, pneumonia, endocarditis, sepsis, and also prevent group B streptococcal disease at the time of delivery and before surgery, etc.

Acknowledgements

The authors are grateful to Sophisticated Instrumentation Centre for Applied Research & Testing (SICART) India, Trivedi Science, Trivedi Global, Inc., and Trivedi Master Wellness for their assistance and support during this work.

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