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The LC-MS and GC-MS Based Isotopic Abundance Ratio Analysis of the Consciousness Energy Healing Treated Silver Sulfadiazine

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Research Article

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Abstract

Silver sulfadiazine is a topical antibiotic helps to prevent and treat wound infections in patients with severe burns. This research was performed to evaluate the impact of the Trivedi Effect®- Consciousness Energy Healing Treatment on the structural properties and the isotopic abundance ratio of silver sulfadiazine using spectroscopic and chromatographic techniques. The test sample silver sulfadiazine sample was divided into control and treated parts. Only the treated part of silver sulfadiazine was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a renowned Biofield Energy Healer, Dahryn Trivedi. The LC-MS spectra of both the samples at retention time (R_t) 4.1 minutes exhibited the mass of the protonated molecular ion peak at *m/z* 251.08 (calculated for $C_{10}H_{11}N_4O_2S^+$, 251.06) was found to be sulfadiazine. The chromatographic peak area of the treated silver sulfadiazine was significantly increased by 20.35% compared to the control sample. The result indicated that the solubility profile of the treated silver sulfadiazine must have increased significantly after the Biofield Energy Treatment compared with the control sample. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated sulfadiazine was significantly increased by 31.88% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of P_{M+1}/P_M and P_{M+2}/P_M in the treated sulfadiazine was significantly increased by 9.74% and 15.13%, respectively compared with the control sample. Hence, ^{13}C, ^{2}H, ^{15}N, ^{17}O, ^{33}S, and ^{34}S contributions from $(C_7H_9N_2O_2S)+$ to *m/z* 186 and 187 in the treated sulfadiazine were significantly increased compared to the control sample. Thus, the isotopic abundance ratios 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 P_{M+2}/P_M ($^{34}S/^{32}S$) in the treated sulfadiazine were significantly increased compared to the control sample. The changes in isotopic abundance and peak area might be due to changes in nuclei possibly *via* the interference of neutrino particles controlled by the Trivedi Effect®-Consciousness Energy Healing Treatment. The increased isotopic abundance ratio of the treated silver sulfadiazine might improve the chemical bond strength, led to increase the stability in the body. This novel silver sulfadiazine would be more efficacious in pharmaceutical formulations which might offer better solubility, bioavailability, and therapeutic response against bacterial infections, superficial and partial thickness burn injuries, etc.

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

Introduction

Silver sulfadiazine is a sulfonamide-based topical antibiotic. It helps to prevent and treat wound infections in patients with severe burns. It has antibacterial and antifungal properties because of the combined activity of silver and sulfadiazine [1]. Silver sulfadiazine, when interacting with the sodium chloride in the body fluids release the silver ions, which released slowly into wounded areas and form the disulfide bonds leading to the structural changes in protein and inactivating thiol-containing enzymes [2]. It inhibits the bacteria by intercalating its DNA thereby interfering in replication and transcription [3,4]. Some of the common side effects associated with the silver sulfadiazine are burning, itching, trouble breathing, pain on the treated skin and systemic absorption lead to adverse effects similar to those of other sulphonamides [5].

The bioavailability of silver sulfadiazine is very poor [<1% (silver), 10% (sulfadiazine)] [6]. The physicochemical properties of a drug molecule have lots of role in the drug solubility, absorption, bioavailability, etc. The drug molecule with poor solubility profile is very limited bioavailability [7]. Therefore, many research going on to improve the quality of the silver sulfadiazine. The Biofield Energy Healing Treatment (the Trivedi Effect®) has an unbelievable impact on the physicochemical properties of pharmaceutical compounds [8-11]. The Trivedi Effect® is a natural and only scientifically proven phenomenon in which an individual can harness this inherently intelligent energy and transfer it anywhere on the planet *via* the possible mediation of neutrinos [12]. A "Biofield" is an electromagnetic energy field which exists surrounding the living body, which is generated by the continuous movement of the electrically charged particles like ions, cells, etc. inside the body. Biofield Energy Healer can harness the energy from the "Universal Energy Field" and can transfer into any living and non-living object(s), the process of treatment is called Biofield Energy Healing Treatment [13,14]. Biofield based Energy Therapies have also been reported with significant outcomes against various disease [15]. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a Complementary and Alternative Medicine (CAM) health care approach along with the other therapies, medicines, and practices such as Tai Chi, hypnotherapy, Reiki, yoga, Qi Gong, etc. [16]. These therapies have been accepted by most of the USA population [17]. The Trivedi Effect®-Consciousness Energy Healing Treatment also altered the characteristic properties of the metals, organic compounds, and ceramic, microbes, crops, nutraceutical compounds and the isotopic abundance ratio of many organic compounds [18-28].

The stable isotope ratio investigation 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]. The 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 appropriate precision [30,31]. The Trivedi Effect®-Biofield Energy Healing Treatment could be an economical approach for designing better pharmaceuticals formulations. 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 and P_{M+2}/P_M in the Consciousness Energy Healing Treated silver sulfadiazine compared to the control sample.

Materials and Methods

Chemicals and Reagents

The test sample silver sulfadiazine was purchased from Tokyo Chemical Industry Co., Ltd., Japan and remaining chemicals used during the experiments were purchased in India.

Consciousness Energy Healing Treatment Strategies

The test sample silver sulfadiazine powder was divided into two parts. One part of the silver sulfadiazine powder sample received no Biofield Energy Treatment is called the control sample. Further, the control sample was treated with a "sham" healer who did not have any knowledge about the Biofield Energy and its treatment. However, the other part of silver sulfadiazine received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 minutes by the renowned Biofield Energy Healer, Dahryn Trivedi, USA, and known as the Biofield Energy Treated silver sulfadiazine. After that, both the samples were kept in sealed conditions and characterized using chromatographic and spectroscopic analytical techniques.

Characterization

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

The LC-MS analysis of both the samples of silver sulfadiazine was carried out with the help of LC-MS ThermoFisher Scientific, the USA equipped with an ion trap detector connected with a triple-stage quadrupole mass spectrometer. The reversed phase column used here was Thermo Scientific Synchronis C18 (Length-250 mm X ID 4.6 mm X 5 micron), maintained at 25˚C. The diluent used

for the sample preparation was methanol. 5 µL of silver sulfadiazine prepared solution was injected, and the analyte was eluted using acetonitrile + 10 mM ammonium acetate (80:20) pumped at a constant flow rate of 0.6 mL/min with a total run time of 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 were recorded.

The natural abundance of each isotope (C, H, N, O, 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 [30,32-34]. The LC-MS based isotopic abundance ratios (P_{M+1}/P_M) for the control and Biofield Energy Treated silver sulfadiazine was calculated using equation 1.

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

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

Gas chromatography-mass spectrometry (GC-MS) analysis

Both the samples of silver sulfadiazine were analyzed with the help of Perkin Elmer Gas chromatograph equipped with a PE-5MS capillary column (30M x 250 micros x 0.250 microns) and coupled to a single quadrupole mass detector was operated with electron impact (EI) ionization in +ve ion mode. The oven temperature was programmed from 75°C (5 min hold) to 280°C (14.5 min hold) @ 10°C/min with a total run time of 40 min. The sample was prepared taking 60 mg of the silver sulfadiazine in 4 ml dimethyl sulfoxide (DMSO) as a diluent. The identification and characterization of analyte were done by GC retention times and by a comparison of the mass spectra of samples. The GC-MS based isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) for both the samples were calculated using equation 1.

Results and Discussion

Liquid Chromatography-Mass Spectrometry (LC-MS)

The liquid chromatography of silver sulfadiazine showed the single major chromatographic peak at the retention time (R_t) of 4.1 minutes in the chromatograms of both the samples (Figure 1). The peak area of the treated silver sulfadiazine was significantly increased by 20.35% compared to the control sample. The result indicated that the solubility profile of the Biofield Energy Treated silver sulfadiazine must have increased significantly after the Biofield Energy Treatment compared with the control sample.

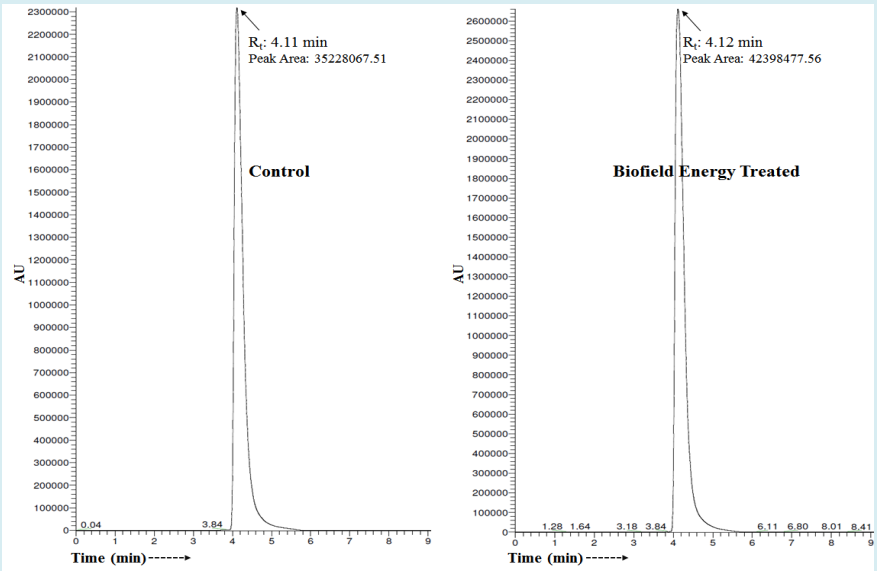

Figure 1: Liquid chromatograms of the control and Biofield Energy Treated silver sulfadiazine.

The mass spectra of both the samples showed the protonated molecular mass peak $[M+H]^+$ at *m/z* 251.08 (calculated for $C_{10}H_{11}N_4O_2S^+$, 251.06) with 100% base peak intensity in the MS spectrum in +ve ion mode was found to be sulfadiazine (Figure 2). The experimental data were well matched with the reported literature data [35]. Along with the molecular ion peak other fragmentation peaks at *m/z* 214.83, 187.17, 173.04, and 156 for $C_7H_{11}N_4O_2S^+$, $C_7H_{11}N_2O_2S^+$, $C_6H_9N_2O_2S^+$, and $C_6H_6NO_2S^+$, respectively in both the control and Biofield Energy Treated sample (Figures 2 and 3).

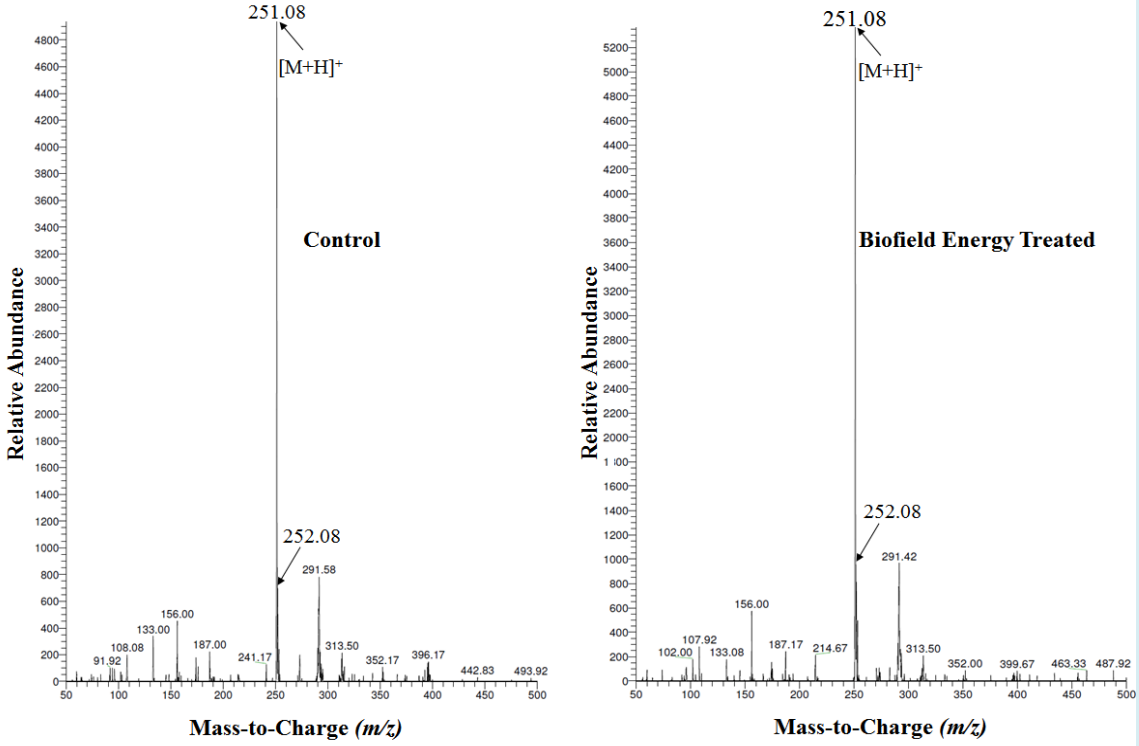

Figure 2: Mass spectra of the control and Biofield Energy Treated sulfadiazine at R_t 4.1 minutes.

The mass spectra of both the samples showed the molecular ion peak at *m/z* 251.08 (calculated for $C_{10}H_{11}N_4O_2S^+$, 251.06) with 100% relative peak intensity. The theoretical calculation of P_{M+1} for sulfadiazine was presented as below:

P (^{13}C) = [(10 x 1.1%) x 100% (the actual size of the M^+ peak)] / 100% = 11%

P (^{2}H) = [(11 x 0.015%) x 100%] / 100%= 0.165%

P (^{15}N) = [(4 x 0.4%) x 100%] / 100% = 1.6%

P (^{17}O) = [(2 x 0.04%) x 100%] / 100% = 0.08%

P (^{33}S) = [(1 x 0.75%) x 100%] / 100% = 0.75%

P_{M+1}, *i.e.* ^{13}C, ^{2}H, ^{15}N, ^{17}O and ^{33}S contributions from $(C_{10}H_{11}N_4O_2S)^+$ to *m/z* 252.08 = 13.6%

From the above calculation, it has been found that ^{13}C, ^{15}N, and ^{33}S have major contribution to *m/z* 252.08. The calculated isotopic abundance (13.6%) is close to the experimental value 11.48% (Table 1).

The LC-MS based isotopic abundance ratio analysis P_M and P_{M+1} for sulfadiazine near *m/z* 251.08 [M^+] and 252.08 [$(M+1)^+$], respectively of both the samples in the ESI-MS spectra (Table 1). The percentage change of the isotopic abundance ratio (P_{M+1}/P_M) in the treated silver sulfadiazine was significantly increased by 31.88% compared with the control sample (Table 1). Therefore, it was concluded that the ^{13}C, ^{2}H, ^{15}N, ^{17}O, and ^{33}S contributions from $(C_{10}H_{11}N_4O_2S)^+$ to *m/z* 252.08 in the treated sulfadiazine were significantly increased compared to the control sample.

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 251.08 (%)	100	100
P_{M+1} at *m/z* 252.08 (%)	11.48	15.14
P_{M+1}/P_M	0.11	0.15
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		31.88

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: The isotopic abundance analysis of the treated sulfadiazine compared to the control sample.

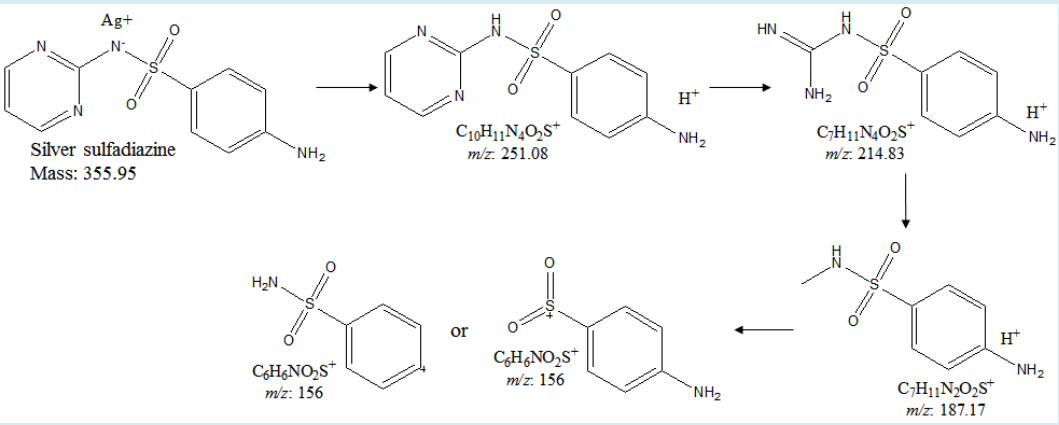

Figure 3: Proposed fragmentation pattern of silver sulfadiazine.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The control and Biofield Energy Treated silver sulfadiazine showed the presence of the sharp chromatographic peak at the R_t 18.41 and 17.62 minutes, respectively in the GC-MS chromatograms (Figures 4 & 5). The R_t indicated a change in polarity of the Biofield Energy Treated sample compared to the control sample. This indicated that the solubility of the treated silver sulfadiazine was altered compared to the control sample, which was supported by the LC-MS data. The GC-MS of silver sulfadiazine did not show the parent molecular ion peak in the mass spectra but, the base peak was observed at *m/z* 185 (calculated for $C_7H_9N_2O_2S^+$, 185.04), along with the lower mass fragment ion peaks (Figures 4 & 5).

The theoretical calculation of P_{M+1} and P_{M+2} for silver sulfadiazine was presented as below:

P (^{13}C) = [(7 x 1.1%) x 100% (the actual size of the M^+ peak)] / 100% = 7.7%

P (^{2}H) = [(9 x 0.015%) x 100%] / 100%= 0.135%

P (^{15}N) = [(2 x 0.4%) x 100%] / 100% = 0.8%

P (^{17}O) = [(2 x 0.04%) x 100%] / 100% = 0.08%

P (^{33}S) = [(1 x 0.75%) x 100%] / 100% = 0.75%

$P_{M+1,}$ *i.e.* ^{13}C, ^{2}H, ^{15}N, ^{17}O, and ^{33}S contributions from $(C_7H_9N_2O_2S)^+$ to *m/z* 185 = 9.47%

Similarly,

P (^{34}S) = [(1 x 4.21%) x 100%] / 100% = 4.21%

$P_{M+2,}$ *i.e.* ^{34}S contributions from $(C_7H_9N_2O_2S)^+$ to *m/z* 186 = 4.21%

From the above calculation, it has been found that ^{13}C, ^{15}N, ^{33}S, and ^{34}S have the major contributions to *m/z* 185 and 186.

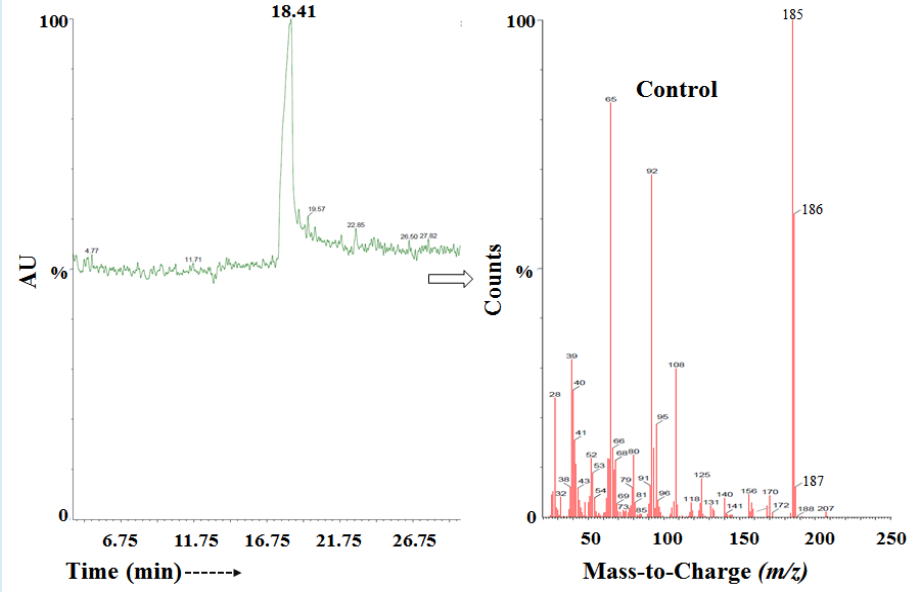

Figure 4: The GC-MS chromatogram and mass spectra of the control sulfadiazine.

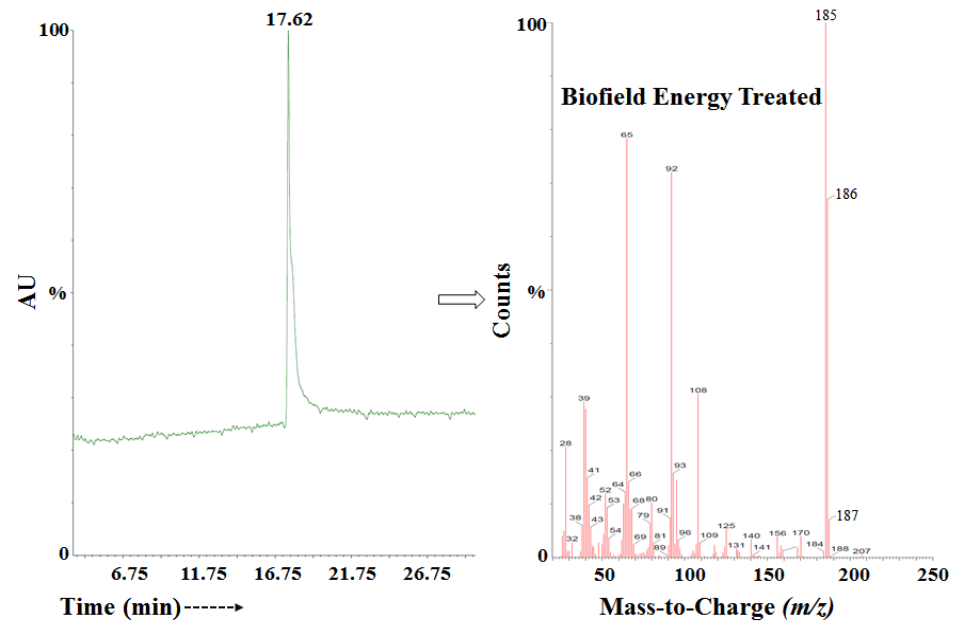

Figure 5: The GC-MS chromatogram and mass spectra of the Biofield Energy Treated sulfadiazine.

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 185 (%)	100.00	100.00
P_{M+1} at *m/z* 186 (%)	61.00	66.94
P_{M+1}/P_M	0.61	0.67
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control sample		9.74
P_{M+2} at *m/z* 187 (%)	6.08	7.00
P_{M+2}/P_M	0.06	0.07
% Change of isotopic abundance ratio (P_{M+2}/P_M) compared to the control sample		15.13

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

Table 2: The isotopic abundance analysis of Biofield Energy Treated sulfadiazine compared to the control samples.

The GC-MS based isotopic abundance ratio analysis of the Biofield Energy Treated samples were calculated compared to the control sample. P_M, P_{M+1}, and P_{M+2} for silver sulfadiazine near *m/z* 185 $[M^+]$, 186 $[(M+1)^+]$, and 187 $[(M+2)^+]$, respectively of the control and Biofield Energy Treated samples in the mass spectra (Table 2). The isotopic abundance ratio of P_{M+1}/P_M and of P_{M+2}/P_M in the treated silver sulfadiazine was significantly increased by 9.74% and 15.13%, respectively compared to the control sample (Table 2). Hence, ^{13}C, ^{2}H, ^{15}N, ^{17}O, ^{33}S, and ^{34}S contributions from $(C_7H_9N_2O_2S)^+$ to *m/z* 186 and 187 in the Biofield Energy Treated sample 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 $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$ or $^{33}S/^{32}S$) and P_{M+2}/P_M ($^{34}S/^{32}S$) in the treated silver sulfadiazine were significantly improved compared to the control sample. It can be hypothesized that the changes in isotopic abundance could be due to changes in nuclei possibly through the interference of neutrino particles *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. Physics tell that the neutrinos change identities which are only possible if the neutrinos possess mass and have the ability to interchange their phase from one phase to another internally. Therefore, the neutrinos have the ability to interact with protons and neutrons in the ucleus, which indicated a close relation between neutrino and the isotope formation [12,30,31]. The improved isotopic abundance ratios $^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$ or $^{33}S/^{32}S$ or $^{34}S/^{32}S$) would highly influence the atomic bond vibration of treated silver sulfadiazine [35]. The increased isotopic abundance ratio of the Consciousness

Energy Healing Treated silver sulfadiazine would stronger the chemical bond and increase the stability, alter the rate of metabolic reactions in the body. The Trivedi Effect®-Consciousness Energy Healing Treated silver sulfadiazine might improve the solubility, bioavailability, pharmacokinetic profile, and therapeutic efficacy, which would be more advantageous in the prevention and treatment of bacterial infections, superficial and partial thickness burn injuries, *etc*.

Conclusions

The silver sulfadiazine showed a significant impact on the peak area and isotopic abundance ratios after the Trivedi Effect®-Consciousness Energy Healing Treatment compared with the control sample. The LC chromatographic peak area of the Consciousness Energy Healing Treated silver sulfadiazine was significantly increased by 20.35% compared to the control sample. The result indicated that the solubility profile of the Consciousness Energy Healing Treated silver sulfadiazine might have increased significantly after the Biofield Energy Treatment compared with the control sample. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the Consciousness Energy Healing Treated sulfadiazine was significantly increased by 31.88% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of P_{M+1}/P_M and P_{M+2}/P_M in the Consciousness Energy Healing Treated sulfadiazine was significantly increased by 9.74% and 15.13%, respectively compared with the control sample. Hence, ^{13}C, ^{2}H, ^{15}N, ^{17}O, ^{33}S, and ^{34}S contributions from $(C_7H_9N_2O_2S)^+$ to *m/z* 186 and 187 in the Consciousness Energy Healing Treated sulfadiazine were significantly increased compared to the control sample. Thus, the isotopic abundance ratios 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 P_{M+2}/P_M ($^{34}S/^{32}S$) in the Consciousness Energy Healing Treated sulfadiazine were significantly increased compared to the control sample. The changes in isotopic abundance and peak area might be due to changes in nuclei possibly *via* the interference of neutrino particles controlled by the Trivedi Effect®-Consciousness Energy Healing Treatment. The increased isotopic abundance ratio of the Consciousness Energy Healing Treated silver sulfadiazine might improve the chemical bond strength, led to increase the stability in the body. This novel silver sulfadiazine would be more efficacious in pharmaceutical formulations which might offer better solubility, bioavailability, and therapeutic response against bacterial infections, superficial and partial thickness burn injuries, *etc*.

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