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Impact of Consciousness Energy Healing Treatment on the Isotopic Abundance Ratio of Sulfamethoxazole Using LC-MS and GC-MS Spectrometry

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Abstract

Sulfamethoxazole is a sulfonamide bacteriostatic antibiotic which is commonly used for the treatment of infections caused by bacteria. In this study, the impact of the Trivedi Effect®-Biofield Energy Healing Treatment on the structural properties and the isotopic abundance ratio of sulfamethoxazole was studied using LC-MS and GC-MS spectroscopy. Sulfamethoxazole sample was divided into two parts, one part of sulfamethoxazole was considered as control (no Biofield Energy Treatment was provided), while the second part was received the Consciousness Energy Healing Treatment remotely by a famous Biofield Energy Healer, Dahryn Trivedi and termed as a treated sample. The LC-MS spectra of both the samples at retention time (R_t) 2.5 minutes exhibited the mass of the deprotonated molecular ion peak at m/z 252 [M-H]$^-$. The peak area of the treated sulfamethoxazole was significantly increased by 42.96% compared to the control sample. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated sulfamethoxazole was significantly decreased by 49.56% compared with the control sample. Thus, ^{13}C, ^2H, ^{15}N, ^{17}O, and ^{33}S contributions from $(C_{10}H_{10}N_3O_3S)_-$ to m/z 253 in the treated sample were significantly decreased compared with the control sample. The GC-MS peak area% of the treated sample was significantly increased by 80.3% compared to the control sample. The GC-MS based isotopic abundance ratio of P_{M+1}/P_M and P_{M+2}/P_M in the treated sulfamethoxazole was significantly altered by 119.53% and -25.48%, respectively compared with the control sample. Hence, ^{13}C, ^2H, ^{15}N, ^{17}O, ^{18}O, ^{33}S, and ^{34}S contributions from $(C_{10}H_{11}N_3O_3S)^+$ to m/z 254 and 255 in the treated sample were significantly altered compared with the control sample. The isotopic abundance ratios 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 P_{M+2}/P_M ($^{18}O/^{16}O$ or $^{34}S/^{32}S$) in the treated sulfamethoxazole were significantly altered compared to the control sample. It can be assumed that the changes in peak area%, isotopic abundance, and mass peak intensities could be due to changes in nuclei possibly through the interference of neutrino particles *via* the Trivedi Effect®. The new form of sulfamethoxazole would be more efficacious pharmaceutical formulations that might offer better solubility, dissolution, absorption, bioavailability and therapeutic response against urinary tract infections, tuberculosis, traveler's diarrhoea, ear infections, shigellosis, bronchitis, and *pneumocystis jiroveci* pneumonia, etc.

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

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Introduction

Sulfamethoxazole is a sulfonamide bacteriostatic antibiotic which is commonly used in combination with trimethoprim for the treatment of infections caused by bacteria. It competitively inhibits bacterial nucleotides and DNA by inhibiting the bacterial synthesis of dihydrofolic acid thereby competing with para-aminobenzoic acid (PABA) for binding to dihydropteroate synthetase [1,2]. Sulfamethoxazole in combination with the trimethoprim is used to treat urinary tract infections, bronchitis, tuberculosis, ear infections, traveler's diarrhea, shigellosis, and *Pneumocystis jiroveci* pneumonia [3,4]. The common adverse effects associated with the sulfamethoxazole therapy are nausea, vomiting, loss of appetite, and skin rashes. It is rapidly absorbed orally as well as absorbed topically. ~70% of sulfamethoxazole is bound to plasma proteins. Bioavailability and stability profile of any pharmaceutical compound depends upon its physicochemical profile [5]. The physicochemical properties have an important role in its dissolution, absorption, and bioavailability to achieve the therapeutic efficacy [6,7].

The Biofield Energy Healing Treatment (the Trivedi Effect®) has a significant impact on the particle size, surface area, and other chemical and thermal behaviour of pharmaceutical/nutraceutical compounds [8-10]. The Trivedi Effect® is a natural and only scientifically established phenomenon in which an individual can harness this inherently intelligent energy and transfer it anywhere on the planet *via* the possible mediation of neutrinos [11]. "Biofield Energy" the electromagnetic energy field which exists surrounding the living beings, generated by the continuous movement of the electrically charged particles like ions, cells, etc. inside the body [12,13]. Biofield based Energy Therapies have significant outcomes against various disease [14]. 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, yoga, Qi Gong, Reiki, hypnotherapy, etc [15]. These CAM therapies have been accepted by most of the U.S.A. population with advantages [16]. The Trivedi Effect®-Consciousness Energy Healing Treatment has the astounding capability to alter the characteristic properties of the several non-living materials and living object(s), *i.e.,* organic compounds [17,18], metals and ceramic [19,20], microbes [21,22], crops [23,24], etc. The Consciousness Energy Healing Treatment has also enhanced the bioavailability [25,26] and isotopic abundance ratio [27,28] of the pharmaceutical compounds.

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 [30,31]. The Trivedi Effect®-Biofield Energy Healing Treatment could be an economical approach for designing better pharmaceuticals formulations. Therefore, in this study, special attention was taken to improve the physicochemical parameters of the pharmaceutical product, e.g., sulfamethoxazole. 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 $^{15}N/^{14}N$ or $^{17}O/^{16}O$ or $^{33}S/^{32}S$) and P_{M+2}/P_M ($^{18}O/^{16}O$ or $^{34}S/^{32}S$) in the Trivedi Effect® - Consciousness Energy Healing Treated sulfamethoxazole compared to the control sample.

Materials and Methods

Chemicals and Reagents

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

Consciousness Energy Healing Treatment Strategies

The sulfamethoxazole powder sample was the test sample divided into two parts and termed as control and treated sample based on the Energy Treatment. The control sample did not receive the Biofield Energy Treatment. Further, the control sample was treated with a "sham" healer who did not have any awareness about the Biofield Energy Treatment. However, the treated sample of sulfamethoxazole was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely for 3 minutes by the renowned Biofield Energy Healer, Dahryn Trivedi, USA. After that, 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 sulfamethoxazole samples was carried out with the help of LC-MS ThermoFisher Scientific (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. Methanol was the diluent used for the sample preparation. 5 μL of sulfamethoxazole solution was

injected, and the analyte was eluted using acetonitrile + 0.1% formic acid (75:25) pumped at a constant flow rate of 0.5 mL/min. Chromatographic separation was achieved using gradient condition and the total run time was 10 min. Peaks were monitored at 254 nm using the PDA detector. The mass spectrometric analysis was performed in -ve ESI mode.

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 sulfamethoxazole was calculated using equation 1.

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

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

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis: GC-MS of the sulfamethoxazole 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 electron impact (EI) ionization in positive mode. The oven temperature was programmed from 75°C (5 min hold) to 280°C (14.5 min hold) @ 10°C / min (total run time 40 min). The sample was prepared taking 60 mg of the sulfamethoxazole in 4 ml acetonitrile and water (1:1) as a diluent. The GC-MS based isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) for the control and Biofield Energy Treated sulfamethoxazole was calculated using equation 1.

Results and Discussion

Liquid Chromatography-Mass Spectrometry (LC-MS)

The chromatogram of both the samples of sulfamethoxazole is shown in Figures 1. Both the chromatograms showed the single major chromatographic peak of sulfamethoxazole at the retention time (R_t) of 2.5 minutes (Figure 1). The peak area of the Biofield Energy Treated sulfamethoxazole was significantly increased by 42.96% compared to the control sample, which indicated that the solubility profile of the Biofield Energy Treated sulfamethoxazole was significantly increased after the Biofield Energy Treatment compared to the control sample.

The sulfamethoxazole was detected with the molecular mass peak [M-H]$^-$ at m/z 252 in the MS spectrum in negative ion mode [35]. The mass spectra of both the samples of sulfamethoxazole (Figure 2) exhibited the mass of the deprotonated molecular ion peak at m/z 252 [M-H]$^-$ (calculated for $C_{10}H_{10}N_3O_3S^-$, 252.04).
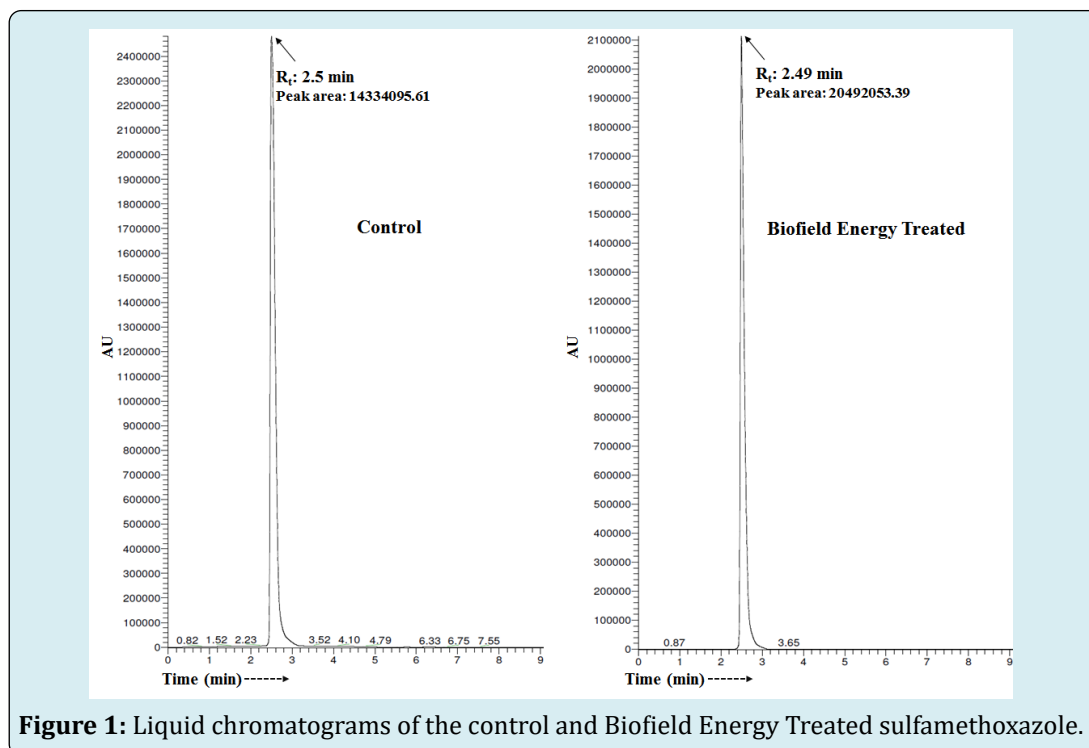

Figure 1: Liquid chromatograms of the control and Biofield Energy Treated sulfamethoxazole.

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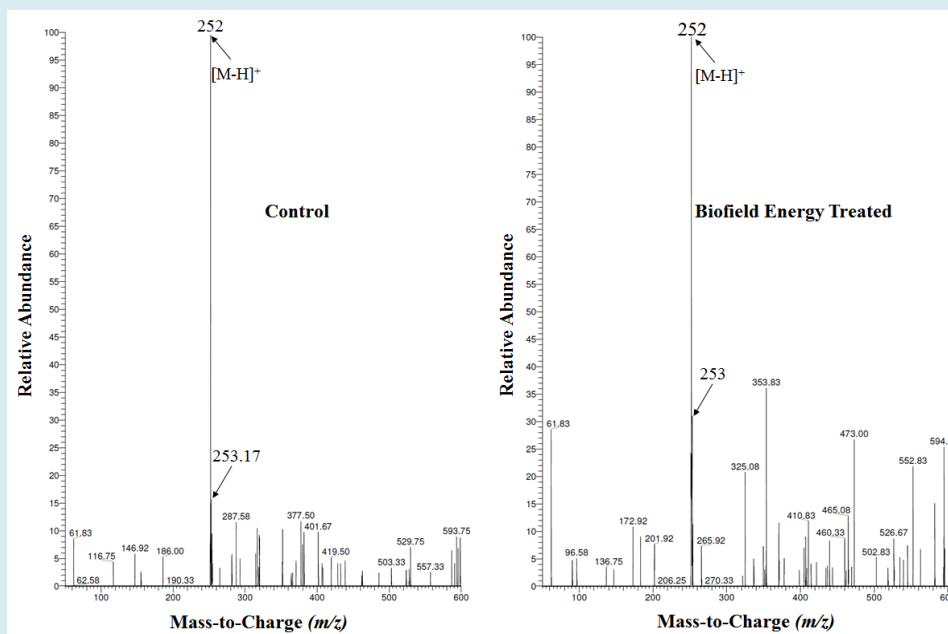
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Figure 2: Mass spectra of the control and Biofield Energy Treated sulfamethoxazole at R$_t$ 2.5 minutes.

The LC-MS spectra of both the samples showed the mass of the molecular ion peak at *m/z* 252 [M-H]$^-$ (calculated for $C_{10}H_{10}N_3O_3S^-$, 252.04) with relative intensity of 100%. The theoretical calculation of P$_{M+1}$ for sulfamethoxazole 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) = [(10 x 0.015%) x 100%] / 100%= 0.15%

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

P (^{17}O) = [(3 x 0.04%) x 100%] / 100% = 0.12%

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 $(C_{10}H_{10}N_3O_3S)^-$ to *m/z* 253 = 13.22%

From the above calculation, it has been found that ^{13}C, ^{15}N, and ^{33}S have major contribution to *m/z* 253.

The LC-MS based isotopic abundance ratio analysis P$_M$ and P$_{M+1}$ for sulfamethoxazole near *m/z* 252 [M$^+$] and 253 [(M+1)$^+$], respectively of the control and Biofield Energy Treated samples in the ESI-MS spectra (Table 1). The change in the isotopic abundance ratio (P$_{M+1}$/P$_M$) in the treated sulfamethoxazole was significantly decreased by 49.56% compared with the control sample (Table 1). Thus, it was concluded that the ^{13}C, ^{2}H, ^{15}N, ^{17}O, and ^{33}S contributions from $(C_{10}H_{10}N_3O_3S)^-$ to *m/z* 253 in the treated sample were significantly decreased compared to the control sample.

Parameter	Control sample	Biofield Energy Treated sample
PM at m/z 252 (%)	100	100
PM+1 at m/z 253 (%)	30.95	15.61
PM+1/PM	0.31	0.16
% Change of isotopic abundance ratio (PM+1/PM) with respect to the control sample		-49.56

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

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.

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

The control and treated sulfamethoxazole showed the

presence of a sharp chromatographic peak at the retention time of 18.7 and 17.35 minutes, respectively in the GC-MS chromatograms (Figures 3 and 4). The peak area% of the treated sample was significantly increased by 15.66%

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compared to the control sample. This indicated that the solubility of the Biofield Energy Treated sulfamethoxazole was significantly increased compared to the control sample. The peak near the R_t of 18 min in both the chromatograms was due to the sulphanilamide present in the sample. The parent molecular ion peak of sulfamethoxazole at m/z 253 $[M]^+$ (calculated for $C_{10}H_{11}N_3O_3S^+$, 253.05) in both the samples, along with the lower mass fragment ion peaks near m/z 156 and 92 (Figures 3 and 4) which were proposed corresponded to the molecular formula $C_6H_6NO_2S^+$ and $C_6H_6N^+$, respectively (Figure 5).

The GC-MS spectra of both the control and treated sulfamethoxazole showed the mass of the molecular ion peak $[M]^+$ at m/z 253 $[M]^+$ (calculated for $C_{10}H_{11}N_3O_3S^+$, 253.05). The theoretical calculation of P_{M+1} and P_{M+2} for sulfamethoxazole was presented as below:

P (^{13}C) = [(10 x 1.1%) x 3.31% (the actual size of the M^+ peak)] / 100% = 0.36%
P (^{2}H) = [(11 x 0.015%) x 3.31%] / 100%= 0.005%
P (^{15}N) = [(3 x 0.4%) x 3.31%] / 100% = 0.04%
P (^{17}O) = [(3 x 0.04%) x 3.31%] / 100% = 0.004%
P (^{33}S) = [(1 x 0.75%) x 3.31%] / 100% = 0.024%
P_{M+1}, *i.e.* ^{13}C, ^{2}H, ^{15}N, ^{17}O, and ^{33}S contributions from $(C_{10}H_{11}N_3O_3S)^+$ to m/z 254 = 0.43%

Similarly,
P (^{18}O) = [(3 x 0.2%) x 3.31%] / 100% = 0.019%
P (^{34}S) = [(1 x 4.21%) x 3.31%] / 100% = 0.14%
P_{M+2}, *i.e.* ^{34}S and ^{18}O contributions from $(C_{10}H_{11}N_3O_3S)^+$ to m/z 255 = 0.16%

From the above calculation, it has been found that ^{13}C, ^{15}N, ^{33}S, and ^{34}S have major contribution to m/z 254 and 255.

Parameter	Control sample	Biofield Energy Treated sample
PM at m/z 253 (%)	3.31	3.59
PM+1 at m/z 254 (%)	0.21	0.5
PM+1/PM	0.06	0.14
% Change of isotopic abundance ratio (PM+1/PM) compared with the control sample		119.53
PM+2 at m/z 255 (%)	1.93	1.56
PM+2/PM	0.58	0.43
% Change of isotopic abundance ratio (PM+1/PM) compared with the control sample		-25.48

Table 2: GC-MS based isotopic abundance analysis results of Biofield Energy Treated sulfamethoxazole compared to the control samples.
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.
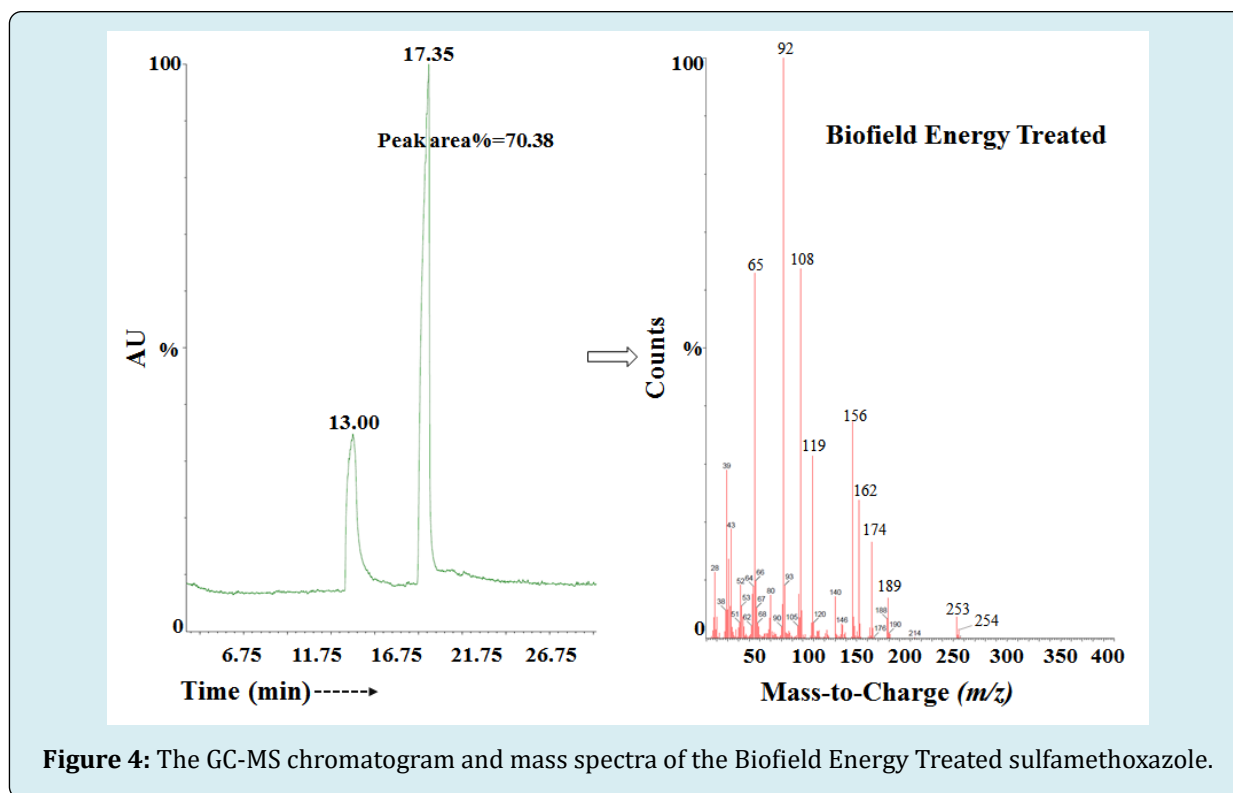

Figure 3: The GC-MS chromatogram and mass spectra of the control sulfamethoxazole.

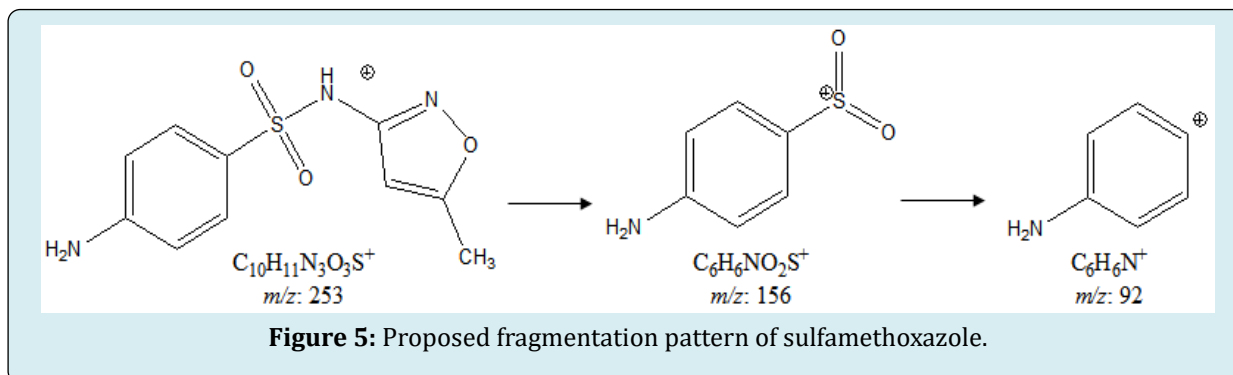**Figure 4:** The GC-MS chromatogram and mass spectra of the Biofield Energy Treated sulfamethoxazole.

Figure 5: Proposed fragmentation pattern of sulfamethoxazole.

The GC-MS based isotopic abundance ratio analysis of the treated sulfamethoxazole samples was calculated compared to the control sample. P_M, P_{M+1}, and P_{M+2} for sulfamethoxazole near m/z 253 $[M^+]$, 254 $[(M+1)^+]$, and 255 $[(M+2)^+]$ were obtained from the observed relative peak intensities from the mass spectra (Table 2). The isotopic abundance ratio of P_{M+1}/P_M in the treated sulfamethoxazole was significantly increased by 119.53% compared with the control sample (Table 2). But, the isotopic abundance ratio of P_{M+2}/P_M in the Biofield Energy Treated sulfamethoxazole was significantly decreased by 25.48% compared with the control sample (Table 2). Hence, ^{13}C, ^2H, ^{15}N, ^{17}O, ^{18}O, ^{33}S, and ^{34}S contributions from $(C_{10}H_{11}N_3O_3S)^+$ to m/z 254 and 255 in the Biofield Energy Treated sample were significantly altered compared to the control sample.

LC-MS and GC-MS study confirmed the structure of the sample as sulfamethoxazole. The isotopic abundance ratios 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 P_{M+2}/P_M ($^{18}O/^{16}O$ or $^{34}S/^{32}S$) in the Biofield Energy Treated sulfamethoxazole were significantly altered compared to the control sample. According to science, 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 nucleus, which indicated a close relation between neutrino and the isotope formation [11,30,31]. The altered isotopic composition in the molecular level of the Trivedi Effect®-Consciousness Energy Healing Treated sulfamethoxazole might have altered the neutron to proton

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ratio in the nucleus. 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®. The overall results concluded that the Consciousness Energy Healing Treatment might create a new form of sulfamethoxazole which would show better solubility, dissolution, absorption, and bioavailability compared with the untreated sample. The Consciousness Energy Healing Treated sulfamethoxazole would be more suitable for the prevention and treatment of various diseases such as urinary tract infections, ear infections, shigellosis, traveler's diarrhea, bronchitis, and *Pneumocystis jiroveci* pneumonia, *etc*.

Conclusion

The Trivedi Effect®-Consciousness Energy Healing Treatment showed a significant impact on the peak area%, isotopic abundance ratios and mass peak intensities of sulfamethoxazole. The LC-MS spectra of both the samples at retention time (R_t) 2.5 minutes exhibited the mass of the deprotonated molecular ion peak at *m/z* 252 [M-H]$^-$. The peak area of the Biofield Energy Treated sulfamethoxazole was significantly increased by 42.96% compared to the control sample. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated sulfamethoxazole was significantly decreased by 49.56% compared with the control sample. Thus, ^{13}C, ^2H, ^{15}N, ^{17}O, and ^{33}S contributions from $(C_{10}H_{10}N_3O_3S)^-$ to *m/z* 253 in the Biofield Energy Treated sample were significantly decreased compared with the control sample. The GC-MS peak area% of the Biofield Energy Treated sample was significantly increased by 80.3% compared to the control sample. The GC-MS based isotopic abundance ratio of P_{M+1}/P_M and P_{M+2}/P_M in the Biofield Energy Treated sulfamethoxazole was significantly altered by 119.53% and -25.48%, respectively compared with the control sample. Hence, ^{13}C, ^2H, ^{15}N, ^{17}O, ^{18}O, ^{33}S, and ^{34}S contributions from $(C_{10}H_{11}N_3O_3S)^+$ to *m/z* 254 and 255 in the Biofield Energy Treated sample were significantly altered compared with the control sample. The isotopic abundance ratios 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 P_{M+2}/P_M ($^{18}O/^{16}O$ or $^{34}S/^{32}S$) in the Biofield Energy Treated sulfamethoxazole were significantly altered compared to the control sample. It can be assumed that the changes in isotopic abundance, and mass peak intensities could be due to changes in nuclei possibly through the interference of neutrino particles *via* the Trivedi Effect® - Consciousness Energy Healing Treatment. The new form of sulfamethoxazole would be better designing novel pharmaceutical formulations that might offer better solubility, dissolution, absorption, bioavailability and therapeutic response against urinary tract infections, ear infections, tuberculosis, traveler's diarrhea, shigellosis, bronchitis, and *Pneumocystis jiroveci* pneumonia, *etc*.

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