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Influence 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 broad spectrum antibiotic especially used for the treatment of infections caused by bacteria. This study was performed to investigate the impact of The Trivedi Effect®-Biofield Energy Healing Treatment on the structural properties and the isotopic abundance ratio of sulfamethoxazole 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 treated with The Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a renowned Biofield Energy Healer, Alice Branton 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 $[M-H]^-$ (calculated for $C_{10}H_{10}N_3O_3S^-$, 252.04). The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated sulfamethoxazole was significantly increased by 44.67% compared with the control sample. Thus, ^{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 increased compared with the control sample. The control and treated sulfamethoxazole showed the presence of a chromatographic peak at the retention time of 16.91 min in the GC-MS chromatograms. The 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 increased by 24.13% and 90.53%, respectively compared with the control sample. Hence, ^{13}C, ^{2}H, ^{15}N, ^{17}O, ^{18}O, ^{33}S, and ^{34}S contributions from $(C_{10}H_{10}N_3O_3S)^+$ to *m/z* 254 and 255 in the Biofield Energy Treated sample were significantly increased compared with the control sample. The isotopic abundance ratios of P_{M+2}/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 ($^{18}O/^{16}O$ 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 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.

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

Introduction

Sulfamethoxazole is a broad spectrum antibiotic especially used for the treatment of infections caused by bacteria. It acts as a bacteriostatic antibacterial agent, which inhibits the bacterial synthesis of dihydrofolic acid by competing with para-aminobenzoic acid (PABA) for binding to dihydropteroate synthetase. Finally, by this mechanism sulfamethoxazole inhibits bacterial nucleotides and DNA synthesis [1,2]. Sulfamethoxazole was used in combination with the trimethoprim to treat urinary tract infections, tuberculosis, traveler's diarrhea, ear infections, bronchitis, shigellosis, and *Pneumocystis jiroveci* pneumonia [3]. However, some common adverse effects associated with the sulfamethoxazole treatment are nausea, vomiting, loss of appetite, and skin rashes. Sulfamethoxazole is rapidly absorbed orally while it is also well-absorbed topically. The free forms of sulfamethoxazole are considered to be the therapeutically active forms. Approximately 70% of sulfamethoxazole are bound to

plasma proteins. Bioavailability and stability profile of any drug depends upon its physicochemical profile [4,5]. Further, physicochemical properties have important role in different pharmaceutical compounds such as in its dissolution, absorption, and bioavailability profile, which have direct influence to achieve maximum biological activities [6,7].

In this scenario, it was observed that Biofield Energy Healing Treatment (The Trivedi Effect®) has the considerable impact on various properties such as particle size, surface area, and other chemical and thermal behaviour of pharmaceutical/nutraceutical [8-10]. 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 [11]. "Biofield Energy" the electromagnetic energy field which exists surrounding the living beings, which can transmit the electromagnetic energy in the form of bio-photons, generated by the continuous movement of the electrically charged particles like 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 [12,13]. Biofield based Energy Therapies have been reported to with 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 yoga, Qi Gong, Tai Chi, hypnotherapy, Reiki, etc. [15].

These therapies have been accepted by most of the U.S.A. population with several advantages [16]. The Trivedi Effect® Consciousness Energy Healing Treatment has been widely reported with 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], crops [21,22], microbes [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. 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 micro molar 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. Hence, 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

Sulfamethoxazole was purchased from Sigma Aldrich, USA. Other chemicals used during the experiments were of analytical grade available in India.

Consciousness Energy Healing Treatment Strategies

The sulfamethoxazole powder was the test sample divided into two parts. One part of sulfamethoxazole powder sample was considered as a control sample (no Biofield Energy Treatment was provided). However, the other part of sulfamethoxazole was treated with The Trivedi Effect®- Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 minutes and known as The Trivedi Effect® Treated or Biofield Energy Treated sulfamethoxazole sample. The Biofield Energy Treatment was provided through the healer's unique energy transmission process by the renowned Biofield Energy Healer, Alice Branton, USA, to the test sample. Further, the control sample was treated with "sham" healer for comparison purpose. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated sulfamethoxazole 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 control and Biofield Energy Treated sulfamethoxazole 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 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. The diluent used for the sample preparation was methanol. 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 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 (*m/z* 50-600) were recorded. The total ion chromatogram and mass spectrum of the individual peak (appeared in LC-MS) 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

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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.

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

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

GC-MS of the control and Biofield Energy Treated sample of sulfamethoxazole 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. 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. Mass spectra were scanned from *m/z* 20 to 400. The identification of analyte was 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 the control and Biofield Energy Treated sulfamethoxazole 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 sample and $IAR_{Control}$ = isotopic abundance ratio in the control sample.

Results and Discussion

Liquid chromatography-mass spectrometry (LC-MS)

The chromatograms and mass spectra of both the samples of sulfamethoxazole are shown in the (Figure 1&2), respectively. The chromatograms of sulfamethoxazole showed the single major chromatographic peak at the retention time (R_t) of 2.5 minutes in case of both the samples (Figure 1). This indicated that the polarity of both the samples was same. ESI-MS of 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) along with other fragmentation peaks in the control sample and Biofield Energy Treated sample (Figure 3).

The LC-MS spectra of both the control and Biofield Energy Treated sulfamethoxazole 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.

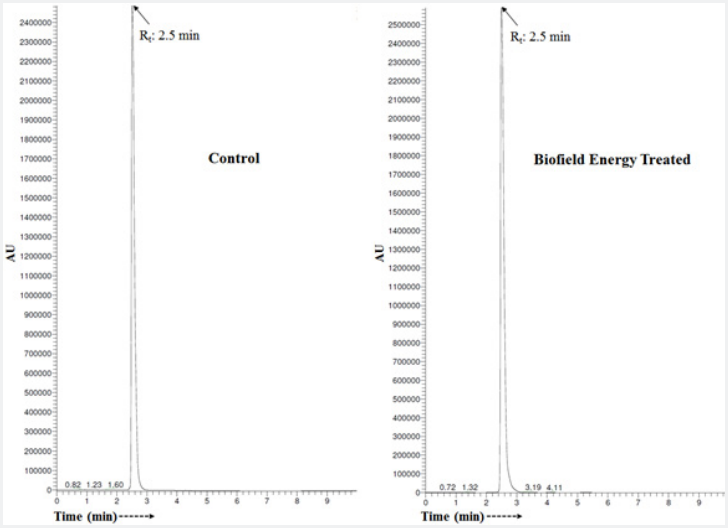

Figure 1: Liquid chromatograms of the control and Biofield Energy Treated sulfamethoxazole.

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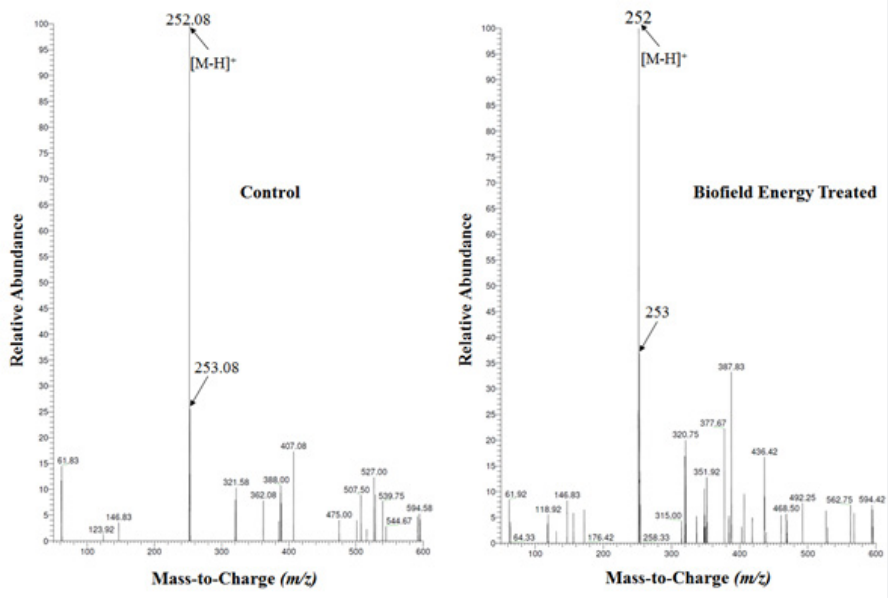

Figure 2: Mass spectra of the control and Biofield Energy Treated sulfamethoxazole at R_t 2.5 minutes.

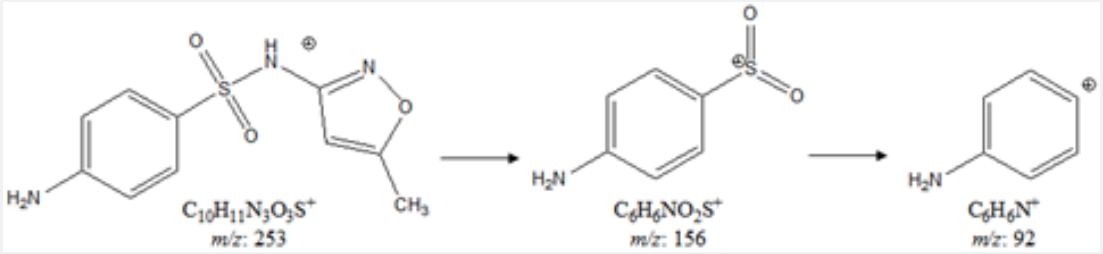

Figure 3: Proposed fragmentation pattern of sulfamethoxazole.

The LC-MS based isotopic abundance ratio analysis P_M and P_{M+1} for sulfamethoxazole near *m/z* 252 and 253, 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 (Table 1). The percentage change of the isotopic abundance ratio (PM+1/PM) in the Biofield Energy Treated sulfamethoxazole was significantly increased by 44.67% 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 $(C_{10}H_{10}N_3O_3S)^-$ to *m/z* 253 in the Biofield Energy Treated sample were significantly increased compared to the control sample.

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

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 252 (%)	100	100
P_{M+1} at *m/z* 253 (%)	25.52	36.92
P_{M+1}/P_M	0.26	0.37
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		44.67

PM: 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.

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Gas chromatography-mass spectrometry (GC-MS) analysis

The control and Biofield Energy Treated sulfamethoxazole showed the presence of a sharp chromatographic peak at the retention time of 16.91 min in the GC-MS chromatograms (Figure 4 & 5). The peak area% of the Biofield Energy Treated sample was significantly increased by 80.3% 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 13 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 the control sample and Biofield Energy Treated sample, along with the lower mass fragment ion peaks near *m/z* 156 and 92 (Figures 4 & 5) which were proposed corresponded to the molecular formula $C_6H_6NO_2S^+$ and $C_6H_6N^+$, respectively (Figure 3). The isotopic abundance ratio depends upon the mass peak intensities of the particular compounds, which was well supported by the LC-MS based isotopic abundance ratio analysis.

The GC-MS spectra of both the control and Biofield Energy 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 2.36% (the actual size of the M+ peak)] / 100% = 0.26%

P (^{2}H) = [(11 x 0.015%) x 2.36%] / 100%= 0.004%

P (^{15}N) = [(3 x 0.4%) x 2.36%] / 100% = 0.03%

P (^{17}O) = [(3 x 0.04%) x 2.36%] / 100% = 0.003%

P (^{33}S) = [(1 x 0.75%) x 2.36%] / 100% = 0.02%

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 = 0.32%

Similarly,

P (^{18}O) = [(3 x 0.2%) x 2.36%] / 100% = 0.014%

P (^{34}S) = [(1 x 4.21%) x 2.36%] / 100% = 0.1%

P_{M+2}, i.e. ^{34}S contributions from $(C_{10}H_{11}N_3O_3S)^+$ to *m/z* 255 = 0.114%

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.

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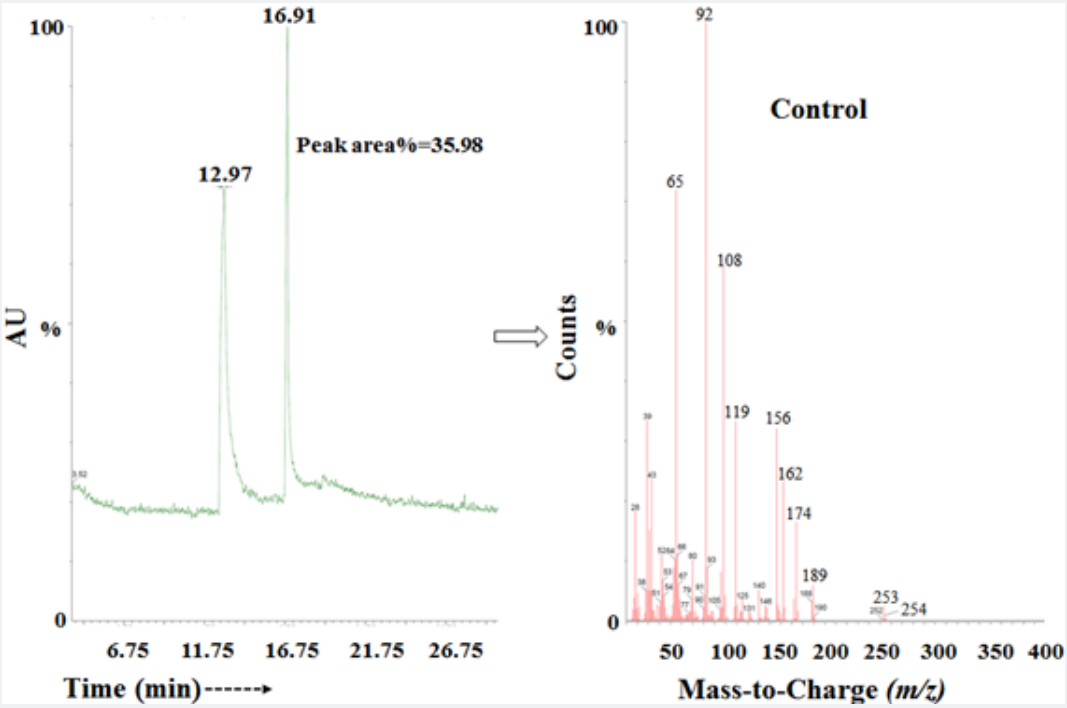

Figure 4: The GC-MS chromatogram and mass spectra of the control sulfamethoxazole.

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 sulfamethoxazole near *m/z* 253, 254, and 255, respectively of the control and Biofield Energy Treated samples, which were obtained from the observed relative peak intensities of $[M^+]$, $[(M+1)^+]$, and $[(M+2)^+]$ peaks, respectively in the mass spectra and are presented in Table 2. The isotopic abundance ratio of P_{M+1}/P_M and of P_{M+2}/P_M in the Biofield Energy

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Treated sulfamethoxazole was significantly increased by 24.13% and 90.53%, respectively compared with the control sample (Table 2). Hence, ^{13}C, ^{2}H, ^{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 increased compared with the control sample.

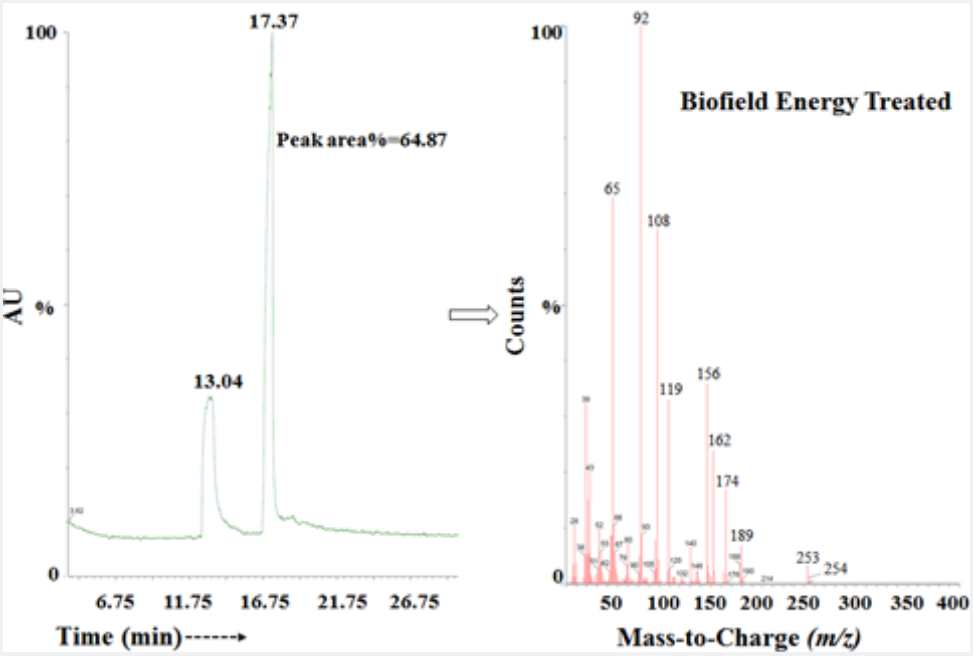

Figure 5: The GC-MS chromatogram and mass spectra of the Biofield Energy Treated sulfamethoxazole.

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

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 253 (%)	2.36	3.27
P_{M+1} at *m/z* 254 (%)	0.25	0.43
P_{M+1}/P_M	0.11	0.13
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		24.13
P_{M+2} at *m/z* 255 (%)	0.5	1.32
P_{M+2}/P_M	0.21	0.4
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		90.53

PM: 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.

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 molecular level of The Trivedi Effect®-Consciousness Energy Healing Treated sulfamethoxazole might have altered the neutron to proton 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® - Consciousness Energy Healing Treatment. The overall

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results concluded that The Trivedi Effect®-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 Trivedi Effect® Treated sulfamethoxazole would be more suitable for the prevention and treatment of various diseases such as urinary tract infections, ear infections, traveler's diarrhea, shigellosis, bronchitis, and *Pneumocystis jiroveci* pneumonia, etc.

Conclusion

The Trivedi Effect®-Consciousness Energy Healing Treatment showed the significant impact on the isotopic abundance ratios and mass peak intensities of sulfamethoxazole. The LC-MS spectra of both the control and Biofield Energy Treated samples at retention time (R_t) 2.5 minutes 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) in the –ve ion mode. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated sulfamethoxazole was significantly increased by 44.67% compared with the control sample. Thus, ^{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 Biofield Energy Treated sample were significantly increased compared with the control sample. The control and Biofield Energy Treated sulfamethoxazole showed the presence of chromatographic peak at the retention time of 16.91 min in the GC-MS chromatograms. The 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 of P_{M+2}/P_M in the Biofield Energy Treated sulfamethoxazole was significantly increased by 24.13% and 90.53%, respectively compared with the control sample. Hence, ^{13}C, ^{2}H, ^{15}N, ^{17}O, ^{18}O, ^{33}S and ^{34}S contributions from $(C_{10}H_{10}N_3O_3S)^+$ to *m/z* 254 and 255 in the Biofield Energy Treated sample were significantly increased compared with the control sample. 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 ($^{18}O/^{16}O$ 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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