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Research Article

Gas Chromatography and Mass Spectroscopy (GC-MS) Based Isotopic Abundance Ratio Evaluation of the Biofield Energy Treated Metronidazole

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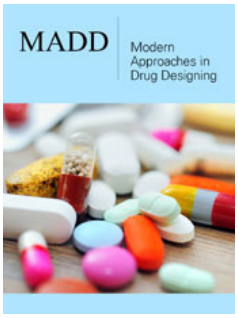

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Abstract

Metronidazole is a common antimicrobial antibiotic, which is used in the treatment of bacterial vaginosis, giardiasis, trichomoniasis, pseudomembranous colitis, etc. This study was performed to evaluate the impact of the Trivedi Effect® on the structural properties, and the isotopic abundance ratio of metronidazole evaluated using GC-MS analytical techniques. The metronidazole sample was divided into control and treated parts. Only the treated part received the Consciousness Energy Healing Treatment remotely by a well-known Biofield Energy Healer, Gopal Nayak. The GC-MS spectra of both the samples of metronidazole at the retention time 16.4 minutes exhibited the molecular ion peak at *m/z* 171 $[M]^+$ (calculated for $C_6H_9N_3O_3^+$, 171.06). The peak area of the Biofield Energy Treated metronidazole was significantly increased by 166.83% compared to the control sample. The isotopic abundance ratio of P_{M+1}/P_M in the treated metronidazole was significantly decreased by 19.72% compared with the control sample. Thus, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_9N_3O_3)^+$ to *m/z* 172 in the treated sample were significantly decreased compared with the control sample. The decreased isotopic composition of the treated metronidazole might be the cause of interference of neutrino *via* the Trivedi Effect®. The isotopic abundance ratios $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{17}O/^{16}O$, and $^{15}N/^{14}N$ would highly influence the atomic bond vibration of treated metronidazole. The decreased isotopic abundance ratio would weaken the chemical bond and increase the solubility of the treated metronidazole. The new form of Biofield Energy Treated metronidazole would be very useful to design more efficacious pharmaceutical formulations that might offer a better therapeutic response against bacterial vaginosis, pseudomembranous colitis, trichomoniasis, giardiasis, pelvic inflammatory disease, brain, and respiratory tract infection, etc.

Keywords: Metronidazole; Consciousness energy healing treatment; Biofield energy; The Trivedi Effect®; GC-MS

Introduction

Metronidazole is used as an antimicrobial antibiotic. It inhibits the microorganism by means of disturbing the genetic material of the microbial cells [1,2]. It is useful in the treatment of bacterial vaginosis, pseudomembranous colitis, giardiasis, trichomoniasis, pelvic inflammatory disease, brain, oral, and respiratory tract infection, aspiration pneumonia, fungating wounds, rosacea, intra-abdominal infections, periodontitis, lung abscess, amoebiasis, etc. It is also used to treat Giardia in animals [2-6]. The side effects associated to the metronidazole therapy are nausea, vomiting, headache, dizziness, diarrhoea, weight loss, metallic taste in the mouth, abdominal pain, stomatitis, thrombophlebitis, hypersensitivity reactions, CNS toxicity, paraesthesia, etc. [2,7]. Metronidazole delivered in the form of a liquid suspension, tablet, capsule, and intravenous injection [7-9]. It is hazardous to the skin and eye on inhalation and ingestion. It is very slightly soluble in water, chloroform, alcohol, dilute acid, and dimethylformamide [10,11].

The dissolution, absorption, and bioavailability of the pharmaceutical compounds have a direct relation with its physicochemical properties [12]. Worldwide pharmaceutical scientists work on the development of pharmaceuticals. The Trivedi Effect®-Consciousness Energy Healing Treatment has a significant impact on the physicochemical properties and bioavailability of pharmaceutical and nutraceutical compounds [13-17]. The Trivedi Effect® is a scientifically proven phenomenon. In which an expert can harness this energy from the

"Universal Energy Field" and transfer it anywhere on the earth *via* the possible mediation of neutrinos [18]. The "Biofield" is the electromagnetic energy field generated by the continuous movement of the electrically charged particles (ions, cells, etc.) inside the body. The method of transfer of the Biofield Energy from the healer to the object is called Biofield Energy Healing Treatment, and it has a significant reported positive outcome [19-22]. The National Center of Complementary and Integrative Health has accepted Energy Therapies as a Complementary and Alternative Medicine (CAM) health care approach in addition to other therapies, medicines, i.e., Reiki, yoga, Qi Gong, Tai Chi, Ayurveda, Chinese herb and medicine, hypnotherapy, etc. [23,24]. These CAM therapies have been accepted by most of the U.S.A. people [24]. Similarly, the Trivedi Effect®-Consciousness Energy Healing Treatment has also been proved scientifically with outstanding performance in the fields of materials science, chemical science, agricultural science, microbiology, cancer research [25-34], etc. Thus, the Trivedi Effect®-Consciousness Energy Healing Treatment could be an economical approach to improve the physicochemical properties of metronidazole. The analysis of stable isotope ratio has various applications in different scientific fields for understanding the isotope effects resulting from the variation of the isotopic composition of the molecule [35,36]. In this study, the isotope ratio analysis can be performed by using the conventional mass spectrometry (MS) techniques, such as gas chromatography-mass spectrometry (GC-MS). The GC-MS can detect the isotope composition in low micromolar concentration with sufficient precision [35,37]. Thus, GC-MS based isotopic abundance ratio analysis of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$ or $^{15}N/^{14}N$) and P_{M+2}/P_M ($^{18}O/^{16}O$) in the metronidazole was evaluated.

Materials and Methods

Chemicals and reagents

The test sample metronidazole was purchased from Tokyo Chemical Industry Co., Ltd., Japan, whereas, the other chemicals used in the experiments were purchased in India.

Consciousness energy healing treatment strategies

The test sample metronidazole powder was divided into two equal parts. One part termed as control and another part as treated samples. The control metronidazole sample did not receive the Biofield Energy Treatment but received treatment from a "sham" healer, who did not have any knowledge about the Biofield Energy Treatment. However, the treated metronidazole was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely for 3 minutes by the renowned Biofield Energy Healer, Gopal Nayak, India. After the treatment, both the samples were kept in sealed conditions and characterized using the GC-MS analytical technique.

Characterization

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: GC-MS of the metronidazole samples were analyzed with the help of Perkin Elmer Gas chromatograph furnished 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 sample was prepared, taking 60mg of the metronidazole in 3ml acetonitrile as a diluent. The experimental abundance of each isotope (C, O, H, and N) 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 [36,38-40].

The GC-MS based isotopic abundance ratios (P_{M+1}/P_M) for the control and treated metronidazole was calculated using equation 1.

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

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

Results and Discussion

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

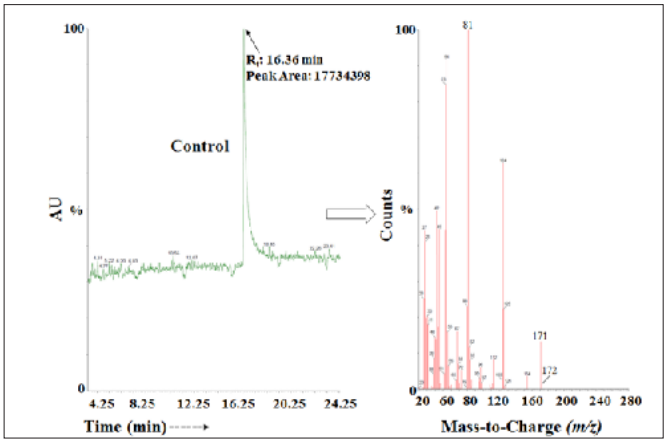

Figure 1: The GC-MS chromatogram and mass spectra of the control metronidazole.

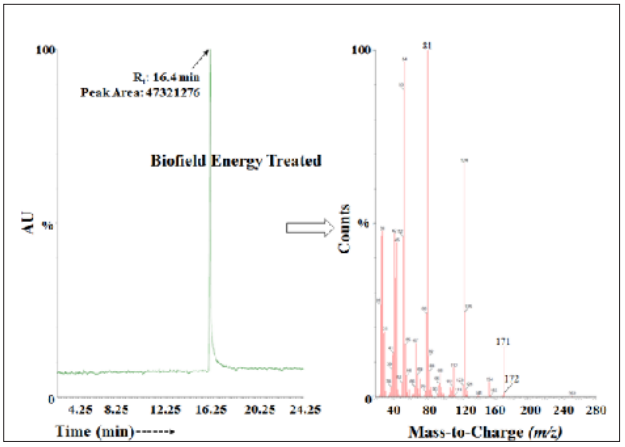

Figure 2: The GC-MS chromatogram and mass spectra of the Biofield energy treated metronidazole.

The GC chromatograms of both the metronidazole samples showed the presence of a single chromatographic peak (Figure 1 & 2) at retention time (R_t) of 16.4 minutes. The peak area of the Biofield Energy Treated metronidazole (47321276) was significantly increased by 166.83% compared to the control sample (17734398). This data indicated that the solubility of the Biofield Energy Treated metronidazole was significantly increased compared to the control sample. The previous study also supported the data and showed that the Biofield Energy Treated metronidazole significantly decreased the crystallite sizes and particle sizes, increased surface area, and thermal stability compared with the control sample [41]. The increased surface area of the treated metronidazole might increase the solubility compared to the control sample, which was supported by the peak area.

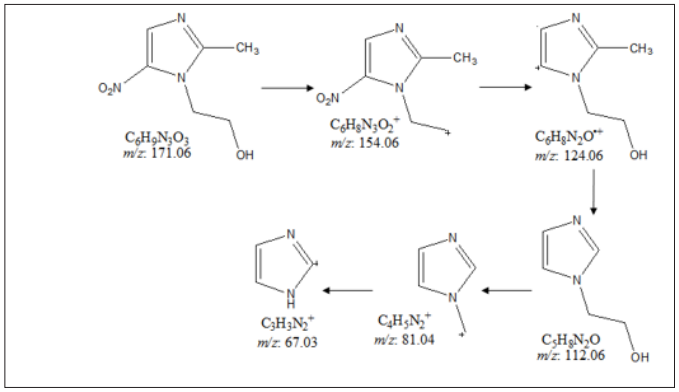

Figure 3: Proposed fragmentation pattern of metronidazole.

Metronidazole shows the molecular mass peak $[M]^+$ at *m/z* 171 in the spectrum in positive ion mode [42]. The parent molecular peak of metronidazole at *m/z* 171 $[M]^+$ (calculated for $C_6H_9N_3O_3^+$, 171.06) along with the fragment peaks near *m/z* 154, 124, 112, 81, and 67 were proposed corresponded to the molecular formula $C_6H_8N_3O_2^+$, $C_6H_8N_2O^+$, $C_5H_8N_2O$, $C_4H_5N_2^+$, and $C_3H_3N_2^+$, respectively (Figures 3). The mass peak intensities influence the isotopic abundance ratio.

The GC-MS based theoretical calculation of P_{M+1} for metronidazole was presented as below:

P (^{13}C) = [(6 x 1.1%) x 14.44% (the actual size of the M^+ peak)] / 100% = 0.95%

P (^{2}H) = [(9 x 0.015%) x 14.44%] / 100%= 0.019%

P (^{15}N) = [(3 x 0.4%) x 14.44%] / 100% = 0.17%

P (^{17}O) = [(3 x 0.04%) x 14.44%] / 100% = 0.017%

P_{M+1}, i.e. ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_9N_3O_3)^+$ to *m/z* 172 = 1.16%

From the above calculation, it has been found that ^{13}C and ^{15}N have major contribution to *m/z* 172. The calculated isotopic abundance (1.16%) was close to the experimentally observed value (1.08%). The GC-MS based isotopic abundance ratio was calculated. The P_M and P_{M+1} for metronidazole near *m/z* 171 and 172, respectively were obtained from the observed relative peak intensities of $[M^+]$ and $[(M+1)^+]$, respectively (Table 1). The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated metronidazole was significantly decreased by 19.72% compared with the control sample (Table 1). Which indicated that the ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_9N_3O_3)^+$ to *m/z* 172 in the Biofield Energy Treated metronidazole were significantly increased compared with the control sample.

Table 1: GC-MS based isotopic abundance analysis results of metronidazole in control and Biofield Energy Treated samples.

Parameter	Control Sample	Biofield Energy Treated Sample
P_M at *m/z* 171 (%)	14.44	14.49
P_M+1 at *m/z* 172 (%)	1.08	0.87
P_M+1/P_M	0.07	0.06
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		-19.72

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.

The isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) in the Biofield Energy Treated metronidazole was significantly decreased compared to the control sample. The natural abundance and relative proportion of the stable isotopes significantly affected by the environment, climate, etc. [43]. The decreased isotopic composition in the molecular level of the Consciousness Energy Healing Treated metronidazole might be due to the alteration in neutron to proton ratio in the nucleus possibly, through the interference of neutrino particles *via* the Trivedi Effect®. 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 [18,36,37]. The isotopic abundance ratios $^2H/^1H$, $^{13}C/^{12}C$, $^{17}O/^{16}O$, and $^{15}N/^{14}N$ would highly influence the atomic bond vibration of treated metronidazole. The decreased isotopic abundance ratio of the Consciousness Energy Healing Treated metronidazole would weaken the chemical bond and increase the solubility in the body. The new form of Biofield Energy Treated metronidazole would be very useful to design more

efficacious pharmaceutical formulations that might offer better therapeutic response against bacterial and protozoal infection in the vagina, stomach, joints, liver, skin, brain, and respiratory tract; also more effective against aspiration pneumonia, intra-abdominal infections, lung abscess, rosacea, fungating wounds, amoebiasis, oral infections, periodontitis, etc.

Conclusion

The Trivedi Effect®-Consciousness Energy Healing Treatment showed a significant impact on the isotopic abundance ratios of metronidazole. The GC-MS spectra of both the samples of metronidazole at the retention time 16.4 minutes exhibited the molecular ion peak at *m/z* 171 $[M]^+$. The peak area of the Biofield Energy Treated metronidazole was significantly increased by 166.83% compared to the control sample. The isotopic abundance ratio of P_{M+1}/P_M in the treated metronidazole was significantly decreased by 19.72% compared with the control sample. Thus, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_9N_3O_3)^+$ to *m/z* 172 in the treated sample were significantly decreased compared with the control sample. The decreased isotopic composition of the treated metronidazole might be the cause of interference of neutrino *via* the Trivedi Effect®. The isotopic abundance ratios $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{17}O/^{16}O$, and $^{15}N/^{14}N$ would highly influence the atomic bond vibration of treated metronidazole. The decreased isotopic abundance ratio would weaken the chemical bond and increase the solubility of the treated metronidazole. The new form of Biofield Energy Treated metronidazole would be very useful to design more efficacious pharmaceutical formulations that might offer better therapeutic response against bacterial and protozoal infection in the vagina, stomach, joints, liver, skin, brain, and respiratory tract; also more effective against aspiration pneumonia, intra-abdominal infections, lung abscess, rosacea, fungating wounds, amoebiasis, oral infections, periodontitis, etc.

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