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Research Article

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

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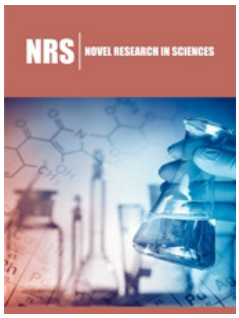

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Abstract

Flutamide is a nonsteroidal antiandrogen (NSAA) which is used to treat the prostate cancer by blocking the action of exogenous testosterone thru binding to the androgen receptor. This study was designed and performed to investigate the impact of The Trivedi Effect®-Biofield Energy Healing Treatment on the structural properties and isotopic abundance ratio of flutamide using LC-MS and GC-MS spectroscopy. Flutamide sample was divided into two parts, one part of flutamide 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) 3.4 minutes exhibited the mass of the deprotonated molecular ion peak at m/z 275.08 $[M-H]^-$ (calculated for $C_{11}H_{10}F_3N_2O_3^-$, 175.06). 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 $^{17}O/^{16}O$) in the treated flutamide was significantly increased by 18.44% compared with the control sample. Thus, ^{13}C, ^2H, ^{15}N, and ^{17}O contributions from $(C_{11}H_{10}F_3N_2O_3)^-$ to *m/z* 276.08 in the treated sample were significantly increased compared with the control sample. The GC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated flutamide was increased by 4.72% compared with the control sample. Hence, ^{13}C, ^2H, ^{15}N, and ^{17}O contributions from $(C_{11}H_{11}F_3N_2O_3)^+$ to *m/z* 277 in the Biofield Energy Treated sample were significantly increased 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 $^{17}O/^{16}O$) in the treated flutamide was 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 treated flutamide would be better designing novel pharmaceutical formulations that might offer better therapeutic response against prostate cancer, androgen-dependent skin and hair conditions including acne, seborrhea, hirsutism, and scalp hair loss, hyperandrogenism, as well useful for feminizing hormone therapy aimed at transgender women

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

Introduction

Flutamide is an acetanilide, nonsteroidal antiandrogen (NSAA), chemically it is 2-methyl-N-[4-nitro-3 (trifluoromethyl) phenyl] propanamide [1-3]. It is orally active as an anti-androgen, which is used primarily to treat men with prostate cancer [4]. It also can be used as an independent drug or is used with other medications as well as with radiation treatments [5]. Testosterone, a natural hormone, helps prostate cancer to grow and spread. The role of flutamide is to inhibit the effects of testosterone, thus slowing the growth and spread of prostate cancer [6]. It is also used extensively in the treatment of androgen-dependent skin and hair conditions in women including acne, seborrhea, hirsutism, and scalp hair loss, as well as in hyperandrogenism. It can be used as a component of feminizing hormone therapy for transgender women [7]. Irregular consumption of this medicine or stopping medications before it completely gets cured could allow cancer to spread more rapidly. Overdose may cause hypoactivity, piloerection, slow respiration, anorexia, ataxia, and/or lacrimation, tranquilization, emesis, and methemoglobinemia [8].

Study of the physicochemical properties of a pharmaceutical product regarding its dissolution and absorption is crucial for the pharmaceutical and nutraceutical formulation [9]. 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 [10-12]. 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 [13]. "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 (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 [14,15]. Biofield based Energy Therapies have been reported with significant outcomes against various disease [16]. 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 in addition to other therapies, medicines, and practices such as yoga, Qi Gong, Tai Chi, hypnotherapy, Reiki, etc. [17]. These therapies have been accepted by most of the U.S.A. population with several advantages [18]. The Trivedi Effect®-Consciousness Energy Healing Treatment has been widely reported with the astounding capability to alter the characteristic properties of the several non-living materials and living object(s), i.e., metals and ceramic [19,20], organic compounds [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 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 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., flutamide. 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 $^{17}O/^{16}O$) in The Trivedi Effect®-Consciousness Energy Healing Treated flutamide compared to the control sample.

Materials and Methods

Chemicals and reagents

Flutamide was purchased from Tokyo Chemical Industry Co., Ltd., Japan. Other chemicals used during the experiments were of analytical grade available in India.

Consciousness energy healing treatment strategies

The flutamide powder was the test sample divided into two parts. One part of flutamide powder sample was considered as a control sample (no Biofield Energy Treatment was provided). However, the other part of flutamide 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 flutamide 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 flutamide 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 flutamide was carried out with the help of LC-MS Thermo Fisher Scientific, the USA equipped with an iron trap detector connected with a triple-stage quadrupole mass spectrometer. The column used here was a reversed phase Thermo Scientific Synchronis C18 (Length-250mmXID 4.6mmX5 micron), maintained at 25 ˚C. The diluent used for the sample preparation was methanol. 10µL of flutamide solution was injected, and the analyte was eluted using 92% acetonitrile + 8% 10 mM ammonium acetate pumped at a constant flow rate of 1mL/min. Chromatographic separation was achieved using gradient condition and the total run time was 10min. Peaks were monitored at 300 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* 50-500) 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,O,H,N, and F) 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 flutamide was calculated.

Percentage (%) change in isotopic abundance ratio = [(IARTreated-IARControl)/IARControl)x100]

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

GC-MS of the control and Biofield Energy Treated sample of flutamide were analyzed with the help of Perkin Elmer Gas chromatograph equipped with a PE-5MS (30Mx250 micros x0.250 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 80 °C (14min hold) to 250 °C (3 min hold) @ 10 °C/min (total run time 25min). The sample was prepared taking 60 mg of the flutamide is in 2ml methanol 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 (PM+1/PM) for the control and Biofield Energy Treated flutamide was calculated.

Percentage (%) change in isotopic abundance ratio = [(IARTreated-IARControl)/IARControl)x100]

Where IARTreated =isotopic abundance ratio in the treated sample and IARControl=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 flutamide are shown in Figures 1&2, respectively. The chromatograms of flutamide showed the single major chromatographic peak at the retention time (R_t) of 3.4 minutes in case of both the samples (Figure 1). This result indicated that the polarity of both the samples was same.

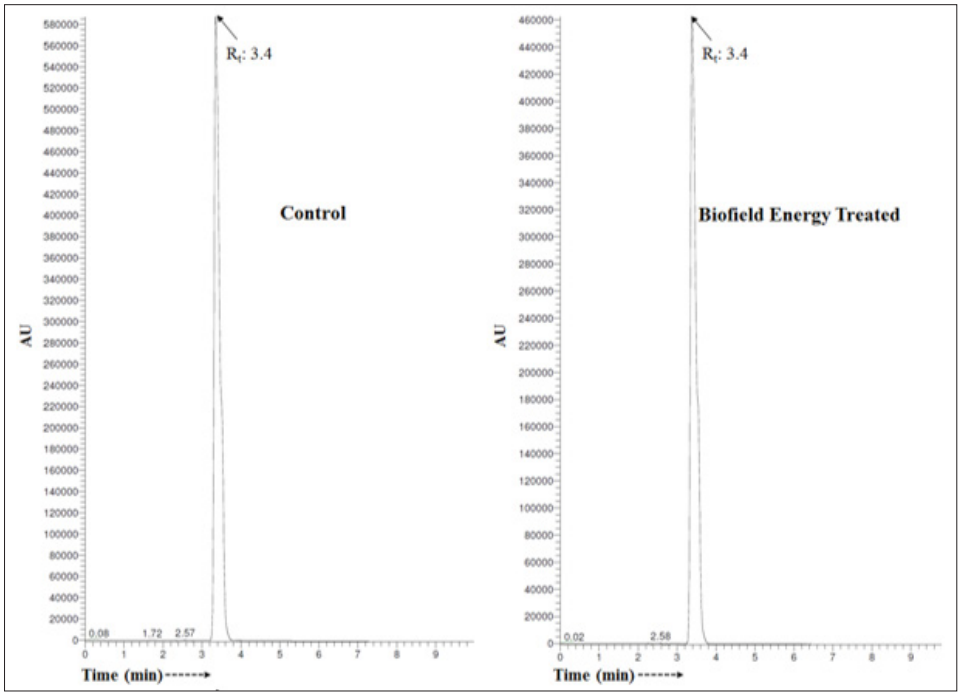

Figure 1: Liquid chromatograms of the control and Biofield Energy Treated flutamide.

ESI-MS of flutamide was detected with the molecular mass peak $[M]^+$ at *m/z* 276 MS spectrum [35]. Here, the mass spectra of both the samples of flutamide (Figure 2) exhibited the mass of the deprotonated molecular ion peak at *m/z* 275.08 $[M-H]^-$ (calculated for $C_{11}H_{10}F_3N_2O_3^-$, 175.06) along with other fragmentation peaks at 256, 202, and 156 in the control sample and Biofield Energy Treated sample (Figure 3).

The LC-MS spectra of both the control and Biofield Energy Treated flutamide showed the mass of the molecular ion peak at *m/z* 275.08 $[M-H]^-$ (calculated for $C_{11}H_{10}F_3N_2O_3^-$ 275.06) with relative intensity of 100%. The theoretical calculation of P_{M+1} for flutamide was presented as below:

P (^{13}C) = [(11 x 1.1%) x100% (the actual size of the M^- peak)]/100%=12.1%

P (^2H) = [(10 x 0.015%)x100%]/100%= 0.15%

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

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

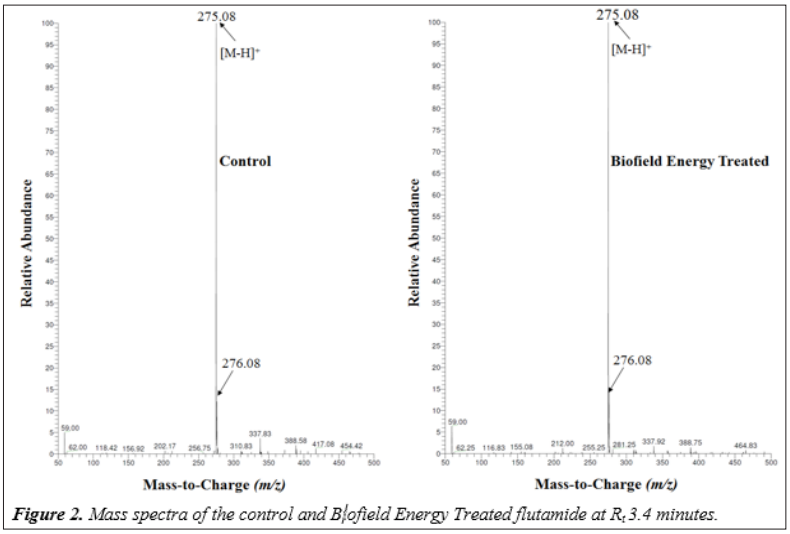

Figure 2. Mass spectra of the control and Biofield Energy Treated flutamide at R_t 3.4 minutes.

Figure 2: Mass spectra of the control and Biofield Energy Treated flutamide at R_t 3.4 minutes.

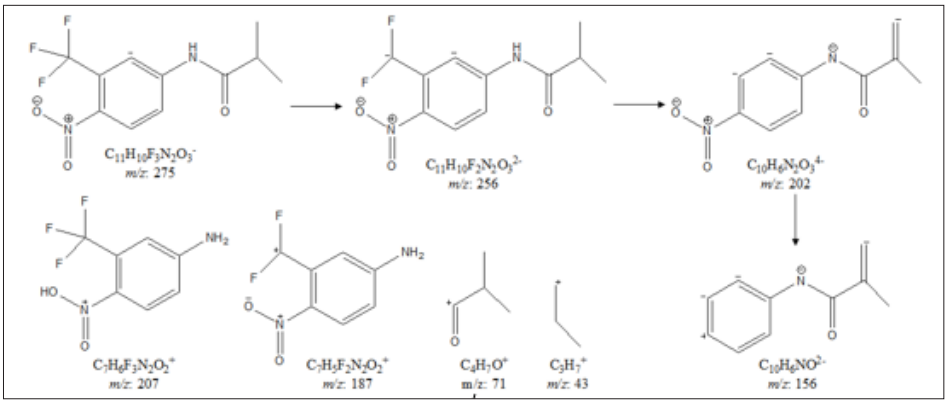

Figure 3: Proposed fragmentation pattern of flutamide.

P_{M+1}, i.e. ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_{11}H_{10}F_3N_2O_3)^-$ to *m/z* 276.08 = 13.17%. The calculated isotope abundance (13.17%) was close to the experimental value 12.15% (Table 1). From the above calculation, it has been found that ^{13}C and ^{15}N have major contribution to *m/z* 276.08. The LC-MS based isotopic abundance ratio analysis P_M and P_{M+1} for flutamide near *m/z* 275.08 and 276.08, 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 (P_{M+1}/P_M) in the Biofield Energy Treated flutamide was significantly increased by 18.44% compared with the control sample (Table 1). Therefore, it was concluded that the ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_{11}H_{10}F_3N_2O_3)^-$ to *m/z* 276.08 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 flutamide compared to the control sample.

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 275.08 (%)	100	100
P_{M+1} at *m/z* 276.08 (%)	12.15	14.39
P_{M+1}/P_M	0.12	0.14
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		18.44

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 Biofield Energy Treated flutamide showed the presence of a single chromatographic peak at the retention time of 16.04 min in the GC-MS chromatograms (Figures 4&5). The parent molecular ion peak of flutamide at *m/z* 276 $[M]^+$ (calculated for $C_{11}H_{10}F_3N_2O_3^+$, 276.08) in the control sample and Biofield Energy Treated sample, along with the fragment ion peaks near *m/z* 207, 187, 71, and 43 (Figures 4&5) which were proposed corresponded to the molecular formula $C_7H_6F_3N_2O_2^+$, $C_7H_5F_2N_2O_2^+$, $C_4H_7O^+$, and C_3H^{7+}, respectively (Figure 3). The isotopic abundance ratio influence by the mass peak intensities, which was well supported by the LC-MS based isotopic abundance ratio analysis.

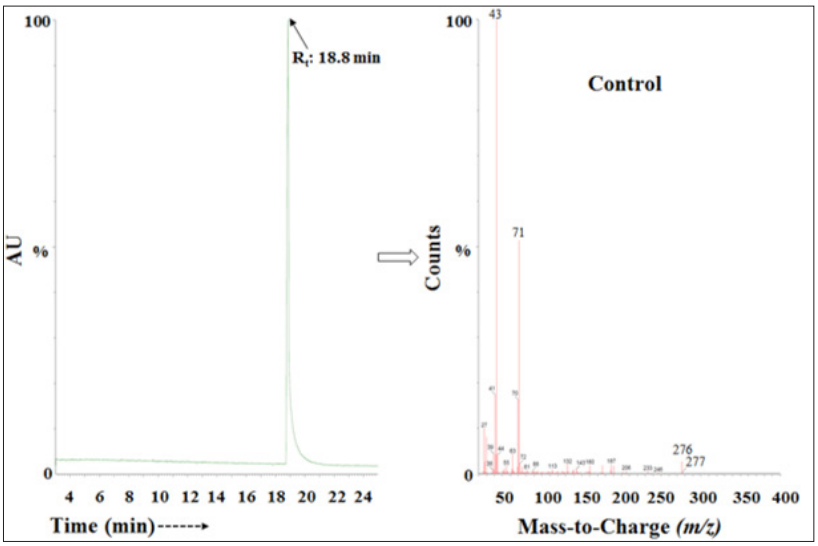

Figure 4: The GC-MS chromatogram and mass spectra of the control flutamide.

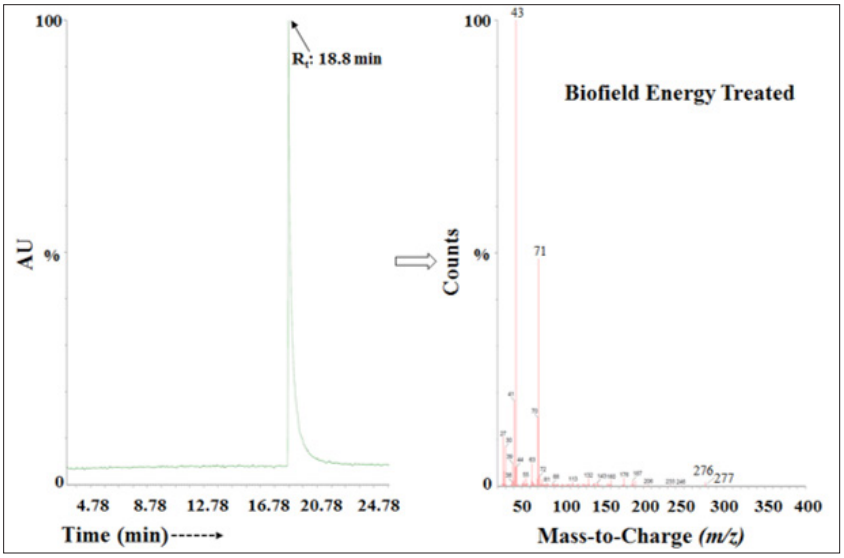

Figure 5: The GC-MS chromatogram and mass spectra of the Biofield Energy Treated flutamide.

The GC-MS spectra of both the control and Biofield Energy Treated flutamide showed the mass of the molecular ion peak $[M]^+$ at *m/z* 276 (calculated for $C_{11}H_{11}F_3N_2O_3^+$, 276.08). The theoretical calculation of P_{M+1} for flutamide was presented as below:

P (^{13}C) = [(11 x 1.1%) x 2.62% (the actual size of the M+ peak)]/100% = 0.32%

P (^{2}H) = [(11 x 0.015%) x 2.62%]/100%= 0.004%

P (^{15}N) = [(2 x 0.4%) x 2.62%]/100% = 0.02%

P (^{17}O) = [(3 x 0.04%) x 2.62%]/100% = 0.003%

P_{M+1}, i.e. ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_{11}H_{11}F_3N_2O_3)^+$ to *m/z* 277 = 0.37%

From the above calculation, it has been found that ^{13}C and ^{15}N have major contribution to *m/z* 277.

The GC-MS based isotopic abundance ratio analysis of the Biofield Energy Treated samples were calculated compared to the control sample. P_M and P_{M+1} for flutamide near *m/z* 276 and 277, 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 mass spectra and are presented in Table 2. The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated flutamide was increased by 4.72% compared with the control sample (Table 2). Hence, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_{11}H_{11}F_3N_2O_3)^+$ to *m/z* 277 in the Biofield Energy Treated sample were increased compared with the control sample.

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

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 276 (%)	2.62	0.86
P_{M+1} at *m/z* 277 (%)	0.32	0.11
P_{M+1}/P_M	0.12	0.13
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		4.72

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.

LC-MS and GC-MS study confirmed the structure of the sample as flutamide. 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 flutamide were significantly altered compared to the control sample. Modern physics tell that, the neutrinos change identities which are only possible if the neutrinos possess mass and can interchange their phase from one phase to another internally. Therefore, the neutrinos can interact with protons and neutrons in the nucleus, which indicated a close relation between neutrino and the isotope formation [12,30,31]. The altered isotopic composition in molecular level of The Trivedi Effect®-Consciousness Energy Healing Treated flutamide 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 new form of flutamide (Biofield Energy Treated) would be very useful to design better pharmaceutical formulations that might offer better therapeutic response against many diseases.

Conclusion

The Trivedi Effect®-Consciousness Energy Healing Treatment showed the significant impact on the isotopic abundance ratios and mass peak intensities of flutamide. The LC-MS spectra of both the control and Biofield Energy Treated samples at retention time (R_t) 3.4 minutes exhibited the mass of the deprotonated molecular ion peak at *m/z* 275.08 $[M-H]^-$ (calculated for $C_{11}H_{10}F_3N_2O_3^-$, 175.06). 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 $^{17}O/^{16}O$) in the Biofield Energy Treated flutamide was significantly increased by 18.44% compared with the control sample. Thus, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_{11}H_{10}F_3N_2O_3)^-$ to *m/z* 276.08 in the Biofield Energy Treated sample were significantly increased compared with the control sample. The GC-MS based isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated flutamide was increased by 4.72% compared with the control sample. Hence, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_{11}H_{11}F_3N_2O_3)^+$ to m/z 277 in the Biofield Energy Treated sample were significantly increased 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 $^{17}O/^{16}O$) in the Biofield Energy Treated flutamide 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 Biofield Energy Treated flutamide would be better designing novel pharmaceutical formulations that might offer better therapeutic response against prostate cancer, androgen-dependent skin and hair conditions including acne, seborrhea, hirsutism, and scalp hair loss, hyperandrogenism, as well useful for feminizing hormone therapy aimed at transgender women.

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