

Isotopic Abundance Ratio Analysis of the Consciousness Energy Healing Treated Flutamide using LC-MS and GC-MS Spectrometry

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Research Article

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Abstract

Flutamide is used to treat prostate cancer, which acts by blocking the action of exogenous testosterone binding through its androgen receptor. This study was designed and performed to investigate the impact of the Trivedi Effect®-Consciousness Energy Healing Treatment on the structural properties and the isotopic abundance ratio of flutamide using LC-MS and GC-MS spectroscopy. Flutamide sample was divided into control and treated parts. Only the treated flutamide received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a famous Biofield Energy Healer, Dahryn Trivedi. The LC-MS spectra of both the samples at retention time (R_1) 3.4 minutes exhibited the mass of the deprotonated molecular ion peak at *m*/*z* 275.08 [M-H]-. The peak area of the treated sample was significantly increased by 92.42% compared to the control sample, which indicated that the solubility profile of the treated sample was significantly increased compared to the control sample. The LC-MS based isotopic abundance ratio of P_{M+1}/PM (²H/¹H or ¹³C/¹²C or ¹⁵N/¹⁴N or ¹⁷O/¹⁶O) in the treated flutamide was significantly increased by 59.48% compared with the control sample. The GC-MS based isotopic abundance ratio of P_{Ma1}/ P_M in the treated flutamide sample was increased by 10.39% compared with the control sample. The results indicated that the 13 C, 2 H, 15 N, and 17 O contributions from (C₁₁H₁₁F₃N₂O₃)+ to *m/z* 277 in the treated sample were significantly increased compared with the control sample. The isotopic abundance ratio of P_{M+1}/P_M (²H/¹H or ¹³C/¹²C or ¹⁵N/¹⁴N or ¹⁷O/¹⁶O) in the treated flutamide was significantly increased compared to the control sample. The changes in the peak area and isotopic abundance could be due to changes in nuclei possibly through the interference of neutrino via the Trivedi Effect®-Consciousness Energy Healing Treatment. The new form of treated flutamide was formed that might have increase the chemical bond strength, stability, solubility, bioavailability, and efficacy, which could be very much useful to design more efficacious pharmaceutical formulations 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; LC-MS; GC-MS

Abbreviations: NSAA: Nonsteroidal Antiandrogen; NCCAM: National Center for Complementary and Alternative Medicine; CAM: Complementary and Alternative Medicine; MS: Mass spectrometry; GC-MS: Gas Chromatography-Mass Spectrometry; LC-MS: Liquid Chromatography Mass Spectrometry; IAR: Isotopic Abundance Ratio; SICART: Sophisticated Instrumentation Centre for Applied Research & Testing; R₊: Retention Time.

Introduction

Flutamide is a nonsteroidal antiandrogen (NSAA) which blocks the action of both endogenous and exogenous testosterone by inhibiting the androgen receptor. Further,

it is a potent inhibitor of testosterone-stimulated prostatic DNA synthesis and it is capable of inhibiting prostatic nuclear uptakeofandrogen [1-3]. Flutamide can be used independently for the treatment or in combination with other medications and radiation treatments [4]. It is used primarily to treat men with prostate cancer [5]. The testosterone hormone aids prostate cancer to grow and spread [6]. It is also used in the treatment of androgen-dependent problems, i.e., acne, seborrhea, hirsutism, scalp hair loss, and hyperandrogenism. It can be used as a constituent of feminizing hormone therapy for transgender women [7]. Overdose of flutamide may cause anorexia, ataxia, piloerection, hypoactivity, slow respiration, and/or lacrimation, emesis, tranquilization, and methemoglobinemia [8].

The physicochemical properties of a pharmaceutical compound in the formulation is very much important and decide the rate of dissolution, absorption, bioavailability, and efficacy in the body [9]. It was observed that the Trivedi Effect®-Consciousness Energy Healing Treatment (Biofield Energy Healing Treatment) has the significant impact on solubility and bioavailability by directly altering the isotope composition, physicochemical and thermal behaviours of various nutraceutical and pharmaceutical compounds [10-12]. The Trivedi Effect[®] is a natural and only technically established phenomenon in which an individual can harness this inherently intelligent energy from the "Universal Energy Field" and transfer it anywhere on the planet via the possible mediation of neutrinos [13]. "Biofield" is the electromagnetic energy field which exists surrounding the living organisms, generated by the continuous movement of the electrically charged particles (ions, cells, etc.) inside the body. It can transmit the electromagnetic energy in the form of bio-photons and this process is called Biofield Energy Healing Treatment [14,15]. Biofield Therapies (Energy Medicine) have been reported with significant outcomes against various disease conditions [16]. The National Center for Complementary and Alternative Medicine (NCCAM) has recognized and accepted Biofield Energy Healing as a Complementary and Alternative Medicine (CAM) health care approach along with other therapies, medicines, and practices such as yoga, Qi Gong, Tai Chi, hypnotherapy, Reiki, etc. [17]. These CAM therapies have been accepted by most of the U.S.A. population with several advantages [18].

The significant outcome of the Trivedi Effect[®]-Consciousness Energy Healing Treatment has been widely reported. It has been reported altering the characteristic properties of the several non-living materials and living object(s), i.e., organic compounds [19,20], metals and ceramic [21,22], polymer [23], crops [24], nutraceuticals [25,26], microbes [27,28], etc.

The stable isotope ratio and its analysis have various

applications in different field of science 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 chromatographymass spectrometry (GC-MS) and liquid chromatographymass spectrometry (LC-MS) in low micromolar concentration with sufficient precision [30,31]. The Trivedi Effect®-Consciousness 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 ¹³C/¹²C or ¹⁵N/¹⁴N or ¹⁷O/¹⁶O) in the Trivedi Effect[®]-Consciousness Energy Healing Treated flutamide compared to the control sample.

Materials and Methods

Chemicals and Reagents

The test sample flutamide was purchased from Tokyo Chemical Industry Co., Ltd., Japan. Whereas, other chemicals and solvents used during the experiments were of analytical grade purchased in India.

Consciousness Energy Healing Treatment Strategies

The flutamide powder sample was divided into two parts. One part of flutamide powder sample was considered as a control sample, which did not receive the Biofield Energy Treatment. Further, the control flutamide was treated with a "sham" healer, did not have any knowledge about the Biofield Energy and its treatment procedure. However, the other part of flutamide was 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 flutamide. After completion of the treatment by the Biofield Energy Healer and "sham" healer, both the samples were kept in sealed conditions and characterized using sophisticated analytical techniques.

Characterization

Liquid Chromatography-Mass Spectrometry (LC-MS) analysis and calculation of iIsotopic 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 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 for the sample preparation. 10 μ L 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 1 mL/min. Chromatographic separation was achieved using gradient condition and the total run time was 10 min. Peaks were monitored at 300 nm using the PDA detector and were 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 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 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 flutamide and $IAR_{Control}$ = isotopic abundance ratio in the control flutamide.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: GC-MS of the control and treated flutamide were

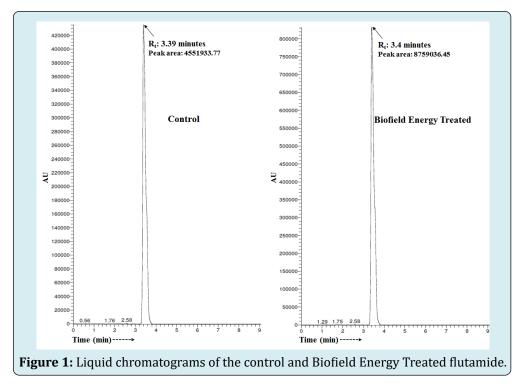
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analyzed with the help of Perkin Elmer Gas chromatograph equipped with a PE-5MS ($30M \times 250$ micros x 0.250 microns) capillary column and coupled to a single quadrupole mass detector was operated with electron impact (EI) ionization (+ve ion mode). The oven temperature was programmed from 80°C (14 min hold) to 250°C (3 min hold) @ 10°C / min (total run time 25 min). The sample was prepared taking 60 mg of the flutamide is in 2 mL 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 (P_{M+1}/P_M) for the control and treated flutamide was calculated.

Results and Discussion

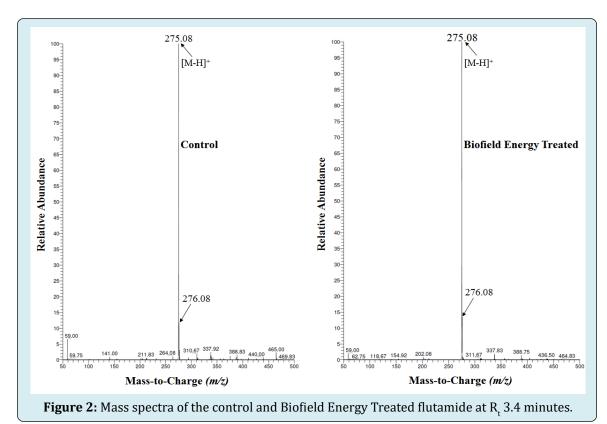
Liquid Chromatography-Mass Spectrometry (LC-MS)

The chromatograms and mass spectra of both the samples of flutamide are shown in Figures 1 and 2, respectively. The control and Biofield Energy Treated flutamide showed the single major chromatographic peak at the retention time (R_1) of 3.39 and 3.4 minutes in the chromatograms (Figure 1). The peak area of the Biofield Energy Treated flutamide was significantly increased by 92.42% compared to the control sample, which indicated that the solubility profile of the Biofield Energy Treated sample was significantly increased after the Biofield Energy Treatment compared to the control sample.



Dahryn Trivedi, et al. Isotopic Abundance Ratio Analysis of the Consciousness Energy Healing Treated Flutamide using LC-MS and GC-MS Spectrometry. J Cancer Oncol 2021, 5(1): 000167.

The molecular mass peak $[M]^+$ of flutamide found to be at m/z 276 in the mass spectrum [35]. In this case, the mass spectra of both the samples (Figure 2) showed the deprotonated molecular ion peak at m/z 275.08 [M-H]⁻ (calculated for C₁₁H₁₀F₃N₂O₃⁻, 175.06) along with other fragmentation peaks (Figure 2).



The LC-MS spectra of both the samples showed the mass of the molecular ion peak at m/z 275.08 [M-H]⁻ (calculated for C₁₁H₁₀F₃N₂O₃⁻, 275.06) with relative intensity of 100%.

The theoretical calculation of $\boldsymbol{P}_{_{M+1}}$ for flutamide was presented as below:

P (¹³C) = [(11 x 1.1%) x 100% (the actual size of the M⁻ peak)] / 100% = 12.1%

P (²H) = [(10 x 0.015%) x 100%] / 100% = 0.15% P (¹⁵N) = [(2 x 0.4%) x 100%] / 100% = 0.8% P (¹⁷O) = [(3 x 0.04%) x 100%] / 100% = 0.12% P_{M+1}, i.e. ¹³C, ²H, ¹⁵N, and ¹⁷O contributions from (C₁₁H₁₀F₃N₂O₃)⁻ to *m*/*z* 276.08 = 13.17%

From the above calculation, it has been found that ¹³C and ¹⁵N have major contributions from $(C_{11}H_{10}F_3N_2O_3)^-$ to m/z 276.08.

Parameter	Control sample	Biofield Energy Treated sample
P _M at <i>m/z</i> 275.08 (%)	100	100
P _{M+1} at <i>m/z</i> 276.08 (%)	8.39	13.41
P _{M+1} /P _M	0.08	0.13
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect		59.48
to the control sample		

Table 1: LC-MS based isotopic abundance analysis results in Biofield Energy Treated flutamide 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.

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 (Table 1). The % change of the isotopic abundance ratio (P_{M+1}/P_M) in the treated flutamide was significantly increased by 59.48% compared with the control sample (Table 1). Therefore, it was concluded that the ¹³C, ²H, ¹⁵N, and ¹⁷O contributions from ($C_{11}H_{10}F_3N_2O_3$)⁻ to m/z 276.08 in the treated flutamide were significantly increased compared to the control sample.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS chromatograms showed the presence of a single chromatographic peak at the retention time of 18.8 minutes in both the samples of flutamide (Figures 3 and 4). The parent molecular ion peak of flutamide at m/z 276 [M]⁺ (calculated for $C_{11}H_{11}F_3N_2O_3^+$, 276.07) in the control sample and Biofield Energy Treated sample, along with the fragment ion peaks near m/z 246, 233, 206, 187, 71, and 43 (Figures 3 and 4) which corresponded to the molecular formula $C_{10}H_9F_3N_2O_2^{2+}$, $C_9H_8F_3N_2O_2^{+}$, $C_7H_5F_3N_2O_2^{2+}$, $C_7H_5F_2N_2O_2^{+}$,

 $C_4H_7O^+$, and $C_3H_7^+$, respectively were proposed (Figure 5).

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 $C_{11}H_{11}F_3N_2O_3^+$, 276.07).

The theoretical calculation of $\boldsymbol{P}_{_{M+1}}$ for flutamide was presented as below:

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

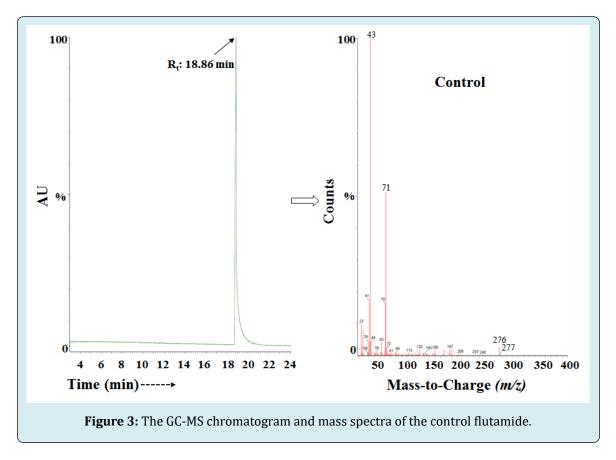
P (²H) = [(11 x 0.015%) x 2.62%] / 100%= 0.004%

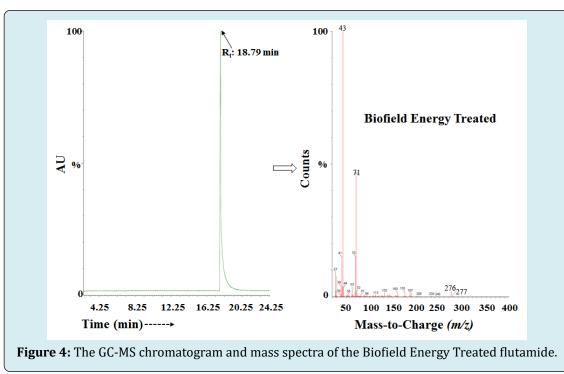
 $P(^{15}N) = [(2 \times 0.4\%) \times 2.62\%] / 100\% = 0.02\%$

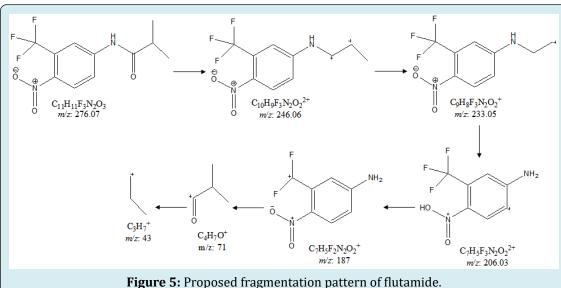
 $P(^{17}O) = [(3 \times 0.04\%) \times 2.62\%] / 100\% = 0.003\%$

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

The calculated isotope abundance (0.37%) was close to the experimental value 0.32% (Table 2). From the above calculation, it has been found that ¹³C and ¹⁵N have major contribution to m/z 277.







Parameter	Control sample	Biofield Energy Treated sample
P _M at <i>m/z</i> 276 (%)	2.62	1.78
P _{M+1} at <i>m/z</i> 277 (%)	0.32	0.24
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		10.39

Table 2: GC-MS based isotopic abundance analysis results of Biofield Energy Treated flutamide 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)⁺]; M: mass of the parent molecule.

The GC-MS based isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated flutamide was significantly increased by 10.39% compared with the control sample (Table 2). Hence, ¹³C, ²H, ¹⁵N, and ¹⁷O 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.

The data confirmed the structure of the sample as flutamide. The peak area and isotopic abundance ratio of P_{M+1}/P_M (²H/¹H or ¹³C/¹²C or ¹⁵N/¹⁴N or ¹⁷O/¹⁶O) in the Biofield Energy Treated flutamide were significantly increased compared to the control sample. According to modern physics, the neutrino is an elementary particle which changes identities. It is 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 capability to 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 the molecular level of the treated flutamide might have altered the neutron to proton ratio in the nucleus. It can be assumed that the alterations in the isotopic abundance could be due to the change in nuclei by the interference of neutrino particles via the Trivedi Effect®-**Consciousness Energy Healing Treatment.**

The improved isotopic abundance ratios ²H/¹H or ¹³C/¹²C or ¹⁵N/¹⁴N or ¹⁷O/¹⁶O would highly influence the atomic bond vibration of treated flutamide. The increased isotopic abundance ratio of the Consciousness Energy Healing Treated flutamide would stronger the chemical bond and increase the stability [36,37]. The Trivedi Effect[®]-Consciousness Energy Healing Treated flutamide might improve the solubility, bioavailability, and therapeutic efficacy compared to the control sample. The new form of Biofield Energy Treated flutamide would be very much useful to design better pharmaceutical formulations 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.

Conclusion

The Trivedi Effect[®]-Consciousness Energy Healing Treatment showed a significant impact on the isotopic abundance ratios and peak area of flutamide. The LC-MS spectra of both the samples at retention time (R_1) 3.4 minutes exhibited the mass of the deprotonated molecular ion peak at m/z 275.08 [M-H]⁻. The peak area of the Biofield Energy Treated flutamide was significantly increased by 92.42% compared to the control sample, which indicated that the solubility profile of the Biofield Energy Treated flutamide was significantly increased compared to the control sample.

The LC-MS based isotopic abundance ratio of P_{M+1}/P_M (²H/¹H or ¹³C/¹²C or ¹⁵N/¹⁴N or ¹⁷O/¹⁶O) in the Biofield Energy Treated flutamide was significantly increased by 59.48% 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 10.39% compared with the control sample. The results indicated that the ¹³C, ²H, ¹⁵N, and ¹⁷O contributions from $(C_{11}H_{11}F_3N_2O_3)^+$ to m/z 277 in the Biofield Energy Treated flutamide were significantly increased compared with the control sample. The isotopic abundance ratio of P_{M+1}/P_M (²H/¹H or ¹³C/¹²C or ¹⁵N/¹⁴N or ¹⁷O/¹⁶O) in the Biofield Energy Treated flutamide was significantly increased compared to the control sample. The changes in the peak area and isotopic abundance could be due to changes in nuclei possibly through the interference of neutrino via the Trivedi Effect®-Consciousness Energy Healing Treatment. The new form of treated flutamide was formed that might have increase the chemical bond strength, stability, solubility, bioavailability, and efficacy, which could be very much useful to design more efficacious pharmaceutical formulations 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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