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Analysis of Isotopic Abundance Ratio of Consciousness Energy Healing Treated Cholecalciferol Using LC-MS and GC-MS Spectrometry

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Abstract

Vitamin D_3 (cholecalciferol) is a fat-soluble vitamin, which widely used for the prevention and treatment of vitamin D deficiency disorders. This study was designed to investigate the impact of The Trivedi Effect®-Biofield Energy Healing Treatment on the structural properties and the isotopic abundance ratio of cholecalciferol using LC-MS and GC-MS spectroscopy. Cholecalciferol sample was divided into two parts, one part of cholecalciferol was considered as a control sample (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 the treated sample. The LC-MS spectra of both the samples at retention time (R_t) ~21.5 minutes exhibited the mass of the molecular ion peak at *m/z* 385.25 (calcd for C27H45O+, 385.35). The LC-MS based isotopic abundance ratio of PM+1/PM in the treated cholecalciferol was increased by 1.9% compared with the control sample. The control and treated cholecalciferol showed the presence of the chromatographic peak at the retention time of ~22.7min in the GC-MS chromatograms. The GC-MS based isotopic abundance ratio of PM+1/PM and PM+2/PM in the treated cholecalciferol was significantly increased by 1.99% and 15.69%, respectively compared with the control sample. Hence, 13C, 2H, 17O and 18O contributions from C27H44O+ to *m/z* 386 and 387 in the Biofield Energy Treated sample were significantly increased compared with the control sample. The isotopic abundance ratios of PM+1/PM (2H/1H or 13C/12C or 17O/16O) and PM+2/PM (18O/16O) in the Biofield Energy Treated cholecalciferol were significantly increased 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 cholecalciferol would be better designing novel pharmaceutical formulations that might offer better therapeutic response against rickets, osteoporosis, arthritis, multiple sclerosis, cancer, diabetes mellitus, mental disorders, cardiovascular diseases, infections, cognitive impairment in older adults, Parkinson's and Alzheimer's diseases, dementia, glucose intolerance, multiple sclerosis, etc.

Keywords: Cholecalciferol; The trivedi effect®; Biofield energy; Consciousness energy healing treatment; LC-MS; GC-MS

Introduction

Vitamin D_3 (cholecalciferol) is majorly found in foods and consume as a dietary supplement to overcome the vitamin D_3 deficiency and associated disease [1]. Vitamin D regulates various functions of muscles, brain, lungs, liver, kidneys, heart, pancreas, large and small intestines, and immune system. Vitamin D receptors are ubiquitously found in most of the body parts, i.e., brain, heart, lungs, kidney, liver, pancreas, large and small intestines, muscles, reproductive organs, nervous system, etc. Vitamin D receptor response elements with hundreds of genes directly or indirectly influence cell-to-cell communication, normal cell growth, cell cycling and proliferation, cell differentiation, maintenance of calcium and phosphorus balance, hormonal balance, neurotransmission, skin health, immune, and cardiovascular functions [1-3]. An insufficient dietary intake or those who fail to produce enough vitamin D_3 in their skin from its precursor, 7-dehydrocholesterol, in response to exposure to ultraviolet light, intestinal malabsorption or chronic liver disease, familial hypophosphatemia, and for the hypocalcaemia that is associated with hypoparathyroidism leads to the vitamin D deficiency [2]. Vitamin D deficiency responsible for several diseases,

e.g., rickets, osteoporosis, arthritis, multiple sclerosis, cancer, diabetes mellitus, mental disorders, cardiovascular diseases, infections, cognitive impairment in older adults, Parkinson's and Alzheimer's diseases, dementia, glucose intolerance, multiple sclerosis, etc. [3,4-6]. As per the literature data, in the USA, 15µg/d (600IU per day) is required for all individuals (males, female, pregnant/lactating women) between the ages of 1 and 70 years old [7]. High dose of vitamin D supplementation may cause the toxicity like hypercalcemia, polyuria, polydipsia, weakness, mental retardation, and insomnia [8]. The stability of vitamin D is more concerned as it is more sensitive to heat and light [9,10]. Vitamin D_3 bioavailability directly affected by various factors such as dietary fiber, genetic factors, and status of vitamin D_3 [11].

In this scenario, several studies revealed that the bioavailability profile of several pharmaceutical compounds, i.e., 25-hydroxyvitamin D_3 [25(OH)D_3], resveratrol, berberine, etc. are significantly altered by means of The Trivedi Effect®-Energy of Consciousness Healing Treatment [12-14]. 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 [15]. The Biofield Energy Healers can harness the energy from the "Universal Energy Field" and can transmit into any living or non-living object(s) [16]. Further, the object(s) respond to the useful way is known as the Biofield Energy Healing Treatment. There are several Biofield based Energy Healing Therapies that are used nowadays against various disease conditions [17,18]. Biofield Energy Healing therapy has been recognized worldwide as a Complementary and Alternative Medicine (CAM) health care approach by National Center of Complementary and Integrative Health (NCCIH) with other therapies, medicines and practices such as Ayurvedic medicine, traditional Chinese herbs and medicines, aromatherapy, yoga, Qi Gong, Tai Chi, chiropractic/osteopathic manipulation, meditation, homeopathy, acupressure, acupuncture, healing touch, hypnotherapy, movement therapy, naturopathy, Reiki, cranial sacral therapy, etc. [19]. These therapies have been accepted by most of the U.S.A. population with several advantages [20]. The Trivedi Effect®- Energy of Consciousness Healing Treatment has the grate potential to transform the characteristic properties of organic compounds [21,22], metals and ceramic [23,24], nutraceuticals and pharmaceuticals [25,26], microorganisms [27,28], and improve the overall productivity of crops [29,30], alteration of the isotopic abundance ratio [31,32]. There are wide applications of study on the natural stable isotope ratio analysis in several fields of sciences to understand the isotope effects resulting from the alterations of the isotopic composition [33-35]. Gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS), are widely used for the analysis of isotope ratio with sufficient precision [34]. 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., cholecalciferol. Hence, the LC-MS and GC-MS were used in this study to characterize the structural properties and to evaluate the isotopic abundance ratio of PM+1/PM ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) and PM+2/PM ($^{18}O/^{16}O$) in The Trivedi Effect®-Consciousness Energy Healing Treated cholecalciferol compared to the control sample.

Materials and Methods

Chemicals and reagents

Cholecalciferol (> 98%) was purchased from Sigma-Aldrich, India. All other chemicals used during the experiments were of analytical grade available in India.

Consciousness energy healing treatment strategies

The cholecalciferol powder was the test sample divided into two parts. One part of cholecalciferol powder sample was considered as a control sample (no Biofield Energy Treatment was provided). However, the other part of cholecalciferol 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 (Biofield Energy Treated) cholecalciferol 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 cholecalciferol 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 cholecalciferol 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-250mm X ID 4.6mm X 5micron), maintained at 25°C. The diluent used for the sample preparation was methanol. 20µL of cholecalciferol solution was injected, and the analyte was eluted using acetonitrile + methanol (80:20) pumped at a constant flow rate of 1.5mL/min. Chromatographic separation was achieved using gradient condition and the total run time was 30min. Peaks were monitored at 300nm using the PDA detector. Mass spectrometric analysis was performed under atmospheric pressure chemical ionization (APCI) +ve ion mode. The total ion chromatogram, peak area% and mass spectrum of the individual peak which was appeared in LC along with the full

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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 (H, C, and O) 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 [35-38]. The LC-MS based isotopic abundance ratios (PM+1/PM) for the control and Biofield Energy Treated cholecalciferol 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 cholecalciferol were analyzed with the help of Perkin Elmer Gas chromatograph equipped with a PE-5MS (30M x 250micros x 0.250microns) 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 150°C (5min hold) to 280°C (17 min hold) @ 10°C /min (total run time 35min). The sample was prepared taking 50mg of the cholecalciferol in 2.5 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 (PM+1/PM and PM+2/PM) for the control and Biofield Energy Treated cholecalciferol 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 of the control and Biofield Energy Treated cholecalciferol are shown in (Figure 1). A single major chromatographic peak of the control cholecalciferol was observed at retention time (R_t) of 21.5 minutes, whereas the peak was observed at 21.8 minutes in the Biofield Energy Treated sample (Figure 1). This indicated that the polarity of both the samples was very close to each other. Similarly, the mass spectra of both the samples of cholecalciferol are shown in (Figure 2). The control and Biofield Energy Treated cholecalciferol were ionized via ESI in +ve ion mode. The mass spectra of the control and Biofield Energy Treated samples at R_t of ~21.5 minutes exhibited the presence of the molecular ion of cholecalciferol ($C_{27}H_{45}O+$) adduct with hydrogen ion (Figure 2) at *m/z* 385.25 (calcd for $C_{27}H_{45}O+$, 385.35). The lower *m/z* showed the presence of the [M-OH] + ion mass peak at *m/z* 367.33 (calcd for $C_{27}H_{43}+$, 367.3) in the control and Biofield Energy Treated cholecalciferol (Figure 2 & 3). The experimental data were well correlated with the literature reported data [39]. The control and Biofield Energy Treated samples showed the mass of a molecular ion at *m/z* 385.25 (calcd for $C_{27}H_{45}O+$, 385.35) with 100% relative abundance in the spectra. The theoretical calculation of isotopic peak PM+1 for the protonated cholecalciferol presented as below:

P (^{13}C) = [(27 x 1.1%) x 100% (the actual size of the M+ peak)] / 100% = 29.7%

P (^{2}H) = [(45 x 0.015%) x 100%] / 100%= 0.675%

P (^{17}O) = [(1 x 0.04%) x 100%] / 100% = 0.04%

PM+1 i. e.^{13}C, ^{2}H, and ^{17}O contributions from $C_{27}H_{45}O+$ to *m/z* 386.25 = 30.42%

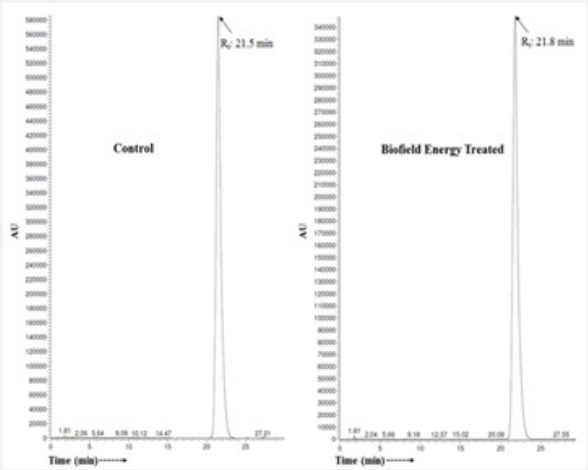

Figure 1: Liquid chromatograms of the control and Biofield Energy Treated cholecalciferol.

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The calculated isotopic abundance of PM+1 value 30.42% was higher to the experimental value (27.3%) (Table 1). From the above calculation, it has been found that 13C has the major contribution to *m/z* 386.25. The LC-MS based isotopic abundance ratio analysis PM and PM+1 for cholecalciferol near *m/z* 385.25 and 386.25, 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 isotopic abundance ratio of PM+1/PM ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) in Consciousness Energy Healing Treated cholecalciferol was increased by 1.9% compared to the control sample (Table 1). Thus, the ^{13}C, ^{2}H, and ^{17}O contributions from $C_{27}H_{45}O+$ to *m/z* 386.25 in the Biofield Energy Treated sample was increased compared to the control sample.

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

Parameter	Control Sample	Biofield Energy Treated Sample
PM at *m/z* 385.25 (%)	100	100
PM+1 at *m/z* 386.25 (%)	27.3	27.82
PM+1/PM	0.27	0.28
% Change of isotopic abundance ratio (PM+1/PM) with respect to the control sample	-	1.9

PM: the relative peak intensity of the parent molecular ion [M+]; PM+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

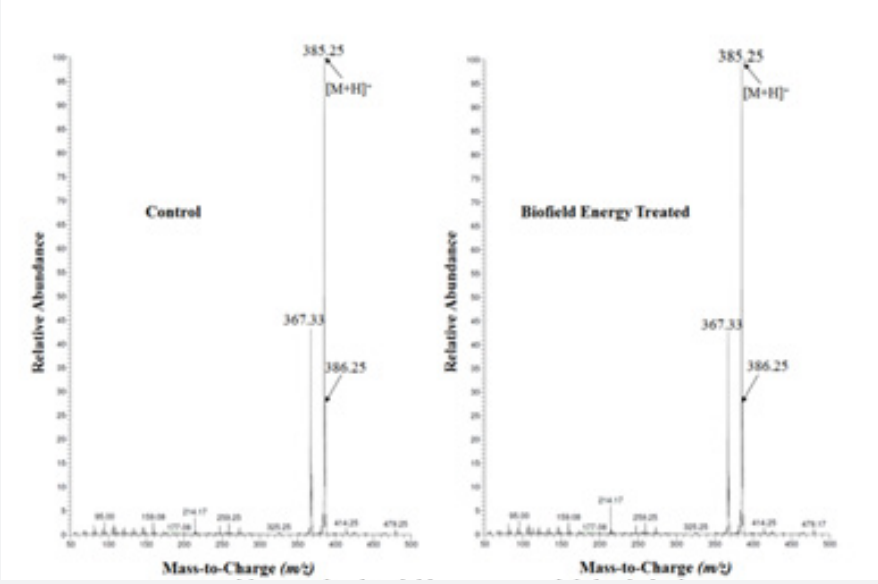

Figure 2: Mass spectra of the control and Biofield Energy Treated cholecalciferol at R_t ~21.5 minutes.

The control and Biofield Energy Treated cholecalciferol showed two major independent peaks in the GC-MS chromatograms (Figure 3). The R_t of the control sample was at 22.02 and 22.69 minutes, whereas Biofield Energy Treated sample showed at 22.06 and 22.75 minutes, which indicated that retention times of both the sample were very close. The results indicated that the polarity of the Biofield Energy Treated cholecalciferol remained close compared to the control sample. The chromatograms of both the control and Biofield Energy Treated showed two peaks might be due to the cis and trans isomers of cholecalciferol. The GC-MS spectra of the control and Biofield Energy Treated samples at R_t of 22.7 minutes exhibited the presence of the molecular ion peak of cholecalciferol ($C_{27}H_{44}O+$) (Figure 4) at *m/z* 385 (calcd for $C_{27}H_{44}O+$, 384.34). The low molecular mass fragmentation peak at *m/z* 367, and 352 for $C_{27}H_{43}+$ and $C_{26}H_{40}+$, respectively in both the control and Biofield Energy Treated cholecalciferol (Figure 4). The mass fragmentation pattern of the Biofield Energy Treated cholecalciferol was similar to that of the control sample. But the mass peak intensities of the Biofield Energy Treated cholecalciferol were altered compared to the control sample. The isotopic abundance ratio depends upon the mass peak intensities of the particular compounds, which was well supported by the LC-

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MS based isotopic abundance ratio analysis. The GC-MS spectra of both the control and Biofield Energy Treated cholecalciferol showed the mass of the molecular ion peak [M]+ at *m/z* 385 (calcd for $C_{27}H_{44}O$+, 384.34). The theoretical calculation of PM+1 and PM+2 for cholecalciferol was presented as below:

P (^{13}C) = [(27 x 1.1%) x 9.06% (the actual size of the M+ peak)] / 100% = 2.69%

P (^{2}H) = [(44 x 0.015%) x 9.06%] / 100%= 0.06%

P (^{17}O) = [(1 x 0.04%) x 9.06%] / 100% = 0.004%

PM+1 i. e.^{13}C, ^{2}H, and ^{17}O contributions from C27H45O+ to *m/z* 386 = 2.75%

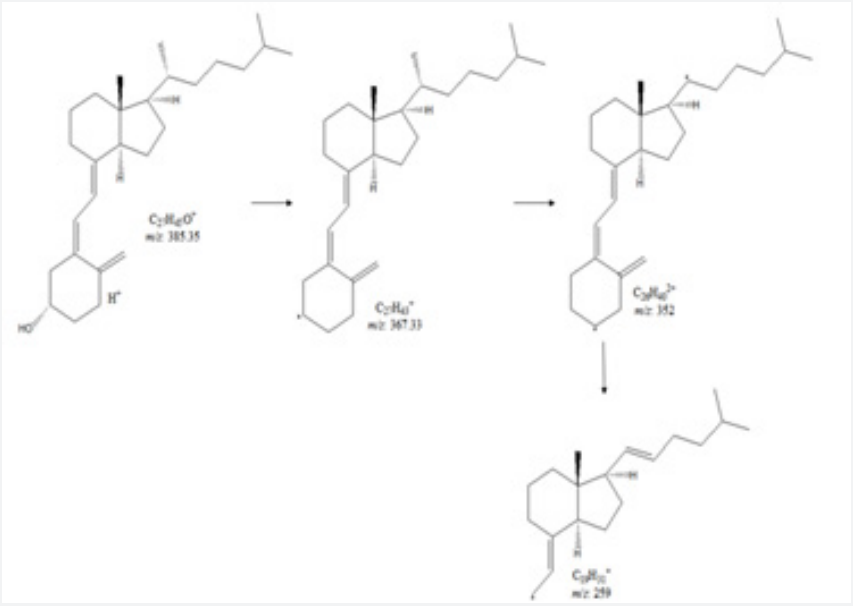

Figure 3: Proposed fragmentation pattern of cholecalciferol.

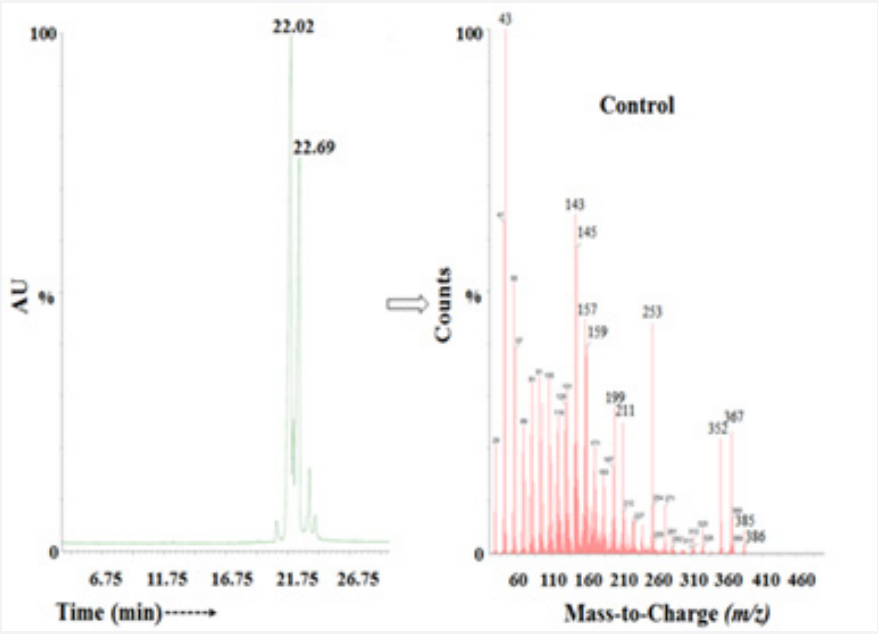

Figure 4: The GC-MS chromatogram and mass spectra of the control cholecalciferol.

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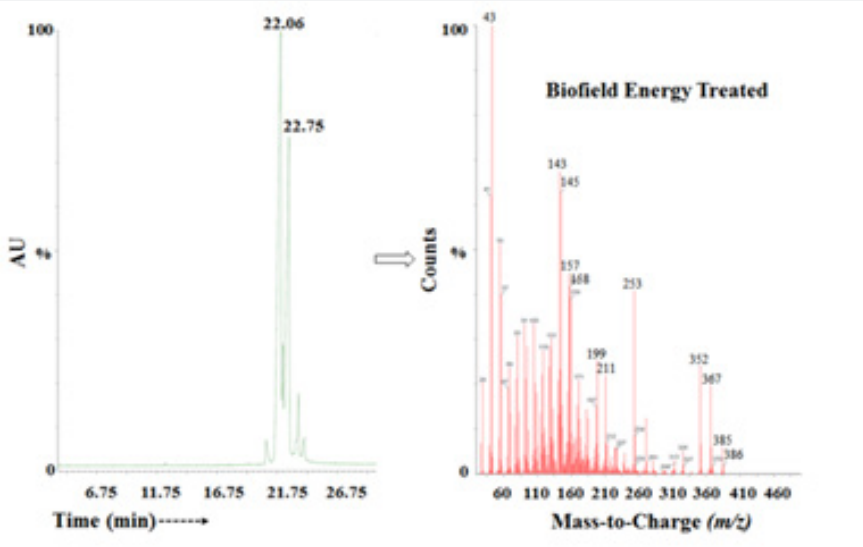

Figure 5: The GC-MS chromatogram and mass spectra of the Biofield Energy Treated cholecalciferol.

Similarly, the theoretical calculation of isotopic peak PM+2 for the protonated cholecalciferol was presented below:

P (^{18}O) = [(1 x 0.20%) x 9.06%] / 100% = 0.02%

PM+2 of ^{18}O contribution from $C_{27}H_{45}O+$ to *m/z* 387 = 0.02%

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

Parameter	Control Sample	Biofield Energy Treated Sample
PM at *m/z* 385 (%)	9.06	6.01
PM+1 at *m/z* 386 (%)	2.69	1.82
PM+1/PM	0.3	0.3
% Change of isotopic abundance ratio (PM+1/PM) with respect to the control sample		1.99
PM+2 at *m/z* 387 (%)	0.43	0.33
PM+2/PM	0.05	0.05
% Change of isotopic abundance ratio (PM+2/PM) with respect to the control sample		15.69

PM: the relative peak intensity of the parent molecular ion [M+]; PM+1: the relative peak intensity of the isotopic molecular ion [(M+1) +]; PM+2: the relative peak intensity of the isotopic molecular ion [(M+2) +]; M: mass of the parent molecule.

The calculated isotopic abundance of PM+1 and PM+2 values were close to the calculated value (Table 2). From the above calculation, it has been found that ^{13}C and ^{18}O have major. The GC-MS based isotopic abundance ratio analysis of the Biofield Energy Treated samples were calculated compared to the control sample. PM, PM+1, and PM+2 for cholecalciferol near *m/z* 385, 386, and 387, 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 PM+1/PM and PM+2/PM in the Biofield Energy Treated cholecalciferol was increased by 1.99% and 15.69%, respectively compared with the control sample (Table 2). Hence, it can be concluded that ^{13}C, ^{2}H, ^{17}O and ^{18}O contributions from $C_{27}H_{44}O+$ to *m/z* 386 and 387 in the Biofield Energy Treated sample were significantly increased compared with the control sample. LC-MS and GC-MS study confirmed the sample as cholecalciferol. The isotopic abundance ratios of PM+1/PM ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) and PM+2/PM ($^{18}O/^{16}O$) in the Biofield Energy Treated cholecalciferol were significantly increased compared to the control sample. According to the physics, the neutrinos change identities which are only possible

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if the neutrinos possess mass and have the ability to 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 [15,34,35]. The altered isotopic composition in molecular level of The Trivedi Effect®-Consciousness Energy Healing Treated cholecalciferol 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 Biofield Energy Treated cholecalciferol would be more suitable for the prevention and treatment of various diseases such as vitamin D deficiency, rickets, osteoporosis, arthritis, diabetes mellitus, multiple sclerosis, cancer, cardiovascular diseases, inflammations, infections, mental disorders, stress, aging, glucose intolerance, Parkinson's and Alzheimer's diseases, dementia, cognitive impairment in older adults, etc.

Conclusion

The Trivedi Effect®-Consciousness Energy Healing Treatment (Biofield Energy Treatment) showed the significant impact on the isotopic abundance ratios of cholecalciferol. The LC-MS spectra of the control and Biofield Energy Treated the samples at retention time (R_t) ~21.5 minutes exhibited the mass of the molecular ion peak at *m/z* 385.25 (calcd for $C_{27}H_{45}O+$, 385.35). The LC-MS based isotopic abundance ratio of PM+1/PM in the Biofield Energy Treated cholecalciferol was increased by 1.9% compared with the control sample. The control and Biofield Energy Treated cholecalciferol showed the presence of the chromatographic peak at the retention time of ~22.7min in the GC-MS chromatograms. The GC-MS based isotopic abundance ratio of PM+1/PM and PM+2/PM in the Biofield Energy Treated cholecalciferol was significantly increased by 1.99% and 15.69%, respectively compared with the control sample. Hence, ^{13}C, ^{2}H, ^{17}O and ^{18}O contributions from $C_{27}H_{44}O+$ to *m/z* 386 and 387 in the Biofield Energy Treated sample were significantly increased compared with the control sample. The isotopic abundance ratios of PM+1/PM ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) and PM+2/PM ($^{18}O/^{16}O$) in the Biofield Energy Treated cholecalciferol were significantly increased 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 cholecalciferol would be better designing novel pharmaceutical formulations that might offer better therapeutic response against rickets, osteoporosis, arthritis, multiple sclerosis, cancer, diabetes mellitus, mental disorders, cardiovascular diseases, infections, cognitive impairment in older adults, Parkinson's and Alzheimer's diseases, dementia, glucose intolerance, multiple sclerosis, etc.

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