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Biofield Energy Treatment and its Impact on the Isotopic Abundance of Cholecalciferol (Vitamin D_3)

Mahendra Kumar Trivedi[1], Alice Branton[1], Dahryn Trivedi[1] and Snehasis Jana[2*]

[1]*Trivedi Global, Inc., Henderson, USA.*

[*2]*Trivedi Science Research Laboratory Pvt. Ltd., Thane (W), Maharashtra, India.*

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ABSTRACT

Cholecalciferol is a fat-soluble vitamin (Vitamin D_3). It is used for the prevention and treatment of rickets, osteoporosis, arthritis, Parkinson's and Alzheimer's diseases, etc. In this study, the LC-MS and GC-MS analytical methods were used to characterize and evaluate the isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M of the Trivedi Effect®-Consciousness Energy Healing Treated cholecalciferol compared to the control sample. The test sample cholecalciferol was divided into control and treated parts. Only, the treated cholecalciferol was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a renowned Biofield Energy Healer, Mahendra Kumar Trivedi. The LC-MS spectra of both the samples at retention time (R_t) ~22 min exhibited the mass of the molecular ion peak at *m/z* 385.25 (called for $C_{27}H_{45}O^+$, 385.35). The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated cholecalciferol was increased by 2.93% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of P_{M+1}/P_M and P_{M+2}/P_M in the treated cholecalciferol was increased by 1.53% and 3.22%, 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 treated cholecalciferol 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 $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) in the treated cholecalciferol were significantly increased compared to the control sample. The increased isotopic composition of the Trivedi Effect®-Consciousness Energy Healing Treated cholecalciferol might have altered the neutron to proton ratio in the nucleus *via* the possible mediation of neutrino. The increased isotopic abundance ratio of the treated cholecalciferol may increase the intra-atomic bond strength, stability, and shelf-life. The new form of cholecalciferol would be more efficacious pharmaceutical/nutraceutical formulations for the prevention and treatment of various diseases such as vitamin D deficiency, rickets, osteoporosis, arthritis, multiple sclerosis, cancer, mental disorders, diabetes mellitus, cardiovascular diseases, hypertension, infections, influenza, cognitive impairment in older adults, Parkinson's and Alzheimer's diseases, dementia, autoimmune disease, glucose intolerance, multiple sclerosis, etc.

Keywords: Consciousness Energy Healing Treatment, The Trivedi Effect®, Cholecalciferol, Isotopic abundance

INTRODUCTION

Cholecalciferol is a fat-soluble vitamin (Vitamin D_3). Humans synthesize vitamin D_3 in the skin on exposure to UV light is biologically inert [2]. It gets activated by hydroxylation in the liver and kidney to 1,25-dihydroxycholecalciferol (calcitriol). Vitamin D binding proteins (DBPs) are found in most of the body parts, *i.e.,* heart, lungs, kidney, liver, pancreas, intestines, muscles, gonads, brain, nervous system, etc. Vitamin D regulates the functions of the muscles, lungs, liver, kidneys, heart, brain, pancreas, intestines, and immune system. 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, neurotransmission, hormonal balance, increase calcium and phosphorus absorption, skin health, immune, and cardiovascular functions [3-5].

Corresponding author: Snehasis Jana, Trivedi Science Research Laboratory Pvt. Ltd., Thane (W), Maharashtra, India, Tel: +91- 022-25811234; E-mail: publication@trivedisrl.com

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people are equally high risk for. The cause of vitamin D deficiency is inadequate exposure to sunlight, high body mass index (>30 kg/m^2), patient taking anticonvulsants and anti-AIDS/HIV medications, fat malabsorption syndromes and bariatric patients, chronic liver disease, hypophosphatemia, and hypocalcemia [2,4]. Vitamin D deficiency may cause abnormalities in calcium, phosphorus, and bone metabolism, rickets, osteoporosis, arthritis, cancer, diabetes mellitus, mental disorders, multiple sclerosis, hypertension, cardiovascular diseases, infections, influenza, cognitive impairment, Parkinson's disease, Alzheimer's diseases, autoimmune disease, dementia, glucose intolerance, multiple sclerosis, etc. [1,2,5-8]. The daily requirements of vitamin D for all individuals between the ages of 1 and 70 years old is 15 µg/d (600 IU per day) [1-4]. High dose of supplementation may cause 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].

Bioavailability of vitamin D_3 directly affected by various factors such as dietary fiber, genetic factors, age, skin color, and status of vitamin D_3 [11]. The Trivedi Effect®-Consciousness Energy Healing Treatment is a form of Energy Therapy that improved the bioavailability profile of several nutraceutical and pharmaceutical compounds [12-14]. The Trivedi Effect® is a scientifically proven Energy Therapy in which a Biofield Energy Healer can harness this inherently intelligent energy from the Universe and transfer it anywhere on the planet through the possible mediation of neutrinos [15]. The Biofield based Energy Healing Therapies nowadays has been used against various disease conditions [17,18]. The Energy Therapy has been recognized worldwide as a Complementary and Alternative Medicine (CAM) health care approach by the National Center of Complementary and Integrative Health (NCCIH) with other therapies, medicines and practices such as Ayurveda, homeopathy, yoga, aromatherapy, Qi Gong, Tai Chi, traditional Chinese herb and medicines, chiropractic/osteopathic manipulation, hypnotherapy, acupressure, acupuncture, movement therapy, naturopathy, Reiki, etc. [19]. The CAM therapies have been well accepted by most of the U.S.A. people [20]. The Trivedi Effect®-Consciousness Energy Healing Treatment also potentially transforms the characteristic properties of polymers, metals, ceramics, pharmaceuticals, nutraceuticals, organic compounds, microbes, cancer cells [21-30], and improve the yield of crops [31,32].

The study of natural stable isotope analysis has a lot of applications in several fields of sciences to understand the isotope effects [33-35]. Liquid chromatography-mass spectrometry (LC-MS) and Gas chromatography-mass spectrometry (GC-MS) is widely used for the analysis of isotope ratio with sufficient precision [34]. In this study, the LC-MS and GC-MS analytical methods were used to characterize the structural properties and evaluated the isotopic abundance ratios of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) of the Trivedi Effect®-Consciousness Energy Healing Treated cholecalciferol compared to the control sample.

MATERIALS & METHODS

Chemicals and reagents

The test sample cholecalciferol (≥ 98%, HPLC) was procured from Sigma-Aldrich, India. Similarly, the other chemicals, i.e., acetonitrile and methanol were purchased from Merck, India.

Consciousness energy healing treatment strategies

The test sample cholecalciferol powder was divided into two parts, i.e., control and treated parts. The control sample did not receive the Trivedi Effect®-Consciousness Energy Healing Treatment. But the control sample was treated with a "sham" healer who did not have any understanding of the Consciousness Energy Healing Treatment. However, the treated part of cholecalciferol was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 min by the renowned Biofield Energy Healer, Mahendra Kumar Trivedi, USA. Finally, both the 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 cholecalciferol 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 MS. 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 used as a diluent for the sample preparation. 20 µL of sample solution was injected, and the analyte was eluted using acetonitrile + methanol (80:20) pumped at a constant flow rate of 1.5 mL/min with the total run time of 30 min. Peaks were monitored at 300 nm using the PDA detector. The mass spectrometric analysis was performed under atmospheric pressure chemical ionization (APCI) +ve ion mode.

The values of the natural isotopic abundance (H, C, and O) of the common elements are obtained from the literature [35-38]. The % change in the isotopic abundance ratio (P_{M+1}/P_M) for the Biofield Energy Treated cholecalciferol compared to the control sample was calculated using the equation (1).

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

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

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis: GC-MS of cholecalciferol was 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. The oven temperature was maintained from 150°C (5 min hold) to 280°C (17 min hold) @ 10°C /min with a total run time of 35 min. The sample was prepared taking 50 mg of the cholecalciferol in 2.5 ml methanol as a diluent. The % change in the isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) for the Biofield Energy Treated cholecalciferol compared to the control sample was calculated using the equation (1).

RESULTS & DISCUSSION

Liquid Chromatography-Mass Spectrometry (LC-MS)

A single major chromatographic peak of the control and treated cholecalciferol was observed at retention time (R_t) of 22.32 and 22.77 min, respectively **(Figure 1)**. This indicated that the polarity of both the samples was very close to each other.

The mass spectra of both the samples corresponding to the R_t ~22 min exhibited the presence of the molecular ion of cholecalciferol ($C_{27}H_{45}O^+$) adduct with hydrogen ion at *m/z* 385.25 (calcd for $C_{27}H_{45}O^+$, 385.35) along with the lower mass peak [M-OH] $^+$at *m/z* 367.33 (calcd for $C_{27}H_{43}^+$, 367.3) **(Figures 2 and 3)**. The experimental data were well matched with the literature data [39].

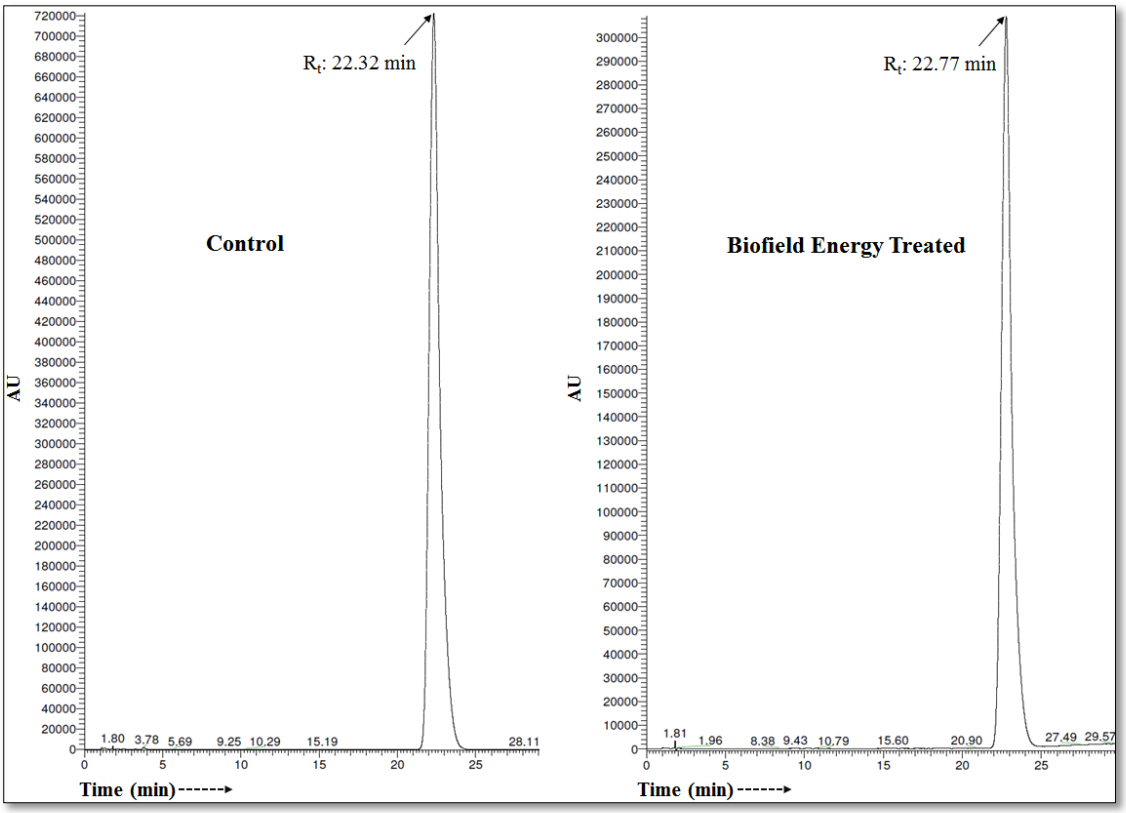

Figure 1. Liquid chromatograms of the control and Biofield Energy Treated cholecalciferol.

The cholecalciferol samples showed the mass of a molecular ion at *m/z* 385.25 with 100% relative abundance in the spectra. The theoretical calculation of isotopic peak P_{M+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%

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

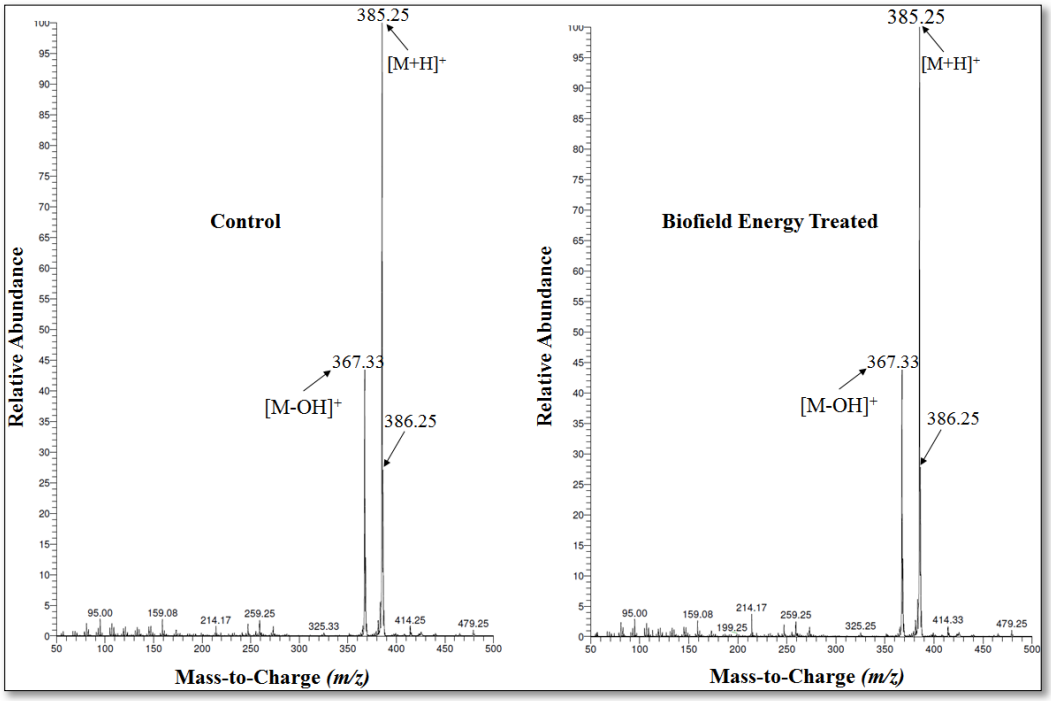

Figure 2. Mass spectra of the control and Biofield Energy Treated cholecalciferol.

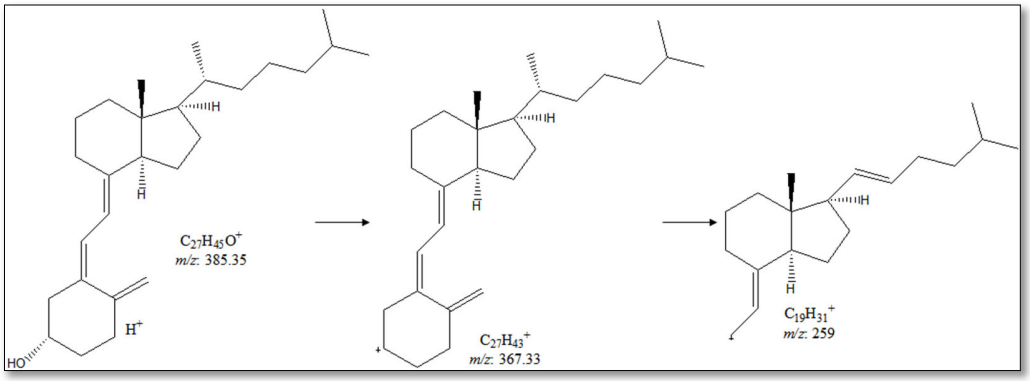

Figure 3. Proposed fragmentation pattern of cholecalciferol.

The calculated isotopic abundance of P_{M+1} value 30.42% was higher to the experimental value (27.76%) **(Table 1)**. From the above calculation, it has been found that ^{13}C has the major contribution to *m/z* 386.25.

The isotopic abundance ratio analysis P_M and P_{M+1} for cholecalciferol near *m/z* 385.25 and 386.25, respectively, 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 P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) in treated cholecalciferol was increased by 2.93% compared to the control sample (Table 1). The result indicated that the ^{13}C, ^2H, and ^{17}O contributions from $C_{27}H_{45}O^+$ to *m/z* 386.25 in the treated cholecalciferol was increased compared to the control sample.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The cholecalciferol samples showed two major independent peaks in the chromatograms **(Figures 4 and 5)**. The R_t of the control cholecalciferol was at 21.9 and 22.56 min, whereas 21.95 and 22.64 min is for the treated cholecalciferol, which indicated that the polarity of both the sample was very close **(Figures 4 and 5)**. The two prominent peaks in the chromatogram might be due to the *cis* and *trans* isomers of cholecalciferol in the sample [40,41].

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
P_M at *m/z* 385.25 (%)	100	100
P_{M+1} at *m/z* 386.25 (%)	26.97	27.76
P_{M+1}/P_M	0.27	0.28
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		2.93

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 molecular ion peak with respect to the R_t of 22 min exhibited the presence of cholecalciferol ($C_{27}H_{44}O^+$) at *m/z* 385 (calcd for $C_{27}H_{44}O^+$, 384.34). The low molecular mass fragmentation peak at *m/z* 367 $[C_{27}H_{43}]^+$ and 352 $[C_{26}H_{40}]^+$ were also observed in both the spectra **(Figures 4 and 5)**. The mass peak intensities of the Biofield Energy Treated cholecalciferol were altered compared to the control sample. The GC-MS spectra of both the cholecalciferol showed the mass of the molecular ion peak $[M]^+$ at *m/z* 385. The theoretical calculation of P_{M+1} and P_{M+2} for cholecalciferol was presented as below:

P (^{13}C) = [(27 x 1.1%) x 7.32% (the actual size of the M^+ peak)] / 100% = 2.17%
P (^{2}H) = [(44 x 0.015%) x 7.32%] / 100%= 0.05%
P (^{17}O) = [(1 x 0.04%) x 7.32%] / 100% = 0.003%
P_{M+1} *i. e.* ^{13}C, ^{2}H, and ^{17}O contributions from $C_{27}H_{45}O^+$ to *m/z* 386 = 2.22%

Similarly, the theoretical calculation of isotopic peak P_{M+2} for the protonated cholecalciferol was presented below:

P (^{18}O) = [(1 x 0.20%) x 7.32%] / 100% = 0.015%
P_{M+2} of ^{18}O contribution from $C_{27}H_{45}O^+$ to *m/z* 387 = 0.015%

The calculated isotopic abundance of P_{M+1} and P_{M+2} values were very close to the calculated value **(Table 2)**. From the above calculation, it has been found that ^{13}C and ^{18}O have major contribution to *m/z* 386 and 387 of cholecalciferol.
The GC-MS based isotopic abundance ratio analysis of the treated sample was calculated compared to the control sample. P_M, P_{M+1}, and P_{M+2} for cholecalciferol near *m/z* 385 $[M^+]$, 386 $[(M+1)^+]$, and 387 $[(M+2)^+]$, respectively of both the samples, which were obtained from the mass spectra. The isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in the treated sample was increased by 1.53% and 3.22%, respectively compared to the control sample **(Table 2)**. Therefore, the ^{13}C, ^{2}H, ^{17}O and ^{18}O contributions from $C_{27}H_{44}O^+$ to *m/z* 386 and 387 in the treated cholecalciferol were significantly increased compared with the control sample.

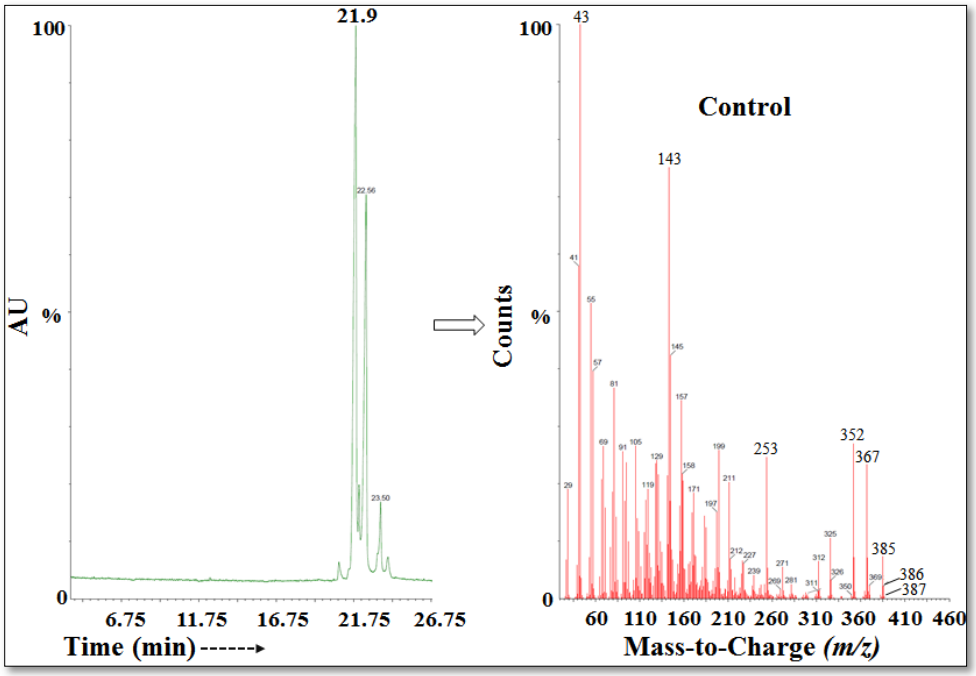

Figure 4. The GC-MS chromatogram and mass spectra of the control cholecalciferol.

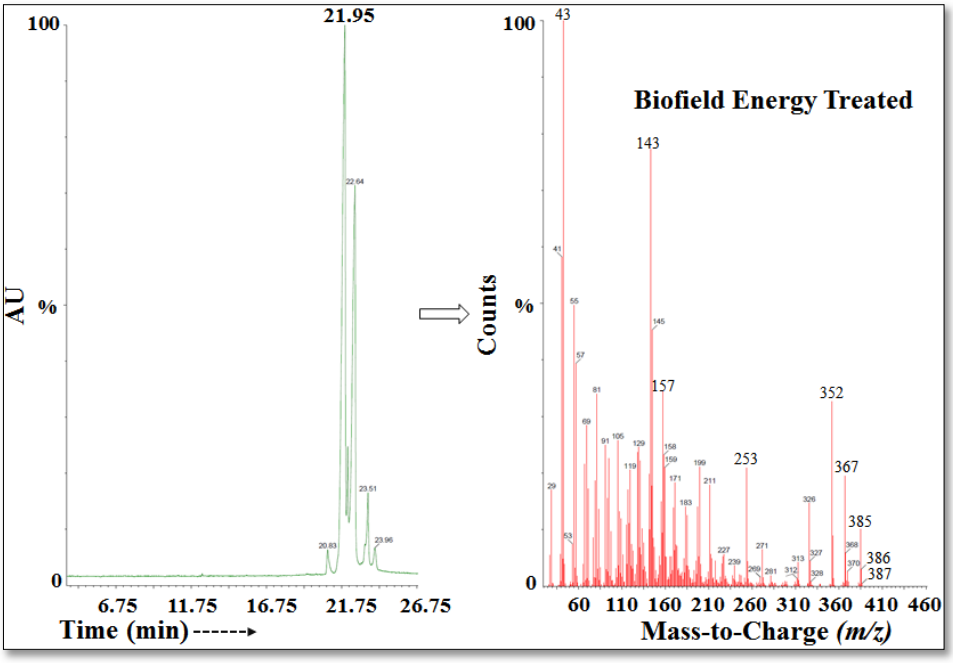

Figure 5. The GC-MS chromatogram and mass spectra of the Biofield Energy Treated cholecalciferol.

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
P_M at *m/z* 385 (%)	7.32	10.10
P_{M+1} at *m/z* 386 (%)	2.12	2.97
P_{M+1}/P_M	0.29	0.29
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control sample		1.53
P_{M+2} at *m/z* 387 (%)	0.33	0.47
P_{M+2}/P_M	0.05	0.05
% Change of isotopic abundance ratio (P_{M+2}/P_M) compared to the control sample		3.22

P_M: the relative peak intensity of the parent molecular ion [M^+]; P_{M+1}: the relative peak intensity of the isotopic molecular ion [$(M+1)^+$]; 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 cholecalciferol. The isotopic abundance ratios of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) in the treated cholecalciferol were increased compared to the control sample. The increased isotopic composition of the Consciousness Energy Healing Treated cholecalciferol might have interacted the neutron to proton ratio in the nucleus *via* the possible mediation of neutrino [15]. Neutrino is a subatomic particle abundantly found in the universe with no electrical charge and a very small mass. The neutrinos have the ability to interact with protons and neutrons in the nucleus, which might have a close relation between neutrino and the isotope formation [15, 34, 35]. The isotopic abundance ratios $^2H/^1H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$ or $^{18}O/^{16}O$ would influence the atomic bond vibration of treated cholecalciferol [42]. The increased isotopic abundance ratio of the treated cholecalciferol may increase the intra-atomic bond strength, increase its stability, and shelf-life. The Trivedi Effect®-Consciousness Energy Healing Treated cholecalciferol would be more stable and suitable for the prevention and treatment of various diseases such as vitamin D deficiency, rickets, arthritis, osteoporosis, multiple sclerosis, cancer, diabetes mellitus, cardiovascular diseases, mental disorders, hypertension, infections, cognitive impairment in older adults, influenza, Parkinson's and Alzheimer's diseases, autoimmune disease, dementia, multiple sclerosis, glucose intolerance, etc.

CONCLUSIONS

The Trivedi Effect®-Consciousness Energy Healing Treatment has shown a significant impact on the isotopic abundance ratios of cholecalciferol. The LC-MS spectra of

both the samples at retention time (R_t) ~22 min 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 P_{M+1}/P_M in the Biofield Energy Treated cholecalciferol was increased by 2.93% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of P_{M+1}/P_M and P_{M+2}/P_M in the Biofield Energy Treated cholecalciferol was increased by 1.53% and 3.22%, 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 cholecalciferol 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 $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) in the Biofield Energy Treated cholecalciferol were significantly increased compared to the control sample. The increased isotopic composition of the Trivedi Effect®-Consciousness Energy Healing Treated cholecalciferol might have altered the neutron to proton ratio in the nucleus *via* the possible mediation of neutrino. The increased isotopic abundance ratio of the Biofield Energy Treated cholecalciferol may increase the intra-atomic bond strength, stability, and shelf-life. The new form of cholecalciferol would be more efficacious pharmaceutical/nutraceutical formulations for the prevention and treatment of various diseases such as vitamin D deficiency, rickets, osteoporosis, arthritis, multiple sclerosis, cancer, mental disorders, diabetes mellitus, cardiovascular diseases, hypertension, infections, influenza, cognitive impairment in older adults, Parkinson's and Alzheimer's diseases, dementia, autoimmune disease, glucose intolerance, multiple sclerosis, etc.

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