Journal of Chemical Science and Engineering
JCSE, 2(3): 110-121
www.scitcentral.com
ISSN: 2642-0406
Original Research Article: Open Access

LC-MS, GC-MS and NMR Spectroscopic Evaluation of Consciousness Energy Healing Treated Cholecalciferol (Vitamin D_3)

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Received June 05, 2019; Accepted June 12, 2019; Published September 24, 2019

ABSTRACT

Cholecalciferol (vitamin D_3) is a fat-soluble vitamin, which is widely used for the prevention and treatment of vitamin D deficiency. The aim of the study was to investigate the impact of the Trivedi Effect®-Consciousness Energy Healing Treatment on the isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) along with the structural properties of vitamin D_3 using advanced spectroscopy methods. Vitamin D_3 sample was divided into two parts, one part of the sample was termed as a control sample, while the other part of the sample received the Trivedi Effect® (Biofield Energy Treatment) remotely by a famous Biofield Energy Healer, Mr. Mahendra Kumar Trivedi termed as the Biofield Energy Treated sample. The liquid chromatography-mass spectrometry (LC-MS) chromatograms of both the cholecalciferol samples showed a single largest peak at the retention time (R_t) 20.6 min and protonated molecular mass peak at *m/z* 385.3 (calcd for $C_{27}H_{45}O^+$, 385.35) in the mass spectra. The LC-MS based 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 ($^{18}O/^{16}O$) were significantly increased by 15.20% and 10.44%, respectively in the treated cholecalciferol compared to the control sample. Thus, the ^{13}C, ^2H and ^{17}O contributions from $C_{27}H_{45}O^+$ to *m/z* 386 and ^{18}O contribution from $C_{21}H_{21}O_6^+$ to *m/z* 387 in the treated sample was significantly increased compared with the control sample. The gas chromatography-mass spectrometry (GC-MS) spectral data showed that the molecular mass peak intensities (*m/z* 384.4) in the treated cholecalciferol at R_t 23.28 and 23.88 min were increased by 4.54% and 3.21%, respectively compared with the control sample. The proton and carbon signals for CH_3, CH_2, CH, C-OH and =C= groups in the ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra of the treated and control samples were similar. The improvement in the isotopic abundance ratios and mass peak intensities of the treated cholecalciferol might be due to the possible mediation of neutrinos via the Trivedi Effect®-Consciousness Energy Healing Treatment. The increased isotopic abundance ratio of the treated cholecalciferol might have a stronger atomic bond, increase the stability and alter the rate of metabolic reactions in the body. Thus, the Biofield Energy Treated cholecalciferol would be more efficacious nutraceutical and pharmaceutical formulations which might provide better therapeutic response counter to deficiency of vitamin D, rickets, osteoporosis, diabetes mellitus, cancer, cardiovascular diseases, infections, etc.

Keywords: Cholecalciferol, The Trivedi Effect®, Energy of consciousness healing treatment, LC-MS, Isotopic abundance, GC-MS, Kinetic isotope effects

INTRODUCTION

Cholecalciferol (vitamin D_3) is found in foods and also in the dietary supplement to overcome vitamin deficiency and associated disease [1]. It has multiple effects on the human body, which regulate the functions of muscles, brain, lungs, liver, kidneys, heart, immune system, pancreas, large and small intestines. Vitamin D receptors are ubiquitously found in most of the body parts. 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]. Deficiency of vitamin D occurs in those who have an insufficient dietary intake or

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Citation: Trivedi MK & Jana S. (2019) LC-MS, GC-MS and NMR Spectroscopic Evaluation of Consciousness Energy Healing Treated Cholecalciferol (Vitamin D_3). J Chem Sci Eng, 2(3): 110-121.

who fail to produce enough vitamin D_3 in their skin from its precursor, 7-dehydrocholesterol, in response to exposure to ultraviolet light. Vitamin D deficiency conditions also caused by intestinal malabsorption or chronic liver disease, familial hypophosphatemia, and for the hypocalcaemia that is associated with hypoparathyroidism [2]. Vitamin D shortage in the body plays a critical role in 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. [3-6]. In the USA, 15 µg/d (600 IU per day) is required for all individuals between all the age group [7]. High dose vitamin D supplementation may cause toxicity like hypercalcemia, polyuria, polydipsia, weakness, mental retardation and insomnia [8]. Vitamin D stability is more concerned as it is a heat and light-sensitive compound [9,10]. The transformation mechanism of vitamin D and the absorption of its active form (vitamin D_3) are very complicated. Vitamin D_3 bioavailability directly affected by various factors such as dietary fiber, genetic factors and the status of vitamin D_3 [11].

Recent studies revealed that the bioavailability profile of several pharmaceutical/nutraceutical compounds, i.e., 25-hydroxyvitamin D_3 [25(OH)D_3], resveratrol, berberine, etc. are significantly altered by means of the Trivedi Effect®-Consciousness Energy Healing Treatment [12-14]. The "Biofield Energy" is a type of electromagnetic energy field generated by continues moment of the charged particles (i.e., cells, ions, etc.) in the human body [15,16]. The Biofield Energy Healers has the ability to harness the energy from the "Universal Energy Field" and can transfer into any object(s) around the globe. The process of treatment to an object is known as the Biofield Energy Healing Treatment. There are several Energy Therapies that are used nowadays against various disease conditions [17,18]. The Energy Therapy has been recognized worldwide as a Complementary and Alternative Medicine health care approach by the National Center of Complementary and Integrative Health with other therapies, medicines and practices such as Qi Gong, Tai Chi, Ayurvedic medicine, traditional Chinese herbs and medicines, aromatherapy, meditation, yoga, chiropractic/osteopathic manipulation, acupressure, homeopathy, acupuncture, healing touch, hypnotherapy, movement therapy, Reiki, cranial sacral therapy, etc. [19]. The Trivedi Effect® has surprizing ability to transform the characteristic properties of metals and ceramic [20, 21], organic compounds [22,23], nutraceuticals [24], pharmaceuticals [25,26], culture medium [27,28] and improve the overall productivity of crops [29], alteration of the isotopic abundance ratio in the organic compounds [30-32] may be through the possible mediation of neutrinos [15].

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]. Thus, the isotopic abundance ratio analysis of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) was performed to evaluate the influence of the Trivedi Effect® on the isotopic abundance ratio in cholecalciferol. Similarly, the LC-MS, GC-MS and NMR (Nuclear Magnetic Resonance) techniques were also used to characterize the structural properties of cholecalciferol.

MATERIALS AND METHODS

Chemicals and reagents

The test sample cholecalciferol (>98%) was purchased from Sigma-Aldrich, India but other chemicals used during the experiments were of analytical grade purchased in India.

Consciousness energy healing treatment strategies

The test sample cholecalciferol was divided into two equal parts. One part of the test cholecalciferol was considered as a control sample, which was not received the Biofield Energy Treatment. Whereas, the second part was received the Consciousness Energy Healing Treatment (Trivedi Effect® for 3 min) remotely under standard laboratory conditions and known as treated cholecalciferol. This Biofield Energy was provided through the healer's unique energy transmission process by a famous Biofield Energy Healer, Mr. Mahendra Kumar Trivedi (USA), to the test item. The control cholecalciferol was treated with a "sham" healer, who did not have any knowledge about the Biofield Energy Treatment. After the treatment both the samples of cholecalciferol were kept in sealed conditions and characterized.

Characterization

Liquid chromatography-mass spectrometry (LC-MS) analysis and calculation of isotopic abundance ratio: The LC-MS analysis of the samples was carried out with the help of LC-Dionex Ultimate 3000, MS-TSQ Endura, USA equipped with a photo-diode array (PDA) detector connected with a triple-stage quadrupole mass spectrometer (Thermo Scientific TSQ Endura, USA) with a Thermo Scientific Ion Max NG source and Atmospheric Pressure chemical ionization (APCI). The analysis was performed on a reversed phase Zorbax SB-C18 100 × 4.6 mm, 3.5 µm in gradient mode in the liquid chromatograph (column temperature 40°C). The mobile phase was ammonium formate and 0.5% formic acid in water (A) and acetonitrile (B) at a constant flow rate of 0.6 mL/min. The injection volume was 10 µL and the total run time was 30 min. Chromatographic separation was achieved using gradient condition as follow: 0 min-50% B, 5 min-90% B, 10 min-100% B, 20 min-100% B, 25 min-50% B and 30 min-50%

B. Peaks were monitored using the PDA detector. The mass spectrometric analysis was performed under +ve ESI mode.

The natural abundance of C, O and H isotope can be predicted from the comparison of the relative abundance 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 [36-39]. The change in the isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) for the control and treated cholecalciferol was calculated using equation (1).

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

Where $IAR_{Treated}$ and $IAR_{Control}$ is the isotopic abundance ratio in the treated and control cholecalciferol, respectively.

Gas chromatography-mass spectrometry (GC-MS) analysis: An Agilent 7890B GC equipped with a silica capillary column HP-5 MS (30 m × 0.25 mm × 0.25 µm) and coupled to a quadrupole detector with pre-filter (5977B, USA) was operated with electron impact (EI) ionization in positive mode at 70 eV. The oven temperature was programmed from 50°C (1 min hold) to 150°C @ 20°C/min to 200°C (6 min hold) @ 25°C/min to 280°C @ 20°C/min (12 min hold). Temperatures of the injector, detector (FID), auxiliary, ion source and quadrupole detector were 230, 250, 280, 230 and 150°C. Cholecalciferol was dissolved in methanol, and 5.0 µL was splitlessly injected with helium as a carrier gas with a flow rate of 2.0 mL/min.

The percent change in peak intensity (I) was calculated using the following equation (2):

$$\% \text{ Change in peak intensity (I)} = \frac{[I_{Treated} - I_{Control}]}{I_{Control}} \times 100 \quad (2)$$

Where, $I_{Control}$ and $I_{Treated}$ are the peak intensity of the control and Biofield Energy Treated sample, respectively.

Nuclear magnetic resonance (NMR) analysis: 1H NMR spectra of cholecalciferol were recorded at at 400 MHz MHz on Agilent-MRDD2 FT-NMR. Approximately 3 mg of the sample was dissolved in DMSO-d6. Chemical shifts (d) were in parts per million (ppm) relative to the solvent's residual proton chemical shift $\{(CD_3)_2SO, \delta=2.5\}$. Similarly, 13C NMR spectra of cholecalciferol were measured at at 100 MHz on Agilent-MRDD2 FT-NMR spectrometer spectrometer at room temperature. The sample was dissolved in DMSO-d6. The solvent's residual carbon chemical shift $\{(CD_3)_2SO, \delta=39.52\}$.

RESULTS AND DISCUSSION

Liquid chromatography-mass spectrometry (LC-MS) analysis and isotopic abundance ratio analysis

The control and treated cholecalciferol showed a sharp chromatographic peak at the retention times (R_t) of 20.63 and 20.62 min, respectively **(Figure 1)**. The % peak area at R_t 20.6 min was 99.58 and 99.54 in control and Biofield Energy Treated sample, respectively. This indicated that the polarity of the Biofield Energy Treated sample remained similar compared to the control cholecalciferol.

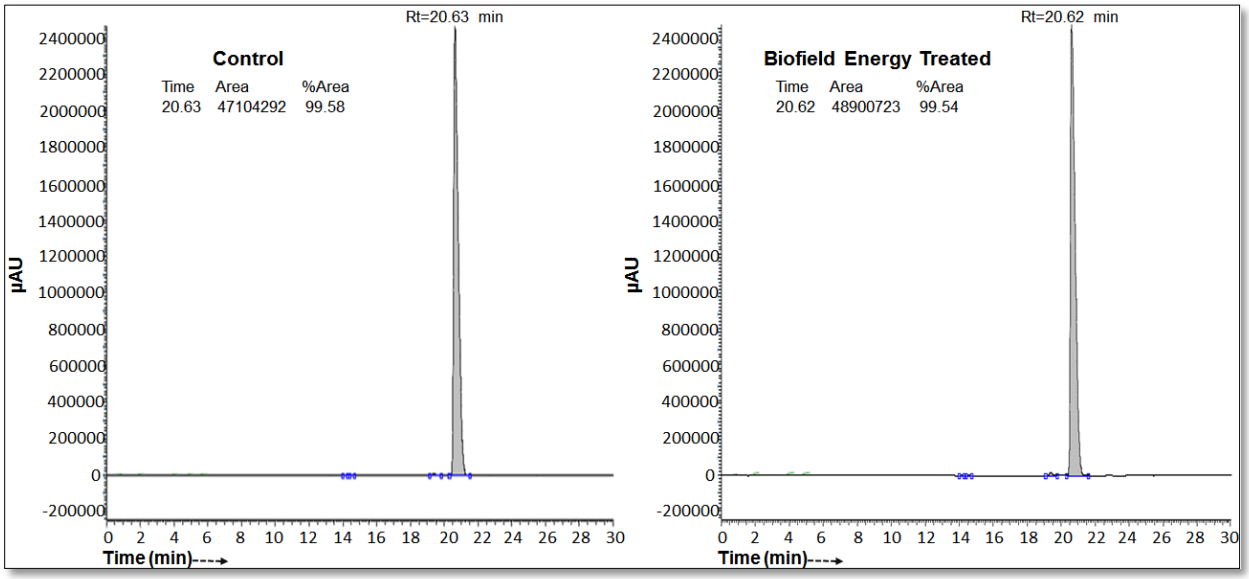

Figure 1. Total ion chromatograms of the control and treated cholecalciferol.

The mass spectra of the control and treated samples at R_t of 20.6 min exhibited the presence of the molecular ion of cholecalciferol ($C_{27}H_{45}O^+$) adduct with hydrogen ion **(Figure 2)** at *m/z* 385.3 (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* 367.3 (calcd for $C_{27}H_{43}^+$, 367.3) in both the cholecalciferol samples **(Figure 2)**.

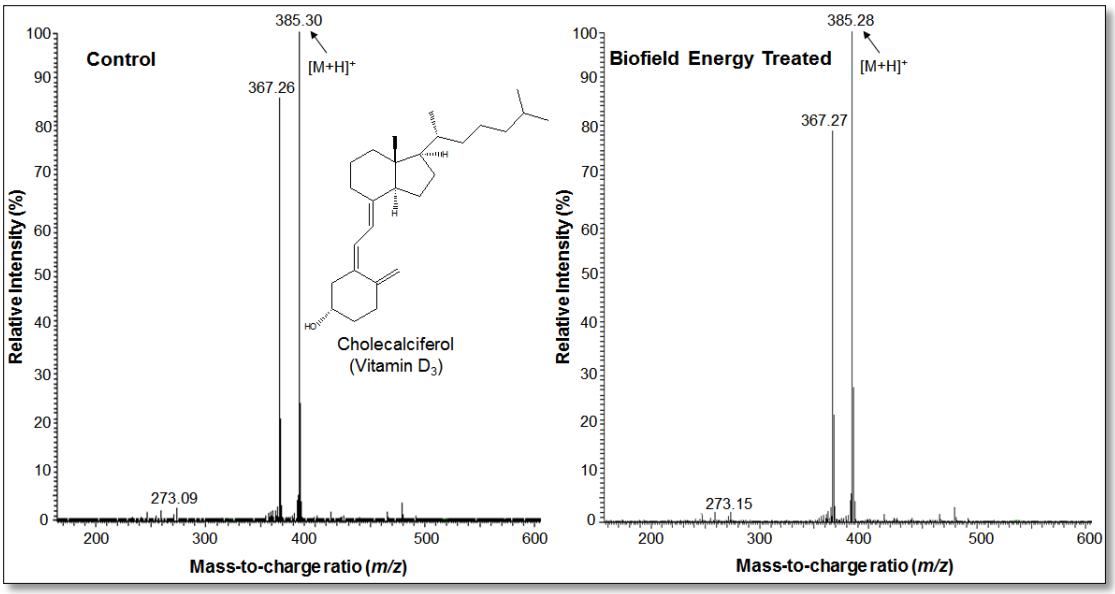

Figure 2. The mass spectra of the control and treated cholecalciferol at R_t 20.6 min in the chromatograms.

The mass spectrum of the Biofield Energy Treated cholecalciferol showed an almost similar type of mass fragmentation pattern of the control sample **(Figure 2)**. The molecular ion peak at *m/z* 385.3 exhibited 100% relative peak intensity in both the mass spectra **(Figure 2)**. The relative peak intensities of the other ion peaks in the Consciousness Energy Healing Treated cholecalciferol were significantly altered compared to the control sample.

The control and treated samples of cholecalciferol showed the mass of a protonated molecular ion at *m/z* 385.3 (calcd for $C_{27}H_{45}O^+$, 385.35) with 100% relative abundance in the mass spectra. The theoretical calculation of isotopic peak PM+1 for the protonated cholecalciferol presented as below:

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

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

P (^{17}O) = [(1 × 0.04%) × 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 = 30.42%

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

P (^{18}O) = [(1 × 0.20%) x 100%] / 100% = 0.2%

P_{M+2} of ^{18}O contribution from $C_{27}H_{45}O^+$ to *m/z* 387 = 0.2%

The calculated isotopic abundance of P_{M+1} value 30.42% was higher to the observed value (23.69%), but the calculated P_{M+2} value 0.2% was lower to the observed value (3.64%) **(Table 1)**. The probability of *A*+1 and *A*+2 elements having an isotope with one and two mass unit heavier, respectively than the most abundant isotope (i.e., ^{13}C, ^{2}H, ^{17}O and ^{18}O) contributions to the mass of the isotopic molecular ion $[M+1]^+$ and $[M+2]^+$. ^{2}H did not contribute much any isotopic *m/z* ratios because of its less natural abundance compared to the natural abundances of C and O isotopes [37,38]. From the calculations, it was observed that ^{13}C, ^{17}O and ^{18}O have the major contributions from cholecalciferol to the isotopic mass peak at *m/z* 386 and 387. Therefore, P_M, P_{M+1} and P_{M+2} of the cholecalciferol at *m/z* 385, 386 and 387 of the control and Biofield Energy Treated samples were obtained from the experimental relative abundance of M^+, $(M+1)^+$ and $(M+2)^+$ peaks, respectively in the mass spectra **(Table 1)**.

The isotopic abundance ratio of P_{M+1}/P_M ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) in the Trivedi Effect®-Consciousness Energy Healing Treated cholecalciferol was significantly increased by 15.20% 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 in the Biofield Energy Treated sample was significantly increased compared to the control sample. Similarly, the isotopic abundance ratio of P_{M+2}/P_M ($^{18}O/^{16}O$) in the Biofield Energy Treated cholecalciferol was significantly increased by 10.44% compared to the control sample **(Table 1)**. Thus, the ^{18}O contribution from $C_{27}H_{45}O^+$ to *m/z* 387 in the Biofield Energy Treated cholecalciferol was also significantly increased compared to the control sample.

Table 1. LC-ESI-MS isotopic abundance ratio analysis of control and treated vitamin D_3.

Parameters	Control sample	Biofield Energy Treated sample
P_M at *m/z* 385 (%)	100	100
P_{M+1} at *m/z* 386 (%)	23.69	27.29
P_{M+1}/P_M	0.2369	0.2729
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control cholecalciferol		15.20
P_{M+2} at *m/z* 387 (%)	3.64	4.02
P_{M+2}/P_M	0.0364	0.0402
% Change of isotopic abundance ratio (P_{M+2}/P_M) compared to the control cholecalciferol		10.44

P_M=the relative peak intensity of the parent molecular ion M^+; P_{M+1}=the relative peak intensity of the isotopic molecular ion $[M+1]^+$, P_{M+2}=the relative peak intensity of the isotopic molecular ion $[M+2]^+$ and M=mass of the parent cholecalciferol molecule

Neutrons and alteration in its number in the molecule lead to the increased or decreased isotopic abundance of the compounds. The changes in atomic/molecular weights are postulated to the changes in atomic mass and charge *via* the possible mediation of neutrinos [15,40-42]. The recent innovation of neutrino oscillations seems to give credence to the postulates of Mr. Mahendra Kumar Trivedi on the Trivedi Effect® [15]. Thus, it can be assumed that the Trivedi Effect®-Consciousness Energy Healing Treatment might be providing the necessary energy for the neutrino oscillations leads to the modification of the fundamental physicochemical properties of a compound [43,44]. The increased isotopic abundance ratios $^2H/^1H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$ or $^{18}O/^{16}O$ would highly influence the atomic bond vibration and increase bond strength of treated cholecalciferol [45]. The alteration in the isotopic abundance ratio of the atoms/molecules cause of the change in the kinetic isotope effects, which is very useful to study the reaction mechanism, understand the enzymatic transition state, and enzyme mechanism that is supportive for designing effective, and specific inhibitors, etc. [34]. Therefore, The Biofield Energy Treated cholecalciferol with improved isotopic abundance ratio (P_{M+1}/P_M and P_{M+2}/P_M) might be advantageous for the better nutraceutical and pharmaceutical formulations.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS chromatograms of the control and Biofield Energy Treated cholecalciferol showed two clear independent peaks **(Figure 3)**. The R_t of the control sample was at 23.28 and 23.88 min, whereas Biofield Energy Treated sample at 23.28 and 23.88 min showed that the R_t of both the sample is very close. Therefore, 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 [46,47].

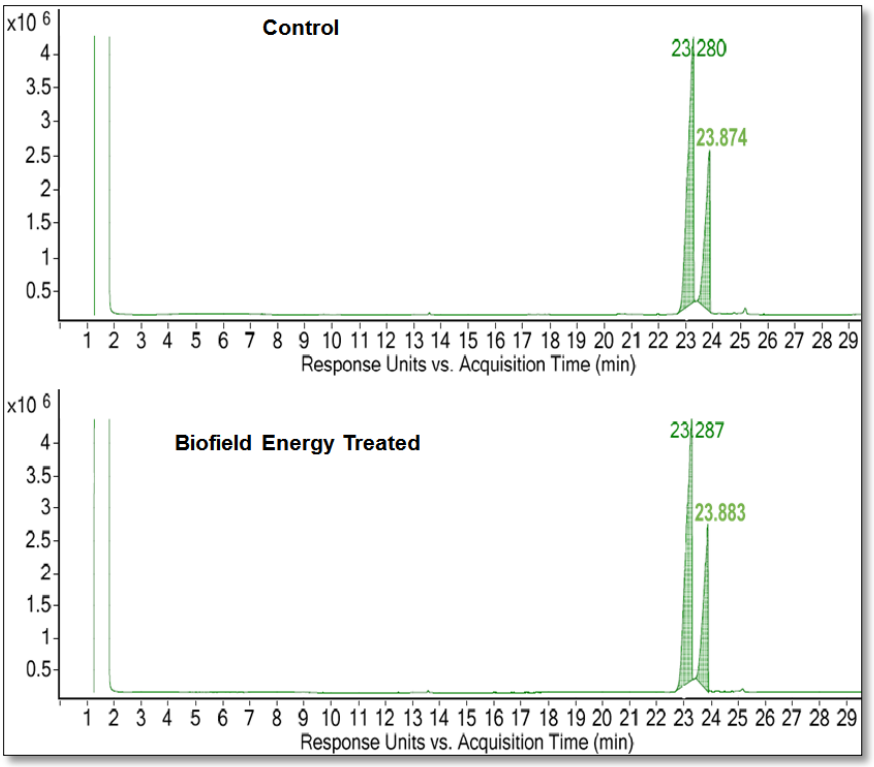

Figure 3. GC chromatograms of the control and treated cholecalciferol.

The GC-MS spectra of the control and treated samples at R_t of 23.28 min exhibited the presence of the molecular ion of cholecalciferol ($C_{27}H_{44}O^+$) **(Figure 4)** at *m/z* 384.4 (calcd for $C_{27}H_{44}O^+$, 384.34). The lower mass fragmentation peak at *m/z* 366, 351.4 and 325.3 for $C_{27}H_{42}^{\bullet+}$, $C_{26}H_{39}^{\bullet\bullet+}$ and $C_{24}H_{37}^+$, respectively in both the spectra **(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 treated cholecalciferol were altered compared to the control sample. The mass peak intensity of the control and treated cholecalciferol were 1384814.88 and 1447749.88, respectively at R_t of 23.28 min. Similarly, the mass peak intensities of the control and Biofield Energy Treated cholecalciferol were 1219705.00 and 1258803.38, respectively at R_t of 23.88 min. The mass peak intensities at R_t 23.28 and 23.88 min in the Biofield Energy Treated sample were increased by 4.54% and 3.21%, respectively compared to the control sample **(Table 2)**. The mass peak intensities were significantly increased which might be possible due to the impact of the Trivedi Effect®-Consciousness Energy Healing Treatment. The Trivedi Effect® is a proved natural phenomenon which has the remarkable potential to alter the isotopic abundance ratios of various compounds through the possible mediation of neutrinos [15,43,44].

Table 2. GC-MS chromatographic and mass spectra analysis at R_t 23.28 and 23.88 min of the control and treated cholecalciferol.

Parameters	Control sample	Biofield Energy Treated sample	% Change
Mass peak (*m/z*=384) intensity at R_t 23.28 min	1384814.88	1447749.88	4.54
Mass peak (*m/z*=384) intensity at R_t 23.88 min	1219705.00	1258803.38	3.21

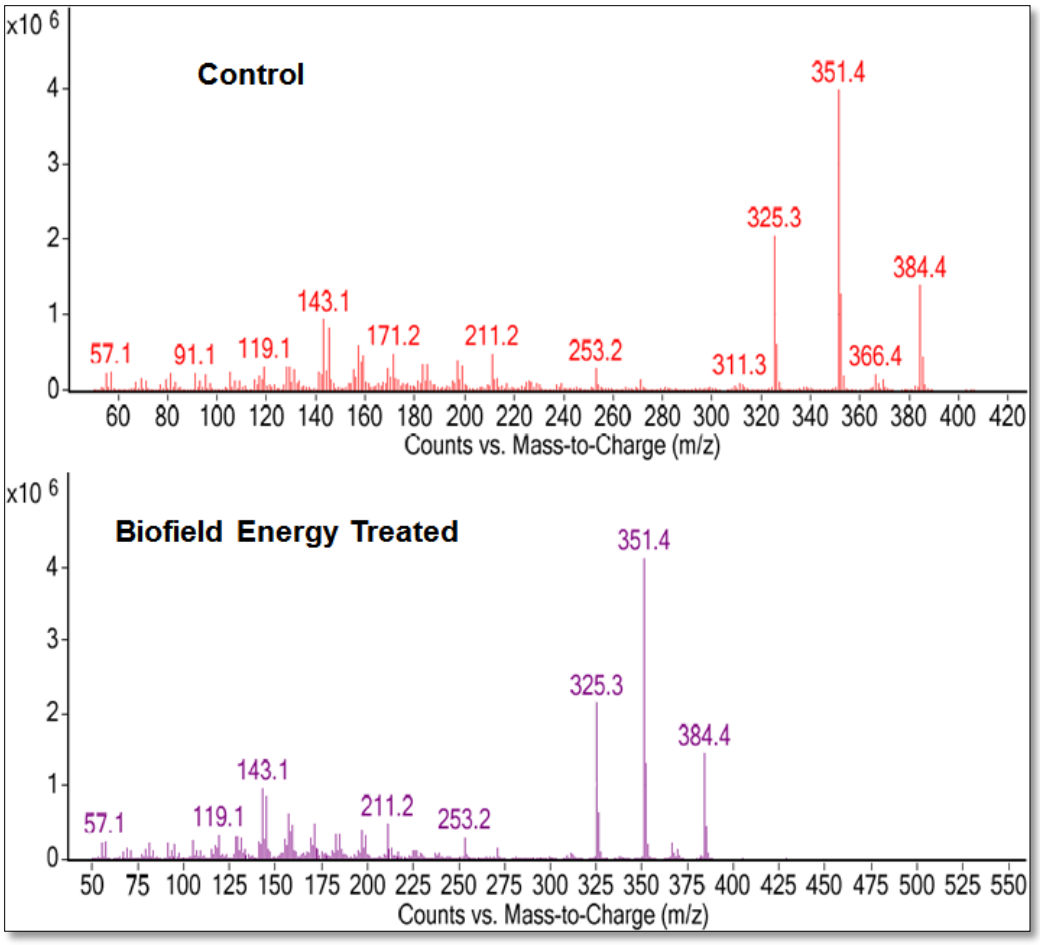

Figure 4. GC-MS spectra of the control and treated cholecalciferol at R_t 23.28 min.

Nuclear magnetic resonance (NMR) spectroscopy analysis

Figures 5 and 6 showed the ^{1}H, and ^{13}C NMR spectra, respectively for the control and Biofield Energy Treated cholecalciferol. The analyzed NMR spectral data of both the samples are presented in **Table 3**. The ^{1}H NMR spectra of both the samples indicated that signals for the protons coupling of CH_3, CH_2, CH and OH protons of cholecalciferol were in the range of δ 0.48 to 6.19 ppm **(Figure 5 and Table 3)**, which were very close to each other. Similarly, the carbon signals for CH_3, CH_2, CH, =C= and C-OH groups in the ^{13}C NMR spectrum **(Figure 6)** were in the range of 11.74-145.45 in both the control and Biofield Energy Treated samples of cholecalciferol **(Table 3)**. The experimental results were closely matched to the reported literature [48]. The ^{1}H and ^{13}C NMR spectral data concluded that there was no structural modification of the treated cholecalciferol compared to the control sample.

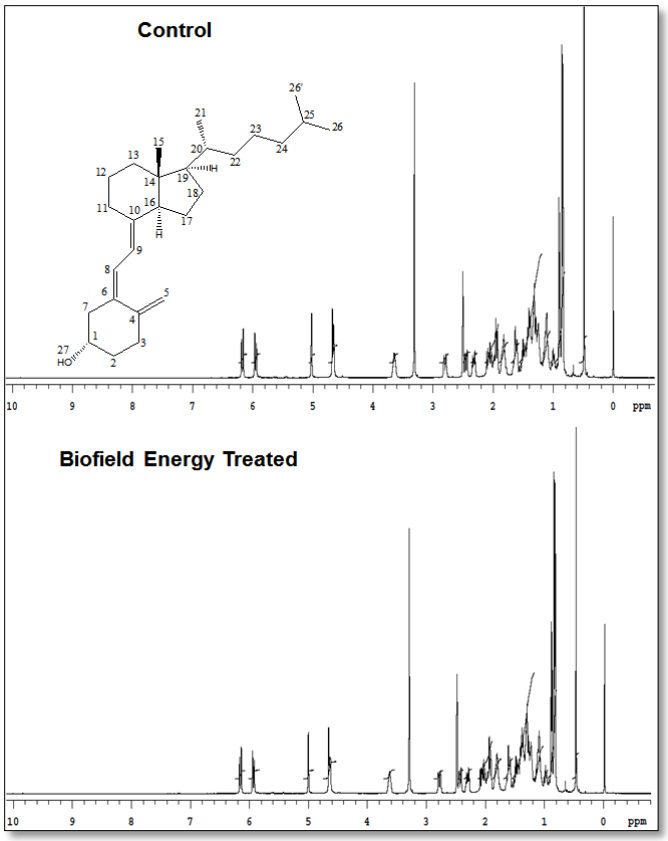

Figure 5. [1]H NMR spectra of the control and treated cholecalciferol.

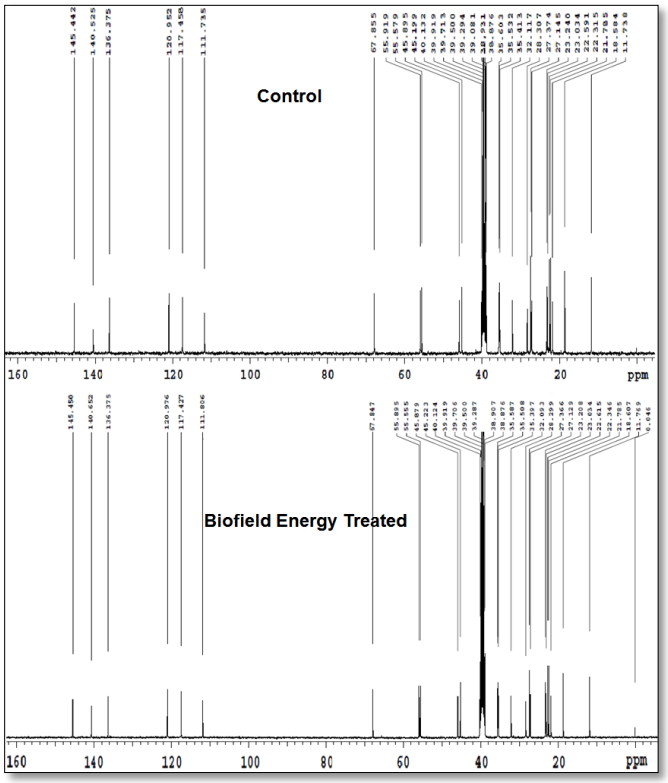

Figure 6. [13]C NMR spectra of the control and treated cholecalciferol.

Table 3. ^{1}H and ^{13}C NMR spectroscopic data of both the control and treated cholecalciferol.

^{1}H & ^{13}C S. No	^{1}H NMR δ (ppm) and Multiplicity		^{13}C NMR δ (ppm)	
	Control	Biofield Energy Treated	Control	Biofield Energy Treated
1	3.65 (m, *J*=20 Hz, H)	3.65 (m, *J*=20 Hz, H)	67.86	67.85
2	1.65 (m, *J*=24 Hz, 2H)	1.65 (m, *J*=24 Hz, 2H)	35.41	35.40
3	1.81 (m, *J*=24 Hz, 2H)	1.81 (m, *J*=24 Hz, 2H)	32.12	32.10
4	--	--	136.38	136.38
5	4.67 (d, *J*=12 Hz, 2H)	d (4.66, *J*=12 Hz, 2H)	111.74	111.81
6	--	--	145.44	145.45
7	1.93-2.11 (m, 2H)	1.93-2.11 (m, 2H)	45.90	45.88
8	6.17 (d, *J*=12 Hz, H)	6.17 (d, *J*=12 Hz, 2H)	120.95	120.98
9	5.95 (d, *J*=12 Hz, H)	5.95 (d, *J*=12 Hz, H)	117.46	117.43
10	--	--	140.53	140.65
11	1.93-2.11 (m, 2H)	1.93-2.11 (m, 2H)	28.31	28.30
12	0.96-1.20 (m, 2H)	0.96-1.20 (m, 2H)	22.31	22.33
13	0.96-1.20 (m, 2H)	0.96-1.20 (m, 2H)	40.13	40.12
14	--	--	45.20	45.22
15	0.48 (S, 3H)	S(0.48, 3H)	11.74	11.77
16	2.78-2.82 (d, *J*=16 Hz, H)	2.78-2.82 (d, *J*=16 Hz, H)	55.92	55.90
17, 18, 22, 23, 24	1.25-1.43 (m, 10H)	1.25-1.43 (m, 10H)	23.03, 27.37, 35.60, 23.24, 39.92	23.03, 27.37, 35.59, 23.21, 39.92
19, 20, 25	2.11-2.50 (m, 3H)	2.11-2.47 (m, 3H)	55.60, 35.53, 27.15	55.91, 35.51, 27.13
21	0.89 (d, *J*=8 Hz, 3H)	0.89 (d, *J*=8 Hz, 3H)	18.58	18.61
26, 26'	0.86 (m, *J*=12 Hz, 6H)	m (0.86, *J*=12 Hz, 6H)	22.59, 21.79	22.61, 21.79
27(OH)	s(5.02)	s(5.02)	--	--

s: singlet; d: doublet; m: multiplet

CONCLUSION

The present experimental data suggest that the Trivedi Effect®-Energy of Consciousness Healing Treatment has shown a significant impact on the isotopic abundance ratios and relative peak intensities of cholecalciferol/vitamin D_3. The LC-MS chromatograms of both the cholecalciferol samples showed a single largest peak at the retention time (R_t) 20.6 min and protonated molecular mass peak at *m/z* 385.3 (calcd for $C_{27}H_{45}O^+$, 385.35) in the mass spectra. The LC-MS based 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 ($^{18}O/^{16}O$) were significantly increased by 15.20% and 10.44%, respectively in the Consciousness Energy Healing Treated cholecalciferol compared to the control sample. Thus, the ^{13}C, ^{2}H, and ^{17}O contributions from $C_{27}H_{45}O^+$ to *m/z* 386 and ^{18}O contribution from $C_{21}H_{21}O_6^+$ to *m/z* 387 in the Consciousness Energy Healing Treated cholecalciferol were significantly increased compared with the control sample. The GC-MS spectral data showed that the molecular mass peak intensities (*m/z* 384.4) in the Consciousness Energy Healing Treated cholecalciferol at R_t 23.28 and 23.88 min were increased by 4.54% and 3.21%, respectively compared with the control sample. The improvement in the

isotopic abundance ratios and mass peak intensities of the Consciousness Energy Healing Treated cholecalciferol might be due to the possible mediation of neutrinos *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. The increased isotopic abundance ratio of the Consciousness Energy Healing Treated cholecalciferol might have a stronger atomic bond, increase the stability, and alter the rate of metabolic reactions in the body. Thus, the Trivedi Effect®-Consciousness Energy Healing Treated vitamin D3 would be more advantageous to develop more efficacious nutraceutical/pharmaceutical formulations which might provide better therapeutic response against 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.

ACKNOWLEDGEMENT

The authors are grateful to GVK Biosciences Pvt. Ltd., Trivedi Science, Trivedi Global, Inc., Trivedi Testimonials and Trivedi Master Wellness for their assistance and support during this work.

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