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Evaluation of the Structural Properties and Isotopic Abundance Ratios of Consciousness Energy Healing Treated Vitamin D₃ Using LC-MS, GC-MS, and NMR Spectroscopy

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Abstract

Vitamin D₃ (cholecalciferol) has multiple effects which regulate the functions of muscles, brain, lungs, liver, kidneys, heart, immune system, pancreas, large and small intestines. 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) and structural properties of vitamin D₃ using LC-MS, GC-MS, and NMR spectroscopy. Vitamin D₃ was divided into two parts, one part of the sample was termed as untreated (control) sample, while the other part of the sample received The Trivedi Effect®-Biofield Energy Healing Treatment remotely by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi and termed as Biofield Energy Treated sample. The LC-ESI-MS analysis of both the samples showed the mass of protonated ion at m/z 385.3 (calcd for $C_{27}H_{45}O^+$, 385.35) at the retention time (R_t) 20.7 minutes. The liquid chromatographic peak area of the treated sample was significantly improved by 16.00% compared to the control sample. Similarly, the isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^12C$ or $^{17}O/^16O$) was very significantly increased by 470.88% in the treated sample compared to the control sample. But, the isotopic abundance ratio of P_{M+2}/P_M ($^{18}O/^16O$) in the treated sample also significantly decreased by 41.81% compared to the control sample. Therefore, the 2H , ^{13}C , and ^{17}O contributions from $C_{27}H_{45}O^+$ to m/z 386 were significantly increased, whereas ^{18}O contribution from $C_{21}H_{21}O_6^+$ to m/z 387 was decreased in the treated sample compared with the control sample. The GC-MS analysis showed that the parent molecular mass peak intensities (m/z 384.4) in the treated sample at R_t 23.04 and 23.65 minutes were significantly increased by 6.11% and 9.62%, 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 NMR spectra of the control and treated samples were similar. The altered isotopic abundance ratios and mass peak intensities might be due to energy produced by The Trivedi Effect®-Consciousness Energy Healing Treatment *via* the possible mediation of neutrinos, which further lead to the change in the kinetic isotope effects and physicochemical properties of the treated sample. Thus, The Trivedi Effect® Treated vitamin D₃ would be advantageous for designing better nutraceutical and pharmaceutical formulations which might provide better therapeutic response against vitamin D deficiency, rickets, osteoporosis, arthritis, multiple sclerosis, diabetes, cancer, cardiovascular diseases, mental disorders, infections, stress, aging, glucose intolerance, multiple sclerosis, Parkinson's and Alzheimer's diseases, dementia, *etc.*

Keywords: Vitamin D₃; The Trivedi Effect®; Energy of Consciousness Healing Treatment; LC-MS; Isotopic abundance; GC-MS; Kinetic isotope effects

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Introduction

Vitamin D₃ is a fat soluble vitamin, scientifically also known as cholecalciferol, found in foods like cod liver oil, mushrooms (if exposed to ultraviolet light), mackerel, halibut, canned sock-eye salmon and also in the dietary supplements^[1-3]. Vitamin D has multiple roles in 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 present in most of the body organs, *i.e.*, brain, heart, lungs, kidney, liver, pancreas, large and small intestines, muscles, reproductive, nervous system, *etc.* Vitamin D receptor response elements with hundreds of genes directly or indirectly influence cell-to-cell communication, normal cell growth, cell differentiation, cell cycling and proliferation, hormonal balance, maintenance of calcium and phosphorus balance, neurotransmission, skin health, immune and cardiovascular function^[1-3]. Insufficient dietary intake, intestinal malabsorption or chronic liver disease, familial hypophosphatemia, hypocalcaemia that is associated with hypoparathyroidism, and those who fail to produce enough vitamin D₃ in their skin from its precursor, 7-dehydrocholesterol, in response to exposure to ultraviolet light lead to vitamin D deficiency. Vitamin D deficiency responsible for several diseases, e.g., rickets, osteoporosis, arthritis, multiple sclerosis, cancer, diabetes mellitus, mental disorders, cardiovascular diseases, inflammations, infections, stress, aging, cognitive impairment in older adults, glucose intolerance, multiple sclerosis, Parkinson's and Alzheimer's diseases, dementia, *etc.*^[1-3,4-6]. The National Academies Press (USA) report suggested that 15 µg/d (600 IU per day) of vitamin D is required for all individuals (males, female, pregnant/lactating women) between the ages of 1 and 70 years old^[7]. The toxicity like hypercalcemia, polyuria, polydipsia, weakness, mental retardation, and insomnia may be likely to cause due to the higher dose of vitamin D supplementation^[8]. Vitamin D₃ is highly heat and light-sensitive compound^[9,10]. Therefore, the stability of vitamin D₃ is more concerned. The mechanism of transformation of vitamin D and its absorption kinetics of active form (vitamin D₃) is very complicated. The bioavailability absorption and bioavailability of vitamin D₃ directly affected by various factors such as dietary fiber, genetic factors, and status of vitamin D₃^[11].

Some of the recent studies revealed that The Trivedi Effect®-Energy of Consciousness Healing Treatment significantly improved the bioavailability of various pharmaceutical/nutraceutical compounds, *i.e.*, resveratrol, berberine, and 25-hydroxyvitamin D₃ in Male Sprague-Dawley rats^[12-14]. Biofield is the electromagnetic field exist around the human body^[15,16]. The Trivedi Effect® is a natural and only scientifically proven phenomenon in which a person can harness this inherently intelligent energy (Universal Energy Field) and transmit it anywhere on the planet. Several Biofield based Energy Healing Therapies used against various human 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, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, homeopathy, acupuncture,

acupressure, healing touch, hypnotherapy, movement therapy, naturopathy, cranial sacral therapy, Reiki, *etc.*^[19]. The Trivedi Effect®-Energy of Consciousness Healing Treatment has astonishing ability to transform the characteristic properties of organic compounds^[20,21], metals and ceramic^[22,23], nutraceuticals^[24,25], pharmaceuticals^[26,27], productivity of crops^[28,29], genetic materials^[30,31] and alteration of the isotopic abundance ratio in the organic compounds^[32,33] may be through the possible mediation of neutrinos^[15]. Several scientific organization is doing extensive research on the natural stable isotopes to understand the isotope effects resulting from the alterations of the isotopic composition, which have lots of implementation in different fields of sciences^[34-36]. The conventional Gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS) techniques are widely used for the study of isotope ratio analysis with sufficient precision^[35]. Considering the various importance of The Trivedi Effect®-Consciousness Energy Healing Treatment and its outstanding impact on various object(s), the isotopic abundance ratio analysis of P_{M+1}/P_M (²H/¹H or ¹³C/¹²C or ¹⁷O/¹⁶O) and P_{M+2}/P_M (¹⁸O/¹⁶O) in vitamin D₃ was performed to evaluate the influence of The Trivedi Effect® on the isotopic abundance ratio and structural properties using the sophisticated LC-MS, GC-MS, and NMR (Nuclear Magnetic Resonance) analytical techniques.

Materials and Methods

Chemicals and Reagents

Vitamin D₃ (>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

Vitamin D₃ sample was divided into two parts. One part of vitamin D₃ was considered as control/untreated which was not subjected to the Biofield Energy Treatment. Whereas, the second part of vitamin D₃ sample was treated with The Trivedi Effect®-Energy of Consciousness Healing Treatment remotely under standard laboratory conditions for 3 minutes by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi (USA) and termed as Biofield Energy Treated sample. This Biofield Energy Treatment was provided through the healer's unique energy transmission process to the test item. Further, the control sample was treated with "sham" healer for the purpose of better comparison. The sham healer did not have any knowledge about the Biofield Energy Treatment. After all, the Biofield Energy Treated and untreated samples of vitamin D₃ were kept in sealed conditions and characterized using LC-MS, GC-MS, and NMR 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 vitamin D₃ 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. The mobile phase was 2mM ammonium formate and 0.5% formic acid in water and acetonitrile at a constant flow rate of 0.6 mL/min. The column temperature was kept constant at 40°C. 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. Mass spectrometric analysis was performed under +ve ESI mode. The total ion chromatogram, peak area% and mass spectrum of the individual peak which was appeared in LC along with the full scan were recorded. The mass peak intensities of the mass spectrum of the individual peak were recorded.

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^[37-40]. The isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) for the control and Biofield Energy Treated vitamin D₃ were calculated.

Percentage (%) change in isotopic abundance ratio of vitamin D₃
 $= [(IAR_{Treated} - IAR_{Control}) / IAR_{Control}] \times 100]$

Where, $IAR_{Treated}$: isotopic abundance ratio in the treated vitamin D₃ and $IAR_{Control}$: isotopic abundance ratio in the control vitamin D₃.

Gas chromatography-mass spectrometry (GC-MS) analysis:

Agilent 7890B Gas chromatograph equipped with a silica capillary column HP-5 MS(30 m x 0.25 mm x 0.25 μm) and coupled to a quadrupole detector with pre-filter (5977B, USA) was operated with electron impact (EI) ionization in positive ion mode at 70 eV. 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. Vitamin D₃ 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. Mass spectra were scanned from m/z 40 to 1050 at a stability of ± 0.1 m/z mass accuracy over 48 hours and mass peak intensities of the mass spectrum of the individual peak were recorded.

Percent change in peak intensity (I) was calculated using following equations:

Percent change in peak intensity (I) = $[(I_{Treated} - I_{Control}) / I_{Control}] \times 100$

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

Nuclear Magnetic Resonance (NMR) Analysis: ¹H NMR spectra of vitamin D₃ were recorded at 400 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 {(CD₃)-

₂SO, δ = 2.5}. ¹H NMR multiplicities were designated as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q), multiplet (m), broad (br), apparent (app). Similarly, ¹³C NMR spectra of vitamin D₃ were measured at 100 MHz on Agilent-MRDD2 FT-NMR spectrometer at room temperature. Approximately 25 mg of the sample was dissolved in DMSO-d₆. Chemical shifts (d) were in parts per million (ppm) relative to the solvent's residual carbon chemical shift {(CD₃)₂SO, δ = 39.52}.

Results and Discussion

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

The control and Biofield Energy Treated vitamin D₃ showed a clear and sharp chromatographic peak at retention times (Rt) 20.73 and 20.71 minutes, respectively (Figure 1). The peak area of the control and Biofield Energy Treated samples at R_t 20.7 minutes was 25243036 and 29282315 in control and Biofield Energy Treated sample, respectively. The peak area of the Biofield Energy Treated sample was significantly improved by 16.00% compared to the control sample. It indicated that the polarity of both the samples remained similar, but the solubility profile of the Biofield Energy Treated sample was significantly improved compared to the control sample. Solubility also plays a major role in the dosage forms of parenteral formulations and one of the important parameters to achieve the improved concentration of drug in systemic circulation for achieving elevated pharmacological response^[41].

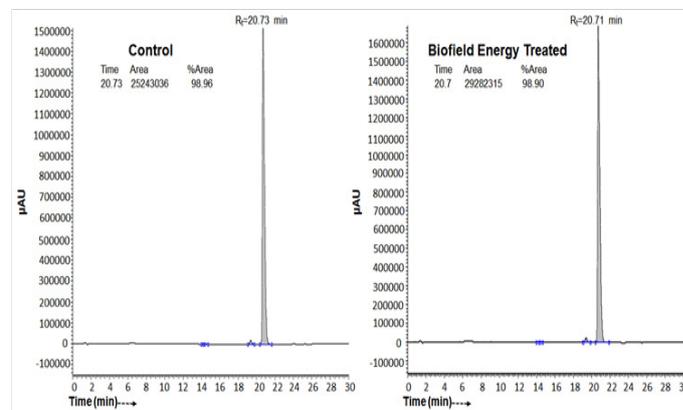


Figure 1: Total ion chromatograms (TIC) of the control and Biofield Energy Treated vitamin D₃.

The ESI-MS mass spectra of the control and Biofield Energy Treated samples at R_t of 20.7 minutes exhibited the molecular ion peak (Figure 2) of vitamin D₃(C₂₇H₄₅O⁺) adduct with hydrogen ion at m/z 385.23 and 385.37, respectively (calcd for C₂₇H₄₅O⁺, 385.35). The major fragmented mass peak [M-OH]⁺ at m/z 367.09 and 367.32 (calcd for C₂₇H₄₃⁺, 367.3) in control and Biofield Energy Treated vitamin D₃, respectively (Figure 2).

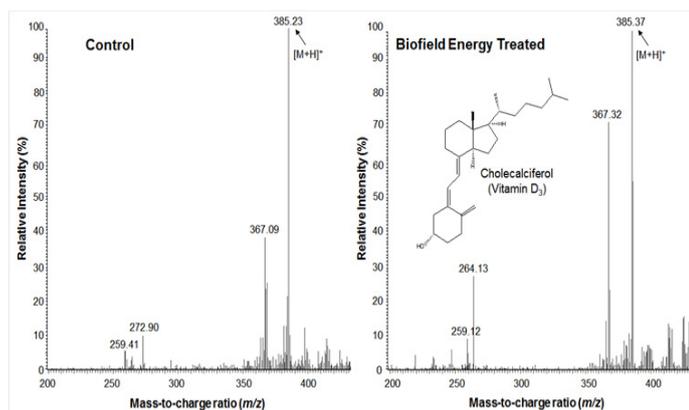


Figure 2: The ESI-MS spectra of the control and Biofield Energy Treated vitamin D₃ at R_t 20.7 minutes in the chromatograms.

The mass fragmentation pattern of the control and Biofield Energy Treated vitamin D₃ in the spectra were the similar type (Figure 2). The parent mass peak at *m/z* 385.3 was the base peak which exhibited 100% relative peak intensity in both the spectra (Figure 2). But, the relative peak intensities of the other ion peaks in the treated vitamin D₃ were significantly altered compared to the control sample.

Isotopic Abundance Ratio Analysis: The control and treated samples of vitamin D₃ showed the mass of a protonated molecular ion at *m/z* 385.3 (calcd for C₂₇H₄₅O⁺, 385.35) with 100% relative abundance in the mass spectra. The theoretical calculation of isotopic peak P_{M+1} for the protonated vitamin D₃ presented below:

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

$$P(^2\text{H}) = [(45 \times 0.015\%) \times 100\%] / 100\% = 0.675\%$$

$$P(^{17}\text{O}) = [(1 \times 0.04\%) \times 100\%] / 100\% = 0.04\%$$

$$P_{M+1} \text{ i.e. } ^{13}\text{C}, ^2\text{H}, \text{ and } ^{17}\text{O} \text{ contributions from } C_{27}H_{45}O^+ \text{ to } m/z \text{ 386} = 30.42\%$$

Similarly, the theoretical calculation of isotopic peak P_{M+2} for the protonated vitamin D₃ presented below:

$$P(^{18}\text{O}) = [(1 \times 0.20\%) \times 100\%] / 100\% = 0.2\%$$

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

The calculated isotopic abundance of P_{M+1} value 30.42% was higher to the observed value (9.72%). But, the calculated P_{M+2} value 0.2% was lower to the observed value (12.03%) (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., ¹³C, ²H, ¹⁷O, and ¹⁸O) contributions to the mass of the isotopic molecular ion [M+1]⁺ and [M+2]⁺. ²H did not contribute much any isotopic *m/z* ratios because of its less natural abundance compared to the abundances of C and O isotopes in nature^[38,39]. But, the contributions of ¹³C, ¹⁷O, and ¹⁸O was major from vitamin D₃ to the isotopic mass peak at *m/z*

386 and 387 confirmed from the calculations. Therefore, P_M, P_{M+1}, and P_{M+2} of the vitamin D₃ 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).

Table 1: LC-ESI-MS isotopic abundance ratio analysis of control and Biofield Energy Treated vitamin D₃

Parameter	Control sample	Biofield Energy Treated sample
P _M at <i>m/z</i> 385 (%)	100	100
P _{M+1} at <i>m/z</i> 386 (%)	9.72	55.49
P _{M+1} /P _M	0.0972	0.5549
% Change of isotopic abundance ratio (P _{M+1} /P _M) with respect to the control vitamin D ₃		470.88
P _{M+2} at <i>m/z</i> 387 (%)	12.03	7.00
P _{M+2} /P _M	0.1203	0.070
% Change of isotopic abundance ratio (P _{M+2} /P _M) with respect to the control vitamin D ₃		-41.81

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 vitamin D₃ molecule.

The isotopic abundance ratio of P_{M+1}/P_M (²H/¹H or ¹³C/¹²C or ¹⁷O/¹⁶O) in treated vitamin D₃ was very significantly increased by 470.88% compared to the control sample (Table 1). This indicated that the ¹³C, ²H, and ¹⁷O contributions from C₂₇H₄₅O⁺ to the isotopic *m/z* 386 in the Biofield Energy Treated vitamin D₃ sample was very significantly increased compared to the control sample. But, the isotopic abundance ratio of P_{M+2}/P_M (¹⁸O/¹⁶O) in the Biofield Energy Treated vitamin D₃ was significantly decreased by 41.81% compared to the control sample (Table 1). Therefore, the ¹⁸O contribution from C₂₇H₄₅O⁺ to the isotopic *m/z* 387 in the Biofield Energy Treated vitamin D₃ was significantly decreased compared with the control sample. Table 1

The recent discovery of neutrino oscillations seems to give credence to the postulates of Mr. Mahendra Kumar Trivedi on The Trivedi Effect[®]^[17]. Neutrinos are one of the most abundant particles in the Universe; however, are very difficult to observe. Neutrino oscillations imply that neutrinos have small but non-zero masses, which have profound implications to our understanding of elementary particle physics and the Universe. Neutrons and alteration in its number in a molecule lead to the increased or decreased isotopic abundance of the compounds. Changes in atomic/molecular weights are postulated to the changes in atomic mass and charge through the possible mediation of neutrinos^[15,42-44]. Neutrinos only interact *via* the weak force, which is indeed very weak and the consequence is significant. If a neutrino is produced, it travels straightly in any matter as if it is traveling in the vacuum and seldom interacts with matter, i.e., a neutrino produced in the atmosphere of Earth can easily travel through the whole Earth^[42]. Thus, it can be assumed that The Trivedi Effect[®]-Consciousness Energy Healing Treatment might provide the necessary energy for the neutrino

oscillations leads to the alteration of the fundamental physico-chemical properties of a compound by the interactions to the subject(s)^[45,46]. The change in the isotopic abundance ratio of the atoms/molecules leads to 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*^[36]. Therefore, The Biofield Energy Treated vitamin D₃ with altered isotopic abundance ratio (P_{M+1}/P_M and P_{M+2}/P_M) would be advantageous for the designing of better nutraceutical and pharmaceutical formulations.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis: The GC chromatograms of vitamin D₃ showed two clear independent peaks both in control, and Biofield Energy Treated samples (Figure 3). The R_t of the control sample was found at 23.03 and 23.64 minutes, whereas Biofield Energy Treated sample at 23.04 and 23.66 minutes. The results indicated that the polarity of the Biofield Energy Treated sample remained close compared to the control sample. May be due to the *cis* and *trans* isomers of vitamin D₃ both the control and Biofield Energy Treated sample showed two peaks the chromatograms^[47,48].

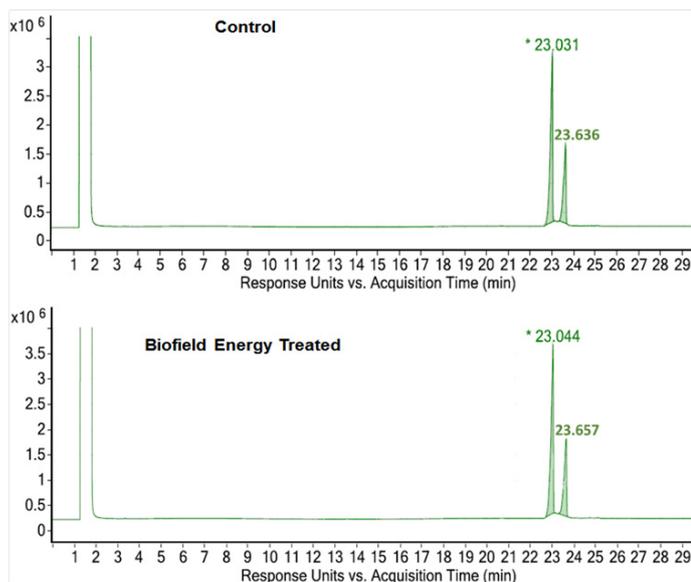


Figure 3: GC chromatograms of the control and Biofield Energy Treated vitamin D₃.

The mass spectra of the control and Biofield Energy Treated vitamin D₃ at R_t of 23.04 minutes exhibited the presence of the molecular ion ($C_{27}H_{44}O^+$) (Figure 4) at m/z 384.4 (calcd for $C_{27}H_{44}O^+$, 384.34). The other mass fragmentation peak at lower m/z 351.4, and 325.3 for $C_{26}H_{39}^{+}$, and $C_{24}H_{37}^+$, respectively in both the control and Biofield Energy Treated vitamin D₃ (Figure 4). The mass fragmentation pattern of both the samples was similar. But the mass peak intensities of the Biofield Energy Treated vitamin D₃ were significantly altered compared to the control sample. The mass peak intensity of the control and Biofield Energy Treated vitamin D₃ were 1127387.63 and 1196286.75, respectively at R_t of 23.04 minutes. Similarly, the mass peak intensities of the control and Biofield Energy Treated vitamin D₃ were 545957.06 and 598460.19, respectively at R_t of 23.65 minutes. The mass peak intensities at R_t 23.04 and 23.65 minutes were significantly increased in the Biofield Energy Treated

sample by 6.11% and 9.62%, respectively (Table 2) compared to the control sample. The mass peak intensities were significantly increased which might be due to the impact of Consciousness Energy Healing Treatment (The Trivedi Effect[®])^[15,45,46]. Fig 3, 4 table 2

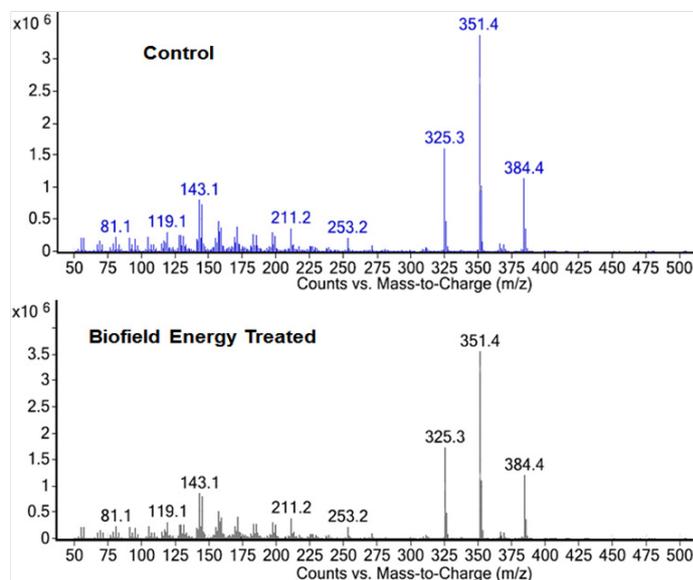


Figure 4: GC-MS spectra of the control and Biofield Energy Treated vitamin D₃ at R_t 23.04 minutes.

Table 2: GC-MS chromatographic and mass spectra analysis at R_t 23.04 and 23.67 minutes of the control and Biofield Energy Treated vitamin D₃.

Parameters	Control sample	Biofield Energy Treated sample	% Change
Masspeak ($m/z=384$) intensity at R _t 23.04 minutes	1127387.63	1196286.75	6.11
Mass peak ($m/z=384$) intensity at R _t 23.65 minutes	545957.06	598460.19	9.62

Nuclear Magnetic Resonance (NMR) Spectroscopy Analysis:

The ¹H and ¹³C NMR spectra shown in Figures 5 and 6, respectively for the control and Biofield Energy Treated vitamin D₃. The analyzed ¹H and ¹³C NMR spectral information are presented in Table 3. The signals for the protons coupling of CH₃, CH₂, CH, and OH protons in both the ¹H NMR spectra of vitamin D₃ were in the range of δ0.48 to 6.17 ppm (Figure 5 and Table 3), which were very close to each other. Similarly, the carbon signals for CH₃, CH₂, CH, =C=, and C-OH groups in both the ¹³C NMR spectra were in the range of 11.72-145.44 (Figure 6 and Table 3). The experimental results were closely matched to the published literature^[48]. The ¹H and ¹³C NMR spectral data indicated that there was no structural modification of The Trivedi Effect[®]-Biofield Energy Treated vitamin D₃ compared to the control sample. Table 3, fig 5, fig 6

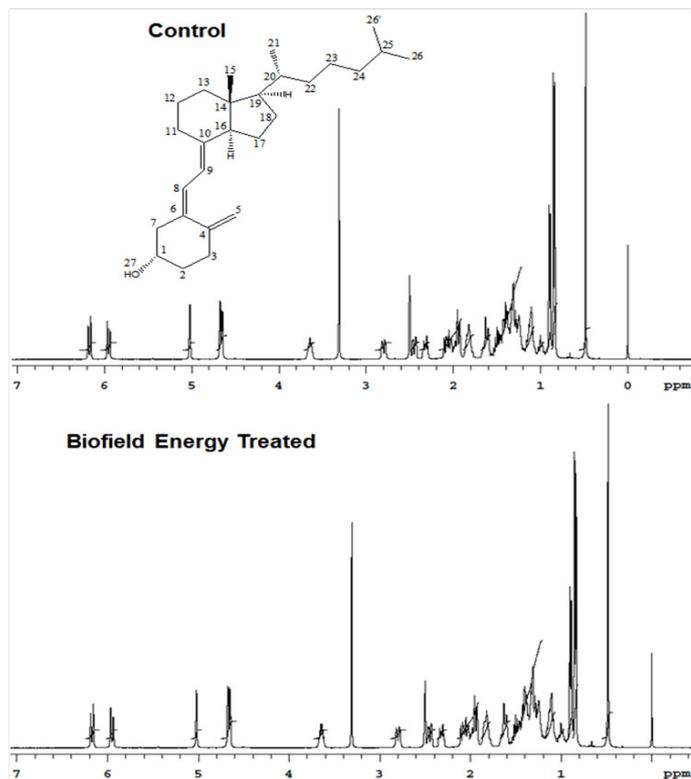


Figure 5: The ¹H NMR spectra of the control and Biofield Energy Treated vitamin D₃.

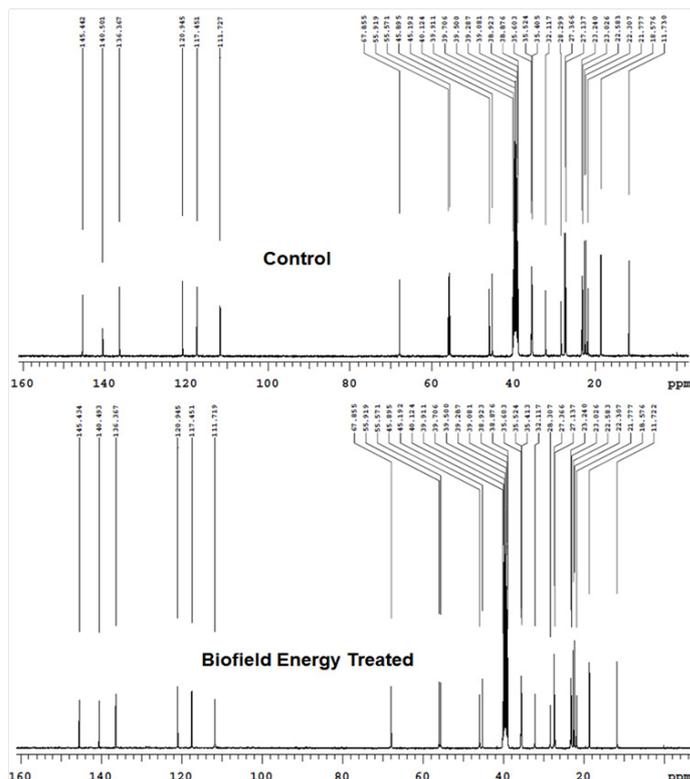


Figure 6: The ¹³C NMR spectra of the control and Biofield Energy Treated vitamin D₃.

Table 3: ¹H and ¹³C NMR spectroscopic data of both the control and Biofield Energy Treated vitamin D₃

¹ H & ¹³ C S. No	¹ H NMR δ (ppm) & Multiplicity		¹³ C NMR δ (ppm)	
	Untreated	Biofield Energy Treated	Untreated	Biofield Energy Treated
1	3.65 (m, <i>J</i> = 20 Hz, H)	3.65 (m, <i>J</i> = 20 Hz, H)	67.86	67.86
2	1.63 (m, <i>J</i> = 24 Hz, 2H)	1.63 (m, <i>J</i> = 24 Hz, 2H)	35.41	35.41
3	1.82 (m, <i>J</i> = 28 Hz, 2H)	1.82 (m, <i>J</i> = 28 Hz, 2H)	32.12	32.12
4	--	--	136.37	136.37
5	4.67 (d, <i>J</i> = 12 Hz, 2H)	4.67 (d, <i>J</i> = 12 Hz, 2H)	111.73	111.72
6	--	--	145.44	145.43
7	1.93-2.11 (m, 2H)	1.93-2.11 (m, 2H)	45.90	45.89
8	6.17 (d, <i>J</i> = 12 Hz, H)	6.17 (d, <i>J</i> = 12 Hz, H)	120.95	120.95
9	5.95 (d, <i>J</i> = 12 Hz, H)	5.95 (d, <i>J</i> = 12 Hz, H)	117.45	117.45
10	--	--	140.51	140.49
11	1.93-2.11 (m, 2H)	1.93-2.11 (m, 2H)	28.30	28.31
12	0.96-1.20 (m, 2H)	0.96-1.20 (m, 2H)	22.31	22.31
13	0.96-1.20 (m, 2H)	0.96-1.20 (m, 2H)	40.12	40.12
14	--	--	45.19	45.19
15	0.48 (s, 3H)	0.48 (s, 3H)	11.73	11.72
16	2.78-2.82 (d, <i>J</i> = 16 Hz, H)	2.78-2.82 (d, <i>J</i> = 16 Hz, H)	55.92	55.92
17, 18, 22, 23, 24	1.25-1.46 (m, 10H)	1.25-1.46 (m, 10H)	23.03, 27.37, 35.60, 23.24, 39.91	23.03, 27.37, 35.60, 23.24, 39.91
19, 20, 25	2.29-2.47 (m, 3H)	2.29-2.47 (m, 3H)	55.57, 35.52, 27.14	55.57, 35.52, 27.14
21	0.87 (d, <i>J</i> = 6 Hz, 3H)	0.87 (d, <i>J</i> = 6 Hz, 3H)	18.58	18.58
26, 26'	0.85 (m, <i>J</i> = 8 Hz, 6H)	0.85 (m, <i>J</i> = 8 Hz, 6H)	22.58, 21.77	22.58, 21.77
27(OH)	5.02 (s)	5.02 (s)	--	--

s- singlet, d-doublet, and m-multiplet.

Conclusions

The Trivedi Effect®-Consciousness Energy Healing Treatment on cholecalciferol/vitamin D₃ by the renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi showed the astonishing significant impact on the isotopic abundance ratios and relative peak intensities. The LC-ESI-MS analysis of both the samples showed the mass of protonated ion at m/z 385.3 (calcd for C₂₇H₄₅O⁺, 385.35) at the retention time (R_t) 20.7 minutes. The liquid chromatographic peak area of the Biofield Energy Treated sample was significantly improved by 16.00% compared to the control sample. Similarly, the isotopic abundance ratio of P_{M+1}/P_M (²H/¹H or ¹³C/¹²C or ¹⁷O/¹⁶O) was very significantly increased by 470.88% in the Biofield Energy Treated sample compared to the control sample. But, the isotopic abundance ratio of P_{M+2}/P_M (¹⁸O/¹⁶O) in the Biofield Energy Treated sample also significantly decreased by 41.81% compared to the control sample. Therefore, the ²H, ¹³C, and ¹⁷O contributions from C₂₇H₄₅O⁺ to m/z 386 was significantly increased, whereas ¹⁸O contribution from C₂₁H₂₁O₆⁺ to m/z 387 was decreased in the Biofield Energy Treated sample compared with the control sample. The GC-MS analysis showed that the parent molecular mass peak intensities (m/z 384.4) in the Biofield Energy Treated sample at R_t 23.04 and 23.65 minutes were significantly increased by 6.11% and 9.62%, respectively compared with the control sample. The proton and carbon signals for CH₃, CH₂, CH, C-OH, and =C= groups in the ¹H and ¹³C NMR spectra of the control and Biofield Energy Treated samples were similar. The altered isotopic abundance ratios and mass peak intensities might be due to energy produced by The Trivedi Effect®-Consciousness Energy Healing Treatment *via* the possible mediation of neutrinos, which further lead to the change in the kinetic isotope effects and physicochemical properties of the Biofield Energy Treated sample. Thus, The Trivedi Effect®-Consciousness Energy Healing Treated vitamin D₃ would be advantageous for designing better nutraceutical and pharmaceutical formulations which might provide better therapeutic response against vitamin D deficiency, rickets, osteoporosis, arthritis, diabetes mellitus, cancer, cardiovascular diseases, mental disorders, inflammations, infections, stress, aging, glucose intolerance, multiple sclerosis, Parkinson's and Alzheimer's diseases, dementia, cognitive impairment in older adults, *etc.*

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