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

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Abstract

Ascorbic acid (vitamin C) is water-soluble essential dietary nutrient adequately available in the food sources, required by both the plants and animals for their essential metabolic process. In this study, the impact of the Trivedi Effect®-Consciousness Energy Healing Treatment on the structural properties and the isotopic abundance ratio of vitamin C was investigated using LC-MS and GC-MS spectroscopy. Vitamin C sample was divided into two parts, i.e., control and treated sample. Only the treated part was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a famous Biofield Energy Healer, Alice Branton. The LC-MS spectra of both the samples at retention time (Rt) 1.8 minutes exhibited the mass of the deprotonated molecular ion peak at *m/z* 175 $[M-H]^-$ (calculated for $C_6H_7O_6$-, 175.02). The peak area of the treated vitamin C was significantly increased by 8.68% compared to the control sample. The LC-MS found isotopic abundance ratio of P_{M+1}/P_M ($^2H/1H$ or $^{13}C/12C$ or $^{17}O/16O$) in the treated vitamin C was significantly decreased by 59.45% compared with the control sample.

Thus, ^{13}C, ^2H, and ^{17}O contributions from $(C_6H_7O_6)$- to *m/z* 176 in the Biofield treated sample were decreased significantly as compared to the control. But the GC-MS based isotopic abundance ratio of P_{M+2}/P_M in the treated vitamin C was significantly increased by 66.67% compared with the control sample. So, 18O contributions from $(C_6H_8O_6)$+ to *m/z* 177 in the treated sample were significantly increased as compared to the control. Isotopic abundance ratio of P_{M+1}/P_M (18O/16O) in the treated vitamin C was significantly altered compared to the control sample. It can be anticipated that the changes in isotopic abundance and mass peak intensities might be attributable to changes in nuclei, possibly through the interference of neutrino particles via the Trivedi Effect®-Consciousness Energy Healing Treatment. The altered isotopic abundance ratios of the treated vitamin C would influence the atomic bond vibration and bond strength, its physical stability, and alter the rate of reactions in the body. The new form of treated vitamin C would be more efficacious novel pharmaceutical formulations that might offer better therapeutic response against scurvy, obesity, cardiovascular diseases (myocardial infarction, stroke, etc.), hypertension, neurodegenerative diseases (Alzheimer's disease), cancer, autoimmune diseases (rheumatoid arthritis), etc.

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

Introduction

Ascorbic acid (vitamin C) is water-soluble essential dietary nutrient adequately available in the food sources, i.e., citrus fruits, tomatoes, red peppers, potatoes, milk, animal liver, oysters, etc. [1,2]. Vitamin C required by both plants and animals in a specific range for the essential metabolic process. Ascorbic acid converts to ascorbate (an ionized form at neutral pH or above pH 5) inside the cells, which is an electron donor and acts as a potent water-soluble antioxidant, protect against oxidative stress [3-5]. Besides, it serves as a cofactor in many enzymatic and few non-enzymatic reactions, helps in the synthesis of collagen, carnitine, neurotransmitters; act as a co-factor in the biosynthesis of catecholamines, L-carnitine, cholesterol, amino acids, and some peptide hormones; helps in the metabolism of microsome, and synthesis and catabolism of tyrosine in the body [2].

It also helps in the wound-healing and prevents bleeding from capillaries. It maintains the internal microenvironment, useful in the prevention and treatment of scurvy, obesity, hypertension, cardiovascular diseases (myocardial infarction,

stroke, etc.), cancer, neurodegenerative diseases (Alzheimer's disease), autoimmune diseases (rheumatoid arthritis), etc. [3, 6-11]. Deficiency of vitamin C leads to scurvy, and other less noticeable signs are the weak immune system, autoimmune disease, gingivitis, slow wound healing, dry and splitting hair, nose bleeding, leaky gut, swollen and painful joints, etc. Long-term vitamin C deficiency leads to cancer, high blood pressure, stroke, gallbladder disease, atherosclerosis, etc. [7-9]. Low plasma concentrations of vitamin C are reported in patients with diabetes, infections, and smokers [12]. The National Academy of Sciences, Food and Nutrition Board reported that the tolerable upper intake level (UL) of vitamin C is 2,000 mg/day. Large doses of vitamin C are toxic and may cause indigestion, diarrhoea, headache, fatigue, disturbed sleep, haemochromatosis, skin rashes, suppress the production of progesterone from the corpus luteum in healthy subjects [2,13].

The stability of vitamin C is the major issue, which degrades during processing, storage, and cooking [13-15]. Some research confirmed that exposure to air and storage temperature significantly affect the stability of vitamin C [14,15]. Dissolution, absorption, bioavailability, and stability of a vitamin C depend upon its physicochemical properties [17]. Improvement of the physicochemical properties of pharmaceutical and nutraceutical products is a challenge to global scientists [17]. In this context, it was observed that the Trivedi Effect® (Biofield Energy Healing Treatment) has the significant impact on various physicochemical properties such as particle size, surface area, thermal behaviour, and bioavailability of pharmaceutical/nutraceutical compounds [18-21]. The Trivedi Effect® is a natural and scientifically proved phenomenon [22]. "Biofield Energy" the electromagnetic energy which exists surrounding all the living beings, which can transmit the electromagnetic energy in the form of bio-photons. It generated by the continuous movement of the electrically charged particles and organs movement (i.e., ions, cells, heat, and blood flow, etc.) inside the body.

Biofield Energy Healing specialists can harness the energy from the "Universal Energy Field" and can transmit into any living and non-living object(s), this process via the possible mediation of neutrinos is called Biofield Energy Healing Treatment [23,24]. Biofield Therapies have been reported with significant positive outcomes against various diseases, human health, and wellness [25]. National Center of Complementary and Integrative Health (NCCIH) has been recognized and accepted Biofield Energy Healing treatment as a Complementary and Alternative Medicine (CAM) health care approach in addition to other therapies, medicines, and practices, i.e., homeopathy, Ayurveda, Chinese herbs and medicines, Reiki, Yoga, etc. [26]. Most of the U.S.A. population has accepted these therapies with significant benefits [27]. The potential of the Trivedi Effect® has been proved with scientific experiment and widely reported with the astonishing results, i.e., altered physical and thermal properties of metals, ceramics, polymers, and organic compounds [28-31], improved productivity of crops [32,33], healing cancer [34], and altered isotopic abundance ratio of pharmaceutical compounds [35].

The study of stable isotope ratio analysis has different applications in multiple scientific fields for understanding the isotope effects resulting from the variation of the isotopic composition of the molecule [36, 37]. This study was performed by using the mass spectrometry (MS) techniques such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) in low micromolar concentration with sufficient precision [37,38]. The Biofield Energy Healing Treatment could be an economical approach for designing better pharmaceuticals formulations. Therefore, LC-MS and GC-MS based structural characterization and 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+1}/P_M ($^{18}O/^{16}O$) in the Trivedi Effect®-Consciousness Energy Healing Treated Vitamin C was evaluated compared to the control sample.

Materials and Methods

Chemicals and reagents

The test sample L-ascorbic acid (vitamin C) was purchased from Alfa Aesar, India. Similarly, other chemicals used during the experiments were purchased in India.

Consciousness energy healing treatment strategies

Vitamin C powder was splited into two parts. One part of the vitamin C powder was considered as a control sample, which did not receive Biofield Energy Treatment. However, the other part of vitamin C was treated with the Trivedi Effect® remotely under standard laboratory conditions for ~3 minutes and known as the treated vitamin C sample. The Biofield Energy Treatment was given by famous Biofield Energy Healer, Alice Branton, USA, through her unique energy transmission process to the test sample. Further, the control sample was treated with a "sham" healer, who did not have any knowledge about the Biofield Energy. After that, both the vitamin C 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 vitamin C was carried out with the help of LC-MS ThermoFisher Scientific (USA), equipped with an ion trap detector and a triple-stage quadrupole mass spectrometer. The column used here was a reversed-phase Thermo Scientific Synchronis C18 (Length-250 mm X ID 4.6 mm X 5 micron), maintained at 25°C. The diluent used for the sample preparation was methanol and water. 10µL of vitamin C solution was injected, and the analyte was eluted using 80% acetonitrile+20% 5mM ammonium acetate (gradient condition) pumped at a constant flow rate of 1 mL/min (total run time 10 min). Peaks were monitored at

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220 nm using the PDA detector. The mass spectrometric analysis was performed under -ve ESI mode. The total ion chromatogram, retention time (R_t), peak area%, and mass spectrum of the individual peak were recorded. The natural abundance of each isotope (C, H, and O) can be predicted from the comparison of the height of the isotope peak than base peak. The values of the natural isotopic abundance of the common elements are taken from the literature [37,39-41]. The LC-MS based isotopic abundance ratio (P_{M+1}/P_M) of the Biofield Energy Treated vitamin C compared to the control sample was calculated as per equation 1.

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

Where IAR = isotopic abundance ratio in the control and treated vitamin C.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS of both the samples of vitamin C were analyzed with the help of Perkin Elmer Gas chromatograph equipped with a PE-5MS (30Mx250microsx0.250microns) capillary column and coupled to a single quadrupole mass detector was operated with electron impact (EI) ionization in positive mode. The oven temperature was programmed from 75°C (5 min hold) to 250°C (2.5min hold) @ 10°C/min (total run time 25min). The sample was prepared taking 100mg of the vitamin C is in 2ml methanol as a diluent. Injection volume was 5µl. The GC-MS based isotopic abundance ratios (P_{M+1}/P_M) of the Biofield Energy Treated vitamin C compared to the control sample was calculated using equation 1.

Results and Discussion

Liquid chromatography-mass spectrometry (LC-MS)

The LC-MS chromatograms and mass spectra of both the control and Biofield Energy Treated vitamin C are depicted in Figures 1 & 2, respectively. The chromatograms of vitamin C showed the major chromatographic peak at the retention time (R_t) of 1.8 minutes for both the case (Figure 1). The peak area of the Biofield Energy Treated vitamin C (10010392.14) was significantly increased by 8.68% compared to the control sample (9210661.92). This indicated that the solubility of the treated vitamin C was increased compared to the control sample.

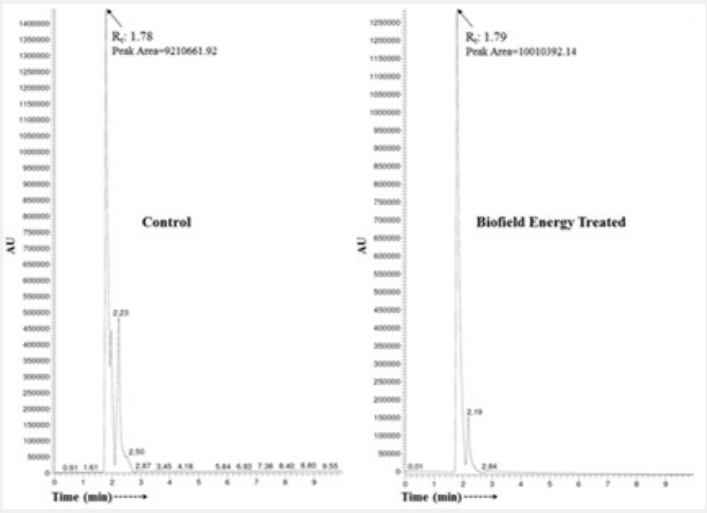

Figure 1: Study of Liquid chromatograms of the control and Biofield Energy Treated vitamin C.

The mass spectrometric analysis was performed in -ve ESI mode. Hence, the mass spectra of both the samples of vitamin C exhibited the mass of the deprotonated molecular ion $[M\text{-}H]^-$ peak at *m/z* 175 (calculated for $C_6H_7O_6$-, 175.02) along with other low molecular fragmentation peaks at 157, 143, 128, 115, 111, 97, and 87 for $C_6H_5O_5$-, $C_6H_7O_4$-, $C_5H_4O_4$-, $C_5H_7O_3$-, $C_5H_3O_3$-, $C_5H_5O_2$-, and $C_4H_7O_2$-, respectively in case of both the samples (Figures 2 & 3). The published literature supported the experimental data [42].

The LC-MS spectra of both the vitamin C samples showed the mass of the molecular ion peak at *m/z* 175 $[M\text{-}H]^-$ with relative intensity of 100%. The theoretical calculation of P_{M+1} for vitamin C was presented as below:

P (^{13}C) = [(6 x 1.1%) x 100% (the actual size of the M+ peak)] / 100% = 6.6%

P (^{2}H) = [(7 x 0.015%) x 100%] / 100%= 0.105%

P (^{17}O) = [(6 x 0.04%) x 100%] / 100% = 0.24%

P_{M+1}, i.e., ^{13}C, ^{2}H, and ^{17}O contributions from ($C_6H_7O_6$)- to *m/z* 176 = 6.95%

Based on the above calculation, it has been found that ^{13}C and ^{17}O have a major contribution to *m/z* 176. The LC-MS based isotopic abundance ratio analysis P_M and P_{M+1} for vitamin C near *m/z* 175 and 176, respectively of the vitamin C, which were obtained from the observed relative peak intensities of $[M^+]$ and

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[(M+1)$^+$] peaks, respectively in the mass spectra (Table 1). The percentage change of the isotopic abundance ratio (P_{M+1}/PM) in the Biofield Energy Treated vitamin C was significantly decreased by 59.45% compared to the control sample (Table 1). Therefore, it was concluded that the ^{13}C, ^{2}H, and ^{17}O contributions from ($C_6H_7O_6$)- to *m/z* 176 in the Biofield Energy Treated sample were significantly decreased compared to the control sample.

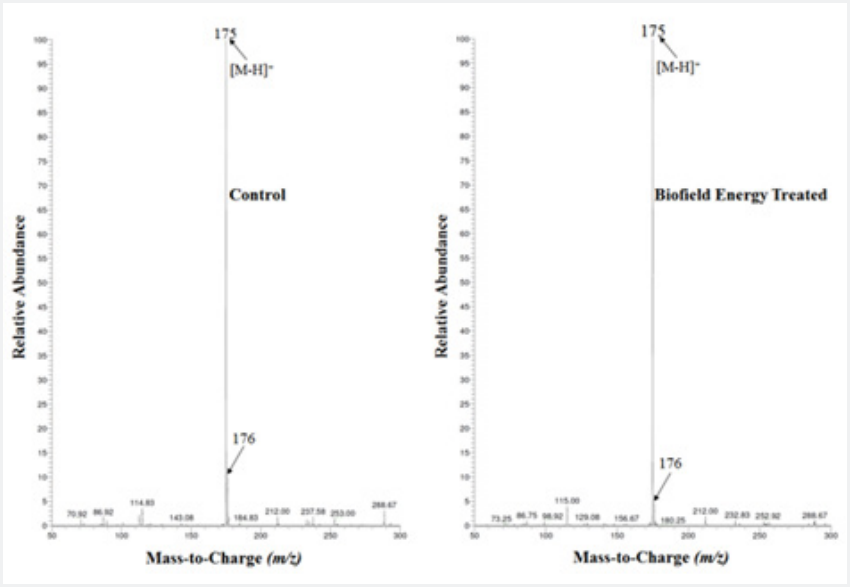

Figure 2: Study of Mass spectra of the control and Biofield Energy Treated vitamin C at R_t 1.8 minutes.

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

Parameter	Control Sample	Biofield Energy Treated Sample
P_M at *m/z* 175 (%)	100	100
P_{M+1} at *m/z* 177 (%)	10.26	4.16
P_{M+1}/P_M	0.10	0.04
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control sample		-59.45

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.

Gas Chromatography-mass spectrometry (GC-MS) analysis

The control and Biofield Energy Treated vitamin C showed several chromatographic peaks in the GC-MS chromatograms (Figures 4 & 5). But the chromatographic peak at R_t of 12.6 minutes in the chromatogram reflected the molecular mass in the spectrum (Figures 4 & 5). The parent molecular ion peak of vitamin C at *m/z* 175 [M]$^+$ (calculated for $C_6H_8O_6$+, 175.02) in both the samples, along with the fragment ion peaks near *m/z* 157, 138, 110, and 70 which were observed (Figures 4 & 5). The isotopic abundance ratio influence by the mass peak intensities and which significantly altered in case of Biofield Energy Treated sample compared to the control sample. The GC-MS spectra of both vitamin C showed the mass of the molecular ion peak [M]$^+$ at *m/z* 175 (calculated for $C_6H_8O_6$+, 176.03). The theoretical calculation of P_{M+2} for vitamin C was presented as below:

P (^{18}O) = [(6 x 0.20%) x 100%] / 100% = 1.2%

P_{M+2} , i.e., ^{18}O contributions from ($C_6H_8O_6$)+ to *m/z* 177 = 1.2%

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From the above calculation, it has been found that 18O have a major contribution to *m/z* 177. The GC-MS based isotopic abundance ratio analysis of the Biofield Energy Treated vitamin C was calculated compared to the control sample. P_M and P_{M+2} for vitamin C near *m/z* 175 and 177, respectively of both the samples, which were obtained from the observed relative peak intensities of [M$^+$] and [(M+2)$^+$] peaks, respectively (Table 2). The isotopic abundance ratio of P_{M+2}/P_M in the Biofield Energy Treated vitamin C was significantly increased by 66.67% compared with the control sample (Table 2). Hence, 18O contributions from ($C_6H_8O_6$)+ to *m/z* 177 in the Biofield Energy Treated sample were significantly increased compared with the control sample.

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

Parameter	Control Sample	Biofield Energy Treated Sample
P_M at m/z 175 (%)	0.05	0.03
P_{M+2} at m/z 177 (%)	0.08	0.08
P_{M+2}/P_M	1.6	2.67
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control sample		66.67

P_M: the relative peak intensity of the parent molecular ion [M^+]; P_{M+2}: the relative peak intensity of the isotopic molecular ion [$(M+2)^+$]; M: mass of the parent molecule.

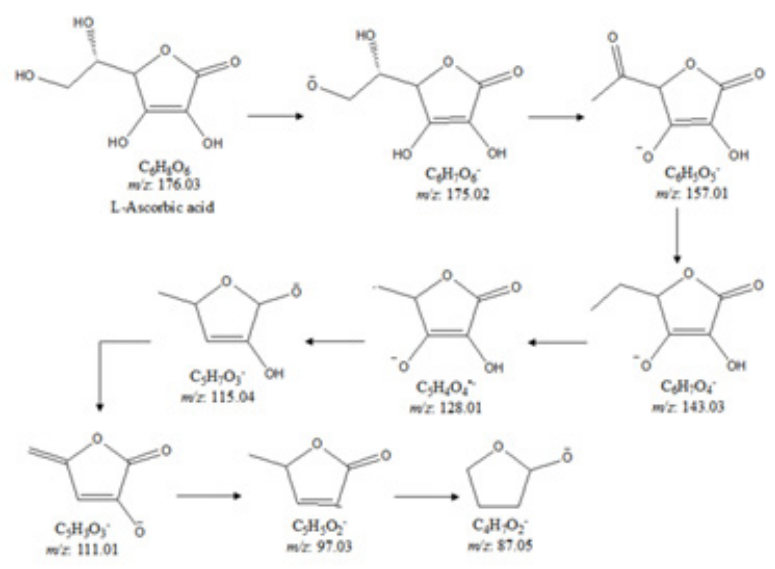

Figure 3: Proposed fragmentation pattern of vitamin C in –ve ion mode.

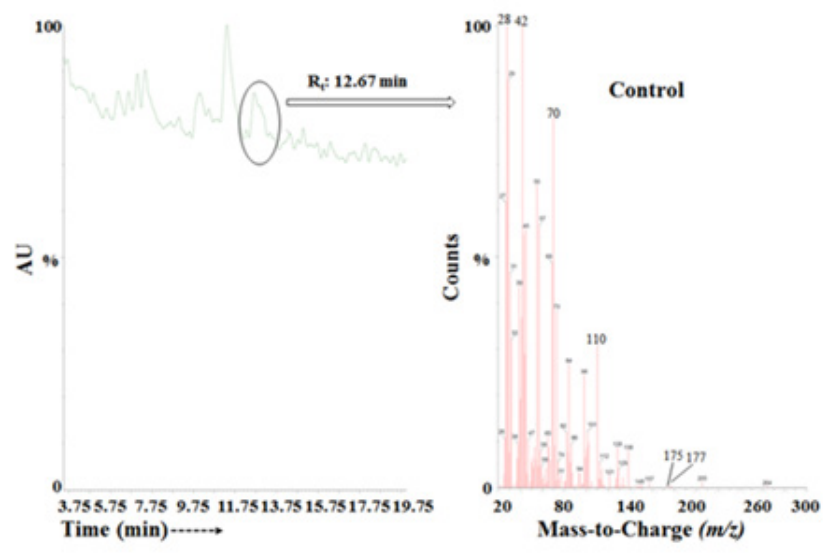

Figure 4: The GC-MS chromatogram and mass spectra of the control vitamin C.

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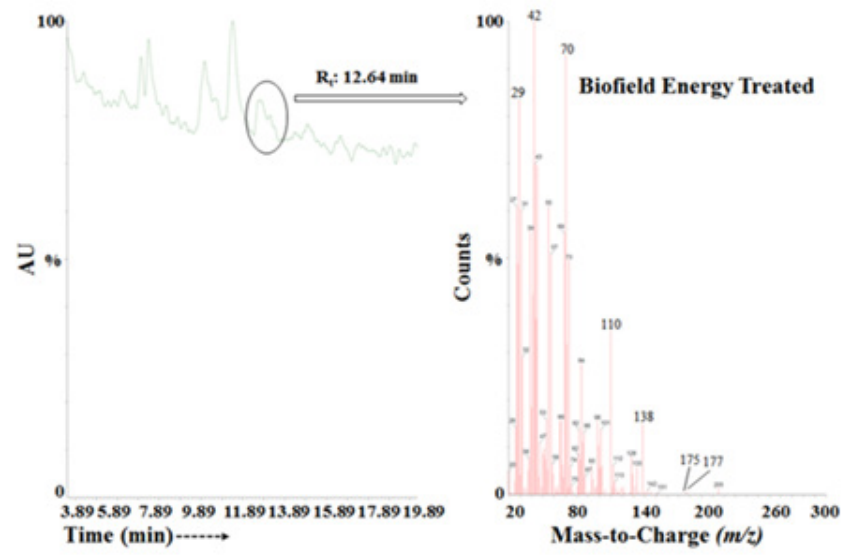

Figure 5: The GC-MS chromatogram and mass spectra of the Biofield Energy Treated vitamin C.

LC-MS and GC-MS study confirmed the structure of the sample as vitamin C. 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 Biofield Energy Treated vitamin C was significantly altered compared to the control sample. The altered isotopic composition in the atomic level of the Trivedi Effect®-Consciousness Energy Healing Treated Vitamin C 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®. As per the literature, the neutrinos change identities which are only possible if the neutrinos possess mass and can interchange their phase from one phase to another internally. Therefore, the neutrinos can interact with protons and neutrons in the nucleus [22,37,38]. The altered isotopic abundance ratios of the treated vitamin C would influence the atomic bond vibration and bond strength, its physical stability, and alter the rate of reactions in the body [43]. The new form of vitamin C would be very useful to design more efficacious pharmaceutical formulations that might offer better therapeutic response against scurvy, obesity, cardiovascular diseases (myocardial infarction, stroke, etc.), hypertension, neurodegenerative diseases (Alzheimer's disease), cancer, autoimmune diseases (rheumatoid arthritis), etc.

Conclusion

The Trivedi Effect® showed the noteworthy impact on the isotopic abundance ratios and mass peak intensities of vitamin C. The LC-MS spectra of both the control and Biofield Energy Treated samples at Rt 1.8 minutes exhibited the mass of the deprotonated molecular ion peak at *m/z* 175 $[M-H]^-$ (calculated for $C_6H_7O_6$-, 175.02). In the Biofield Treated vitamin C group, the peak area was significantly increased by 8.68% compared to the control sample. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M ($^2H/1H$ or $^{13}C/12C$ or $^{17}O/16O$) in the Biofield Energy Treated vitamin C was significantly decreased by 59.45% compared with the control sample. Thus, ^{13}C, ^2H, and ^{17}O contributions from $(C_6H_7O_6)$- to *m/z* 176 in the Biofield Energy Treated sample were significantly decreased compared with the control sample. But the GC-MS based isotopic abundance ratio of P_{M+2}/P_M in the Biofield Energy Treated vitamin C was significantly increased by 66.67% compared with the control sample. So, ^{18}O contributions from $(C_6H_8O_6)$+ to *m/z* 177 in the Biofield Energy Treated sample were significantly increased as compared to the control. The isotopic abundance ratio of P_{M+1}/P_M ($^{18}O/16O$) in the treated vitamin C was significantly altered compared to the control sample. It can be anticipated that the changes in isotopic abundance and mass peak intensities might be attributable to changes in nuclei, possibly through the interference of neutrino particles via the Trivedi Effect® - Consciousness Energy Healing Treatment. The altered isotopic abundance ratios of the treated vitamin C would influence the atomic bond vibration and bond strength, its physical stability, and alter the rate of reactions in the body. The new form of treated vitamin C would be more efficacious novel pharmaceutical formulations that might offer better therapeutic response against scurvy, obesity, cardiovascular diseases (myocardial infarction, stroke, etc.), hypertension, neurodegenerative diseases (Alzheimer's disease), cancer, autoimmune diseases (rheumatoid arthritis), etc.

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