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

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ABSTRACT

Pyridoxine (vitamin B_6) is commonly found in foods and dietary supplements, has an important role in physiologic development. In this study, the influence of the Trivedi Effect®-Biofield Energy Healing Treatment on the structural properties and the isotopic abundance ratio of vitamin B_6 were evaluated using LC-MS and GC-MS. Vitamin B_6 sample was divided into two parts and termed as control and treated samples. Only the treated vitamin B_6 was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a well-known Biofield Energy Healer, Dahryn Trivedi. The LC-MS spectra of both the vitamin B_6 samples at retention time 2.3 min exhibited the mass of the protonated molecular ion peak $[M+H]^+$ at *m/z* 170 (calculated for $C_8H_{12}NO_3^+$; 170.08) in the MS spectrum. The peak area% of the treated vitamin B_6 was increased by 1.32% compared to the control sample. The isotopic abundance ratio of P_{M+1}/P_M in the treated vitamin B_6 was significantly decreased by 36.94% compared with the control sample. Thus, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_8H_{12}NO_3)^+$ to *m/z* 171 in the treated sample were significantly decreased compared with the control sample. Similarly, in the GC-MS chromatograms, the peak area of the treated sample was increased by 4.54% compared to the control sample. Similarly, the mass peak intensity of the treated vitamin B_6 at *m/z* 153 was significantly increased by 66.69% compared to the control sample. The isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) and peak area in the treated vitamin B_6 was significantly decreased compared to the control sample. The decreased isotopic abundance ratio of the treated vitamin B_6 may decrease the intra-atomic bond strength, decrease its physical stability. The increased peak area of the treated vitamin B_6 might be responsible for the increase in solubility and bioavailability compared to the control sample. The new form of vitamin B_6 would be better for designing more efficacious pharmaceutical formulations for the prevention and treatment of vitamin B_6 deficiency, anaemia, seizures, cardiovascular disease, tuberculosis, Alzheimer's disease, cancer, anxiety, hypertension, asthma, depression, dysmenorrhea, breast pain, etc.

Keywords: The Trivedi Effect®, Biofield Energy, Consciousness Energy Healing Treatment, Vitamin B_6, Isotopic abundance

INTRODUCTION

Pyridoxine (vitamin B_6) is a water-soluble vitamin and has an important role in physiologic development [1]. Vitamin B_6 is naturally found as pyridoxal, pyridoxine, and pyridoxamine; in the human body, all the forms converted into pyridoxal phosphate (the active form of vitamin B_6) [2]. Vitamin B_6 commonly found in foods (fish, poultry, meat, tofu, chickpeas, nuts, bananas, whole grains, spinach, etc.) and dietary supplements [2,3]. Pyridoxal phosphate acts as a coenzyme for more than 100 enzymatic reactions; biosynthesis of neurotransmitters; maintaining healthy levels of homocysteine, the amino acid in the blood; gluconeogenesis, glycogenolysis, immune function, hemoglobin formation; involved in the metabolism of amino acid, carbohydrates, and lipids [2-5]. Pyridoxine

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hydrochloride is a salt form of vitamin B_6 commonly used in the pharmaceutical industries [6].

Vitamin B_6 generally used for the prevention and treatment of vitamin B_6 deficiency diseases, metabolic disorder, Alzheimer's disease, sideroblastic anemia, anxiety, asthma, cardiovascular disease, pulmonary tuberculosis, diabetes, hyperhomocysteinaemia, cancer, pyridoxine-dependent epilepsy, depression, attention deficit hyperactivity disorder, dysmenorrhea, post-partum lactation suppression, McArdle's disease, osteoporosis, nausea and vomiting in pregnancy, problems from isoniazid, mushroom poisoning, etc. [1,3-8]. Vitamin B_6 rarely shows side effects, but it can interact with the drugs like valproic acid, carbamazepine, phenytoin, cycloserine, and theophylline, which might adversely affect vitamin B_6 levels in the body [3,5]. Vitamin B_6 is very sensitive to light. It is soluble in water and alcohol, sparingly soluble acetone and insoluble in ether and chloroform. When it heated to decomposition, it emits very toxic oxide fumes of nitrogen and hydrogen chloride [9].

The physical and chemical properties of any drug material play a vital role in the solubility, absorption, bioavailability, etc. Improvement of the quality of drug with respect to the solubility, stability, and bioavailability is the biggest challenge for the pharmaceutical scientists. The Trivedi Effect®-Consciousness Energy Healing Treatment in larger extent has the incredible impact on the particle size, surface area, crystal size, thermal properties, and bioavailability of pharmaceutical/nutraceutical compounds [10-14]. Whereas, the Trivedi Effect® is a natural and only scientifically proven phenomenon in which an individual can control and make use of this inherently intelligent energy anywhere on the planet *via* the possible mediation of neutrinos [15].

In the human body the continuous moment of the ions, cells, blood, heart, etc. form a quantum of energy matrix around the body is called the "Biofield Energy" [16,17]. Biofield Energy Healers can pass this energy into any living or nonliving object(s) anywhere on the planet, and the overall process is called Biofield Energy Healing Treatment [17,18]. This treatment has significant positive outcomes against various disease [19]. The National Center of Complementary and Integrative Health has recognized and accepted Biofield Energy Healing as a Complementary and Alternative Medicine (CAM) health care approach along with the other therapies, medicines, and practices, such as yoga, Ayurveda, Qi Gong, Tai Chi, etc. [20,21]. Most of the USA population has accepted these CAM therapies [22]. Similarly, the Trivedi Effect® also have reported astonishing scientific results altering the physicochemical and thermal properties of the several non-living and living object(s), i.e., organic compounds, metals, polymers, ceramic, crops, microbes, cancer cells [23-30], etc. The Trivedi Effect®-Consciousness Energy Healing Treatment has also enhanced the isotopic abundance ratio of organic compounds [31,32].

In order to understand the isotope effects resulting from the variation of the isotopic composition of the molecule, the stable isotope ratio analysis is important and it has many applications in different field of science [33,34]. The isotope ratio analysis usually performed by using the gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) in low micromolar concentration with sufficient precision [34,35]. Therefore, the structural properties characterization and the isotopic abundance ratio analysis of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) in the Trivedi Effect®-Consciousness Energy Healing Treated vitamin B_6 was performed compared to the control sample using LC-MS and GC-MS analytical techniques.

MATERIALS AND METHODS

Chemicals and Reagents

The test sample pyridoxine hydrochloride (100%) was purchased from TCI, Japan and remaining chemicals used in the experiments were purchased in India.

❖ Consciousness Energy Healing Treatment Strategies

The test sample pyridoxine hydrochloride powder sample was divided into two parts. One part of the sample was called the control sample, which did not receive the Trivedi Effect®-Consciousness Energy Healing Treatment, but was treated with a "sham" healer who did not have any knowledge about the Biofield Energy Treatment. However, the other part called the Biofield Energy Treated pyridoxine received the Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 min through the healer's unique energy transmission process by the renowned Biofield Energy Healer, Dahryn Trivedi, USA. After the treatment both the vitamin B_6 samples were kept in sealed conditions and characterized using sophisticated analytical techniques.

❖ Characterization

➢ Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis and Calculation of Isotopic Abundance Ratio

The LC-MS analysis of vitamin B_6 was carried out with the help of LC-MS ThermoFisher Scientific (USA) equipped with an ion trap detector connected with a triple-stage quadrupole mass spectrometer. The reversed phase column Thermo Scientific Synchronis C18 (Length-250 mm X ID 4.6 mm X 5 micron), maintained at 25˚C was used. Methanol and water were used as diluent for the sample preparation. 10 µL of vitamin B_6 solution was injected, and the analyte was eluted using acetonitrile + 5 mM ammonium acetate (80:20) pumped at a constant flow rate of 1 mL/min. Chromatographic separation was achieved using

gradient condition and the total run time was 10 min. Peaks were monitored at 220 nm using the PDA detector. The mass spectrometric analysis was performed under +ve ESI mode.

The values of the natural isotopic abundance of the common elements are obtained from the literature [34,36-38]. The LC-MS based isotopic abundance ratio (P_{M+1}/P_M) for both the samples was calculated using equation 1.

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

Where $IAR_{Control}$ and $IAR_{Treated}$ is the isotopic abundance ratio in the treated and control vitamin B_6 sample, respectively.

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

GC-MS of the vitamin B_6 samples were 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 programmed from 75°C (5 min hold) to 250°C (2.5 min hold) @ 10°C /min (total run time 25 min). The sample was prepared taking 100 mg of the vitamin B_6 in 4 ml methanol as a diluent. Injection volume was 5 µl. The % change in the peak area and mass peak intensities of the treated vitamin B_6 was calculated compared to the control sample using the following equation 2:

$$\% \text{ Change} = \frac{[\text{Treated} - €\text{control}]}{€\text{control}} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

- **Liquid chromatography-mass spectrometry (LC-MS)**

The hydrochloride salt of vitamin B_6 samples showed a single major chromatographic peak at retention time (R_t) of 2.3 min **(Figure 1)**. The peak area% of the Biofield Energy Treated vitamin B_6 was increased by 1.32% compared to the control sample. The mass spectra of both the samples exhibited the protonated molecular mass peak $[M+H]^+$ at *m/z* 170 (calculated for $C_8H_{12}NO_3^+$, 170.08) with 100% base peak intensity in the MS spectrum in +ve ion mode was confirmed to be vitamin B_6 **(Figures 2 and 3)**, which was reported in the literature [39]. Further, other lower-mass peaks at *m/z* 152, 134, 125, and 94 for $C_8H_{10}NO_2^+$, $C_8H_8NO^{\bullet+}$, $C_7H_{11}NO^{2+}$, and $C_6H_8N^+$, respectively in both the samples **(Figures 2 and 3)**.

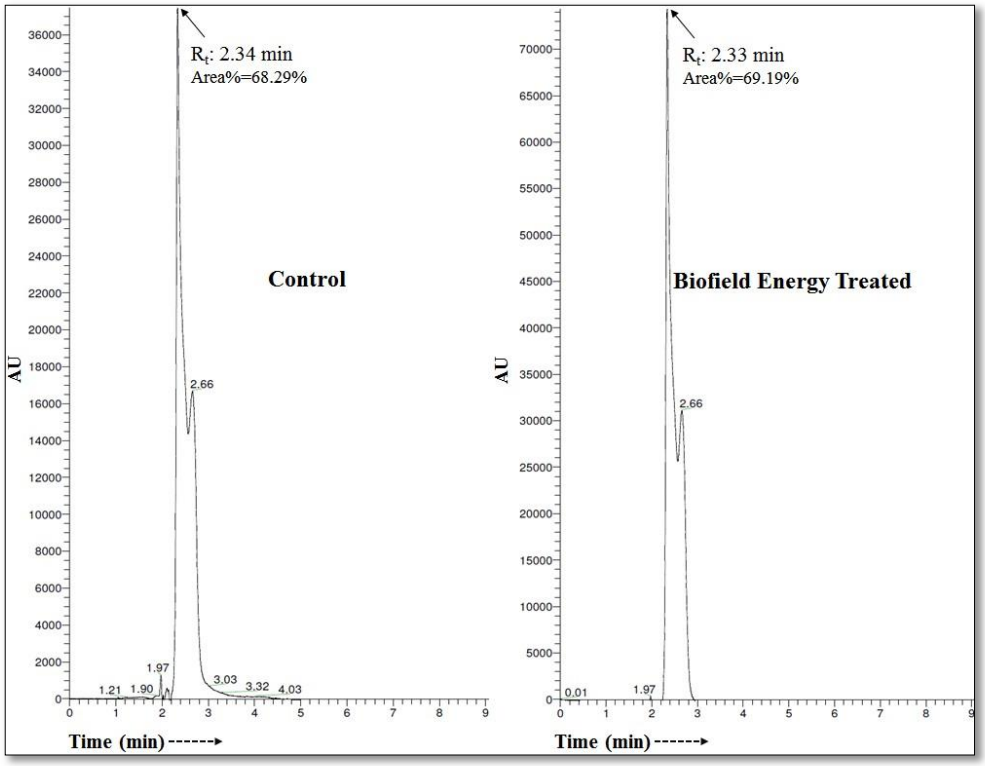

Figure 1. Liquid chromatograms of the control and Biofield Energy Treated vitamin B_6.

The LC-MS based theoretical calculation of P_{M+1} for vitamin B_6 was presented as below:

P (^{13}C) = [(8 x 1.1%) x 100% (the actual size of the M^+ peak)] / 100% = 8.8%
P (^{2}H) = [(12 x 0.015%) x 100%] / 100% = 0.18%
P (^{15}N) = [(1 x 0.40%) x 100%] / 100% = 0.4%
P (^{17}O) = [(3 x 0.04%) x 100%] / 100% = 0.12%
P_{M+1} i.e., ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $C_8H_{12}NO_3^+$ to *m/z* 171 = 9.5%

From the above calculation, it has been found that ^{13}C and ^{15}N have major contribution to *m/z* 171.

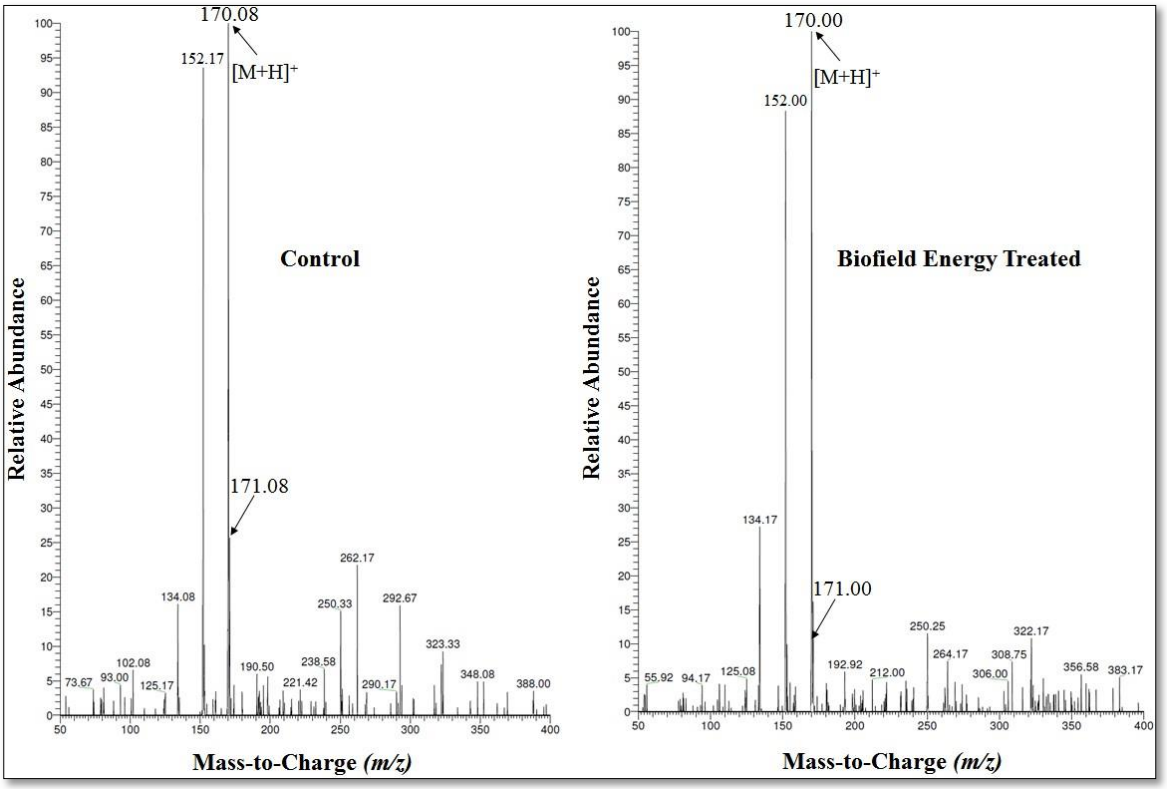

Figure 2. Mass spectra of the control and Biofield Energy Treated vitamin B_6 at R_t 2.3 min.

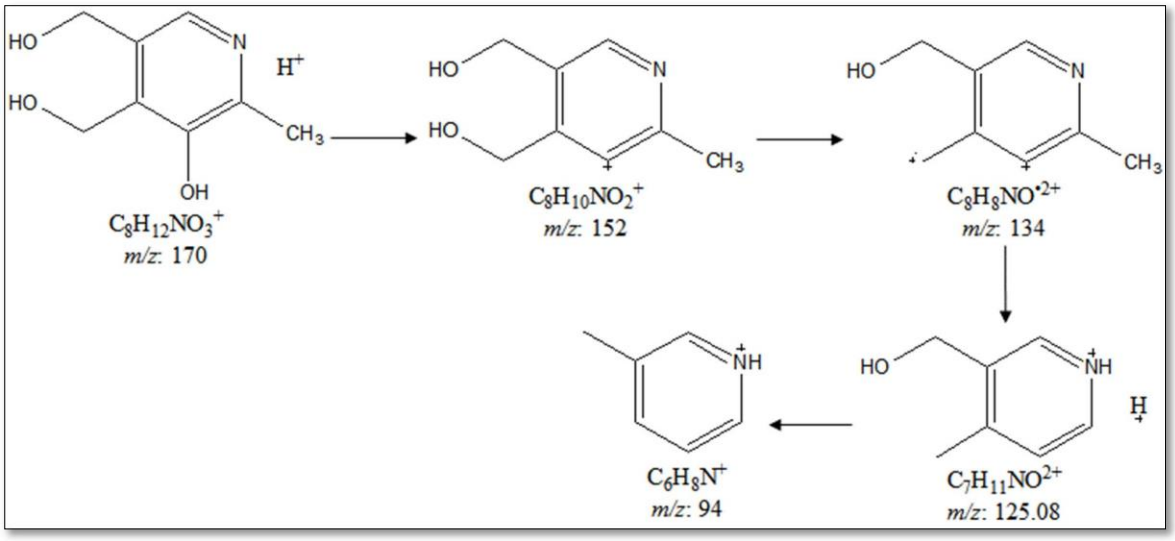

Figure 3. Proposed fragmentation pattern of vitamin B_6.

The LC-MS based isotopic abundance ratio analysis P_M and P_{M+1} for vitamin B_6 near *m/z* 170 [M^+] and 171[$(M+1)^+$], respectively of both the samples **(Table 1)**. The isotopic abundance ratio P_{M+1}/P_M in the Biofield Energy Treated vitamin B_6 was significantly decreased by 36.94% compared with the control sample **(Table 1)**. Therefore, it was concluded that the ^{13}C, ^{2}H, ^{15}N, ^{17}O contributions from $(C_8H_{12}NO_3)^+$ to *m/z* 171 in the Biofield Energy Treated vitamin B_6 was significantly increased compared to the control sample.

Table 1. LC-MS based isotopic abundance analysis results in Biofield Energy Treated vitamin B_6 compared to the control sample.

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 170 (%)	100.00	100.00
P_{M+1} at *m/z* 171 (%)	25.66	16.18
P_{M+1}/P_M	0.26	0.16
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		-36.94

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

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

The GC-MS chromatograms of both the vitamin B_6 samples are shown in **Figures 4 and 5**. The chromatographic peak was obtained at R_t 16.9 min in both the sample, but peak area of the Biofield Energy Treated sample (36903272) was increased by 4.54% (Table 2) compared to the control sample (35301072). This indicated that the solubility of the Biofield Energy Treated vitamin B_6 was increased. The data was supported by one of the recently published articles, in which the data showed that the particle sizes of the vitamin B_6 were significantly decreased and the surface area was significantly increased after the Biofield Energy Treatment [40].

The corresponding mass spectra for the chromatographic peak at R_t of ~17 min of both the samples of vitamin B_6 exhibited the presence of the dehydrated molecular ion peak at *m/z* 151 (calcd for $C_8H_9NO_2^{\bullet+}$, 151.06) **(Figures 4 and 5)**. The other mass fragmentation peak at lower *m/z* 124 ($C_7H_{10}NO^{2+}$), 106 ($C_7H_8N^+$), 94 ($C_6H_8N^+$) were also observed in both vitamin B_6 mass spectra **(Figures 4 and 5)**. But the mass peak intensities of the treated sample were significantly altered compared to the control vitamin B_6. The mass peak intensity of the Biofield Energy Treated vitamin B_6 at *m/z* 153 was significantly increased by 66.69% compared to the control sample **(Table 2)**.

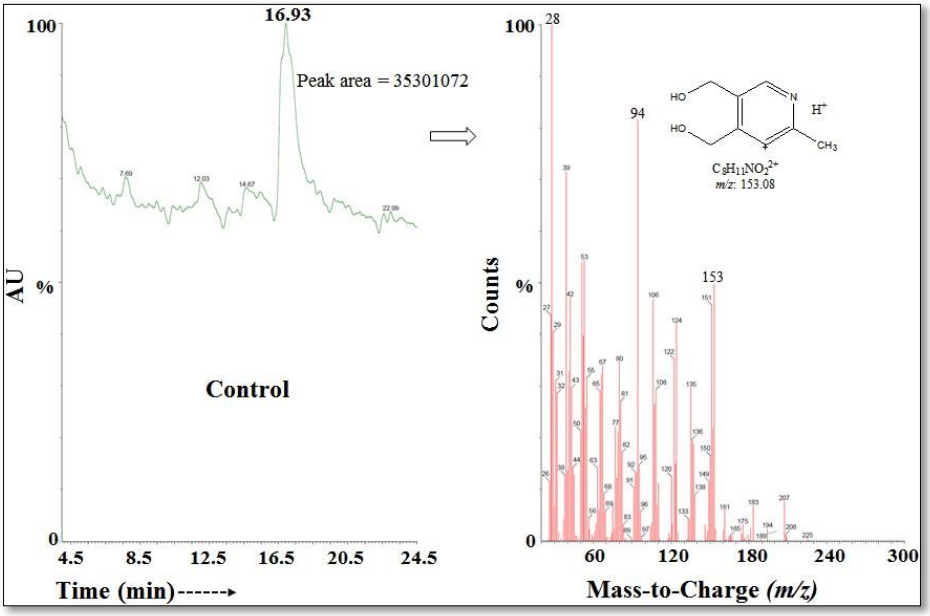

Figure 4. The GC-MS chromatogram and mass spectra of the control vitamin B_6.

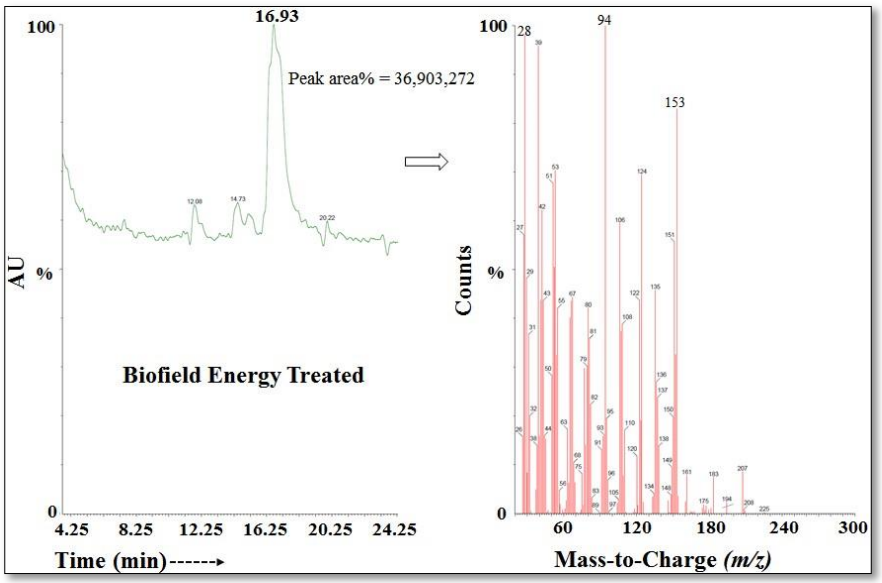

Figure 5. The GC-MS chromatogram and mass spectra of the Biofield Energy Treated vitamin B_6.

Table 2. GC-MS chromatographic and mass spectral analysis of the control and Biofield Energy Treated vitamin B_6.

Parameters	Control sample	Biofield Energy Treated sample	% Change
Peak area	3,53,01,072	3,69,03,272	4.54
Mass peak (*m/z* = 153) intensity	49.78	82.98	66.69

LC-MS and GC-MS structural analysis confirmed the sample as vitamin B_6. The isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) in the Biofield Energy Treated vitamin B_6 was significantly decreased compared to the control sample. Similarly, the peak area and mass peak intensity of the vitamin B_6 sample was improved after the Biofield Energy Treatment. All these characteristic changes in vitamin B_6 could be due to changes in nuclei possibly through the interference of neutrino particles *via* the Trivedi Effect®-Consciousness Energy Healing Treatment [15]. Neutrino is a subatomic particle with no charge and small mass, which is one of the most abundant particles in the universe. These neutrinos have the ability to interact with protons and neutrons in the nucleus and the formation of isotopes [15,34,35]. The decreased 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 vitamin B_6 [41]. The decreased isotopic abundance ratio of the treated vitamin B_6 may decrease the intra-atomic bond strength, decrease its physical stability. The Trivedi Effect®-Consciousness Energy Healing Treated vitamin B_6 would be more soluble and bioavailable compared to the control sample. It would be helpful to design more efficacious nutraceutical and pharmaceutical formulations for the prevention and treatment of vitamin B_6 deficiency disorders, sideroblastic anemia, premenstrual syndrome, seizures, cardiovascular disease, tuberculosis, metabolic disorders, Alzheimer's disease, cancer, hyperhomocysteinemia, anxiety, hypertension, asthma, depression, dysmenorrhea, akathisia, angioplasty, birth outcomes, cognitive function, hyperkinetic cerebral dysfunction syndrome, carpal tunnel syndrome, breast pain, lactation suppression, McArdle's disease, autism, osteoporosis, Tardive dyskinesia, etc.

CONCLUSIONS

The Trivedi Effect®-Consciousness Energy Healing Treatment showed a significant impact on the isotopic abundance ratio, peak area, and mass peak intensities of vitamin B_6. The LC-MS spectra of both the control and Biofield Energy Treated vitamin B_6 samples have same retention time, 2.3 min exhibited the mass of the protonated molecular ion peak $[M+H]^+$ at *m/z* 170 in the MS spectrum. The peak area% of the Biofield Energy Treated vitamin B_6 was increased by 1.32% compared to the control sample. The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated vitamin B_6 was significantly decreased by 36.94% compared with the control sample. Thus, ^{13}C, ^2H, ^{15}N, and ^{17}O contributions from $(C_8H_{12}NO_3)^+$ to *m/z* 171 in the Biofield Energy Treated sample were significantly decreased compared with the control sample. Similarly, in the GC-MS chromatograms, the peak area of the Biofield Energy Treated sample was increased by 4.54% compared to the control sample. The mass peak intensity of the treated vitamin B_6 at *m/z* 153 was significantly increased by 66.69% compared to

the control sample. The isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) and peak area in the treated vitamin B_6 was significantly decreased compared to the control sample. It changes in isotopic abundance, peak area, and mass peak intensities could be due to changes in nuclei possibly *via* the interference of neutrino particles controlled by the Trivedi Effect®. The decreased isotopic abundance ratio of the treated vitamin B_6 may decrease the intra-atomic bond strength, decrease its physical stability. The increased peak area of the treated vitamin B_6 might be responsible for the increase in solubility and bioavailability compared to the control sample. The new form of vitamin B_6 would be better for designing more efficacious pharmaceutical formulations for the prevention and treatment of vitamin B_6 deficiency, hereditary sideroblastic anemia, premenstrual syndrome, pyridoxine-dependency seizures, febrile seizures, cardiovascular disease, pulmonary tuberculosis, metabolic disorders, Alzheimer's disease, cancer, hyperhomocysteinemia, anxiety, hypertension, asthma, depression, attention deficit hyperactivity disorder, dysmenorrhea, akathisia, angioplasty, cognitive function, hyperkinetic cerebral dysfunction syndrome, carpal tunnel syndrome, breast pain, pregnancy-induced nausea and vomiting in pregnancy, lactation suppression, McArdle's disease, autism, osteoporosis, Tardive dyskinesia, stroke recurrence, etc.

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