

## 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<sub>6</sub>) is commonly found in foods and dietary supplements, has an important role in physiologic development. In this study, the influence of the Trivedi Effect<sup>®</sup>-Biofield Energy Healing Treatment on the structural properties and the isotopic abundance ratio of vitamin B<sub>6</sub> were evaluated using LC-MS and GC-MS. Vitamin B<sub>6</sub> sample was divided into two parts and termed as control and treated samples. Only the treated vitamin B<sub>6</sub> was received the Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment remotely by a well-known Biofield Energy Healer, Dahryn Trivedi. The LC-MS spectra of both the vitamin B<sub>6</sub> samples at retention time 2.3 min exhibited the mass of the protonated molecular ion peak [M+H]<sup>+</sup> at *m/z* 170 (calculated for C<sub>8</sub>H<sub>12</sub>NO<sub>3</sub><sup>+</sup>; 170.08) in the MS spectrum. The peak area% of the treated vitamin B<sub>6</sub> was increased by 1.32% compared to the control sample. The isotopic abundance ratio of P<sub>M+1</sub>/P<sub>M</sub> in the treated vitamin B<sub>6</sub> was significantly decreased by 36.94% compared with the control sample. Thus, <sup>13</sup>C, <sup>2</sup>H, <sup>15</sup>N, and <sup>17</sup>O contributions from (C<sub>8</sub>H<sub>12</sub>NO<sub>3</sub>)<sup>+</sup> 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<sub>6</sub> at *m/z* 153 was significantly increased by 66.69% compared to the control sample. The isotopic abundance ratio of P<sub>M+1</sub>/P<sub>M</sub> (<sup>2</sup>H/<sup>1</sup>H or <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N or <sup>17</sup>O/<sup>16</sup>O) and peak area in the treated vitamin B<sub>6</sub> was significantly decreased compared to the control sample. The decreased isotopic abundance ratio of the treated vitamin B<sub>6</sub> may decrease the intra-atomic bond strength, decrease its physical stability. The increased peak area of the treated vitamin B<sub>6</sub> might be responsible for the increase in solubility and bioavailability compared to the control sample. The new form of vitamin B<sub>6</sub> would be better for designing more efficacious pharmaceutical formulations for the prevention and treatment of vitamin B<sub>6</sub> deficiency, anaemia, seizures, cardiovascular disease, tuberculosis, Alzheimer's disease, cancer, anxiety, hypertension, asthma, depression, dysmenorrhea, breast pain, etc.

**Keywords:** The Trivedi Effect<sup>®</sup>, Biofield Energy, Consciousness Energy Healing Treatment, Vitamin B<sub>6</sub>, Isotopic abundance

### INTRODUCTION

Pyridoxine (vitamin B<sub>6</sub>) is a water-soluble vitamin and has an important role in physiologic development [1]. Vitamin B<sub>6</sub> 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<sub>6</sub>) [2]. Vitamin B<sub>6</sub> 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<sub>6</sub> commonly used in the pharmaceutical industries [6].

Vitamin B<sub>6</sub> generally used for the prevention and treatment of vitamin B<sub>6</sub> 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<sub>6</sub> 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<sub>6</sub> levels in the body [3,5]. Vitamin B<sub>6</sub> 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<sup>®</sup>-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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup>-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<sub>M+1</sub>/P<sub>M</sub> (<sup>2</sup>H/<sup>1</sup>H or <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N or <sup>17</sup>O/<sup>16</sup>O) in the Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treated vitamin B<sub>6</sub> 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<sup>®</sup>-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<sub>6</sub> 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<sub>6</sub> 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<sub>6</sub> 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.

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

Where  $IAR_{\text{Control}}$  and  $IAR_{\text{Treated}}$  is the isotopic abundance ratio in the treated and control vitamin B<sub>6</sub> sample, respectively.

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

GC-MS of the vitamin B<sub>6</sub> samples were analyzed with the help of Perkin Elmer Gas chromatograph equipped with a PE-5MS (30M x 250 microns 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

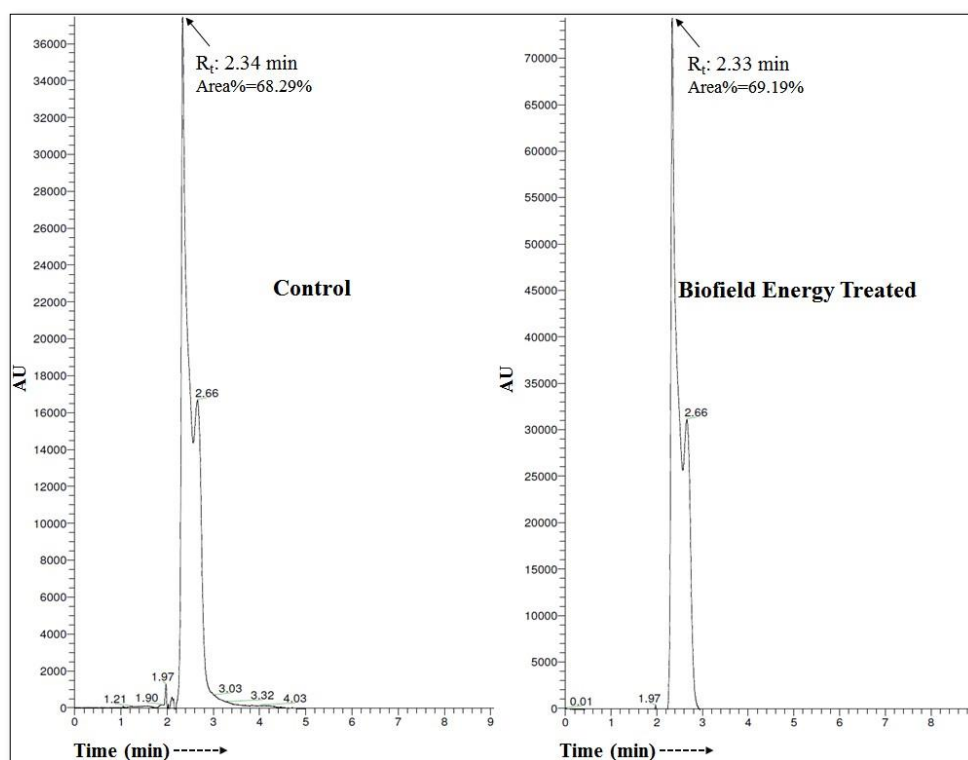
sample was prepared taking 100 mg of the vitamin B<sub>6</sub> in 4 ml methanol as a diluent. Injection volume was 5 µl. The % change in the peak area and mass peak intensities of the treated vitamin B<sub>6</sub> was calculated compared to the control sample using the following equation 2:

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

## RESULTS AND DISCUSSION

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

The hydrochloride salt of vitamin B<sub>6</sub> 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<sub>6</sub> 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<sub>6</sub> (**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  $C_8H_{10}NO_2^+$ ,  $C_8H_8NO^+$ ,  $C_7H_{11}NO^{2+}$ , 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<sub>6</sub>.

The LC-MS based theoretical calculation of  $P_{M+1}$  for vitamin B<sub>6</sub> is presented as below:

$$P(^{13}\text{C}) = [(8 \times 1.1\%) \times 100\% \text{ (the actual size of the } M^+ \text{ peak)}] / 100\% = 8.8\%$$

$$P(^2\text{H}) = [(12 \times 0.015\%) \times 100\%] / 100\% = 0.18\%$$

$$P(^{15}\text{N}) = [(1 \times 0.40\%) \times 100\%] / 100\% = 0.4\%$$

$$P(^{17}\text{O}) = [(3 \times 0.04\%) \times 100\%] / 100\% = 0.12\%$$

$P_{M+1}$  i.e.,  $^{13}\text{C}$ ,  $^2\text{H}$ ,  $^{15}\text{N}$ , and  $^{17}\text{O}$  contributions from  $\text{C}_8\text{H}_{12}\text{NO}_3^+$  to  $m/z$  171 = 9.5%

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

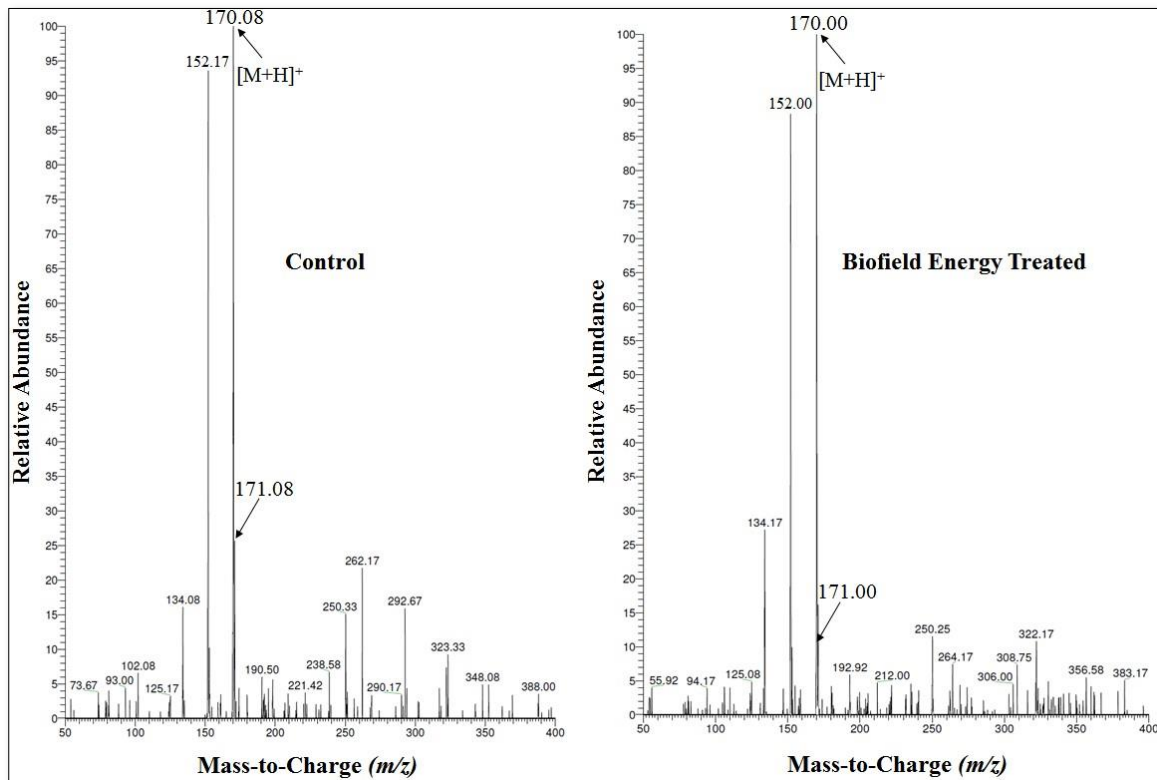


Figure 2. Mass spectra of the control and Biofield Energy Treated vitamin B<sub>6</sub> at R<sub>t</sub> 2.3 min.

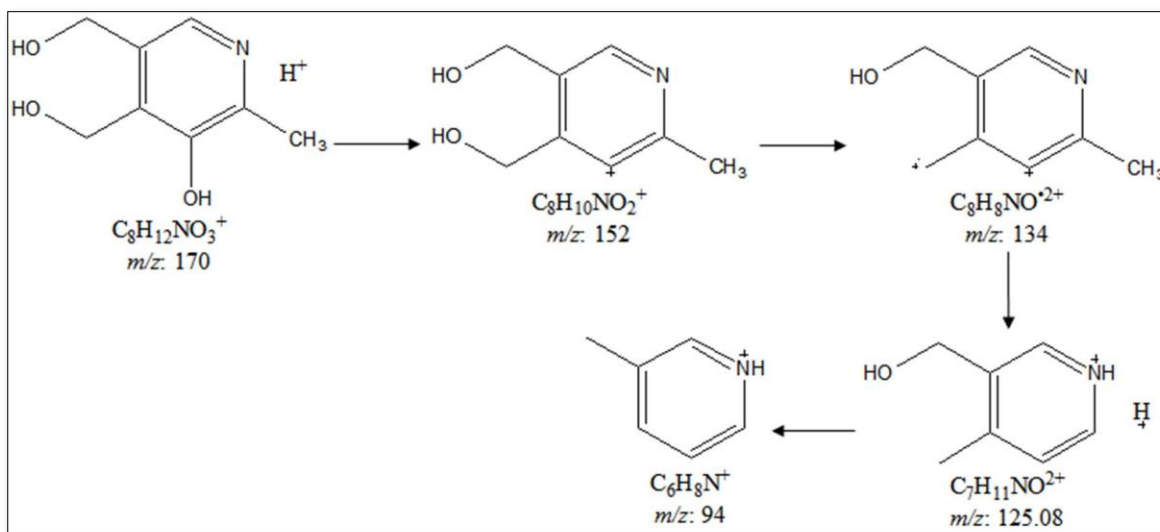


Figure 3. Proposed fragmentation pattern of vitamin B<sub>6</sub>.

The LC-MS based isotopic abundance ratio analysis  $P_M$  and  $P_{M+1}$  for vitamin B<sub>6</sub> near  $m/z$  170 [ $M^+$ ] and 171[( $M+1$ )<sup>+</sup>], respectively of both the samples (**Table 1**). The isotopic abundance ratio  $P_{M+1}/P_M$  in the Biofield Energy Treated vitamin B<sub>6</sub> was significantly decreased by 36.94% compared

with the control sample (**Table 1**). Therefore, it was concluded that the <sup>13</sup>C, <sup>2</sup>H, <sup>15</sup>N, <sup>17</sup>O contributions from (C<sub>8</sub>H<sub>12</sub>NO<sub>3</sub>)<sup>+</sup> to  $m/z$  171 in the Biofield Energy Treated vitamin B<sub>6</sub> was significantly increased compared to the control sample.

**Table 1.** LC-MS based isotopic abundance analysis results in Biofield Energy Treated vitamin B<sub>6</sub> 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

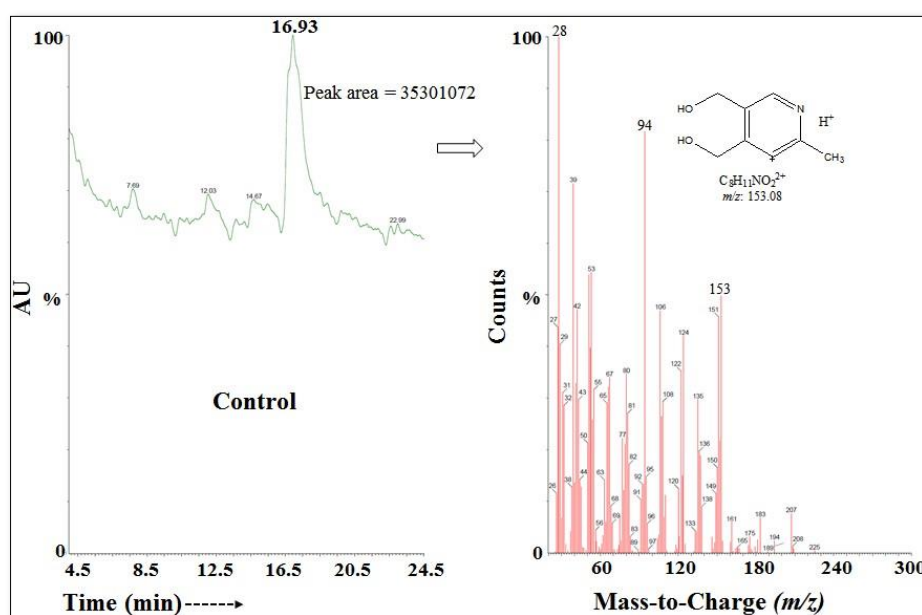
$P_M$ : the relative peak intensity of the parent molecular ion [ $M^+$ ];  $P_{M+1}$ : the relative peak intensity of the isotopic molecular ion [( $M+1$ )<sup>+</sup>],  $P_{M+2}$ : the relative peak intensity of the isotopic molecular ion [( $M+2$ )<sup>+</sup>],  $M$ : mass of the parent molecule

### ➤ Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS chromatograms of both the vitamin B<sub>6</sub> 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<sub>6</sub> 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<sub>6</sub> 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<sub>6</sub> exhibited the presence of the dehydrated molecular ion peak at  $m/z$  151 (calcd for C<sub>8</sub>H<sub>9</sub>NO<sub>2</sub><sup>+</sup>, 151.06) (**Figures 4 and 5**). The other mass fragmentation peak at lower  $m/z$  124 (C<sub>7</sub>H<sub>10</sub>NO<sup>2+</sup>), 106 (C<sub>7</sub>H<sub>8</sub>N<sup>+</sup>), 94 (C<sub>6</sub>H<sub>8</sub>N<sup>+</sup>) were also observed in both vitamin B<sub>6</sub> mass spectra (**Figures 4 and 5**). But the mass peak intensities of the treated sample were significantly altered compared to the control vitamin B<sub>6</sub>. The mass peak intensity of the Biofield Energy Treated vitamin B<sub>6</sub> 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<sub>6</sub>.



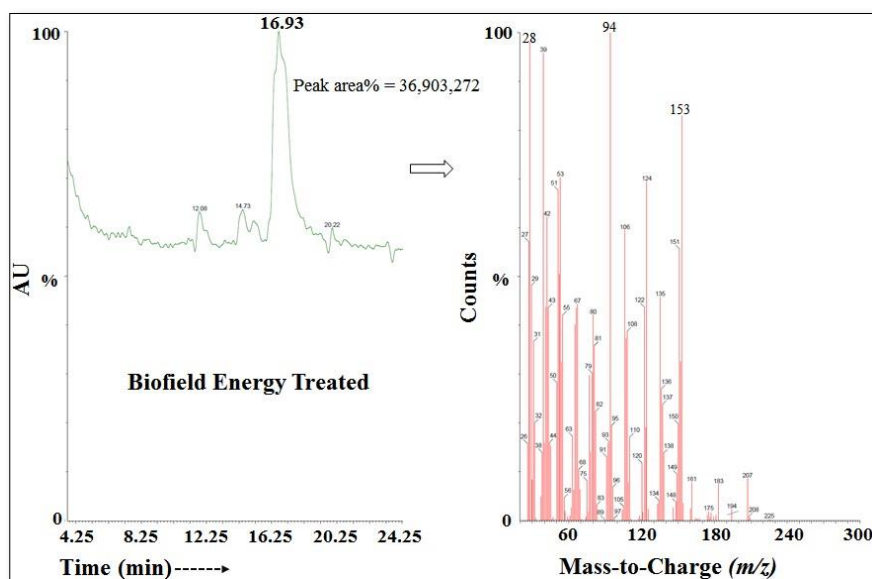


Figure 5. The GC-MS chromatogram and mass spectra of the Biofield Energy Treated vitamin B<sub>6</sub>.

Table 2. GC-MS chromatographic and mass spectral analysis of the control and Biofield Energy Treated vitamin B<sub>6</sub>.

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<sub>6</sub>. 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<sub>6</sub> was significantly decreased compared to the control sample. Similarly, the peak area and mass peak intensity of the vitamin B<sub>6</sub> sample was improved after the Biofield Energy Treatment. All these characteristic changes in vitamin B<sub>6</sub> could be due to changes in nuclei possibly through the interference of neutrino particles *via* the Trivedi Effect<sup>®</sup>-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<sub>6</sub> [41]. The decreased isotopic abundance ratio of the treated vitamin B<sub>6</sub> may decrease the intra-atomic bond strength, decrease its physical stability. The Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treated vitamin B<sub>6</sub> 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<sub>6</sub> 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<sup>®</sup>-Consciousness Energy Healing Treatment showed a significant impact on the isotopic abundance ratio, peak area, and mass peak intensities of vitamin B<sub>6</sub>. The LC-MS spectra of both the control and Biofield Energy Treated vitamin B<sub>6</sub> 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<sub>6</sub> 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<sub>6</sub> 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<sub>6</sub> at  $m/z$  153 was significantly increased by 66.69% compared to

the control sample. The isotopic abundance ratio of  $P_{M+1}/P_M$  ( $^2\text{H}/^1\text{H}$  or  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  or  $^{17}\text{O}/^{16}\text{O}$ ) and peak area in the treated vitamin B<sub>6</sub> 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<sup>®</sup>. The decreased isotopic abundance ratio of the treated vitamin B<sub>6</sub> may decrease the intra-atomic bond strength, decrease its physical stability. The increased peak area of the treated vitamin B<sub>6</sub> might be responsible for the increase in solubility and bioavailability compared to the control sample. The new form of vitamin B<sub>6</sub> would be better for designing more efficacious pharmaceutical formulations for the prevention and treatment of vitamin B<sub>6</sub> 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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