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Research Article

Impact of Biofield Energy Healing Treatment on the Isotopic Abundance Ratio Analysis of Pyridoxine HCl Using LC-MS and GC-MS Spectrometry

Alice Branton[1], Mahendra Kumar Trivedi[1], Dahryn Trivedi[1] and Snehasis Jana[2]*

[1]Trivedi Global Inc., Henderson, USA

[2]Trivedi Science Research Laboratory Pvt. Ltd., Thane (West), Maharashtra, India

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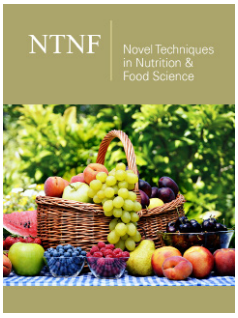

***Corresponding author:** Snehasis Jana, Trivedi Science Research Laboratory Pvt Ltd, Thane (West), Maharashtra,India.

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Abstract

Pyridoxine (vitamin B_6) is a water-soluble vitamin commonly found in food and also provided using dietary supplements. It plays an important role in the biosynthesis of neurotransmitters and maintaining healthy levels of homocysteine, the amino acid in the blood, gluconeogenesis, immune function, haemoglobin formation, etc. This study was performed to determine the impact of the Trivedi Effect®-Biofield Energy Healing Treatment on the structural properties and the isotopic abundance ratio of pyridoxine hydrochloride using LC-MS and GC-MS spectroscopy. Pyridoxine HCl sample was divided into two parts, one part of pyridoxine was considered as control (no Biofield Energy Treatment was provided), while the second part was treated with the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a renowned Biofield Energy Healer, Alice Branton and termed as a treated sample. The LC-MS spectra of both the control and Biofield Energy Treated pyridoxine hydrochloride samples at retention time 2.33 minutes exhibited the mass of the protonated molecular ion 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 found to be pyridoxine. The LC-MS based isotopic abundance ratios of PM+1/PM and PM+2/PM in the treated pyridoxine were significantly increased by 20.45% and 116.67%, respectively compared with the control sample. Thus, ^{13}C, ^{2}H, ^{15}N, ^{17}O, and ^{18}O contributions from $(C_8H_{12}NO_3)^+$ to *m/z* 171 and 172 in the treated sample were significantly increased compared with the control sample. Similarly, in the GC-MS chromatograms, the peak area% of the treated sample was increased by 4.06% compared to the control sample. But the mass peak intensity of the treated pyridoxine at *m/z* 151 was significantly decreased by 14.16% compared to the control sample. The isotopic abundance ratios of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$), P_{M+2}/P_M ($^{18}O/^{16}S$), and peak area% in the treated pyridoxine were significantly increased compared to the control sample. It can be assumed that the 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®-Consciousness Energy Healing Treatment. The new form of pyridoxine HCl would be better for designing novel pharmaceutical formulations which would be more soluble, absorbable, and bioavailable, that might offer better therapeutic response 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: Pyridoxine HCl; The trivedi effect®; Biofield energy; Consciousness energy healing treatment; LC-MS; GC-MS

Introduction

Pyridoxine (vitamin B_6) is a water-soluble vitamin commonly found in food and also provided using dietary supplements [1]. Vitamin B_6 naturally available in food sources like fish, meat, poultry, tofu, chickpeas, avocados, nuts, whole grains, bananas, spinach, etc. [2]. Inside the body for more than 100 enzymatic reactions, it acts like cofactors or prosthetic groups and also plays an important role in the biosynthesis of neurotransmitters and maintaining healthy levels of homocysteine, the amino acid in the blood, gluconeogenesis, glycogenolysis, immune function (lymphocyte and interleukin-2 production), and haemoglobin formation [3-5]. The active form of vitamin B_6, pyridoxal 5' phosphate (PLP) and pyridoxamine 5' phosphate (PMP) are the coenzymes which involved in the metabolism of amino acid, carbohydrates, and lipids. Pyridoxine hydrochloride is the commonly used salt form of vitamin B_6 [6]. Vitamin B_6 generally used in vitamin supplements and also as a component of multivitamin preparations for the prevention and treatment of vitamin B_6 deficiency, sideroblastic anaemia, metabolic disorder,

Alzheimer's disease, pyridoxine-dependent epilepsy, pulmonary tuberculosis, hyperhomocysteinaemia, cancer, cardiovascular disease, anxiety, asthma, depression, attention deficit hyperactivity disorder (ADHD), dysmenorrhoea, diabetes, post-partum lactation suppression, McArdle's disease, osteoporosis, problems from isoniazid, mushroom poisoning, etc. [1,3-8]. Vitamin B_6 rarely shows side effects like a headache, sleepiness, numbness, sensory neuropathy (ataxia), etc. It can interact with many medications, i.e., antiepileptic drugs (valproic acid, carbamazepine, phenytoin, etc.), cycloserine, and theophylline, which might adversely affect vitamin B_6 levels [3,5]. Pyridoxine HCl is light-sensitive material and degrades slowly when exposed to light. It is soluble in water and alcohol; sparingly soluble acetone; insoluble in ether and chloroform. When it heated to decomposition, it emits very toxic oxide fumes of nitrogen and hydrogen chloride [9].

Intrinsic physicochemical properties play a vital role in the drug solubility, absorption, bioavailability, etc. In this scenario, it was observed that Biofield Energy Healing Treatment (The Trivedi Effect®) has the incredible impact on the particle size, surface area, and thermal behaviour of pharmaceutical/nutraceutical compounds [10-14]. The Trivedi Effect® is a natural and only scientifically proven phenomenon in which a person can harness this inherently intelligent energy and transmit it anywhere on the planet *via* the possible mediation of neutrinos [15].

The human body contains the electrically charged particles (ions, cells, etc.), which form a quantum of energy matrix that surrounds the body is called the "Biofield Energy" [16,17]. Biofield Energy Healers can harness the inherently intelligent energy from the "universal energy field" and can transmit into any living or nonliving object(s) anywhere on the planet, and the overall process is called Biofield Energy Healing Treatment [17,18]. Biofield based Energy Therapies have been reported with significant outcomes against various disease [19]. National Center of Complementary and Integrative Health (NCCIH) 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 [20,21]. CAM as a group of assorted medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine. Most of the USA population has accepted these therapies [22]. Similarly, The Trivedi Effect®-Consciousness Energy Healing Treatment also well recognized and reported with powerful scientific data showing astonishing capability to alter the characteristic properties of the several non-living materials and living object(s), i.e., organic compounds, metals, polymers, and ceramic [23-26], crops [27,28], microbes [29], cancer cells [30], etc. The Trivedi Effect® Treatment has also enhanced the bioavailability of pharmaceutical compounds [31,32] and the isotopic abundance ratio of organic compounds [13,14].

The stable isotope ratio analysis and its detailed study have many applications in the different field of science for understanding the isotope effects resulting from the variation of the isotopic composition of the molecule [33,34]. Isotope ratio analysis usually performed by using the conventional 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 [34,35]. The Trivedi Effect®-Biofield Energy Healing Treatment could be an economical approach for designing better nutraceutical and pharmaceutical formulations. Therefore, LC-MS and GC-MS were used in this study to characterize the structural properties and evaluate the isotopic abundance ratio analysis of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) in The Trivedi Effect®-Consciousness Energy Healing Treated pyridoxine HCl compared to the control sample.

Materials and Methods

Chemicals and reagents

Pyridoxine hydrochloride ($C_8H_{12}ClNO_3$; 100%) was purchased from TCI, Japan. All other chemicals used during the experiments were of analytical grade available in India.

Consciousness energy healing treatment strategies

The pyridoxine HCl powder considered for the research was divided into two parts. One part of pyridoxine HCl was treated with The Trivedi Effect®-Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 minutes and known as The Trivedi Effect® Treated or Biofield Energy Treated pyridoxine HCl sample. The Biofield Energy Treatment was provided through the healer's unique energy transmission process by the renowned Biofield Energy Healer, Alice Branton, USA, to the test sample. However, the other part of pyridoxine HCl powder was considered as a control sample, where no Biofield Energy Treatment was provided. Further, the control sample was treated with "sham" healer for comparison purpose. The sham healer did not have any knowledge about the Biofield Energy Treatment. After that, the Biofield Energy Treated and untreated pyridoxine HCl 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 the control and Biofield Energy Treated pyridoxine HCl was carried out with the help of LC-MS ThermoFisher Scientific, the USA equipped with an iron trap detector connected with a triple-stage quadrupole mass spectrometer. The column used here was a reversed phase Thermo Scientific Synchronis C18 (Length-250mm X ID 4.6mm X 5micron), maintained at 25 ˚C. The diluent used for the sample preparation was methanol and water. 10µL of pyridoxine HCl solution was injected, and the analyte was eluted using acetonitrile +5mM ammonium acetate (80:20) pumped at a constant flow rate of 1mL/min. Chromatographic separation was achieved using gradient condition and the total run time was 10min. Peaks were monitored at 220nm using the PDA detector. The 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 (*m/z* 50-400) were recorded. The total ion chromatogram and mass spectrum of the individual peak were recorded.

The natural abundance of each isotope (C,H,N, and O) can be predicted from the comparison of the height 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 [34,36-38]. The LC-MS based isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) for the control and Biofield Energy Treated pyridoxine HCl was calculated.

Percentage (%) change in isotopic abundance ratio=[(IARTreated-IARControl)/IARControl) x100]

Where IARTreated=isotopic abundance ratio in the treated sample and IARControl=isotopic abundance ratio in the control sample.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS of the control and Biofield Energy Treated sample of pyridoxine HCl were analyzed with the help of Perkin Elmer Gas chromatograph equipped with a PE-5MS (30Mx250 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. Oven temperature was programmed from 75 °C (5min hold) to 250 °C (2.5min hold) @ 10 °C/min (total run time 25min). The sample was prepared taking 100mg of the pyridoxine HCl in 4ml methanol as a diluent. Injection volume was *5µl*. Mass spectra were scanned from *m/z* 20 to 400. The identification and characterization of analyte were done by GC retention times and by a comparison of the mass spectra of samples.

Results and Discussion

Liquid Chromatography-Mass Spectrometry (LC-MS)

Pyridoxine HCl showed the single major chromatographic peak in the LC-MS chromatograms in case of both the control sample and Biofield Energy Treated samples at retention time (R_t) of 2.33 minutes (Figure 1). The R_t of both the samples was same, so it indicated that the polarity of both the samples was close to each other. Similarly, the ESI-MS 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 pyridoxine HCl (Figures 2 & 3). The experimental data were well matched with the reported literature data [39]. Along with the molecular ion peak at *m/z* 170 [M+H] + other lower-mass peaks at *m/z* 152, 134, 124, 106, and 94 for $C_8H_{10}NO_2$+, $C_8H_8NO^{\bullet+}$, $C_7H_{10}NO^+$, $C_7H_8N^{\bullet+}$, and $C_6H_8N^+$, respectively in both the samples (Figures 2 & 3).

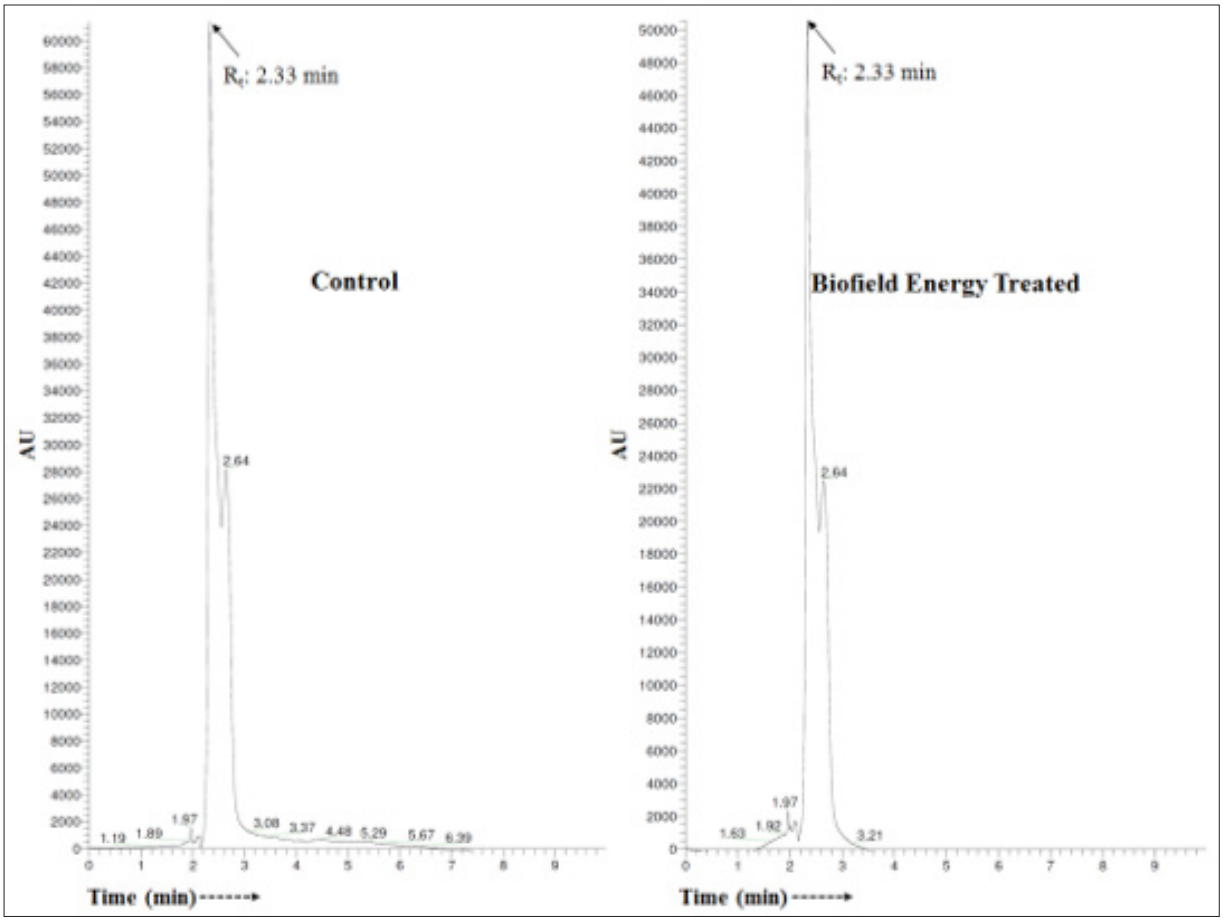

Figure 1: Liquid chromatograms of the control and biofield energy treated pyridoxine HCl.

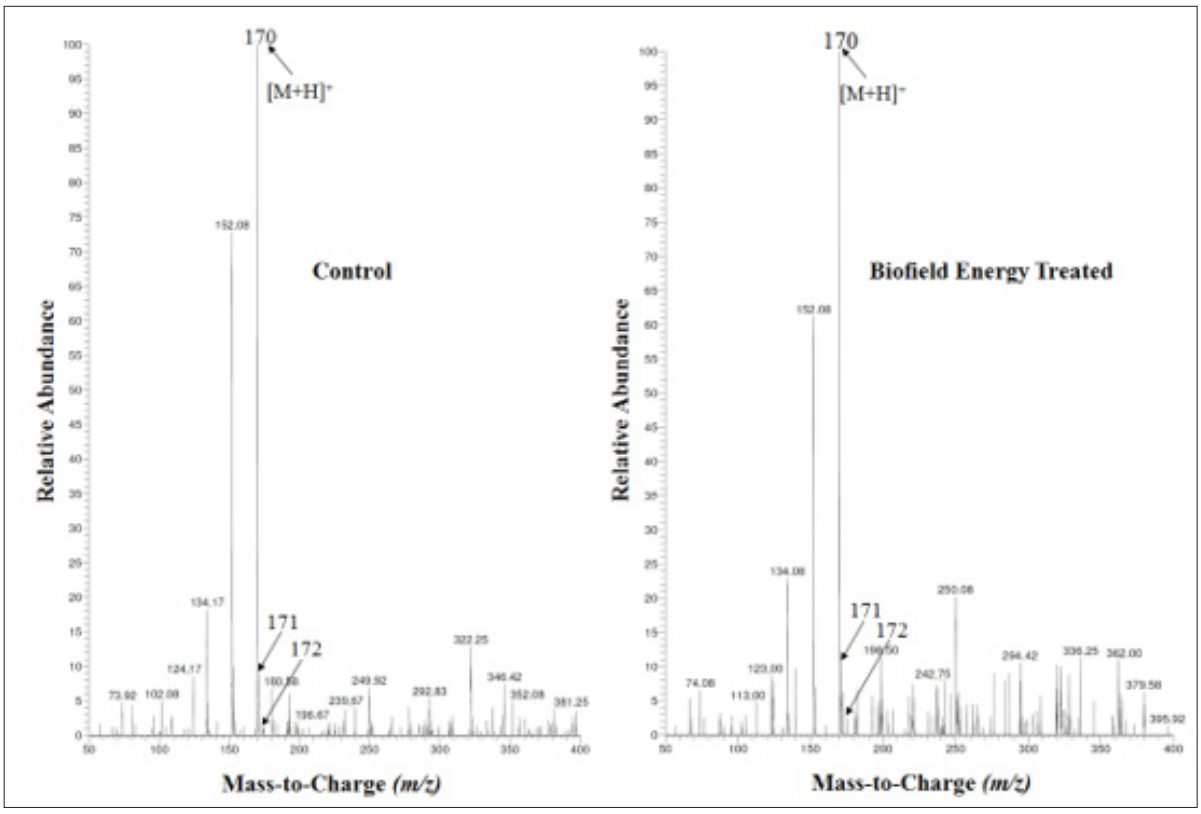

Figure 2: Mass spectra of the control and biofield energy treated pyridoxine at R_t 2.33 minutes.

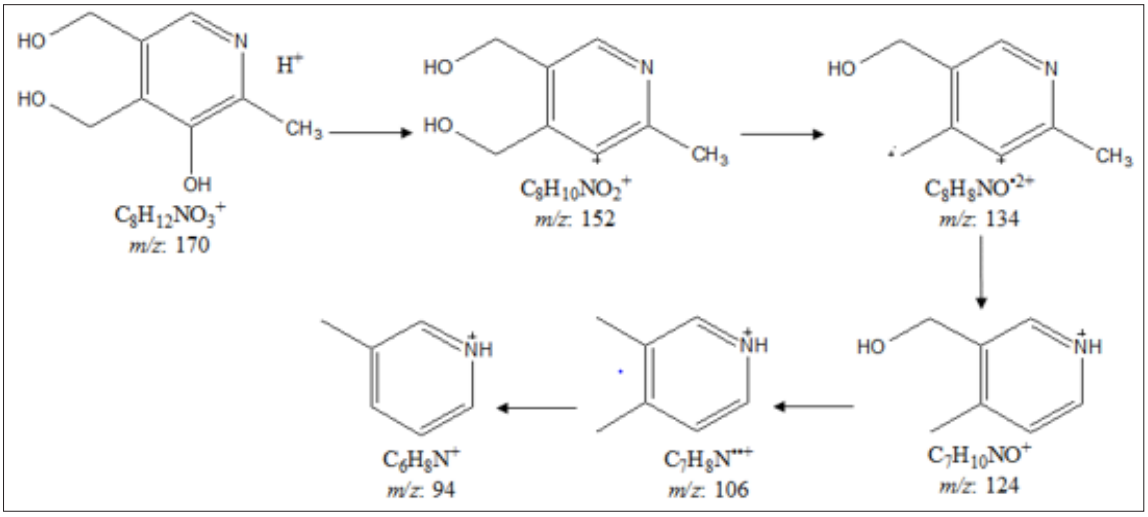

Figure 3: Proposed fragmentation pattern of pyridoxine.

The LC-MS spectra of both the control and Biofield Energy Treated pyridoxine HCl showed the mass of the molecular ion peak at *m/z* 170(calculated for $C_8H_{12}NO_3^+$, 170.08) with 100% relative peak intensity. The theoretical calculation of PM+1 for pyridoxine was presented as below:

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

P (^2H) = [(12x0.015%) x100%]/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, ^2H, ^{15}N, and ^{17}O contributions from $C_8H_{12}NO_3$+ to *m/z* 171 = 9.5%

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

P (^{18}O) = [(3 x 0.20%) x 100%] / 100% = 0.6%

P_{M+2} of ^{18}O contribution from $C_8H_{12}NO_3$+ to *m/z* 172 = 0.6%

From the above calculation, it has been found that ^{13}C, ^{15}N, and ^{18}O have major contribution to *m/z* 171 and 172. The calculated isotopic abundances were close to the experimental value (Table 1).

The LC-MS based isotopic abundance ratios analysis P_M, P_{M+1}, and P_{M+2} for pyridoxine near *m/z* 170, 171, and 172, respectively of the control and Biofield Energy Treated samples, which were obtained from the observed relative peak intensities of $[M^+]$, $[(M+1)^+]$, and $[(M+2)^+]$ peaks, respectively in the ESI-MS spectra (Table 1). The isotopic abundance ratios P_{M+1}/P_M and P_{M+2}/P_M in the Biofield Energy Treated pyridoxine HCl was significantly increased by 20.45% and 116.67%, respectively compared with the control sample (Table 1). Therefore, it was concluded that the ^{13}C, ^{2}H, ^{15}N, ^{17}O, and ^{18}O contributions from $(C_8H_{12}NO_3)^+$ to *m/z* 171 and 172 in the Biofield Energy Treated sample were significantly increased compared to the control sample.

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

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 170 (%)	100	100
P_{M+1} at *m/z* 171 (%)	8.8	10.6
P_{M+1}/P_M	0.09	0.11
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		20.45
P_{M+1} at *m/z* 172 (%)	1.2	2.6
P_{M+2}/P_M	0.01	0.03
% Change of isotopic abundance ratio (P_{M+2}/P_M) with respect to the control sample		116.67

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 the control and Biofield Energy Treated samples of pyridoxine HCl are shown in (Figures 4&5). The chromatographic peak was 16.9 minutes in the control sample, whereas it was at 17.51 minutes in the Biofield Energy Treated sample. Both the chromatograms showed the close R_t in the chromatograms, but peak area% of the Biofield Energy Treated sample (80.25%) was increased by 4.06% (Table 2) compared to the control sample (77.12%).

The GC-MS spectra of the control and Biofield Energy Treated pyridoxine at R_t of ~17 minutes exhibited the presence of the dehydrated molecular ion (Figures 4&5) peak at *m/z* 151 (calcd for $C_8H_9NO_2^{\bullet+}$, 151.06). The other mass fragmentation peak at lower *m/z* 122, 106, 94 corresponding to the molecular formula $C_7H_8NO^+$, $C_7H_8N^+$, $C_6H_8N^+$ were also observed in both control and Biofield Energy Treated pyridoxine (Figures 4 & 5). The mass fragmentation patterns of Biofield Energy Treated pyridoxine was similar compared to the control sample. But the mass peak intensities of the Biofield Energy Treated pyridoxine were significantly altered compared to the control pyridoxine. The mass peak intensity of the Biofield Energy Treated pyridoxine at *m/z* 151 was significantly decreased by 14.16% compared to the control sample (Table 2).

Table 2: GC-MS chromatographic and mass spectra analysis at R_t ~17 minutes of the control and Biofield Energy Treated pyridoxine HCl.

Parameters	Control sample	Biofield Energy Treated sample	% Change
Peak area%	77.12	80.25	4.06
Mass peak (*m/z* = 151) intensity	50.08	42.99	-14.16

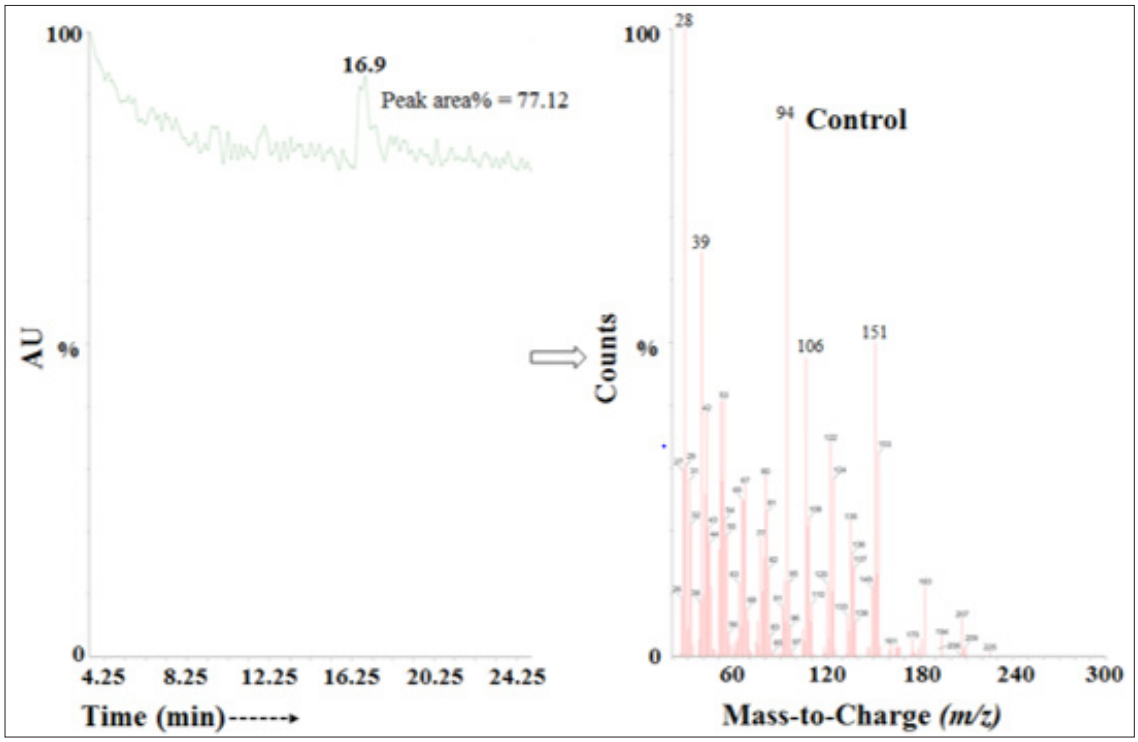

Figure 4: The GC-MS chromatogram and mass spectra of the control pyridoxine HCl.

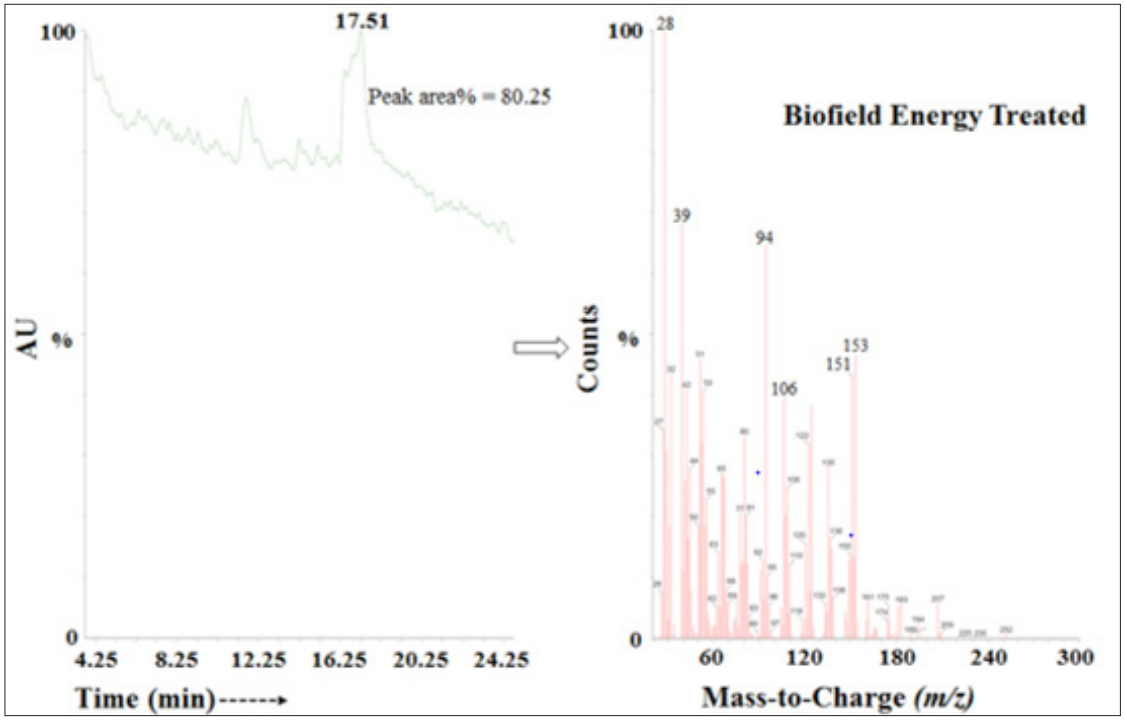

Figure 5: The GC-MS chromatogram and mass spectra of the biofield energy treated pyridoxine HCl.

LC-MS and GC-MS analysis confirmed the structure of pyridoxine HCl. The isotopic abundance ratios of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) in the Biofield Energy Treated pyridoxine HCl were significantly improved compared to the control sample. Similarly, the peak area% of the Biofield Energy Treated sample was improved compared to the control sample. The neutrinos change identities which are only possible if the neutrinos possess mass and have the ability to interchange their phase from one phase to another. Therefore, the neutrinos have the ability to interact with protons and neutrons in the nucleus,

which indicated a close relationship between neutrino and the isotope formation [15,34,35]. The improved isotopic composition in the Biofield Energy Treated pyridoxine HCl might have influenced 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®-Consciousness Energy Healing Treatment. The overall results concluded that The Trivedi Effect®-Consciousness Energy Healing Treatment might have created a new form of pyridoxine HCl which would be more soluble, absorbable, and bioavailable compared to the control sample. It would be more suitable for the prevention and treatment of vitamin B_6 deficiency, hereditary sideroblastic anaemia, 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, birth outcomes, cognitive function, hyperkinetic cerebral dysfunction syndrome, carpal tunnel syndrome, breast pain, pregnancy-induced nausea and vomiting, lactation suppression, McArdle's disease, autism, osteoporosis, Tardive dyskinesia, stroke recurrence, etc.

Conclusion

The Trivedi Effect®-Consciousness Energy Healing Treatment showed the significant impact on the isotopic abundance ratios, peak area%, and mass peak intensities of pyridoxine HCl. The LC-MS spectra of both the control and Biofield Energy Treated pyridoxine hydrochloride samples at retention time (R_t) 2.33 minutes exhibited the mass of the protonated molecular ion 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 found to be pyridoxine HCl. The LC-MS based isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in the Biofield Energy Treated pyridoxine HCl were significantly increased by 20.45% and 116.67%, respectively compared with the control sample. Thus, ^{13}C, ^{2}H, ^{15}N, ^{17}O, and ^{18}O contributions from $(C_8H_{12}NO_3)+$ to *m/z* 171 and 172 in the Biofield Energy Treated sample were significantly increased compared with the control sample. Similarly, the control and treated pyridoxine HCl showed the presence of the chromatographic peak at R_t of ~17 minutes in the GC-MS chromatograms. The peak area% of the Biofield Energy Treated sample (80.25%) was increased by 4.06% compared to the control sample (77.12%). But, the mass peak intensity of the Biofield Energy Treated pyridoxine at *m/z* 151 ($C_8H_9NO_2^{\bullet+}$) was significantly decreased by 14.16% compared to the control sample. The isotopic abundance ratios of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$), P_{M+2}/P_M ($^{18}O/^{16}S$), and peak area% in the treated pyridoxine HCl were significantly increased compared to the control sample. It can be assumed that the 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® - Consciousness Energy Healing Treatment. The new form of pyridoxine HCl would be better for designing novel pharmaceutical formulations which would be more soluble, absorbable, and bioavailable, that might offer better therapeutic response for the prevention and treatment of vitamin B_6 deficiency, hereditary sideroblastic anaemia, 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, lactation suppression, McArdle's disease, autism, osteoporosis, Tardive dyskinesia, stroke recurrence, etc.

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