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Annals of Chromatography and Separation Techniques

Research Article

Evaluation of the Impact of Consciousness Energy Healing Treatment on the Structural Properties and Isotopic Abundance Ratio of Vitamin C using LC-MS, GC-MS, and NMR Spectroscopy

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Article Information

Received date: Apr 29, 2019

Accepted date: May 09, 2019

Published date: May 14, 2019

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Keywords L-ascorbic acid; The Trivedi Effect®; Biofield Energy; Consciousness Energy Healing Treatment; LC-MS; GC-MS; NMR

Abstract

Ascorbic Acid (Vitamin C) is very much important for the essential metabolic reactions in the body. This study was performed to investigate the impact of the Trivedi Effect®-Consciousness Energy Healing Treatment on the structural properties and isotopic abundance of vitamin C using LC-MS, GC-MS, and NMR spectroscopy. The test sample vitamin C was divided into two parts. One part was considered as control (no Biofield Treatment was provided), while second part received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a famous Biofield Energy Healer, Mr. Mahendra Kumar Trivedi and termed as treated vitamin C. The LC-ESI-MS spectra of both the samples of vitamin C at the retention time 1.8 minutes exhibited the mass of the protonated molecular ion at m/z 176 $[M+H]^+$ (calculated for $C_6H_9O_6^+$, 177.04) in both the sample. Similarly, the fragmented ion peaks near m/z 158.91, 141.04, 129.19, and 95.10 correspond to the molecular formula $C_6H_7O_5^+$, $C_6H_5O_4^+$, $C_5H_5O_4^+$, and $C_5H_3O_2^+$, respectively were proposed for both the samples. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated vitamin C was significantly increased by 119.57% compared with the control sample. Thus, ^{13}C, ^{2}H, and ^{17}O contributions from $(C_6H_8O_6)^+$ to m/z 177.99 in the treated vitamin C were significantly increased compared with the control sample. On the contrary, the isotopic abundance ratio of P_{M+2}/P_M in the treated vitamin C was significantly decreased by 15.07% compared with the control sample. Therefore, ^{18}O contributions from $(C_6H_8O_6)^+$ to m/z 179.05 in the treated vitamin C was significantly decreased compared with the control sample. The GC-MS analysis indicated that the intensities for the mass peak at m/z 95 and 140 were significantly increased by 90.91% and 97.09%, respectively in the treated vitamin C compared to the control sample. The isotopic abundance ratios of P_{M+1}/P_M ($^{13}C/^{12}C$ or $^{2}H/^{1}H$ or $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) along with the mass peak intensities in the treated vitamin C might have altered the physicochemical properties compared to the untreated vitamin C. The increased isotopic abundance ratio of treated ascorbic acid would stronger the chemical bond, increase the stability, shift the chemical equilibrium constants, and alter the rate of metabolic reactions in the body. The Trivedi Effect®-Biofield Energy Healing Treated vitamin C would be very useful to design better nutraceutical/pharmaceutical formulations which might offer better therapeutic response against scurvy, cancer, obesity, cardiovascular, neurodegenerative, and autoimmune diseases.

Introduction

Ascorbic acid is also known as vitamin C (Figure 1) and is plenty available in the citrus fruits, tomatoes, red peppers, potatoes, animal liver, oysters, milk, etc. [1,2]. Vitamin C required in a certain range for the essential metabolic reactions both in plants and animals. It converts to ascorbate (ionized form) in neutral pH or above pH 5 in the cells of the body [3]. It is an electron donor acts as a potent water-soluble highly effective antioxidant, protect against oxidative stress [3-5]. It acts as a cofactor in many enzymatic reactions and few non-enzymatic reactions. Besides, it performs other physiological functions include the synthesis of carnitine, collagen, neurotransmitters;

OPEN ACCESS

ISSN: 2473-0696

How to cite this article Mahendra Kumar Trivedi. Evaluation of the Impact of Consciousness Energy Healing Treatment on the Structural Properties and Isotopic Abundance Ratio of Vitamin C using LC-MS, GC-MS, and NMR Spectroscopy. Ann Chromatogr Sep Tech. 2019; 5(1): 1036.

metabolism of microsome, and synthesis and catabolism of tyrosine in the body [2]. Vitamin C helps in the synthesis of collagen which is very important in the wound-healing and prevents bleeding from capillaries. It maintain the internal microenvironment determined by the redox balance, proven to be effective in the prevention and treatment of scurvy, obesity, cancer, hypertension, cardiovascular diseases (myocardial infarction, stroke, etc.), neurodegenerative diseases (Alzheimer's disease), autoimmune diseases (rheumatoid arthritis), etc. [3, 6-11] (Figure 1).

Vitamins are essential for our health and are present in almost all the foods we consume. Vitamin supplements such as multivitamin formulations are available for the prevention and treatment of vitamin deficiency diseases. Deficiency of vitamin C brings out the complications in the body, such as "scurvy" indicate bleeding gums, spongy gums, bleeding from all mucous membranes weakness, fatigue, and brown spots on the skin (thighs and legs). Other less noticeable signs but are still very serious of vitamin C deficiency such as weak immune system, gingivitis, slow wound healing, dry and splitting hair, nose bleeding, leaky gut, autoimmune disease, swollen and painful joints, etc. Long term vitamin C deficiency leads to cancer, high blood pressure, stroke, gallbladder disease, atherosclerosis, etc. [7-9]. As per the Food and Nutrition Board of the National Academy of Sciences; the tolerable upper intake level (UL) of vitamin C is 2,000 mg/day. Relatively large doses of vitamin C may cause indigestion, diarrhoea, headache, skin rashes, fatigue, disturbed sleep, haemochromatosis, suppress the production of progesterone from the corpus luteum in healthy subjects [2,12]. Low plasma concentrations are reported in patients with diabetes, infections, and smokers, but the relative contribution of diet and stress to these situations is uncertain [14].

Vitamin C degrades during the packaging, storage, and cooking of blended foods (maize, soya, etc.) is the prime limitations associated with it. Some of the research studies confirmed that exposure to air and storage temperature condition significantly affects the stability of the vitamin C. The stability of vitamin C is the major issue during processing, storage, and cooking [13-16]. Dissolution, absorption, bioavailability, and stability of a pharmaceutical compound depend upon the physicochemical properties [17], and to improve the quality of these parameters is a constant approach by the pharmaceutical scientists. The Trivedi Effect®-Consciousness Energy Healing Treatment is an economical approach and has the significant impact on the physicochemical, spectral, and thermal properties of pharmaceuticals and nutraceuticals, through the possible mediation of neutrinos [18-20]. It is also proved that with the help of Biofield Energy Healing Treatment (the Trivedi Effect®) significantly altered the isotopic abundance ratios of the pharmaceutical compounds [21, 22].

"Biofield Energy" the electromagnetic energy surrounded by every human body, which can discharge the electromagnetic energy in the form of bio-photons, generated by the continuous movement of the electrically charged particles (ions, cells, etc.) inside the body. The Biofield Energy Healing experts have the capability to harness the energy from the environment or the "Universal Energy Field" and can transfer into any living or non-living object(s), this process is called Biofield Energy Healing Treatment [23-25]. The Biofield based Energy Therapies have been reported to with significant outcomes against various disease [26]. The National Center of Complementary and Integrative Health has recognized and accepted Energy Healing Treatment as a Complementary and Alternative Medicine health care approach in addition to other therapies, medicines, and practices such as yoga, Qi Gong, Tai Chi, hypnotherapy, Reiki, etc. [27,28]. These therapies have been accepted by most of the U.S.A. population with several advantages [28]. The Trivedi Effect®-Consciousness Energy Healing Treatment had been widely accepted treatment and reported scientifically with significant outcome in different fields of pharmaceuticals and nutraceuticals [18-22, 29-31], materials science [32,33], agricultural science [34,35], microbiology [35,36], cancer research [37,38], etc. The isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M were significantly altered in the Biofield Energy Treated pharmaceutical compounds such as 1,4-dichlorobenzene [21], o- and m-nitrophenol [22], 4-bromoaniline [39], and 2,4-dichlorophenol [40]. Thus, the Trivedi Effect® can be an economical approach and solution to the practical problem of thermal stability of vitamin C by altering the isotope composition to improve the physicochemical parameters for designing better pharmaceuticals and nutraceutical formulations. The stable isotope ratio analysis has various applications in different scientific fields for considerate the isotope effects resultant from the variation of the isotopic composition of the molecule [41,42]. Isotope ratio analysis can be 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 [41, 43]. Hence, LC-MS, GC-MS, and NMR (Nuclear Magnetic Resonance) were used in this study to characterize the structural properties of the treated and untreated vitamin C. Consequently, LC-MS based 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+2}/P_M ($^{18}O/^{16}O$) in both of the samples of vitamin C were aimed to investigate the impact of the Trivedi Effect® on the isotopic abundance ratio in vitamin C.

Materials and Methods

Chemicals and reagents

The test sample vitamin C (Alfa Aesar) and other chemicals, i.e, acetonitrile (Merck), formic acid (Merck), methanol (advent), purified water (Evoqua) were of analytical grade purchased in India.

Consciousness energy healing treatment strategies

The test sample of vitamin C was divided into two parts and termed as control and treated vitamin C. The control sample did not receive the Biofield Energy Treatment; while the treated vitamin C received the Trivedi Effect®-Consciousness Energy Healing Treatment by a famous Biofield Energy Healer, Mr. Mahendra Kumar Trivedi (USA). Further, the control sample was treated with a "sham" healer who did not have any knowledge about the Biofield Energy Treatment. The Biofield Energy Treated and control samples were kept in sealed conditions and characterized using sophisticated analytical techniques.

Table 1: Isotopic abundance analysis results of vitamin C in control and treated samples

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 176.98 (%)	100	100
P_{M+1} at *m/z* 177.99 (%)	2.81	6.17
P_{M+1}/P_M	0.0281	0.0617
% Change of isotopic abundance ratio (PM+1/PM) compared to the control sample		119.57
P_{M+2} at *m/z* 179.05 (%)	1.46	1.24
P_{M+2}/P_M	0.0146	0.0124
% Change of isotopic abundance ratio (P_{M+2}/P_M) compared to the control sample		-15.07

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

Table 2: The GC retention time (R_t) and mass peak intensity values for both the control and treated vitamin C.

Peak	R_t (min)	Intensity at *m/z* 95	Intensity at *m/z* 140
Control	5.64	420197.44	56469.96
Biofield Treated Sample	5.64	802183.56	111296.63
% Change	0	90.91	97.09

Characterization

Liquid chromatography-mass spectrometry (lc-ms) analysis and calculation of isotopic abundance ratio: The LC-MS analysis of both the vitamin C samples were carried out with the help of LC-Dionex Ultimate 3000, MS-TSQ Endura, USA [21,22]. The column used here was a reversed phase Zorbax SB-C18 100X4.6mm, 3.5µm, maintained at 40˚C. 10 µL of vitamin C solution in methanol was injected and the analyte was eluted using 2 mM ammonium formate in water with 0.5% formic acid (mobile phase A) and acetonitrile (mobile phase B) pumped at a constant flow rate of 0.6 mL/min. Chromatographic separation was achieved using gradient condition as follow: 0.1 min-5%B, 5.0 min-5%B, 15.0 min-60%, 20.0 min-75%B, 25.0 min-95%B, 35.0 min-95%B, 40.0 min-5%B and 45.0 min-5% B and the total run time was 45 min. Peaks were monitored at 250 nm using the PDA detector with electro spray ionization (ESI) in positive mode. The peak area%, total ion chromatogram and mass spectrum of the individual peak which was appeared in LC were recorded.

The natural abundance of each isotope (C, O, and H) was predicted comparing the height of the isotope peak to the base peak. The natural isotopic abundance values of the common elements are obtained from the literature [42, 44-46]. The isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) for the control and Biofield Energy Treated vitamin C was calculated [21,22].

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

Where $IAR_{Treated}$ = isotopic abundance ratio in the treated vitamin C and $IAR_{Control}$ = isotopic abundance ratio in the control sample.

Gas chromatography-mass spectrometry (GC-MS) analysis: GC-MS of both the samples of vitamin C were analyzed with the help of Agilent 7890B Gas chromatograph and coupled to a quadrupole detector with pre-filter (5977B, USA) was operated with electron impact (EI) ionization in positive mode. 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) [22].

The total ion chromatogram and mass spectrum of the individual peak (appeared in GC-MS) were recorded. The % change in GC-MS mass peak intensity (I) was calculated using the following equation 1:

$$\% \text{ change in peak intensity} = [I_{Treated} - I_{Control}]/I_{Control} \times 100 \quad (1)$$

Where $I_{Control}$ and $I_{Treated}$ are the peak intensity of the control and treated vitamin C, respectively.

Nuclear magnetic resonance (nmr) analysis: ^1H and ^{13}C NMR analysis of the vitamin C was conducted at 400 MHz and 100 MHz, respectively using Agilent-MRDD2 FT-NMR spectrometer at room temperature using TMS as an internal standard [21,22]. Chemical shifts (δ) were in parts per million (ppm) relative to the solvent's residual proton chemical shift (DMSO-d_6, δ = 2.50 ppm) and solvent's residual carbon chemical shift (DMSO-d_6, δ = 39.52 ppm).

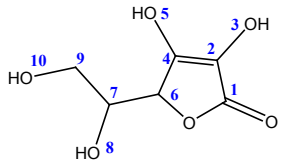
Results and Discussion

Liquid chromatography-mass spectrometry (LC-MS)

The LC chromatograms and mass spectra of both the samples of vitamin C are shown in Figures 2 and 3, respectively. The LC chromatograms of both the sample of vitamin C revealed the presence of a single peak at the retention time (R_t) 1.8 minutes (Figure 2). Thus, the results indicated that the polarity of the vitamin C remained the same in both the sample.

As per the literature LC-ESI-MS spectrum in negative ion mode produced a molecular mass peak at m/z 175.4 [47]. The ESI-MS spectra of both the samples of vitamin C (Figure 3) at the retention time 1.8 minutes exhibited the mass of the protonated molecular ion at m/z 176.98 $[M+H]^+$ (calculated for $C_6H_9O_6^+$, 177.04) in the control sample and at m/z 176.99 $[M+H]^+$ in the Biofield Energy Treated vitamin C, along with the fragment ion peaks near m/z 158.91, 141.04, 129.19, and 95.10 which were corresponded to the molecular formula

Table 3: NMR assignments of the control and treated vitamin C



Position	^{1}H NMR δ (ppm) & Multiplicity		^{13}C NMR δ (ppm)	
	Untreated	Treated	Untreated	Treated
1	--	--	170.63	170.62
2	--	--	117.97	117.96
3	10.98 (s, H)	10.98 (s, H)	--	--
4	--	--	152.90	152.90
5	8.27 (S, H)	8.27 (S, H)	--	--
6	4.71(S, H)	4.71 (S, H)	74.59	74.58
7	3.72 (m, J = 18.8 Hz, H)	3.72 (m, J = 4.8 Hz, H)	68.40	68.39
8,10	4.86 (d, J = 6 Hz, H)	4.86 (d, J = 5.2 Hz, H)	--	--
9	3.43(m, J = 29.6 Hz, 2H)	3.43 (m, J = 30.4 Hz, 2H)	61.97	61.96

s: singlet; d: doublet; m: multiple

$C_6H_7O_5^+$, $C_6H_5O_4^+$, $C_5H_5O_4^+$, and $C_5H_3O_2^+$, respectively in both the samples (Figure 3,4).

The ESI-MS spectra of both the samples showed the mass of the molecular ion peak at m/z 176.98 ($C_6H_8O_6^+$) showing relative intensity of 100%. The theoretical calculation of P_{M+1} for vitamin C were 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) = [(8 x 0.015%) x 100%] / 100%= 0.12%

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 $(C_6H_8O_6)^+$ to m/z 177.99 = 6.96%

From the calculation, it was found that ^{13}C and ^{17}O have major contribution to m/z 177.99.

Similarly, the theoretical calculation of P_{M+2} for vitamin C were 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 179.05 = 1.2%

From the above calculation, it has been found that 18O have major contribution to m/z 179.05.

The isotopic abundance ratio analysis of vitamin C in the control and Biofield Energy Treated samples were calculated for its molecular mass at m/z 176.98. P_M (m/z 176.98), P_{M+1} (m/z 177.99), and P_{M+2} (m/z 179.05) were achieved from the observed relative peak intensities of $[M^+]$, $[(M+1)^+]$, and $[(M+2)^+]$ peaks, respectively in the ESI-MS spectra and are presented in Table 1. The % change of the isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) in the treated vitamin C compared with the control sample are shown in Table 1. The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated vitamin C was significantly increased by 119.57% compared with the control sample (Table 1). So, ^{13}C, ^{2}H, and ^{17}O contributions from $(C_6H_8O_6)^+$ to m/z 177.99 in the Biofield Energy Treated vitamin C were significantly increased compared with the control sample. The isotopic abundance ratio of P_{M+2}/P_M in the treated vitamin C was significantly decreased by 15.07% compared with the control sample (Table 1). So, ^{18}O contributions from $(C_6H_8O_6)^+$ to m/z 179.05 in the Biofield Energy Treated vitamin C were significantly decreased compared with the control sample.

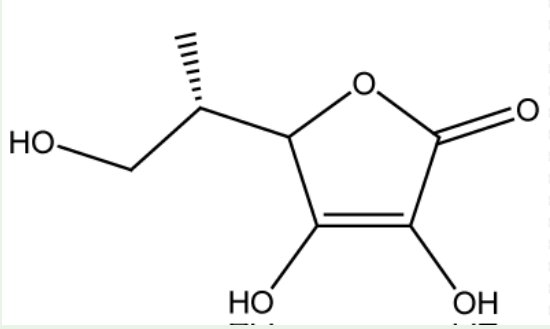


Figure 1: Structure of vitamin C

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC chromatograms of the control and Biofield Energy Treated samples of vitamin C showed the presence of several chromatographic peaks (Figure 5). The retention times of the Biofield Energy Treated vitamin Care similar to those of the control sample. Parent molecular

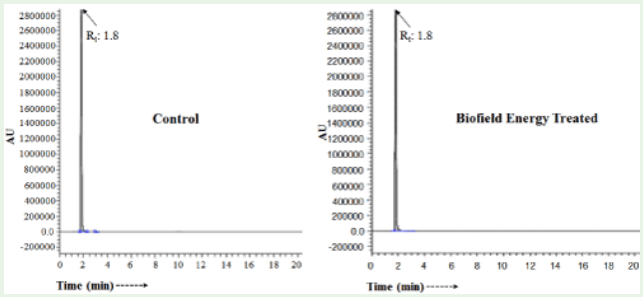


Figure 2: Liquid chromatograms of the control and treated vitamin C

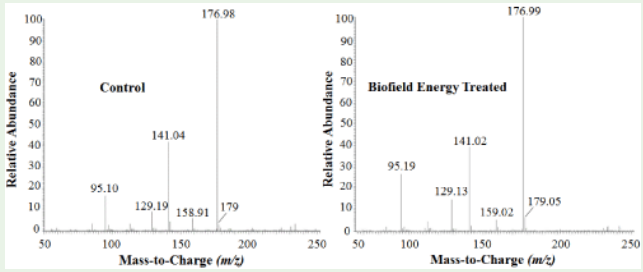


Figure 3: Mass spectra of the control and treated vitamin Cat Rt 1.8 minutes.

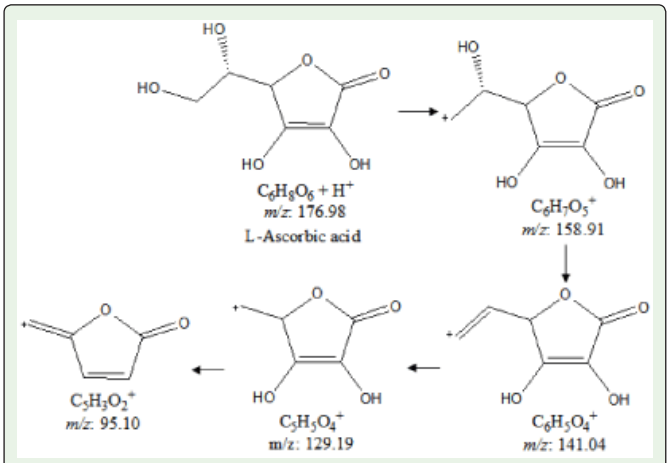


Figure 4: Proposed fragmentation pathway of vitamin C.

peak of vitamin C did not observe in any of the mass spectra of control and Biofield Energy Treated samples. Molecular fragments at m/z 95.0 [M$^+$] (calculated for $C_5H_3O_2^+$, 95.01) and m/z 140.0 [M$^+$] (calculated for $C_6H_4O_4^+$, 140.01) of the control (Figure 5A) and Biofield Energy Treated (Figure 5B) vitamin C were proposed for R_t of 5.64 minutes from both the mass spectra. The results indicated that the polarity of both samples is same, but the mass peak intensities are widely different in the Biofield Energy Treated vitamin C compared to the control sample. The intensities for the mass peak at m/z 95 and 140 were significantly increased by 90.91% and 97.09%, respectively in the Biofield Energy Treated vitamin C compared to the control sample (Table 2). The mass peak intensities influence the isotopic abundance ratio, which was well supported by the LC-MS, based isotopic abundance ratio analysis.

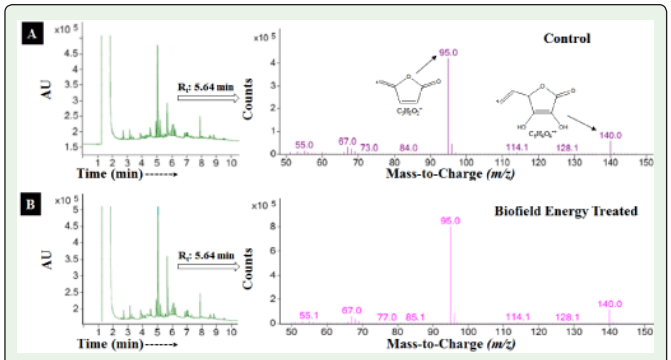


Figure 5: The GC-MS chromatogram of the Control (A) and treated (B) sample of L-vitamin C.

Nuclear Magnetic Resonance Spectroscopy (^{1}H &^{13}C NMR)

The ^{1}H and ^{13}C NMR of the control and Biofield Energy Treated vitamin C were carried out, and the results are reports in Table 3. The characteristic proton signals for aromatic protons, -CH_2, -CH, and -OH groups of vitamin C in the ^{1}H NMR of spectra of both the control and Biofield Energy Treated samples was described in Table 3. Similarly, the characteristic carbon signals for -C=O, quaternary carbon (aromatic), -CH (aromatic and aliphatic),-CH_2 (aliphatic), -COH (aromatic and aliphatic) groups of vitamin C in the ^{13}C NMR of spectra of the both the control and Biofield Energy Treated samples were described in Table 3. The experimental data well matched with the literature data [48]. The results indicated that there was no such significant alternation in the characteristic proton and carbon signals for vitamin C in the ^{1}H and ^{13}C NMR spectrum of the Biofield Energy Treated vitamin C compared with the control sample.

Finally, LC-MS, GC-MS, and NMR study confirmed the structure of vitamin C. The LC-MS based isotopic abundance ratios of P_{M+1}/P_M (^{2}H/^{1}H 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 were significantly altered compared to the control sample. Modern physics explained that 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 internally. Therefore, the neutrinos have the ability to interact with protons and neutrons in the nucleus, which indicated a close relation between neutrino and the isotope formation [20, 44,45]. The altered isotopic composition in the molecular level of the Trivedi Effect®-Consciousness Energy Healing Treated vitamin C might be due to the alteration in 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 natural abundance and relative proportion of the stable isotopes in organs and tissues significantly affected by the environment, climate, etc. The isotopic abundance ratios ^{2}H/^{1}H, ^{13}C/^{12}C, ^{17}O/^{16}O, and ^{18}O/^{16}Owould highly influence the atomic bond vibration of treated ascorbic acid [49]. The increased isotopic abundance ratio of the Consciousness Energy Healing Treated ascorbic acid would stronger the chemical bond, increase the

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stability, shift the chemical equilibrium constants, and alter the rate of metabolic reactions in the body.

Conclusions

The experimental results revealed that the Trivedi Effect®-Consciousness Energy Healing Treatment has the significant impact on the isotopic abundance ratios and mass peak intensities of vitamin C. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated vitamin C was significantly increased by 119.57% compared with the control sample. Thus, ^{13}C, ^{2}H, and ^{17}O contributions from $(C_6H_8O_6)^+$ to m/z 177.99 in the treated vitamin C were significantly increased compared with the control sample. On the contrary, the isotopic abundance ratio of P_{M+2}/P_M in the treated vitamin C was significantly decreased by 15.07% compared with the control sample. Therefore, 18O contributions from $(C_6H_8O_6)^+$ to m/z 179.05 in the Biofield Energy Treated vitamin C was significantly decreased compared with the control sample. The GC-MS indicated that the intensities for the mass peak at m/z 95 and 140 were significantly increased by 90.91% and 97.09%, respectively in the treated vitamin C compared to the control sample. The isotopic abundance ratios of P_{M+1}/P_M ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$) and P_{M+2}/P_M ($^{18}O/^{16}O$) along with the mass peak intensities in the Biofield Energy Treated vitamin C might have altered the physicochemical properties compared to the untreated vitamin C. The isotopic abundance ratios $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{17}O/^{16}O$, and $^{18}O/^{16}O$ highly influence the atomic bond vibration of the Biofield Energy Treated ascorbic acid. The increased isotopic abundance ratio of ascorbic acid would stronger the chemical bond, increase stability, shift the chemical equilibrium constants, and alter the rate of metabolic reactions in the body. The Trivedi Effect® treated vitamin C would be very useful to design better nutraceutical/pharmaceutical formulations which might offer better therapeutic response against scurvy, cancer, obesity, cardiovascular diseases (myocardial infarction, stroke, etc.), hypertension, neurodegenerative diseases (Alzheimer's disease), autoimmune diseases (rheumatoid arthritis), etc.

Acknowledgements

The authors are grateful to GVK Biosciences Pvt. Ltd., Trivedi Science, Trivedi Global, Inc., Trivedi Testimonials, and Trivedi Master Wellness for their assistance and support during this work.

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Citation: Mahendra Kumar Trivedi. Evaluation of the Impact of Consciousness Energy Healing Treatment on the Structural Properties and Isotopic Abundance Ratio of Vitamin C using LC-MS, GC-MS, and NMR Spectroscopy. Ann Chromatogr Sep Tech. 2019; 5(1): 1036.

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