



# LC-MS and GC-MS Based Isotopic Abundance Ratio Analysis of The Consciousness Energy Healing Treated Ascorbic Acid

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#### Abstract

Ascorbic acid is required by both plants and animals for the essential metabolic process. It is sufficiently available in the citrus fruits, tomatoes, potatoes, red peppers, milk, oysters, animal liver, etc. In this study, the LC-MS and GC-MS were used to characterize the structural properties and isotopic abundance ratio to evaluate the impact of the Trivedi Effect®-Consciousness Energy Healing Treatment on L-ascorbic acid compared to the control sample. The test sample was divided into two parts, one part of ascorbic acid was considered as a control sample, while the second part only received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a renowned Biofield Energy Healer, Dahryn Trivedi and termed as a Biofield Energy Treated sample. The LC-MS spectra of both the control and Treated samples at retention time (R.) 1.8minutes exhibited the mass of the deprotonated molecular ion peak at m/z 175 [M-H]<sup>-</sup> (calculated for C<sub>e</sub>H<sub>2</sub>O<sub>e</sub>-, 175.02). The peak area of the treated sample (3607446.27) was significantly decreased by 65.05% compared to the control sample (10320794.8). The LC-MS based 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>17</sup>O/<sup>16</sup>O) in the treated ascorbic acid was significantly increased by 119.71% compared with the control sample. Thus, <sup>13</sup>C, <sup>2</sup>H, and <sup>17</sup>O contributions from  $(C_{H_0})$  to m/z 176 in the treated sample were significantly increased compared with the control sample. The gas chromatographic peak area of the treated sample (12.74) was significantly decreased by 31.1% compared to the control sample (18.49). Similarly, the GC-MS based isotopic abundance ratio of  $P_{M+2}/P_M$  in the treated ascorbic acid was significantly increased by 28.74% compared with the control sample. So, 180 contributions from  $(C_6H_8O_6)^{+}$  to m/z 177 in the Biofield Energy Treated sample were significantly increased compared with the control sample. The isotopic abundance ratio of  $P_{M+1}/P_{M}$  $({}^{18}O/{}^{16}O)$  in the treated ascorbic acid was significantly increased compared to the control sample. The increased isotopic composition of the Trivedi Effect®-Consciousness Energy Healing Treated ascorbic acid might have altered the neutron to proton ratio in the nucleus. 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 increased isotopic abundance ratio and peak area of the treated ascorbic acid may increase the intra-atomic bond strength, increase its stability. This new form of ascorbic acid would be very useful to design the better pharmaceutical formulations that might offer better therapeutic response against scurvy, obesity, cardiovascular diseases, hypertension, Alzheimer's disease, cancer, rheumatoid arthritis, etc.

Keywords: Ascorbic acid; The Trivedi Effect<sup>®</sup>; Biofield energy; Consciousness energy healing treatment; LC-MS; GC-MS

## Introduction

Ascorbic acid (vitamin C) is required by both plants and animals for the essential metabolic process. It is sufficiently available in the citrus fruits, tomatoes, potatoes, red peppers, milk, oysters, animal liver, etc. [1,2]. Ascorbic acid converts to ascorbate at neutral pH or above pH 5 inside the cells and acts as a potent water-soluble antioxidant [3-5]. It is a cofactor in many enzymatic and non-enzymatic reactions, which helps in the synthesis of collagen, carnitine, neurotransmitters; wound healing and prevent bleeding from capillaries, metabolism of microsome, and synthesis and catabolism of tyrosine in the body [2]. It is useful in the prevention and treatment of scurvy, hypertension, obesity, cancer, cardiovascular diseases, Alzheimer's disease, rheumatoid arthritis, etc. [3,6-11]. Deficiency of ascorbic acid leads to scurvy, gingivitis, nose bleeding, weak immune system, autoimmune disease, slow

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wound healing, dry and splitting hair, leaky gut, swollen and painful joints, cancer, high blood pressure, stroke, gallbladder disease, atherosclerosis, etc. [7-9]. Low plasma concentrations of ascorbic acid are observed in the case of diabetes, infections, and smoking [12]. Vitamin C supplements are available for the prevention and treatment of vitamin deficiency diseases. The tolerable upper intake level of ascorbic acid is 2,000mg/day and excess of ascorbic acid in the body may cause indigestion, diarrhea, headache, fatigue, disturbed sleep, haemochromatosis, skin rashes, suppress the production of progesterone from the corpus luteum in healthy subjects [2,13].

While cooking, processing, packaging, storage, and cooking of blended foods, ascorbic acid degrades is a primary problem [13-15]. Exposure to air and temperature significantly affect the stability of ascorbic acid [14,16]. Dissolution, absorption, bioavailability, and stability of a pharmaceutical compound depend upon the physicochemical properties of any pharmaceutical compounds [17]. Improvement of the physicochemical properties of pharmaceutical and nutraceutical products is a challenging task [17]. But it was observed that Biofield Energy Healing Treatment (the Trivedi Effect<sup>®</sup>) has a significant impact on various physicochemical properties and bioavailability of pharmaceutical/nutraceutical compounds [18-21]. The Trivedi Effect® is a natural and only scientifically established phenomenon in which an individual can harness this inherently intelligent energy and transmit it anywhere on the globe through the possible mediation of neutrinos [22]. "Biofield Energy" the electromagnetic energy field which exists surrounding the living beings, which can transmit 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. Biofield Energy Healing specialists can harness the energy from the Universe and can transmit into any living and non-living object(s), this process is called Biofield Energy Treatment [23,24]. Biofield based Energy Therapies have been reported with significant outcomes against various disease, human health, and wellness [25]. The National Center of Complementary and Integrative Health (NCCIH) has been recognized and accepted Biofield Energy Healing treatment as a Complementary and Alternative Medicine (CAM) health care approach along with other therapies, medicines, and practices [26]. Most of the U.S.A. people have adopted these therapy [27]. The Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment has also reported with altering the physical and thermal properties of metals, ceramics, polymers, and organic compounds [28-31], improved productivity of crops [32,33], healing cancer [34], and altered isotopic abundance [31,35].

The study of stable isotope ratio analysis has various applications in different scientific fields of science [36,37]. This study can be performed by using the conventional gas chromatographymass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) in low micromolar concentration with sufficient precision [37,38]. The Trivedi Effect<sup>®</sup>-Biofield Energy Healing Treatment could be an economical approach for designing better pharmaceuticals formulations. Thus, the LC-MS and GC-MS were used to characterize the structural properties and isotopic abundance ratio to evaluate the impact of the Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment on L-ascorbic acid compared to the control sample.

#### **Materials and Methods**

#### **Chemicals and reagents**

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

#### **Consciousness energy healing treatment strategies**

The test sample vitamin C powder was divided into control and Biofield Energy Treated parts. To the control sample, no Biofield Energy Treatment was provided but was treated with a "sham" healer who did not have any understanding about the Biofield Energy Treatment. However, the treated sample of ascorbic acid was received the Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 minutes by the renowned Biofield Energy Healer, Dahryn Trivedi, USA. After that, both the treated and control ascorbic acid samples were kept in sealed conditions and characterized using chromatographic and spectroscopic analytical techniques.

#### Characterization

Liquid chromatography-mass spectrometry (lc-ms) analysis and calculation of isotopic abundance ratio: The LC-MS analysis of both the ascorbic acid samples were carried out with the help of LC-MS ThermoFisher Scientific, the USA equipped with an ion 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 5 micron), maintained at 25°C. The diluent used for the sample preparation was methanol and water. 10µL of the ascorbic acid solution was injected, and the analyte was eluted using 80% acetonitrile +20% 5mM ammonium acetate pumped at a constant flow rate of 1mL/ min. 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 values of the natural isotopic abundance of the common elements are obtained from the literature [37,39,40]. The LC-MS based isotopic abundance ratios ( $P_{M+1}/P_M$ ) for both the ascorbic acid samples were calculated using equation (1).

% Change in isotopic abundance ratio=
$$\begin{bmatrix} (IAR Treated-IAR Control) \\ IAR Control \\ IAR Control \end{bmatrix} (1)$$

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 both the samples of ascorbic acid 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 (5min hold) to 250°C (2.5min hold) @ 10°C /min (total run time 25min). The sample was prepared taking 100mg of the ascorbic acid is in 2ml methanol as a diluent. Injection

volume was 5µl. The GC-MS based isotopic abundance ratios ( $P_{M+1}/P_{M}$ ) for the control and Biofield Energy Treated ascorbic acid was calculated using equation (1).

### **Results and Discussion**

#### Liquid Chromatography-Mass Spectrometry (LC-MS)



Figure 1: Liquid chromatograms of the control and Biofield Energy Treated ascorbic acid.

The LC-MS chromatograms and mass spectra of both the control and treated samples of ascorbic acid are shown in Figures 1 & 2, respectively. The major chromatographic peak was observed at the retention time ( $R_1$ ) of 1.8 minutes for both the case (Figure 1). But the peak area of the Biofield Energy Treated sample (3607446.27) was significantly decreased by 65.05% compared to the control sample (10320794.8). This indicated that the solubility of the Biofield Energy Treated ascorbic acid was significantly decreased. The data was supported by one of the recently published articles, in which the particle sizes of the ascorbic acid were significantly increased, and the surface area was significantly decreased after the Biofield Energy Treatment by the healer Dahryn Trivedi [41].





The mass spectra of both the samples of ascorbic acid exhibited the mass of the deprotonated molecular ion  $[M-H]^{-}$  peak at m/z 175 (calculated for  $C_6H_7O_6^{-}$ , 175.02) along with other low molecular

fragmentation peaks at 143, 115, 111, 87, and 70 for  $C_6H_7O_4$ ,  $C_5H_7O_3$ ,  $C_5H_3O_3$ ,  $C_4H_7O_2$ , and  $C_4H_6O^-$  (Figures 2 & 3). The published literature supported the experimental data [42].



Figure 3: Proposed fragmentation pattern of ascorbic acid in -ve ion mode.

The LC-MS spectra of both the ascorbic acid showed the mass of the molecular ion peak at m/z 175[M-H]<sup>-</sup> (calculated for  $C_6H_7O_6^-$ , 175.02) with 100% relative intensity. The theoretical calculation of  $P_{M+1}$  for ascorbic acid was presented as below:

P (<sup>13</sup>C) = [(6 x 1.1%) x 100% (the actual size of the M<sup>+</sup> peak)]/100% = 6.6%

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

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

 $\rm P_{M+1,}$  i.e.  $^{13}$  C,  $^{2}$  H, and  $^{17}$  O contributions from (C\_6H\_7O\_6)^- to m/z 176 = 6.95%

From the above calculation, it has been found that <sup>13</sup>C and <sup>17</sup>O have a major contribution to m/z 176. The LC-MS based isotopic abundance ratio analysis PM (m/z 175) and  $P_{M+1}$  (m/z 176) of both the samples were obtained from the observed relative peak intensities of [M<sup>+</sup>] and [(M+1)<sup>+</sup>] peaks, respectively in the mass spectra (Table 1). The percentage change of the isotopic abundance ratio (P<sub>M+1</sub>/PM) in the Biofield Energy Treated ascorbic acid was significantly increased by 119.71% compared to the control sample (Table 1). Therefore, the <sup>13</sup>C, <sup>2</sup>H, and <sup>17</sup>O contributions from (C<sub>6</sub>H<sub>7</sub>O<sub>6</sub>)<sup>-</sup> to m/z 176 in the Biofield Energy Treated sample were significantly increased compared to the control sample.

Table	<b>1:</b> LC-MS	based	isotopic	abundance	analysis	results	in 1	Biofield	Energy	Treated	ascorbic	acid	compared	to	the
contro	l sample.														

Parameter	Control sample	Biofield Energy Treated sample				
P <sub>M</sub> at m/z 175 (%)	100	100				
P <sub>M+1</sub> at m/z 176 (%)	4.16	9.14				
P <sub>M+1</sub> /PM	0.04	0.09				
% Change of isotopic abundance ratio $(P_{M+1}/P_M)$ with respect to the control sample 119.71						
PM: the relative peak intensity of the parent molecular ion $[M+]$ ; $P_{M+1}$ : the relative peak intensity of the isotopic molecular ion $[(M+1)^+]$ , M: mass of the parent molecule						

# Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The chromatographic peak at Rt of 12.7 minutes of both the chromatogram reflected the molecular ion peak of ascorbic acid at

m/z 175 [M]<sup>+</sup> (calculated for  $C_6H_8O_6^+$ , 175.02) in their respective spectrum (Figures 4 & 5). The gas chromatographic peak area of the treated sample (12.74) was significantly decreased by 31.1% compared to the control sample (18.49). The data was supported by the LC-MS data.



Figure 4: The GC-MS chromatogram and mass spectra of the control ascorbic acid.



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

The mass peak intensities were significantly altered in the case of Biofield Energy Treated sample compared to the control sample. The GC-MS spectra of both the samples showed the mass of the molecular ion peak [M]<sup>+</sup> at m/z 175 (calculated for  $C_6H_8O_6^{+}$ , 176.03). The theoretical calculation of  $P_{M+2}$  for ascorbic acid was presented as below:

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

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

From the above calculation, it has been found that  $^{18}\text{O}$  have a major contribution to m/z 177. The GC-MS based isotopic

abundance ratio analysis of the Biofield Energy Treated ascorbic acid samples were calculated compared to the control sample.  $P_M$ and  $P_{M+2}$  for ascorbic acid near m/z 175 and 177, respectively of the control and Biofield Energy Treated samples, which were obtained from the observed relative peak intensities of [M<sup>+</sup>] and [(M+2)<sup>+</sup>] peaks, respectively (Table 2). The isotopic abundance ratio of  $P_{M+2}/P_M$  in the Biofield Energy Treated ascorbic acid was significantly increased by 28.74% compared with the control sample (Table 2). Hence, <sup>18</sup>O contributions from ( $C_6H_8O_6$ )<sup>+</sup> to m/z 177 in the Biofield Energy Treated sample were significantly increased compared with the control sample.

Parameter	Control sample	Biofield Energy Treated sample			
P <sub>M</sub> at m/z 175 (%)	0.05	0.04			
P <sub>M+2</sub> at m/z 177 (%)	0.08	0.08			
P <sub>M+2</sub> /P <sub>M</sub>	1.6	2.06			
% Change of isotopic abundance ratio $(P_{M+1}/P_M)$ with respect to the control sample 28.74					
PM: the relative peak intensity of the parent molecular ion $[M+]$ ; $P_{M+2}$ : the relative peak intensity of the isotopic molecular ion $[(M+2)^*]$ ; M: mass of the parent molecule.					

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

LC-MS and GC-MS study confirmed the structure of the sample as ascorbic acid. The isotopic abundance ratios of  $P_{M+1}/P_{M}$  (<sup>2</sup>H/<sup>1</sup>H or  $^{13}\text{C}/^{12}\text{C}$  or  $^{17}\text{O}/^{16}\text{O})$  and  $P_{_{M+2}}/$   $P_{_{M}}$  ( $^{18}\text{O}/^{16}\text{O})$  in the Biofield Energy Treated ascorbic acid were significantly increased compared to the control sample. The increased isotopic composition of the Trivedi Effect®-Consciousness Energy Healing Treated ascorbic acid might have altered the neutron to proton ratio in the nucleus. 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 neutrinos can interact with protons and neutrons in the nucleus, which indicated a close relationship between neutrino and the isotope formation [22,37,38]. The isotopic abundance ratios  $^{2}H/^{1}H$  or  $^{13}C/^{12}C$ or  ${}^{17}0/{}^{16}0$  or  ${}^{18}0/{}^{16}0$  would influence the atomic bond vibration of treated ascorbic acid [43]. The increased isotopic abundance ratio and peak area of the treated ascorbic acid may increase the intra-atomic bond strength, increase its stability. This new form of ascorbic acid would be very useful to design the better pharmaceutical formulations that might offer better therapeutic response against scurvy, obesity, cardiovascular diseases, hypertension, Alzheimer's disease, cancer, rheumatoid arthritis, etc.

#### Conclusions

The Trivedi Effect®-Consciousness Energy Healing Treatment showed a noteworthy impact on the isotopic abundance ratios and peak area of the ascorbic acid. The LC-MS peak area of the treated sample was significantly decreased by 65.05% compared to the control sample. The LC-MS based isotopic abundance ratio of  $P_{M+1}/P_M$  (<sup>2</sup>H/<sup>1</sup>H or <sup>13</sup>C/<sup>12</sup>C or <sup>17</sup>O/<sup>16</sup>O) in the Consciousness Energy Healing Treated ascorbic acid was significantly increased by 119.71% compared with the control sample. Thus, <sup>13</sup>C, <sup>2</sup>H, and <sup>17</sup>O contributions from  $(C_6H_7O_6)^2$  to m/z 176 in the Consciousness Energy Healing Treated sample were significantly increased compared with the control sample. The gas chromatographic peak area of the Consciousness Energy Healing Treated sample (12.74) was significantly decreased by 31.1% compared to the control sample (18.49). Similarly, the GC-MS based isotopic abundance ratio of  $P_{M+2}/P_M$  in the Consciousness Energy Healing Treated ascorbic acid was significantly increased by 28.74% compared with the control sample. So, <sup>18</sup>O contributions from  $(C_6H_8O_6)^+$  to m/z 177 in the Biofield Energy Treated sample were significantly increased compared with the control sample. The isotopic abundance ratio

of  $P_{M+1}/P_M$  (<sup>18</sup>O/<sup>16</sup>O) in the Consciousness Energy Healing Treated ascorbic acid was significantly increased compared to the control sample. The increased isotopic composition of the Consciousness Energy Healing Treated ascorbic acid might have altered the neutron to proton ratio in the nucleus. The changes in isotopic abundance could be due to changes in nuclei possibly through the interference of neutrino particles via the Trivedi Effect<sup>®</sup>-Consciousness Energy Healing Treatment. The increased isotopic abundance ratio and peak area of the Consciousness Energy Healing Treated ascorbic acid may increase the intra-atomic bond strength, increase its stability. The new form of ascorbic acid would be very useful to design better pharmaceutical formulations that might offer better therapeutic response against scurvy, obesity, cardiovascular diseases, hypertension, Alzheimer's disease, cancer, rheumatoid arthritis, etc.

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