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Consciousness Energy Healing Treatment Impacted the Isotopic Abundance Ratio of 6-Mercaptopurine (6-MP)

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

[1]*Trivedi Global, Inc., Henderson, USA*

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

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

Abstract

6-mercaptopurine (6-MP) is an antimetabolite antineoplastic chemotherapy drug. In this research work, the impact of the Trivedi Effect® on the structural properties and the isotopic abundance ratio of 6-MP were evaluated. The 6-MP test sample was divided into two-parts and termed as control and Biofield Energy Treated sample. The treated 6-MP only received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a well-known Biofield Energy Healer, Mahendra Kumar Trivedi. The LC-MS spectra of both the 6-MP samples at retention time (R_t) 2.2 minutes showed the mass of the protonated molecular ion peak at *m/z* 173 $[M+H]^+$. The peak area of the treated 6-MP was significantly increased by 92.07% compared to the control sample. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated 6-MP was significantly increased by 34.79% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in the treated 6-MP was significantly increased by 40.78% and 377.01% compared with the control sample. Thus, ^{13}C, ^{2}H, ^{15}N, ^{33}S, and ^{18}O contributions from $(C_5H_5N_4S)^+$ to *m/z* 153 and 154 in the Biofield Energy Treated 6-MP were significantly increased as compared to the control 6-MP. The isotopic abundance ratio of P_{M+1}/PM ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{33}S/^{32}S$) and P_{M+2}/P_M ($^{34}S/^{32}S$) in the treated 6-MP was significantly improved compared to the control sample. The significant increase in the peak area and isotopic abundance could be due to the interference of neutrino particles in the nucleus *via* the Trivedi Effect®. The increased isotopic abundance ratio of the treated 6-MP would improve the chemical bond strength, increase the physical and chemical stability of 6-MP in the body. The Biofield Energy Treated 6-MP would be better designing more efficacious pharmaceutical formulations that might offer increased bioavailability and therapeutic response against acute lymphocytic leukemia, Crohn's disease, chronic myeloid leukemia, and ulcerative colitis, etc.

Keywords: 6-Mercaptopurine; Biofield Energy; The Trivedi Effect®; Consciousness Energy Healing Treatment; LC-MS; GC-MS

Introduction

6-mercaptopurine (6-MP) is an antimetabolite antineoplastic chemotherapy drug. It is most effective at killing tumour cells that are rapidly dividing by interfering with the nucleic acid synthesis by inhibiting purine metabolism [1,2]. It is used as an anticancer and an immunosuppressive agent, i.e., myeloid leukaemia, lymphocytic leukaemia, ulcerative colitis, and Crohn's disease [2-5]. It has been approved for medical use in the U.S.A. (1953) and also listed as an essential medicine by the WHO [6]. The common side effects related to the mercaptopurine use are immune and bone marrow suppression, liver toxicity, diarrhoea, loss of appetite, mouth sores, fatigue, weakness, fever, sore throat, hair loss, red spots on the skin, darkening of the skin, yellowing of eyes or skin, bloody stools, bloody or dark urine, painful or difficult urination, genetic polymorphisms, etc. [7-9]. Mercaptopurine delivered in the form of a tablet and liquid suspension [10-12]. It is soluble in hot alcohol and dilute alkali solutions; slightly soluble in dilute sulfuric acid; insoluble in water, chloroform, acetone, and diethyl ether [12].

The physicochemical properties of the pharmaceutical compound determine the quality, stability, solubility, and bioavailability [13]. The Trivedi Effect® has been scientifically proved with the significant impact on particle size, surface area, and bioavailability of pharmaceutical and nutraceutical compounds [14-18]. The Trivedi Effect® is a well-proven phenomenon in which a healer can harness this inherently intelligent energy from the Universe and transfer it anywhere on the planet through the possible mediation of neutrinos [19]. The "Biofield" is an electromagnetic energy field which exists surrounding the living beings, which generated by the continuous movement of the charged particles (i.e., ions, cells, blood flow, etc.) in the body [20-22]. The "Biofield" based Energy Therapies reported having significant positive outcomes against various disease [23]. The

National Centre of Complementary and Integrative Health has approved the Biofield Energy Therapies as a Complementary and Alternative Medicine (CAM) health care approach in addition to other therapies, medicines, and practices *viz*. Ayurveda, homeopathy, hypnotherapy, yoga, Reiki, healing touch, Tai Chi, Qi Gong, etc. [24,25]. In similar way, the Trivedi Effect®-Consciousness Energy Healing Treatment also has a significant effect on the metals, ceramics, polymers, organic materials, crops, microbes, biotechnology, cancer cells, bone health, etc. [26-40].

This indicated that the Trivedi Effect®-Consciousness Energy Healing Treatment could be an economical approach to improve the physicochemical properties of 6-MP. The study of stable isotope ratio and its composition helps to understand the atomic bond strength, physicochemical, and thermal properties of the compound [41,42]. Isotope ratio analysis can be performed with the help of gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) in low micromolar concentration with sufficient precision [41,43]. In this study, the structural characterization and isotopic abundance ratio analysis of P_{M+1}/P_M (2H/1H or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{33}S/^{32}S$) and P_{M+2}/P_M ($^{33}S/^{32}S$) in the Consciousness Energy Healing Treated 6-MP was evaluated compared to the control sample using LC-MS and GC-MS analytical techniques.

Materials and Methods

Chemicals and Reagents

The chemicals and reagents are purchased in India and abroad. The 6-MP powder sample was procured from Tokyo Chemical Industry Co., Ltd., Japan, but the other chemicals were procured in India.

Consciousness Energy Healing Treatment Strategies

The 6-MP powder was divided into two equal parts, i.e., the control part and the treated part. The control 6-MP powder sample did not get the Biofield Energy Treatment, but the sample has received treatment from a "sham" healer, who did not have any knowledge related to Biofield. However, the treated 6-MP was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely for 3 minutes by the well-known Biofield Energy Healer, Mahendra Kumar Trivedi, USA. The Energy Treatment was provided through the Mahendra Kumar Trivedi's unique energy transmission process. After the treatment, both the 6-MP samples were kept in the sealed conditions and characterized using LC-MS and GC-MS analytical techniques.

Characterization

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

The LC-MS of the 6-MP was performed in LC-MS ThermoFisher Scientific (USA), equipped with a triple-stage quadrupole mass spectrometer. A reversed phase Thermo Scientific Synchronis C18 (Length-250 mm X ID 4.6 mm X 5 micron) column was used. For the sample preparation, water and acetonitrile was used as diluent. 10 µL of the 6-MP solution was injected, and the analyte was eluted in gradient mode using 0.1% formic acid in water (mobile phase A; 10%), and acetonitrile (mobile phase B; 95%) pumped at a constant flow rate of 0.5 mL/min. The chromatographic peaks were monitored at 300 nm using the PDA detector, and the mass spectrometric analysis was performed in +ve ESI mode.

The natural abundance of each isotope (C, O, H, N, and S) was predicted by comparing the intensity of the isotope peak with the base peak. The values of the natural isotopic abundance of the elements are obtained from the literature [42,43-46]. The % change in the isotopic abundance ratio (P_{M+1}/P_M) was calculated with the help of equation 1.

$$\%\ change\ in\ isotopic\ abundance\ ratio\ =\ \left[\left(IARTreated\ -\ IARControl\right)/\ IARControl\right)\right]\ x\ 100$$

Where IAR: isotopic abundance ratio in the control and treated sample.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS of the 6-MP was analyzed using Perkin Elmer GC equipped with a PE-5MS (30M x 250 micros x 0.250 microns) capillary column and coupled to a single quadrupole mass detector. It was operated with electron impact (EI) ionization method in positive ion mode. The % change in isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) was calculated using equation 1.

Results and Discussion

Liquid Chromatography-Mass Spectrometry (LC-MS)

A single chromatographic peak was observed in both the control and treated 6-MP chromatograms at the retention time (R_t) of 2.2 minutes (Figure 1). In the Biofield Energy Treated 6-MP, the peak area (28285376.87) was significantly increased by 92.07% as compared to the control 6-MP (14726473.48). This indicated that the solubility of the treated 6-MP was significantly improved compared with the control sample. The data were strongly supported by the recently published article in which the Consciousness Energy Healing Treatment significantly decreased the particle size and increased the surface area of 6-MP [14]. This may improve the solubility, bioavailability, and therapeutic efficacy of the treated 6-MP compared to the control sample.

As per the literature 6-MP generally shows the protonated molecular mass $[M+H]^+$ peak at *m/z* 153 in positive ion mode [47]. The mass spectra of 6-MP (Figure 2) exhibited the protonated molecular ion peak at *m/z* 173 $[M+H]^+$ (calculated for $C_5H_5N_4S^+$, 153.18) along with the fragmentation peak $C_5H_3N_4^+$ (*m/z* 119) and $C_4H_7N_2^+$ (*m/z* 82) in case of both the samples (Figure 3).

The 6-MP showed the molecular ion $[M+H]^+$ peak at *m/z* 173 (calculated for $C_5H_5N_4S^+$, 153.18) with relative intensity of 100%. The theoretical calculation of P_{M+1} for 6-MP was presented as below:

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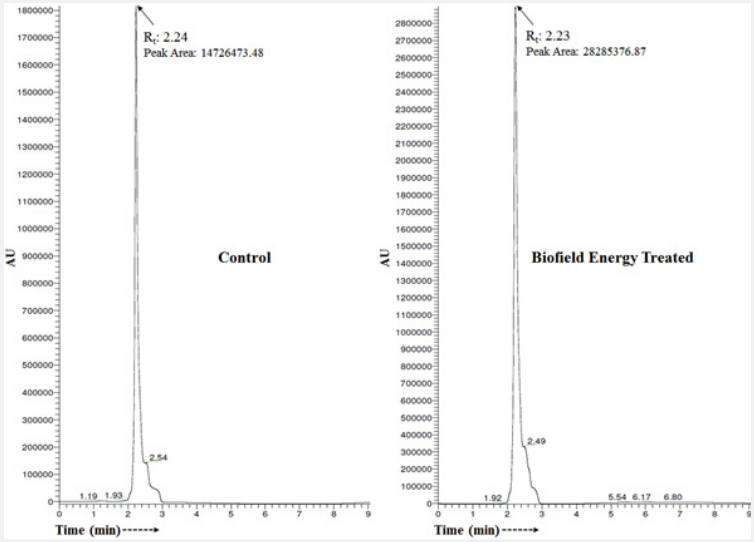

Figure 1: Liquid chromatograms of the control and treated 6-MP.

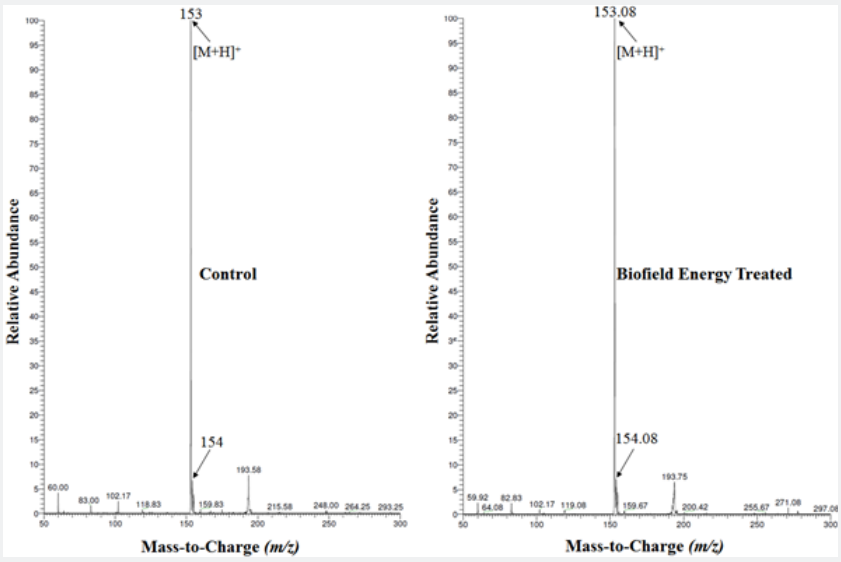

Figure 2: Mass spectra of the control and treated 6-MP at R_t 2.2 minutes.

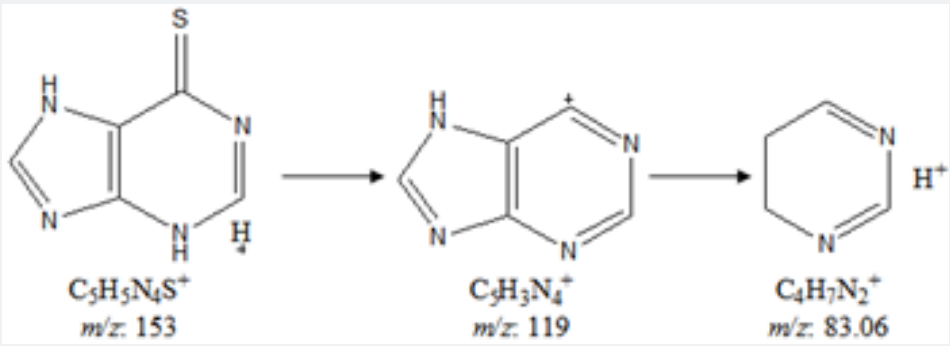

Figure 3: Proposed fragmentation pattern of 6-MP.

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P (^{13}C) = [(5 x 1.1%) x 100% (the actual size of the M^+ peak)] / 100% = 5.5%

P (^{2}H) = [(5 x 0.015%) x 100%] / 100%= 0.075%

P (^{15}N) = [(4 x 0.4%) x 100%] / 100% = 1.6%

P (^{33}S) = [(1 x 0.08%) x 100%] / 100% = 0.08%

P_{M+1}, i.e. ^{13}C, ^{2}H, ^{15}N, and ^{33}S contributions from $(C_5H_5N_4S)^+$ to *m/z* 154= 7.26%

The calculated isotope abundance was close to the experimental value (Table 1). It has been found that ^{13}C and ^{15}N have major contribution to m/z 154.

The LC-MS based isotopic abundance ratio analysis PM and PM+1 of the 6-MP at were obtained from the observed relative peak intensities of $[M^+]$ and $[(M+1)^+]$, respectively in the ESI-MS spectra (Table 1) of both the samples. The isotopic abundance ratio P_{M+1}/P_M in the treated 6-MP was significantly increased by 34.79% compared with the control sample (Table 1). Thus, it was concluded that the ^{13}C, ^{2}H, ^{15}N, and ^{33}S contributions from $(C_5H_5N_4S)^+$ to *m/z* 154 in the treated 6-MP were significantly decreased as compared to the control 6-MP.

Table 1: LC-MS based isotopic abundance analysis results of the treated 6-MP compared to the control sample.

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 153 (%)	100	100
P_{M+1} at *m/z* 154 (%)	5.26	7.09
P_{M+1}/P_M	0.05	0.07
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control sample		34.79

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

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

A single chromatographic peak at the retention time of 17.46 and 16.17 minutes in the chromatogram control and treated 6-MP (Figures 4 and 5). The parent molecular ion peak of 6-MP at *m/z* 152 $[M]^+$ (calculated for $C_5H_5N_4S^+$, 152.02) was observed in both the samples, along with the fragment ion peaks (Figures 3-5).

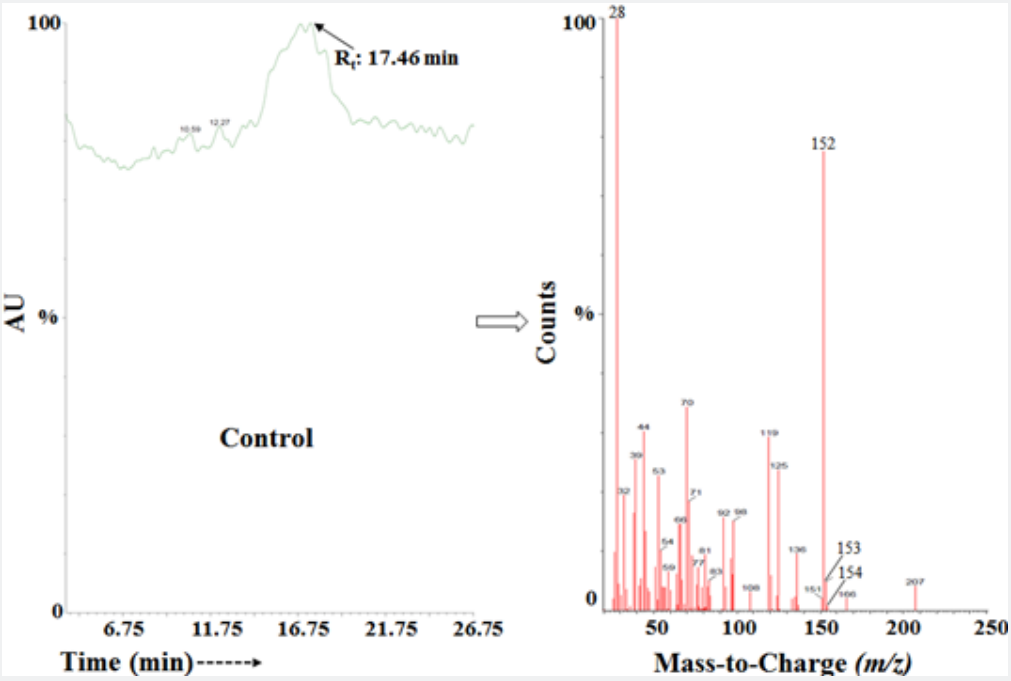

Figure 4: The GC-MS chromatogram and mass spectra of the control 6-MP.

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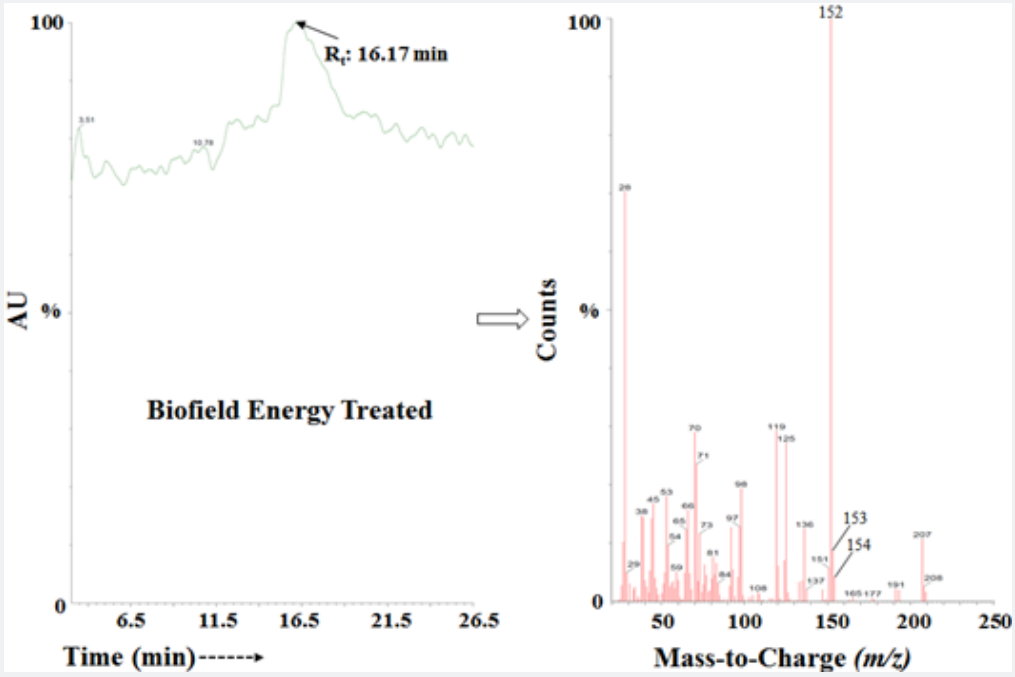

Figure 5: The GC-MS chromatogram and mass spectra of the Biofield Energy Treated 6-MP.

The theoretical calculation of P_{M+1} for 6-MP was done with respect to the peak intensity of the molecular ion peak $[M]^+$ at *m/z* 152.

P (^{13}C) = [(5 x 1.1%) x 77% (the actual size of the M^+ peak)] / 100% = 4.24%

P (^{2}H) = [(4 x 0.015%) x 77%] / 100%= 0.05%

P (^{15}N) = [(4 x 0.4%) x 77%] / 100% = 1.23%

P (^{33}S) = [(1 x 0.08%) x 77%] / 100% = 0.06%

P_{M+1}, i.e. ^{13}C, ^{2}H, ^{15}N, and ^{33}S contributions from $(C_5H_5N4S)^+$ to m/z 153 = 5.58%

Based on the above calculation, it has been observed that ^{13}C and ^{15}N have major contribution to *m/z* 153. The calculated isotopic abundances (5.58) was close to the experimental value 4.79 (Table 2).

Table 2: GC-MS based isotopic abundance analysis results of 6-MP in control and Biofield Energy Treated samples.

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 152 (%)	77.6	100
P_{M+1} at *m/z* 153 (%)	4.79	8.69
P_{M+1}/P_M	0.06	0.09
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control sample		40.78
P_{M+1} at *m/z* 154 (%)	0.68	4.18
P_{M+1}/P_M	0.01	0.04
% Change of isotopic abundance ratio (P_{M+2}/P_M) compared to the control sample		377.01
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.		

Similarly, the theoretical calculation of P_{M+2} for 6-MP was presented as below:

P (^{34}S) = [(1 x 4.21%) x 77%] / 100% = 3.24%

P_{M+2}, i.e. ^{34}S contributions from $(C_5H_5N_4S)^+$ to *m/z* 154 = 4.21%

From the above calculation, it has been found that only ^{34}S have the major contribution to *m/z* 173.

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The GC-MS based isotopic abundance P_M, P_{M+1}, and P_{M+2} for the mercaptopurine were obtained from the observed relative peak intensities of [M^+], [$(M+1)^+$], and [$(M+2)^+$], respectively (Table 2). The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated 6-MP was significantly increased by 40.78% compared with the control sample (Table 2). Similarly, the isotopic abundance ratio of P_{M+2}/P_M in the treated 6-MP was significantly increased by 377.01% compared with the control sample (Table 2). Thus, ^{34}S contributions from $(C_5H_5N_4S)^+$ to *m/z* 154 in the treated 6-MP was significantly increased compared with the control sample.

The structure of the 6-MP was confirmed from the spectral characterization. The isotopic abundance ratios of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{33}S/^{32}S$) and P_{M+2}/P_M ($^{34}S/^{32}S$) in the treated 6-MP were significantly improved compared to the control sample. The changes in isotopic abundance could be due to possibly the changes in nuclei, the interference of neutrino particles *via* the Trivedi Effect®. The neutrinos have the ability to interact with both protons and neutrons in the nucleus, which indicated a close relation between neutrino and the isotope formation [19,42,43]. The increased isotopic abundance ratios would influence the atomic bond vibration of treated 6-MP. The increased isotopic abundance ratio of the Biofield Treated 6-MP may increase the intra-atomic bond strength, its physical stability, and alter the rate reactions in the body [48]. The Consciousness Energy Healing Treated 6-MP would be very useful to design better pharmaceutical formulations that might offer better therapeutic response against chronic myeloid leukemia, acute lymphocytic leukemia, ulcerative colitis, and Crohn's disease, etc.

Conclusion

Based on the outcomes, it was observed that the LC-MS spectra of both the 6-MP samples at Rt 2.2 minutes showed the mass of the protonated molecular ion peak at *m/z* 173 $[M+H]^+$. The peak area of the Biofield Treated 6-MP was significantly increased by 92.07% as compared to the control 6-MP. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the Biofield Treated 6-MP was significantly increased by 34.79% as compared with the control 6-MP. Similarly, the GC-MS based isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in the Biofield Energy Treated 6-MP was significantly increased by 40.78% and 377.01% compared with the control sample. Thus, 13C, 2H, 15N, 33S, and 18O contributions from $(C_5H_5N_4S)^+$ to *m/z* 153 and 154 in the Biofield Energy Treated sample were significantly increased compared with the control 6-MP. The isotopic abundance ratio of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{33}S/^{32}S$) and P_{M+2}/P_M ($^{34}S/^{32}S$) in the Biofield Energy Treated 6-MP was significantly improved compared to the control 6-MP. The significant increase in the peak area and isotopic abundance could be due to the interference of neutrino particles in the nucleus via the Trivedi Effect®-Consciousness Energy Treatment. The increased isotopic abundance ratio of the Biofield Energy Treated 6-MP would improve the chemical bond strength, increase the physical and chemical stability of 6-MP in the body. The Biofield Energy Treated 6-MP would be better designing more efficacious pharmaceutical formulations that might offer increased bioavailability and therapeutic response against acute lymphocytic leukemia, Crohn's disease, chronic myeloid leukemia, and ulcerative colitis, etc.

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