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Impact of Consciousness Energy Healing Treatment on the Structure and Isotopic Abundance Ratio of 6-Mercaptopurine

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Abstract

6-Mercaptopurine is an anti-cancer chemotherapy drug classified as an antimetabolite. In this study, the impact of the Trivedi Effect® (Consciousness Energy Healing Treatment) on the structural properties and the isotopic abundance ratio of 6-mercaptopurine were evaluated using LC-MS and GC-MS spectroscopy. The test sample 6-mercaptopurine was divided and termed as control and Biofield Energy Treated sample. The treated part of the sample only received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a renowned Biofield Energy Healer, Dahryn Trivedi. The LC-MS spectra of both the samples at retention time (R_t) 2.2 minutes exhibited the mass of the protonated molecular ion peak at *m/z* 173 $[M+H]^+$ (calculated for $C_5H_5N_4S^+$, 153.18). The peak area of the treated 6-mercaptopurine was significantly increased by 94.62% compared to the control sample. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the treated mercaptopurine was significantly increased by 107.92% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of P_{M+2}/P_M in the treated 6-mercaptopurine was significantly increased by 25.78% 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* 154 in the treated sample were significantly increased compared with the control sample. The isotopic abundance ratio of P_{M+1}/P_M ($^{2}H/^{1}H$ 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-mercaptopurine was significantly improved compared to the control sample. The significant increase in the peak area, isotopic abundance and mass peak intensities could be due to changes in nuclei possibly happened due to the interference of neutrino particles *via* the Trivedi Effect®. The increased isotopic abundance ratio of the treated sample would improve the strength of the chemical bond, increase the physical and chemical stability of 6-mercaptopurine in the body. The nove l6-mercaptopurine would be better designing more efficacious pharmaceutical formulations that might offer better more bioavailability and therapeutic response against acute lymphocytic leukemia, chronic myeloid leukemia, Crohn's disease, and ulcerative colitis, etc.

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

Introduction

6-mercaptopurine is an anti-cancer chemotherapy drug classified as an "antimetabolite". It interferes with the nucleic acid synthesis by inhibiting purine metabolism in the tumour cells[1,2]. It is used for the treatment of cancer and autoimmune diseases, *i.e.*, lymphocytic leukaemia, myeloid leukaemia, ulcerative colitis, and Crohn's disease[2-5]. Since from 1953 it was approved for medical use in the U.S.A. and also listed as Essential Medicines by the World Health Organization (WHO)[6]. Very common side effects associated with the mercaptopurine therapy are immune suppression, bone marrow suppression, liver toxicity, diarrhoea, nausea, vomiting, loss of appetite, stomach and abdominal pain, mouth sores, fatigue, weakness, fever, sore throat, pinpoint red spots on the skin, skin rash, darkening of the skin, yellowing of eyes or skin,

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hair loss, bloody stools, black or tarry stools, bloody urine, dark urine, painful or difficult urination, and genetic polymorphisms [7-9]. The dosage forms of mercaptopurine are the tablet and liquid suspension[10-12]. The solubility of mercaptopurine is very poor. It is insoluble in water, chloroform, acetone, and diethyl ether; whereas slightly soluble in dilute sulfuric acid; soluble in hot alcohol and dilute alkali solutions[12].

The physicochemical properties are the key factors which determine the quality, stability, solubility, and bioavailability of any pharmaceutical and nutraceutical compounds[13]. The Biofield Energy Healing Treatment (the Trivedi Effect®) has been proved with the significant effect on particle size, surface area, and bioavailability of pharmaceutical and nutraceutical compounds[14-18]. The Trivedi Effect® is an accepted scientifically proven phenomenon in which a skilled person can harness this inherently intelligent energy from the Universe and transfer it anywhere on the planet *via* the possible mediation of neutrinos[19]. The "Biofield" is an electromagnetic energy field which exists surrounding all the living beings. It is generated by the continuous movement of the electrically charged particles (*i.e*., ions, cells, blood flow, *etc*.) inside the body[20-22]. The "Biofield" based Energy Therapies have been reported with significant outcomes against various disease[23]. The National Center of Complementary and Integrative Health has recognized and accepted Biofield Energy Therapies as a Complementary and Alternative Medicine (CAM) health care approach in addition to other therapies, medicines, and practices such as hypnotherapy, yoga, Reiki, Tai Chi, Qi Gong, *etc*.[24,25]. The Trivedi Effect®-Consciousness Energy Healing Treatment has also been reported altering significant change in the metals, chemicals, ceramics, polymers, crops, microbes, biotechnology, skin health, bone health, cancer cell line[26-40], *etc*. This indicated that the Trivedi Effect®-Consciousness Energy Healing Treatment could be an economical approach to overcome some of the practical problems associated with 6-mercaptopurine with respect to the physicochemical properties. The stable isotope ratio analysis and isotope effects have a significant impact on atomic bond strength, physicochemical, and thermal of the molecule[41,42]. Isotope ratio analysis can be performed with the help of 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]. In this study, the structural properties and the 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-mercaptopurine was evaluated compared to the control sample using LC-MS and GC-MS analytical techniques.

Materials and Methods

Chemicals and Reagents

The test sample 6-mercaptopurine was purchased from Tokyo Chemical Industry Co., Ltd., Japan and the other chemicals were of analytical grade purchased in India.

Consciousness Energy Healing Treatment Strategies

The test sample 6-mercaptopurine powder was equally divided into two equal part and termed as a control and the treated sample. The control 6-mercaptopurine powder sample did not receive the Biofield Energy Treatment but the sample was treated with a "sham" healer who did not have any knowledge about the Biofield Energy Treatment. However, the treated 6-mercaptopurine was treated with the Trivedi Effect®-Consciousness Energy Healing Treatment remotely for 3 minutes by the well-known Biofield Energy Healer, Dahryn Trivedi, USA. The Energy Treatment was provided through the Biofield Energy Healer's unique energy transmission process. After the treatment, the Biofield Energy Treated and untreated 6-mercaptopurine were kept in the sealed conditions and characterized using LC-MS and GC-MS analytical techniques.

Characterization

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysisand Calculation of Isotopic Abundance Ratio: The LC-MS analysis of the 6-mercaptopurine was carried out with the help of LC-MS Thermo Fisher Scientific, the USA equipped with a triple-stage quadrupole mass spectrometer. The column used here was a reversed phase Thermo Scientific Synchronis C18 (Length-250 mm XID 4.6 mm X 5 micron). The diluent used for the sample preparation was water and acetonitrile. 10 µL of the 6-mercaptopurine solution was injected, and the analyte was eluted 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. Peaks were monitored at 300 nm using the PDA detector. The mass spectrometric analysis was performed under +ve ESI mode.

The natural abundance of each isotope (C, O, H, N, and S) was predicted comparing 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 [42,43-46]. The LC-MS based isotopic abundance ratios (P_{M+1}/P_M) for both the samples was calculated.

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

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

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis: The GC-MS of the 6-mercaptopurine was 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 ion mode. The GC-MS based isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) for the control and Biofield Energy Treated 6-mercaptopurine was calculated using equation 1.

Results and Discussion

Liquid Chromatography-Mass Spectrometry (LC-MS): The chromatograms of 6-mercaptopurine showed the single major chromatographic peak at the retention time (R_t) of 2.2 minutesin case of both the samples. The peak area of the Biofield Energy Treated 6-mercaptopurine was significantly increased by

94.62% compared to the control sample (Figure 1). The results indicated that the solubility of the treated 6-mercaptopurine was significantly improved after the Consciousness Energy Healing Treatment compared to the control sample. This may improve the bioavailability of the treated 6-mercaptopurine compared to the control sample. The results were strongly supported by the recently published article in which the Consciousness Energy Healing Treatment significantly improved the particle size and surface area properties of 6-mercaptopurine [47].

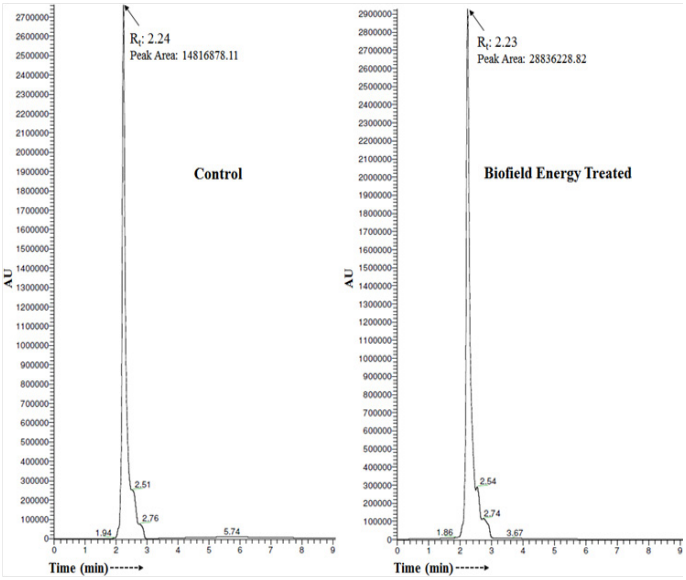

Figure 1: Liquid chromatograms of the control and treated6-mercaptopurine.

6-Mercaptopurine generally shows the molecular mass peak $[M]^+$ at *m/z* 152 in positive ion mode[48]. The 6-mercaptopurine (Figure 2) exhibited the mass of 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), $C_4H_6N_2^+$ (*m/z*=82), and $C_3H_6N_2^+$ (*m/z*=70) of in both the samples (Figure 3).

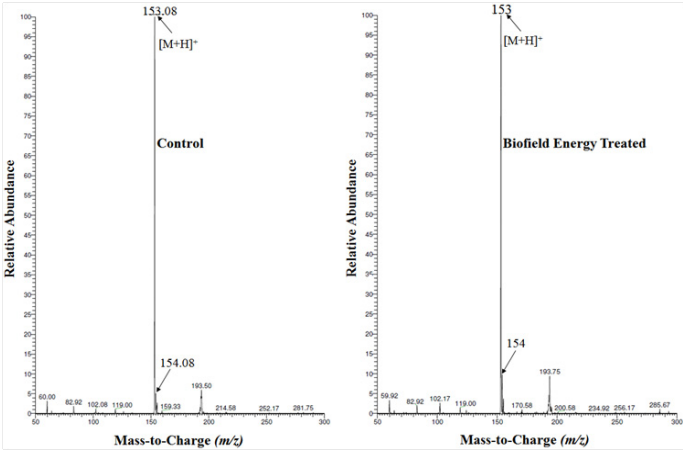

Figure 2: Mass spectra of the control and treated 6-mercaptopurine at R_t 2.2 minutes.

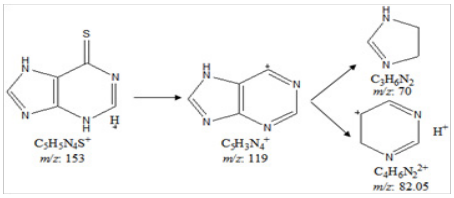

Figure 3: Proposed fragmentation pattern of 6-mercaptopurine.

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

$P(^{13}C)$ = [(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 (7.26%) was close to the experimental value 4.29% (Table 1). From the above calculation, 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 P_M and P_{M+1} of the 6-mercaptopurine at *m/z* 153 and 154, respectively which were obtained from the observed relative peak intensities of $[M^+]$ and $[(M+1)^+]$ peaks, 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-mercaptopurine was significantly increased by 107.92% compared with the control sample (Table 1). Therefore, 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 Biofield Energy Treated 6-mercaptopurine were significantly decreased compared to the control sample.

Table 1: LC-MS based isotopic abundance analysis results of 6-mercaptopurine in the treated sample 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 (%)	4.29	8.92
P_{M+1}/P_M	0.04	0.09
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control sample		107.92

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: The GC-MS of both the samples showed asingle chromatographic peak at the retention time of 16.04 minutes in the chromatogram (Figures 4 and 5). The parent molecular ion peak of mercaptopurine at *m/z* 152 $[M]^+$ (calculated for $C_5H_5N_4S^+$, 152.02) in both the samples, along with the fragment ion peaks (Figures 3-5).

The GC-MS spectra of both the samples of mercaptopurine showed the mass of the molecular ion peak $[M]^+$ at *m/z* 152 (calculated for $C_5H_4N_4S^+$, 152.02). The theoretical calculation of P_{M+1} for 6-mercaptopurine was presented as below:

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

$P(^{2}H)$ = [(4 x 0.015%) x 100%] / 100%= 0.06%

$P(^{15}N)$ = [(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* 153 = 7.24%

From the above calculation, it has been found that ^{13}C and ^{15}N have major contribution to *m/z* 153. The calculated isotopic abundances (7.24) was close to the experimental value 7.9 (Table 2).

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

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

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 34S have the major contribution to *m/z* 173. The calculated isotopic abundances (4.21) was close to the experimental value 3.53 (Table 2).

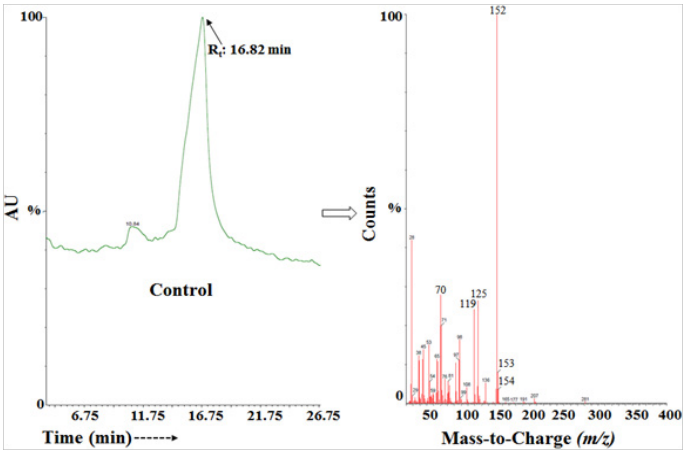

Figure 5: The GC-MS chromatogram and mass spectra of the treated 6-mercaptopurine.

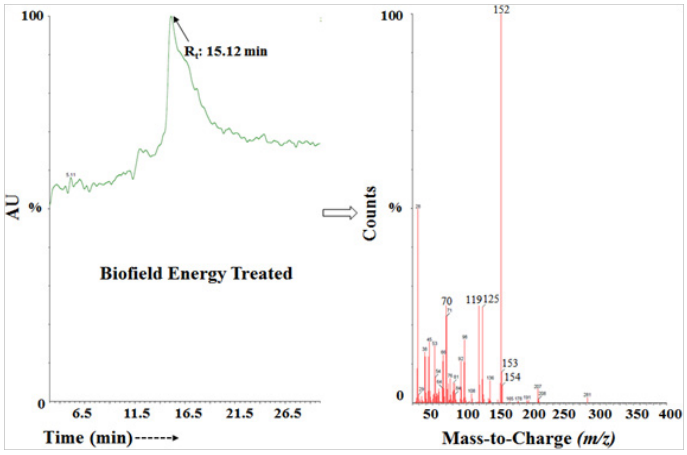

Figure 4: The GC-MS chromatogram and mass spectra of the control 6-mercaptopurine.

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

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 152 (%)	100.00	100.00
P_{M+1} at *m/z* 153 (%)	7.90	7.90
P_{M+1}/P_M	0.08	0.08
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control sample		0.0
P_{M+1} at *m/z* 154 (%)	3.53	4.44
P_{M+1}/P_M	0.04	0.04
% Change of isotopic abundance ratio (P_{M+2}/P_M) compared to the control sample		25.78

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.

The GC-MS based isotopic abundance P_M,P_{M+1}, and P_{M+2} for the mercaptopurine near *m/z* 152, 153, and 154, respectively of both the samples, which were obtained from the observed relative peak intensities of $[M^+]$, $[(M+1)^+]$, and $[(M+2)^+]$, respectively in the mass spectra (Table 2). The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated 6-mercaptopurine did not alter compared with the control sample (Table 2). However, the isotopic abundance ratio of P_{M+2}/P_M in the treated 6-mercaptopurine was significantly increased by 25.78% compared with the control sample (Table 2). Thus, ^{34}S contributions from $(C_5H_5N_4S)^+$ to *m/z* 154 in the Biofield Energy Treated sample were significantly increased compared with the control sample.

The spectral characterization helped to confirm the structure of the sample as 6-mercaptopurine. 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 Biofield Energy Treated 6-mercaptopurine were significantly altered compared to the control sample. It can be hypothesized that the changes in isotopic abundance could be due to changes in nuclei possibly through the interfer-

ence of neutrino particles *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. The neutrinos change identities and it is only possible if the neutrinos possess mass. 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[19,42,43]. The altered isotopic composition of the Consciousness Energy Healing Treated 6-mercaptopurine might be due to the alteration in neutron to proton ratio in the nucleus. The Biofield Energy Treatment may highly influence the atomic bond vibration and its atomic spectra of treated 6-mercaptopurine. The increased isotopic abundance ratio of the treated 6-mercaptopurine would stronger the chemical bond, increase the stability, and alter the rate reactions in the body. The Biofield Energy Treated 6-mercaptopurine would be very useful to design better pharmaceutical formulations that might offer better therapeutic response against acute lymphocytic leukemia, chronic myeloid leukemia, Crohn's disease, and ulcerative colitis, *etc*.

Conclusions

The Trivedi Effect® - Consciousness Energy Healing Treatment showed a significant impact on the peak area, isotopic abundance ratios and mass peak intensities of 6-mercaptopurine. The LC-MS spectra of both the samples at R_t 2.2 minutes exhibited the mass of the protonated molecular ion peak at *m/z* 173 $[M+H]^+$. The peak area of the Biofield Energy Treated 6-mercaptopurine was significantly increased by 94.62% compared to the control sample. The LC-MS based isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated mercaptopurine was significantly increased by 107.92% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of P_{M+2}/P_M in the Biofield Energy Treated 6-mercaptopurine was significantly increased by 25.78% 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* 154 in the Biofield Energy Treated sample were significantly increased compared with the control sample. The isotopic abundance ratio of P_{M+1}/P_M ($^{2}H/^{1}H$ 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 Biofield Energy Treated 6-mercaptopurine was significantly improved compared to the control sample. The significant increase in the peak area, isotopic abundance and mass peak intensities could be due to changes in nuclei possibly happened due to the interference of neutrino particles *via* the Trivedi Effect®. The increased isotopic abundance ratio of the Biofield Energy Treated sample would improve the strength of the chemical bond, increase the physical and chemical stability of 6-mercaptopurine in the body. The novel Biofield Energy Treated 6-mercaptopurine would be better designing more efficacious pharmaceutical formulations that might offer better more bioavailability and therapeutic response against acute lymphocytic leukemia, chronic myeloid leukemia, Crohn's disease, and ulcerative colitis, *etc*.

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