



# Isotopic Abundance Ratio Analysis of 1,2,3-Trimethoxybenzene (TMB) After Biofield Energy Treatment (The Trivedi Effect<sup>®</sup>) Using Gas Chromatography-Mass Spectrometry

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**Abstract:** 1,2,3-Trimethoxybenzene (TMB) is one of the most versatile chemical used for the synthesis of several pharmaceuticals, dyes, polymers, organic compounds, etc. The stable isotope ratio analysis has increased attention day-by-days in several fields such as agricultural, food authenticity, biochemistry, medical research, etc. The current study was aimed to evaluate the effect of the biofield energy treatment on the isotopic abundance ratios of <sup>13</sup>C/<sup>12</sup>C or <sup>2</sup>H/<sup>1</sup>H or <sup>17</sup>O/<sup>16</sup>O ( $P_{M+1}/P_M$ ) and <sup>18</sup>O/<sup>16</sup>O ( $P_{M+2}/P_M$ ) in TMB using Gas chromatography - mass spectrometry (GC-MS) technique. TMB was divided into two parts - one part was denoted as control and another part was referred as biofield energy treated sample that was received through Mr. Trivedi's unique biofield energy (The Trivedi Effect<sup>®</sup>). The GC-MS of the biofield treated TMB was characterized at different time intervals considered as T1, T2, T3, and T4 to examine the impact of the biofield energy treatment on isotopic abundance ratio with respect to the time. The GC-MS spectra of the both control and biofield treated TMB exhibited the presence of molecular ion peak [ $M^+$ ] at  $m/z$  168 (calculated 168.08 for C<sub>9</sub>H<sub>12</sub>O<sub>3</sub>) along with similar pattern of fragmentation. The relative peak intensities of the fragmented ions in the biofield treated TMB, particularly at T2 and T3 was altered from the control sample. The isotopic abundance ratio analysis in the biofield treated TMB exhibited that the isotopic abundance ratio of  $P_{M+1}/P_M$  in the biofield treated TMB at T2 and T3 was significantly enhanced by 128.13 and 117.99%, respectively with respect to the control sample. Consequently, the percentage change in isotopic abundance ratio of  $P_{M+2}/P_M$  was significantly increased in the biofield treated TMB at T2 and T3 by 125.93 and 116.67%, respectively as compared with the control TMB. The isotopic abundance ratios ( $P_{M+1}/P_M$  and  $P_{M+2}/P_M$ ) in the biofield treated TMB at T1 and T4 was altered with respect to the control TMB. In summary, <sup>13</sup>C, <sup>2</sup>H, and <sup>17</sup>O contributions from (C<sub>9</sub>H<sub>12</sub>O<sub>3</sub>)<sup>+</sup> to  $m/z$  169 and <sup>18</sup>O contribution from (C<sub>9</sub>H<sub>12</sub>O<sub>3</sub>)<sup>+</sup> to  $m/z$  170 for the biofield treated TMB, particularly at T2 and T3 were significantly improved and biofield treated TMB might exhibit changed isotope effects as compared to the control sample. The biofield treated TMB might assist to develop new chemicals and pharmaceuticals through using its kinetic isotope effects like understanding the reaction mechanism, the enzymatic transition state and all aspects of enzyme mechanisms.

**Keywords:** Biofield Energy Treatment, The Trivedi Effect<sup>®</sup>, 1,2,3-Trimethoxybenzene, Gas Chromatograph - Mass Spectrometry, Isotopic Abundance Ratio, Isotope Effects, Kinetic Isotope Effect

## 1. Introduction

Stable Isotope Ratio Analysis (SIRA) *i.e.* analysis of

natural abundance variations in stable isotopes include <sup>2</sup>H, <sup>13</sup>C, <sup>15</sup>N, <sup>18</sup>O, <sup>34</sup>S, <sup>37</sup>Cl, etc. is a potential method for the measurement of the flow of materials and energy both within and among organisms. This method is commonly used in

agricultural, food authenticity, biochemistry, metabolism, medical research, environmental pollution, archaeology, etc. [1-4]. Isotope effects *i.e.* minute differences in physical and chemical properties of the molecule are the resultant for the variation in isotopic abundance ratio between isotopic forms of the molecule. Isotope effects play a vital role in thermal motion, molecular spectra, chemical reactions (reaction rate and bond strength), physicochemical properties, chemical equilibria, etc. [5-8]. The isotopic composition of the isotopic molecules is measured through isotope amount ratios or isotope amount fractions. Mass spectrometry (MS) technique is the major choice for the isotope ratio analysis, although infrared (IR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and neutron activation analysis (NAA) can also offer response for the isotopes of an element [9, 10]. Isotope ratio mass spectrometer (IRMS), multiple-collector inductively coupled plasma mass spectrometry are commonly used for the measurement of the ratio of natural isotopic abundances in the molecules having molar isotope enrichments at below 0.1%. But, a conventional scanning mass spectrometer for *e.g.* GC-MS can be used for isotope ratio measurement at low micromolar concentration levels with appropriate precision, once the molar isotope enrichment levels of the molecule are above 0.1%. Literature reported that the peak height (*i.e.* relative intensity) in the mass spectra is directly proportional to the relative isotopic abundance of the sample [2, 3, 8-14]. SIRA is also useful for the determination of the pharmacokinetic profile or mode of action of a drug substance, bioavailability of the drug products, the release profile for the drug delivery systems and also used for the assessment in relation to patient-specific drug treatment [2].

1,2,3-Trimethoxybenzene (TMB) is an organic compound containing three methoxy groups in benzene ring (Figure 1) having a molecular formula  $C_9H_{12}O_3$  and molecular weight of 168.19.

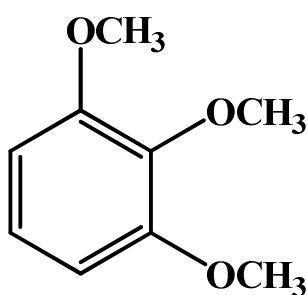


Figure 1. Structure of 1,2,3-Trimethoxybenzene (TMB).

TMB can be used as chemical intermediate to synthesize the target molecules of pharmaceuticals, pesticides, antioxidant, perfumes and other several organic compounds. Literature reported that methoxylated phenol family compounds have bacteriostatic and floral preservative properties. These compounds can be used in pharmaceuticals (analgesics, antiseptics), biocides, formulating insect attractants, flavoring agents, etc. They are also useful in the preparation of antioxidants, stabilizers, UV absorbers for

plastics and rubbers [15-18]. Trimethoxybenzene based compounds are potentially toxic in nature and cause numerous health problems. They are found in many hazardous waste sites, which have been included as national priority list proposed by the environmental protection agency [19, 20].

Several kind of literature described that biofield is a dynamic electromagnetic field within and surrounding the human body carry information for regulating the organism. The biofield energy can be harnessed from the earth, the “universal energy field” and can be applied by healing practitioner in order to attain the significant effects. This process is known as biofield energy treatment [21-23]. Mr. Trivedi is one of the well-known healing practitioner and has amazing capability to modify the characteristic properties of several organic compounds [24-26], pharmaceuticals [27, 28], nutraceuticals [29], metals and ceramic in materials science [30, 31], and improve the overall productivity of crops [32, 33] as well as to modulate the efficacy of the various living cells [34-38]. A number of literatures demonstrated that biofield energy treatment has astounding capability for changing the isotopic abundance ratio in the organic compounds [39-42]. Spectroscopic and thermal analysis of TMB revealed that the physicochemical, structural and thermal properties of TMB were significantly altered due to the biofield energy treatment. Consequently, the observed findings were increased crystallite size and low thermal stability that might improve the volatilization of treated TMB and the alteration in C-O bond of the biofield treated TMB due to the changes in force constant [15]. Hence, it was hypothesized that alteration of the physicochemical, structural and thermal properties of biofield treated TMB might be due to the effect of the changes on the isotopic abundance ratio in biofield treated TMB. Thus, stable isotope ratio analysis of the both control and biofield treated TMB using GC-MS was carried out to explore the influence of the biofield energy treatment on the isotopic abundance ratios of  $^{13}C/^{12}C$  or  $^2H/^1H$  or  $^{17}O/^{16}O$  ( $P_{M+1}/P_M$ ) and  $^{18}O/^{16}O$  ( $P_{M+2}/P_M$ ) in TMB.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

1,2,3-Trimethoxybenzene (TMB) was procured from S D Fine Chemicals Ltd., India. All the other chemicals used in this experiment were analytical grade purchased from local vendors.

### 2.2. Biofield Energy Treatment

TMB was divided into two portions: one was denoted as untreated or control and other part was considered as biofield energy treated sample. The sample for the biofield treatment was handed over to Mr. Trivedi in a sealed condition. The biofield energy treatment was provided by Mr. Trivedi (also known as The Trivedi Effect<sup>®</sup>) through his unique energy transmission process to the test product in a sealed pack under laboratory conditions for 5 minutes without touching

the sample. After treatment, control and the biofield treated samples were stored at standard laboratory condition and characterized by GC-MS. The biofield treated TMB was analyzed in different time intervals denoted as T1, T2, T3, and T4.

### 2.3. Gas Chromatograph - Mass Spectrometry (GC-MS)

GC-MS analysis was performed on Perkin Elmer/Auto system XL with Turbo mass, USA. The GC-MS method was followed by previously published work [42]. The GC-MS was accomplished in a silica capillary column prepared with a quadrupole detector with pre-filter, one of the fastest, widest mass ranges available for any GC-MS. The mass spectrometer was operated in an electron ionization (EI) positive/negative, and chemical ionization mode at the electron ionization energy of 70 eV. Mass range: 10-650 Daltons (amu), stability:  $\pm 0.1$  m/z mass accuracy over 48 hours. The identification of analytes were completed by

retention time and by a comparison of the mass spectra of identified substances with references.

### 2.4. Method for the Calculation of Isotopic Abundance Ratio from the GC-MS Spectra

The isotopic abundances of the elements are basically considered into three types: *A elements* having only one natural isotope in appreciable abundance; *A + 1 elements* (For e.g. C, N and H) containing two isotopes – one isotope is one nominal mass unit heavier than the most abundant isotope, and *A + 2 elements* (For e.g. O, Cl, S, Si, and Br) having an isotope that has two mass unit heavier than the most abundant isotope [7, 13, 43]. The natural abundance of each isotope can be predicted from the comparison of the height of the isotope peak with respect to the base peak, *i.e.* relative intensity in the mass spectra. The values of the natural isotopic abundance of some elements are obtained from the literature and presented in the Table 1 [2, 7, 8, 43, 44].

**Table 1.** The isotopic composition (*i.e.* the natural isotopic abundance) of the elements.

Element	Symbol	Mass	% Natural Abundance	A+1 Factor	A+2 Factor
Hydrogen	$^1\text{H}$	1	99.9885		
	$^2\text{H}$	2	0.0115	$0.015n_{\text{H}}$	
Carbon	$^{12}\text{C}$	12	98.892		
	$^{13}\text{C}$	13	1.108	$1.1n_{\text{C}}$	
Oxygen	$^{16}\text{O}$	16	99.762		
	$^{17}\text{O}$	17	0.038	$0.04n_{\text{O}}$	
	$^{18}\text{O}$	18	0.200		$0.20n_{\text{O}}$
Nitrogen	$^{14}\text{N}$	14	99.60		
	$^{15}\text{N}$	15	0.40	$0.40n_{\text{N}}$	
Chlorine	$^{35}\text{Cl}$	35	75.78		
	$^{37}\text{Cl}$	37	24.22		$32.50n_{\text{Cl}}$

A represents element, n represents the number of the element (*i.e.* C, H, O, N, etc.)

The following method that was adopted from literature [7, 8, 11-14] was used for calculating the isotopic abundance ratio in the current study:

$P_{\text{M}}$  stands for the relative peak intensity of the parent molecular ion  $[\text{M}^+]$  expressed in percentage. In other way, it indicates the probability to have *A elements* (for e.g.  $^{12}\text{C}$ ,  $^1\text{H}$ ,  $^{16}\text{O}$ ,  $^{14}\text{N}$ , etc.) contributions to the mass of the parent molecular ion  $[\text{M}^+]$ .

$P_{\text{M}+1}$  represents the relative peak intensity of the isotopic molecular ion  $[(\text{M}+1)^+]$  expressed in percentage  
 $= (\text{no. of } ^{13}\text{C} \times 1.1\%) + (\text{no. of } ^{15}\text{N} \times 0.40\%) + (\text{no. of } ^2\text{H} \times 0.015\%) + (\text{no. of } ^{17}\text{O} \times 0.04\%)$

*i.e.* the probability to have *A + 1 elements* (for e.g.  $^{13}\text{C}$ ,  $^2\text{H}$ ,  $^{15}\text{N}$ , etc.) contributions to the mass of the isotopic molecular ion  $[(\text{M}+1)^+]$

$P_{\text{M}+2}$  represents the relative peak intensity of the isotopic molecular ion  $[(\text{M}+2)^+]$  expressed in the percentage  
 $= (\text{no. of } ^{18}\text{O} \times 0.20\%) + (\text{no. of } ^{37}\text{Cl} \times 32.50\%)$

*i.e.* the probability to have *A + 2 elements* (for e.g.  $^{18}\text{O}$ ,  $^{37}\text{Cl}$ ,  $^{34}\text{S}$ , etc.) contributions to the mass of isotopic molecular ion  $[(\text{M}+2)^+]$

Isotopic abundance ratio for *A + 1 elements* =  $P_{\text{M}+1}/P_{\text{M}}$

Similarly, isotopic abundance ratio for *A + 2 elements* =

$P_{\text{M}+2}/P_{\text{M}}$

Percentage (%) change in isotopic abundance ratio =  $[(\text{IAR}_{\text{Treated}} - \text{IAR}_{\text{Control}}) / \text{IAR}_{\text{Control}}] \times 100$ ,

Where,  $\text{IAR}_{\text{Treated}}$  = isotopic abundance ratio in the treated sample and  $\text{IAR}_{\text{Control}}$  = isotopic abundance ratio in the control sample.

## 3. Results and Discussion

### 3.1. GC-MS Analysis

The GC-MS spectra of the control and biofield treated TMB are presented in the Figures 2-4.

The GC-MS spectrum of the control TMB (Figure 2) indicated the presence of molecular ion peak  $[\text{M}^+]$  at  $m/z$  168 (calculated 168.08 for  $\text{C}_9\text{H}_{12}\text{O}_3$ ) along with eight major fragmented peaks in lower  $m/z$  region at the retention time ( $R_t$ ) of 12.73 min. This fragmentation pattern of TMB was well matched with the literature [45]. The fragmented peaks at  $m/z$  153, 125, 110, 95, 93, 77, 65, and 39 might be due to  $\text{C}_8\text{H}_9\text{O}_3$ ,  $\text{C}_7\text{H}_9\text{O}_2^+$ ,  $\text{C}_7\text{H}_{10}\text{O}^+$ ,  $\text{C}_6\text{H}_7\text{O}^+$ ,  $\text{C}_6\text{H}_5\text{O}$ ,  $\text{C}_6\text{H}_5^+$ ,  $\text{C}_5\text{H}_5^+$  and  $\text{C}_3\text{H}_3^+$  ions, respectively as shown in Figure 2. The GC-MS spectra of the biofield treated TMB at T1, T2, T3, and T4 as shown in Figures 3 and 4 exhibited molecular ion peak

[M<sup>+</sup>] at  $m/z$  168 at  $R_t$  of 12.72 min (T1 to T3) and 12.77 (T4) min, respectively. The biofield treated TMB showed similar  $R_t$  and the same pattern of fragmentation as shown in the

control sample. Only, the relative peak intensities of the fragmented ions of the biofield treated TMB were altered as compared with the control sample.

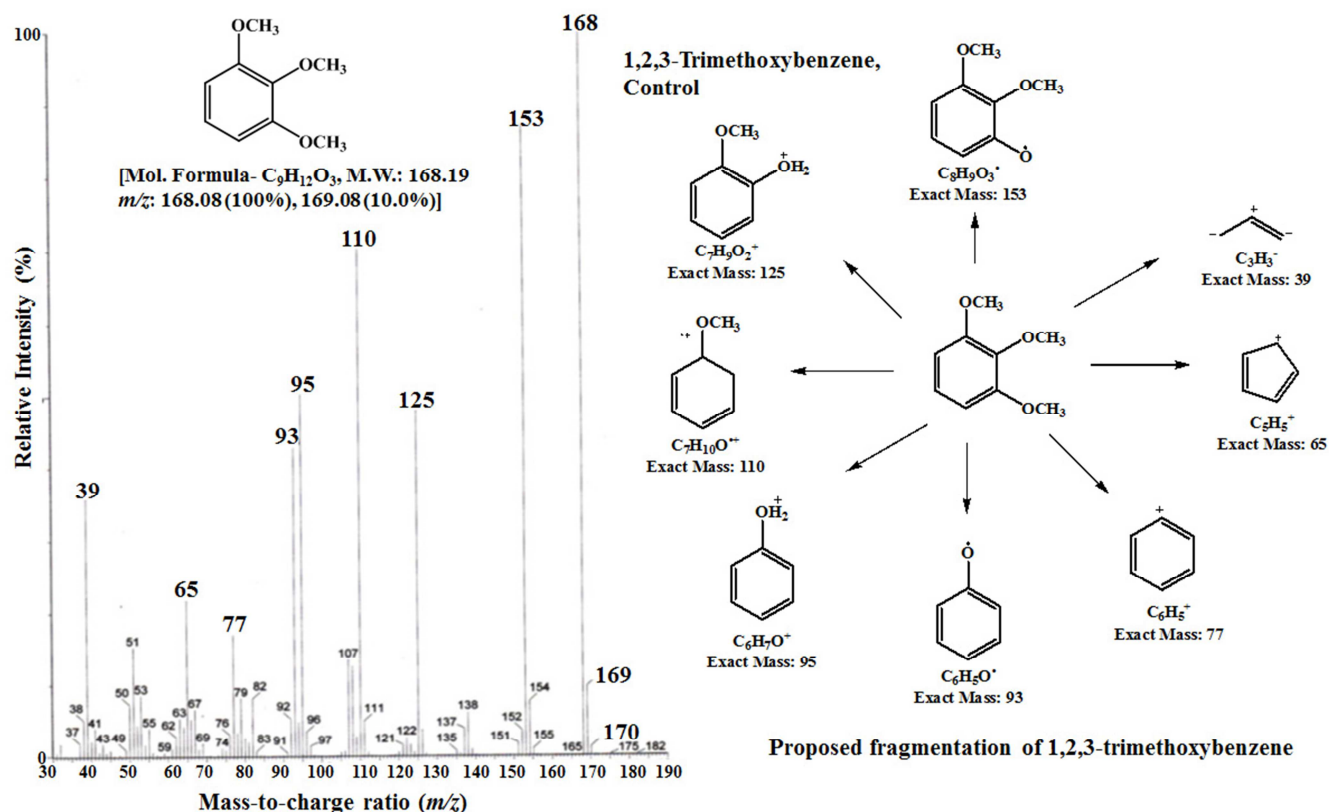


Figure 2. GC-MS spectrum and proposed fragmentation of the control 1,2,3-Trimethoxybenzene (TMB).

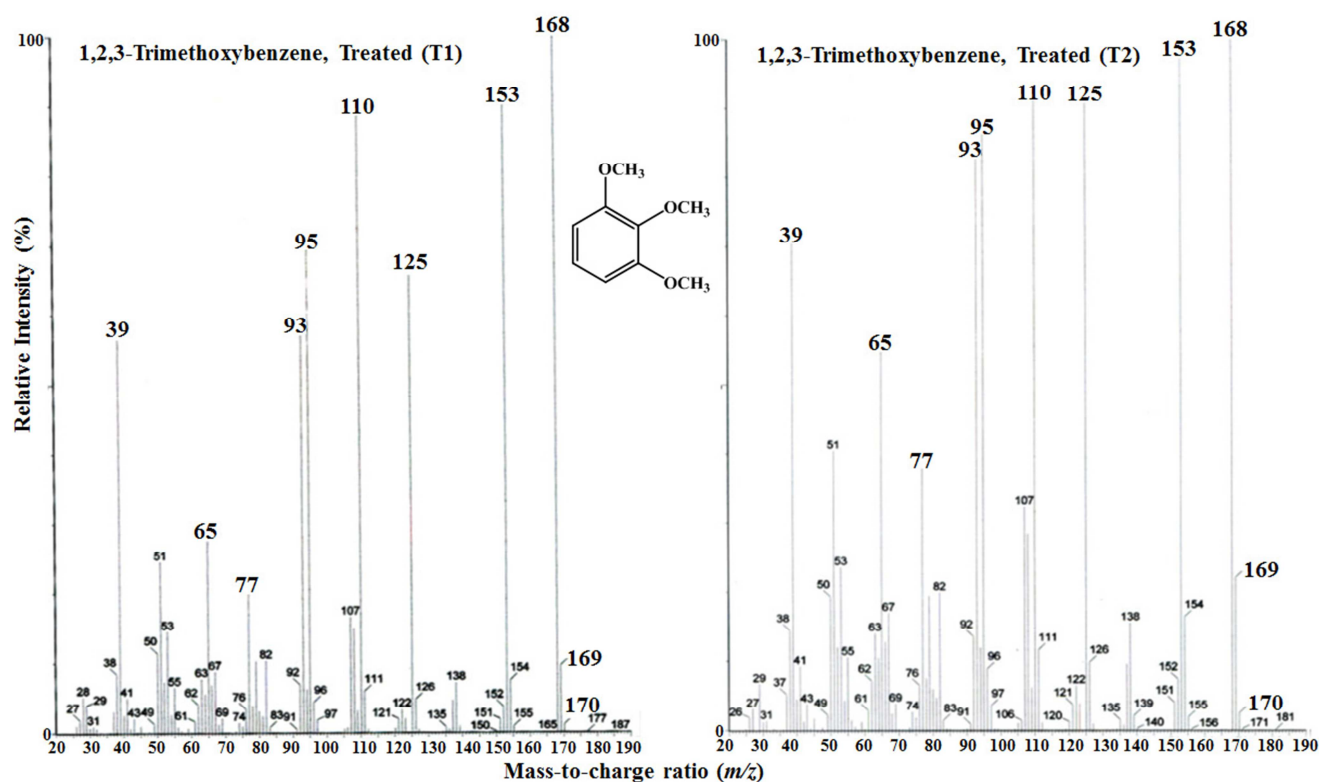


Figure 3. GC-MS spectra of the biofield energy treated 1,2,3-Trimethoxybenzene (TMB) at T1 and T2.

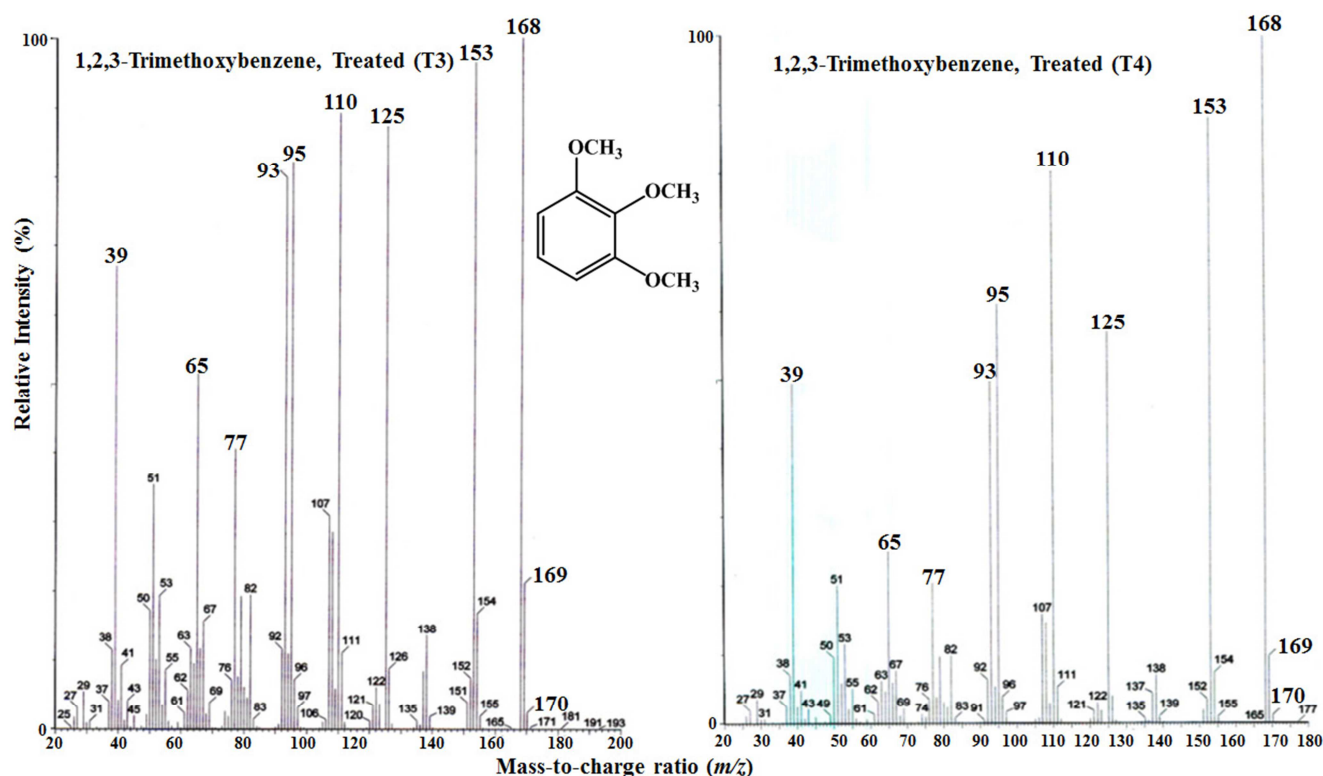


Figure 4. GC-MS spectra of the biofield energy treated 1,2,3-Trimethoxybenzene (TMB) at T3 and T4.

### 3.2. Analysis of Isotopic Abundance Ratio

TMB has the molecular formula of  $C_9H_{12}O_3$  and the molecular ion  $[M^+]$  peak showed 100% relative intensity.  $P_{M+1}$  and  $P_{M+2}$  can be calculated theoretically according to the method described in the materials and method (section 2.4).

$P(^{13}C) = [(9 \times 1.1\%) \times 100\% \text{ (the actual size of the } M^+ \text{ peak)}] / 100\% = 9.9\%$

$P(^2H) = [(12 \times 0.015\%) \times 100\%] / 100\% = 0.18\%$

$P(^{17}O) = [(3 \times 0.04\%) \times 100\%] / 100\% = 0.12\%$

$P_{M+1}$  i.e.  $^{13}C$ ,  $^2H$ ,  $^{17}O$  contributions from  $(C_9H_{12}O_3)^+$  to  $m/z$  169 = 10.2%

From the above calculation, it has been found that  $^{13}C$  has major contribution to  $m/z$  169.

In the similar approach,  $P_{M+2}$  can be calculated as follow:

$P(^{18}O) = [(3 \times 0.20\%) \times 100\%] / 100\% = 0.60\%$

So,  $P_{M+2}$  i.e.  $^{18}O$  contribution from  $(C_9H_{12}O_3)^+$  to  $m/z$  170 = 0.60%.

$P_M$ ,  $P_{M+1}$ ,  $P_{M+2}$  for the control and biofield energy treated TMB at  $m/z$  168, 169 and 170, respectively were achieved from the observed relative peak intensities of  $[M^+]$ ,  $[(M+1)^+]$ , and  $[(M+2)^+]$  peaks in the GC-MS spectra, respectively and are presented in the Table 2.

Table 2. Isotopic abundance analysis result of the control and biofield energy treated 1,2,3-Trimethoxybenzene (TMB).

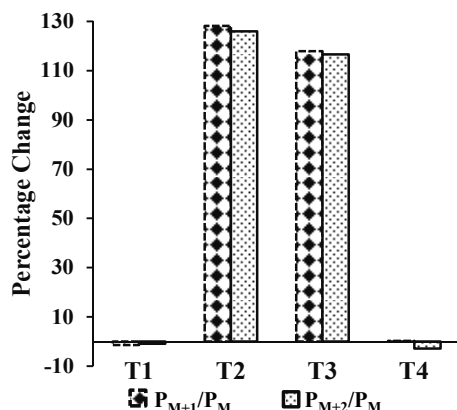
Parameter	Control TMB	Biofield Energy Treated TMB			
		T1	T2	T3	T4
$P_M$ at $m/z$ 168 (%)	100	100	100	100	100
$P_{M+1}$ at $m/z$ 169 (%)	9.67	9.54	22.06	21.08	9.70
$P_{M+1}/P_M$	0.0967	0.0954	0.2206	0.2108	0.0970
% Change of isotopic abundance ratio ( $P_{M+1}/P_M$ )		-1.34	128.13	117.99	0.31
$P_{M+2}$ at $m/z$ 170 (%)	1.08	1.07	2.44	2.34	1.05
$P_{M+2}/P_M$	0.0108	0.0107	0.0244	0.0234	0.0105
% Change of isotopic abundance ratio ( $P_{M+2}/P_M$ )		-0.93	125.93	116.67	-2.78

T1, T2, T3, and T4: Biofield energy treated sample analyzed at different time intervals;  $P_M$  = 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  $[(M+2)^+]$  and  $M$  = mass of the parent molecule.

By comparing the theoretical values as calculated above and the experimental values as shown in the Table 2, it can be suggested that  $^{13}C$  and  $^{18}O$  might have major contributions from  $(C_9H_{12}O_3)^+$  to  $m/z$  169 and 170 respectively. The percentage change of the isotopic abundance ratios ( $P_{M+1}/P_M$

and  $P_{M+2}/P_M$ ) in the biofield treated TMB with respect to the control TMB is shown in Table 2 and Figure 5. The isotopic abundance ratio of  $P_{M+1}/P_M$  in the biofield treated TMB at T2 and T3 was significantly increased by 128.13 and 117.99%, respectively with respect to the control sample. But, the

isotopic abundance ratio in T1 was slightly decreased by 1.34%, whether in case of T4, it was increased by 0.31% with respect to the control TMB.



**Figure 5.** Percent change of isotopic abundance ratios of  $P_{M+1}/P_M$  and  $P_{M+2}/P_M$  in the biofield energy treated 1,2,3-Trimethoxybenzene (TMB) as compared to the control sample.

Consequently, the percentage change in the isotopic abundance ratio of  $P_{M+2}/P_M$  was significantly enhanced in the biofield treated TMB at T2 and T3 by 125.93 and 116.67%, respectively with respect to the control sample. On the other hand, the isotopic abundance of  $P_{M+1}/P_M$  in the biofield treated at T1 and T4 was decreased by 0.93 and 2.78%, respectively as compared to the control TMB. Thus,  $^{13}\text{C}$ ,  $^2\text{H}$ , and  $^{17}\text{O}$  contributions from  $(\text{C}_9\text{H}_{12}\text{O}_3)^+$  to  $m/z$  169 and  $^{18}\text{O}$  contribution from  $(\text{C}_9\text{H}_{12}\text{O}_3)^+$  to  $m/z$  170 for the biofield treated TMB, particularly at T2 and T3 were significantly changed as compared to the control sample.

From the results, it has been found that isotopic abundance ratio in biofield treated TMB was increased at first with time and was found to be highest in T2. Surprisingly, when biofield treated TMB was stored for a long time in laboratory condition, the isotopic abundance ratio in T3 was decreased slowly. But after the long time (T4), the isotopic abundance ratio in biofield treated TMB was dropped suddenly, even lower than the control. This result indicated that the biofield energy treatment might be active for alteration of the isotopic abundance ratio in TMB for a period of time after receiving the treatment.

There is a very close relationship between the neutrino physics and rare isotope physics through the nucleosynthesis of various elements. Thus, neutrinos that are produced through the nuclear reactions in the Sun, cosmic rays, and collapsing stars/supernovae might lead to the variation in the

isotopic composition in the molecule [46, 47]. Neutrinos are electrically neutral particles and the most probable carrier of the hidden mass in the Universe. Neutrino flux can freely move between human and environment without affected by the electromagnetic forces can freely flow between human and environment [48-50]. For this reason, the neutrinos have the capability to interact with protons and neutrons in the nucleus. Recently, literature mentioned that biofield energy might have effect on the variations of isotopic composition in water molecule [23]. It can be postulated that Trivedi's unique biofield energy might have the ability to transform the behavior at atomic and molecular level by varying the neutron to proton ratio in the nucleus possibly through the introducing neutrino particles inside the compound. Based on this hypothesis, it is presumed that the possible reason for the alteration of the isotopic abundance ratios ( $P_{M+1}/P_M$  and  $P_{M+2}/P_M$ ) in the biofield treated TMB might be due to the involvement of neutrino particles through biofield energy treatment.

The amount of the electronic, vibration, rotational and translation energies constitutes the energy of a compound. The change of the isotopic abundance ratio of the molecule does not affect electronic, translational, and rotational energies of the molecule, but significantly alters the vibrational energy [6, 9]. The vibrational energy of a diatomic molecule depends on the reduced mass ( $\mu$ ) for a diatomic molecule as shown in the below [6, 9]:

$$E_0 = \frac{h}{4\pi} \sqrt{\frac{f}{\mu}}$$

Where  $E_0$  = the vibrational energy of a harmonic oscillator at absolute zero or zero point energy

$f$  = force constant

$\mu$  = reduced mass =  $\frac{m_a m_b}{m_a + m_b}$ ,  $m_a$  and  $m_b$  are the masses of the constituent atoms.

The possible isotopic bond formation in the TMB molecule and their effect on the vibrational energy of TMB are presented in the Table 3. From the Table 3, it has been observed that alteration of  $^{12}\text{C}$  with  $^{13}\text{C}$  for C-C bond,  $^1\text{H}$  with  $^2\text{H}$  for C-H bond,  $^{16}\text{O}$  with  $^{18}\text{O}$  for C-O bond have much effect on the vibrational energy of the molecule. The isotope effect is principally due to the ground state vibrational energies as shown in the Table 3.

**Table 3.** Possible isotopic bond and their effect in the vibrational energy in 1,2,3-Trimethoxybenzene (TMB) molecule.

Entry No.	Probable isotopic bond	Isotope type	Reduced mass ( $\mu$ )	Zero point vibrational energy ( $E_0$ )
1	$^{12}\text{C}-^{12}\text{C}$	Lighter	6.00	Higher
2	$^{13}\text{C}-^{12}\text{C}$	Heavier	6.26	Smaller
3	$^1\text{H}-^{12}\text{C}$	Lighter	0.92	Higher
4	$^1\text{H}-^{13}\text{C}$	Heavier	0.93	Smaller
5	$^2\text{H}-^{12}\text{C}$	Heavier	1.04	Smaller
6	$^{12}\text{C}-^{16}\text{O}$	Lighter	6.86	Higher
7	$^{13}\text{C}-^{16}\text{O}$	Heavier	7.17	Smaller
8	$^{12}\text{C}-^{17}\text{O}$	Heavier	7.03	Smaller
9	$^{12}\text{C}-^{18}\text{O}$	Heavier	7.20	Smaller



The isotopic abundance ratio analysis clearly indicated that the isotopic abundance ratios of  $^{13}\text{C}/^{12}\text{C}$  or  $^2\text{H}/^1\text{H}$  ( $P_{M+1}/P_M$ ) and  $^{18}\text{O}/^{16}\text{O}$  ( $P_{M+2}/P_M$ ) in the biofield treated TMB (particularly at T2 and T3) was significantly increased as compared to the control TMB. Hence, biofield treated TMB might exhibit different isotope effects such as physicochemical properties, the rate of reaction, binding energy than lighter molecules from the control sample. Isotope effects have a role in the thermal decomposition of the molecules [51-53]. So, the alteration in the isotopic abundance ratio in the molecule might have an effect on the thermal properties of the molecule. Thus, biofield treated TMB (particularly at T2 and T3) might have altered physicochemical and thermal properties than control TMB. Hence, the current findings concluded that the increased isotopic abundance ratio in TMB due to the biofield energy treatment might be responsible for alteration in volatilization and force constant of the biofield treated TMB [15]. Kinetic isotope effect (KIE) *i.e.* the alteration in the rate of a chemical reaction occurs due to the alteration in the isotopic abundance ratio of one of the atoms in the reactants. KIE is a very powerful technique to study the reaction mechanism, to alleviate the transition state of the rate-determining step of the reaction and for understanding the enzymatic transition state and all aspects of enzyme mechanism [6, 9, 54, 55]. Thus, biofield treated TMB might have altered physicochemical and thermal properties, different reaction rate, selectivity and binding energy.

## 4. Conclusions

The current findings revealed that biofield energy treatment had amazing ability for changing the isotopic abundance ratio in TMB. The GC-MS spectra of the control and biofield treated TMB exhibited the presence of molecular ion peak  $[M^+]$  at  $m/z$  168 (calculated 168.08 for  $\text{C}_9\text{H}_{12}\text{O}_3$ ) along with the similar pattern of fragmentation. The relative peak intensities of the fragmented ions in biofield treated TMB (particularly at T2 and T3) was different from the control sample. The isotopic abundance ratio analysis in TMB exhibited that the isotopic abundance ratio of  $P_{M+1}/P_M$  in the biofield treated TMB at T2 and T3 was significantly increased by 128.13 and 117.99%, respectively with respect to the control sample. Consequently, the percentage change in the isotopic abundance ratio of  $P_{M+2}/P_M$  was significantly enhanced in the biofield treated TMB at T2 and T3 by 125.93 and 116.67%, respectively with respect to the control TMB. Briefly,  $^{13}\text{C}$ ,  $^2\text{H}$ , and  $^{17}\text{O}$  contributions from  $(\text{C}_9\text{H}_{12}\text{O}_3)^+$  to  $m/z$  169 and  $^{18}\text{O}$  contribution from  $(\text{C}_9\text{H}_{12}\text{O}_3)^+$  to  $m/z$  170 for the biofield treated TMB, particularly at T2 and T3 were significantly changed that might show altered isotope effects as compared with the control sample. For this reason, TMB after biofield energy treatment exhibited different physicochemical and thermal properties as well as the rate of chemical reactions, binding energy. By using these isotope effects, the biofield treated TMB could be more useful in

research purpose and might help to introduce the new chemicals and pharmaceuticals.

## Abbreviations

A: Element; GC-MS: Gas chromatography-mass spectrometry; KIE: Kinetic isotope effect; M: Mass of the parent molecule;  $m/z$ : Mass-to-charge ratio; n: Number of the element;  $P_M$ : The relative peak intensity of the parent molecular ion  $[M^+]$ ;  $P_{M+1}$ : The relative peak intensity of isotopic molecular ion  $[(M+1)^+]$ ;  $P_{M+2}$ : The relative peak intensity of isotopic molecular ion  $[(M+2)^+]$ ;  $R_t$ : Retention time; TMB: 1,2,3-Trimethoxybenzene.

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