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Gas Chromatography-Mass Spectrometric Analysis of Isotopic Abundance of ^{13}C , ^2H , and ^{18}O in Biofield Energy Treated *p*-tertiary Butylphenol (PTBP)

Mahendra Kumar Trivedi¹, Alice Branton¹, Dahryn Trivedi¹, Gopal Nayak¹, Parthasarathi Panda², Snehasis Jana^{2,*}

¹Trivedi Global Inc., Henderson, Nevada, USA

²Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, Madhya Pradesh, India

Email address:

publication@trivedisrl.com (S. Jana)

*Corresponding author

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Abstract: *p*-tert-Butylphenol (PTBP) is a phenolic monomer used in the synthesis of numerous industrially useful chemicals. The current research work aimed to evaluate the effect of the biofield energy treatment on the isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in PTBP using gas chromatography - mass spectrometry (GC-MS). The sample, PTBP was distributed into two parts - one part was designated as control PTBP and another part was considered as biofield energy treated PTBP. The biofield energy treatment was achieved through unique biofield energy transmission process by Mr. Trivedi (also known as The Trivedi Effect[®]). T1, T2, T3, and T4 were indicated to the different time interval analysis of the biofield treated PTBP. The GC-MS spectra of the both control and biofield treated PTBP showed the presence of molecular ion peak $[M^+]$ at m/z 150 (calculated 150.10 for $\text{C}_{10}\text{H}_{14}\text{O}$) along with eight major fragmented peaks at m/z 135, 107, 95, 91, 77, 65, 41, and 39, which might be due to $\text{C}_{10}\text{H}_{15}^+$, $\text{C}_7\text{H}_7\text{O}^+$ or $\text{C}_8\text{H}_{11}^+$, $\text{C}_6\text{H}_7\text{O}^+$, C_7H_7^+ , C_6H_5^+ , C_5H_5^+ , C_3H_5^+ , and $\text{C}_3\text{H}_3^{++}$ ions, respectively. The relative intensities of the parent molecule and other fragmented ions of the biofield treated PTBP were altered as compared to the control PTBP. The percentage in the isotopic abundance ratio of P_{M+1}/P_M was enhanced in the biofield treated PTBP at T2, T3 and T4 by 1.60%, 3.57%, and 120.13%, respectively while it was decreased by 4.14% in the treated sample at T1 with respect to the control PTBP. Consequently, the isotopic abundance ratio of P_{M+2}/P_M was increased in the biofield treated PTBP at T1, T3, and T4 by 1.28%, 2.56%, and 123.08%, respectively with respect to the control sample. On the other hand, it was reduced in the biofield treated sample at T2 by 1.28% as compared to the control PTBP. Concisely, ^{13}C , ^2H , and ^{17}O contributions from $(\text{C}_{10}\text{H}_{14}\text{O})^+$ to m/z 151 and ^{18}O contribution from $(\text{C}_{10}\text{H}_{14}\text{O})^+$ to m/z 152 in the biofield treated PTBP were changed with respect to the control sample and was found to have time dependent effect. The biofield energy treated PTBP might display isotope effects such as different physicochemical and thermal properties, rate of the reaction, selectivity and binding energy due to the changed isotopic abundance ratio as compared to the control sample. Biofield treated PTBP could be valuable for the designing new chemicals and pharmaceuticals through using its kinetic isotope effects.

Keywords: Biofield Energy Treatment, The Trivedi Effect[®], PTBP, Gas Chromatography - Mass Spectrometry, Isotopic Abundance Ratio, Isotope Effects, Kinetic Isotope Effect

1. Introduction

p-tert-Butylphenol (PTBP) or chemically known as 4-(1,1-dimethylethyl) phenol is a phenol derivative, $\text{C}_{10}\text{H}_{14}\text{O}$ and is

widely used as a monomer in the synthesis of several industrial chemicals. PTBP is an important chemical for the manufacturing of polycarbonate polymers, phenolic resins, epoxy resins, and perfumery intermediates [1-5]. Literature reported a wide industrial applications of PTBP-

formaldehyde resins, for e.g. as glues and laminates, adhesives, surface coatings in plywood and building industries, as tackifiers and vulcanisation agent in tyre industry; as good insulating materials used in electronic and electric appliances; as binders for glass and mineral fibers in the production of heat, noise, and fire-insulating materials; printing inks, etc. [1-5]. PTBP is useful for the production of *p*-tert-butylcyclohexanol and tri-aryl phosphate esters used as flame retardants and plasticizers [3]. Besides these applications, PTBP has other useful applications such as soap-antioxidant, pour point depressant, plasticizer, germicidal agent in detergent disinfectant, fumigant, insecticide, de-emulsifier for oil, etc. [2]. PTBP is a quintessential reactant for the condensation reaction with formaldehyde to produce calixarenes-cyclic oligomers used as supramolecular catalysts, enzyme mimetics, etc. [6, 7]. The uses of PTBP is constrained due to its adverse effects on skin leading to contact dermatitis and contact eczema [1, 8-10]. As PTBP is the potent phenolic depigmentation agent, it causes patchy depigmentation of the skin due to the loss of functional melanocytes through apoptosis [8, 11].

Now-a-days analysis of natural abundance variations in the stable isotopes (or also known as stable isotope ratio analysis) is increasing attention in various scientific fields such as agricultural, biochemistry, metabolism, medical research, environmental pollution, archaeology, etc. for the measurement of the flow of materials and energy both within and among the organisms [12-14]. The alteration in isotopic abundance ratio between the isotopic forms of the molecule produces isotope effects *i.e.* the differences in physical and chemical properties of the molecule [15-17]. Literature mentioned that conventional mass spectrometry (MS) technique such as GC-MS is the main choice for isotope ratio analysis with sufficient precision. But specialized instruments like isotope ratio mass spectrometer, multiple collector inductively coupled plasma mass spectrometry are required when the molecules have molar isotope enrichments at below 0.1% [13, 14, 17].

The biofield is a massless electromagnetic field that surrounds, permeates and affects the human body. Healing practitioner has the ability to harness the energy from the earth, the “universal energy field” and can be transmitted the biofield energy into any living or non-living object(s) around the Globe with the purpose of improving the wholeness within the objects. This is known as biofield energy treatment or energy medicine and is recognized as complementary and alternative medicine (CAM) [18, 19]. The Trivedi Effect®-biofield energy healing has recently drawn attention in the various scientific fields, such as medical science [20], biotechnology [21, 22], microbiology [23, 24], organic chemistry [25, 26], pharmaceutical [27], nutraceutical [28], materials science [29, 30], and agricultural [31, 32] due to its outstanding applicability to modify the characteristic properties of the living and non-living substances. Number of literatures [33-36] indicated that biofield energy treatment (also known as The Trivedi Effect®) might be a potential method for alteration of the isotopic abundance ratio in the

organic compounds. An altered physicochemical and thermal properties such as increased crystallite size, enhanced thermal stability was observed in the biofield energy treated PTBP as compared to the control sample through the spectroscopic and thermal study [37]. The toxicity, hazards effects and human health effects by the chemical or material are very closely associated with its intrinsic physicochemical properties [38]. Hence, the isotopic abundance ratio (P_{M+1}/P_M and P_{M+2}/P_M) analysis in both of the control and biofield treated PTBP was performed in the current research work through gas chromatography-mass spectrometry.

2. Materials and Methods

2.1. Chemicals

PTBP was procured from Sisco Research Laboratories Pvt. Ltd., India. All the other chemicals and reagents used analytical grade in this experiment were purchased from local vendors.

2.2. Biofield Energy Treatment Modalities

The sample PTBP was distributed into two parts: one was denoted as control, where no treatment was given. The other part of the sample represented as biofield energy treated sample, which was handed over to Mr. Trivedi for the biofield energy treatment in a sealed condition. The biofield energy treatment was provided by Mr. Trivedi (also known as The Trivedi Effect®) through his unique energy transmission process to the test item 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 investigated by GC-MS. The biofield treated PTBP was analyzed in different time intervals designated as T1, T2, T3, and T4 in order to understand the influence of the biofield energy treatment on isotopic abundance ratio with respect to the time.

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

The GC-MS analysis was conducted on Perkin Elmer/Auto system XL with Turbo mass, USA. A silica capillary column equipped with a quadrupole detector with pre-filter, one of the fastest, widest mass ranges was applied for the analysis. The mass spectrometer was worked 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: ± 0.1 m/z mass accuracy over 48 hours. The analytes were identified by retention time and through the comparison of the mass spectra of the identified substances with references.

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

A comprehensive literature review [39-42] revealed that the relative intensity of the peak in the mass spectra is directly proportional to the relative isotopic abundance of the

molecule and the method used for the analysis of the isotopic abundance ratio of the molecule is mentioned below:

P_M stands for the relative peak intensity of the parent molecular ion $[M]^+$ expressed in percentage. In other way, it indicates the probability to A elements having only one natural isotope in appreciable abundance (for e.g. ^{12}C , ^1H , ^{16}O , ^{14}N , etc.) contributions to the mass of the parent molecular ion $[M]^+$.

P_{M+1} represents the relative peak intensity of the isotopic molecular ion $[(M+1)^+]$ 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 $A + 1$ elements having an isotope that has one mass unit heavier than the most abundant isotope (for e.g. ^{13}C , ^2H , ^{15}N , etc.) contributions to the mass of the isotopic molecular ion $[(M+1)^+]$.

P_{M+2} represents the relative peak intensity of the isotopic molecular ion $[(M+2)^+]$ 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 having an isotope that has two mass unit heavier than the most abundant isotope (for e.g. ^{18}O , ^{37}Cl , ^{34}S , etc.) contributions to the mass of isotopic molecular ion $[(M+2)^+]$. The value of the natural isotopic abundance of the some elements are obtained from several literatures and presented in the Table 1 [13, 42-44].

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

Isotopic abundance ratio for $A + 1$ elements $= P_{M+1}/P_M$

Similarly, isotopic abundance ratio for $A + 2$ elements $= P_{M+2}/P_M$

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

Where, IAR_{Treated} = isotopic abundance ratio in the treated

sample and IAR_{Control} = isotopic abundance ratio in the control sample.

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	^1H	1	99.9885	0.015 n_{H}	
	^2H	2	0.0115		
Carbon	^{12}C	12	98.892	1.1 n_{C}	
	^{13}C	13	1.108		
Oxygen	^{16}O	16	99.762	0.04 n_{O}	0.20 n_{O}
	^{17}O	17	0.038		
	^{18}O	18	0.200		
Nitrogen	^{14}N	14	99.60	0.40 n_{N}	
	^{15}N	15	0.40		
Chlorine	^{35}Cl	35	75.78		32.50 n_{Cl}
	^{37}Cl	37	24.22		

3. Results and Discussion

3.1. GC-MS Analysis

The GC-MS spectrum of the control PTBP (Figure 1) indicated the presence of molecular ion peak $[M]^+$ at m/z 150 (calculated 150.10 for $\text{C}_{10}\text{H}_{14}\text{O}$) at the retention time (R_t) of 12.48 min. Eight major fragmented peaks in lower m/z region at m/z 135, 107, 95, 91, 77, 65, 41, and 39 were observed in the control TPBP due to the breaking up of PTBP, corresponded to the following ions: $\text{C}_{10}\text{H}_{15}^+$ (*tert*-Butylbenzene), $\text{C}_7\text{H}_7\text{O}^+$ (*p*-cresol) or $\text{C}_8\text{H}_{11}^+$ (Ethyl benzene), $\text{C}_6\text{H}_7\text{O}^+$ (Phenol), C_7H_7^+ (Toluene), C_6H_5^+ (Benzene), C_5H_5^+ , C_3H_5^+ , and $\text{C}_3\text{H}_3^{++}$ ions, respectively (Figure 1). The GCMS spectrum along with fragmentation pattern of PTBP as shown in the Figure 1 was well accorded with the literature [45].

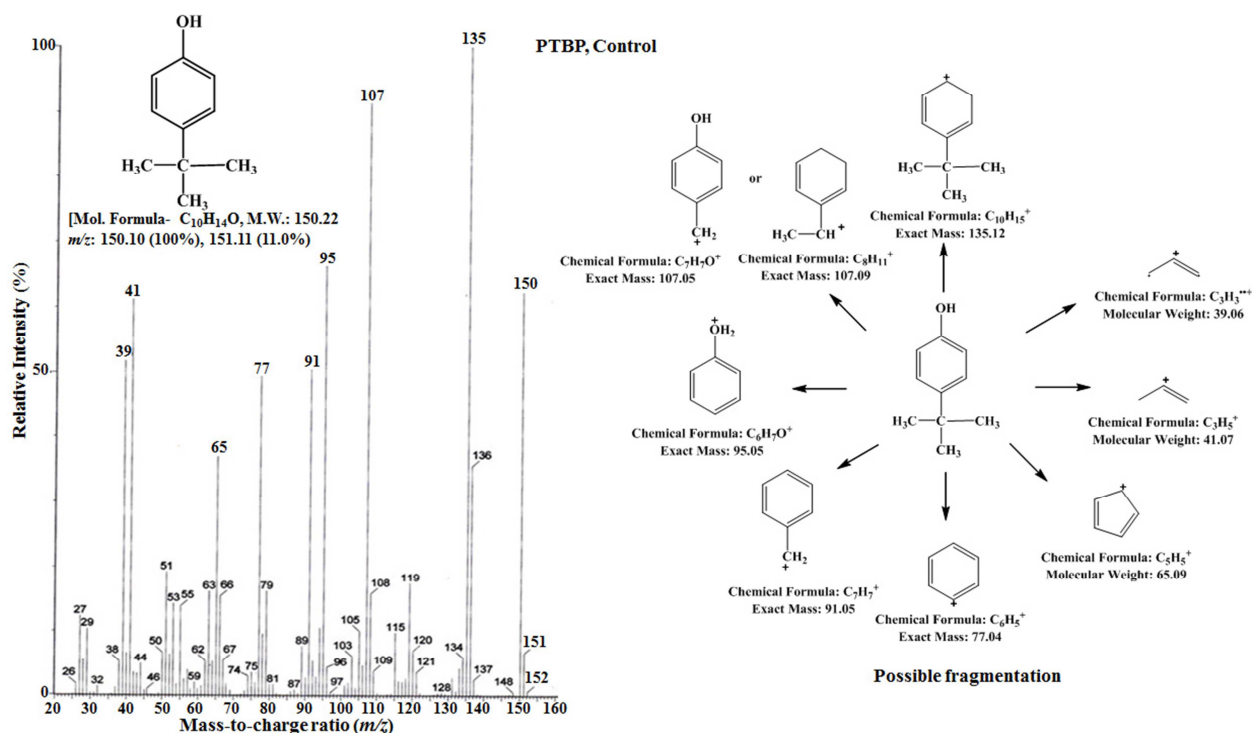


Figure 1. GC-MS spectrum and possible fragmentation of the control *p*-tertiary butylphenol (PTBP).

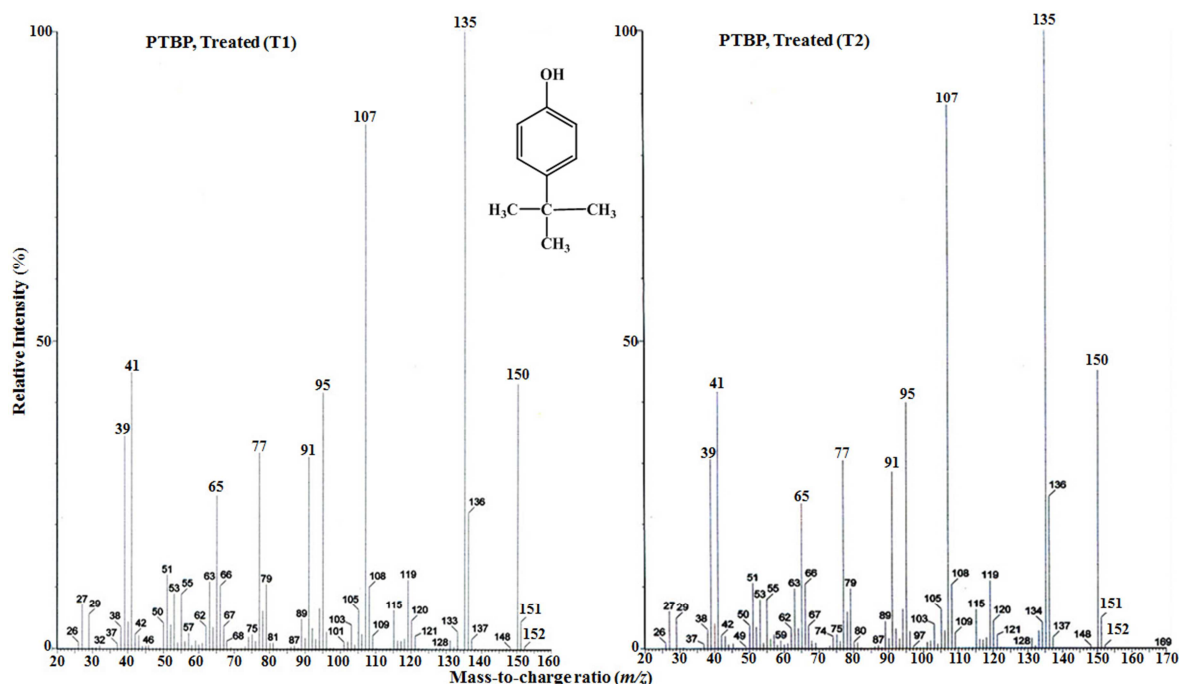


Figure 2. GC-MS spectra of the biofield energy treated *p*-tertiary butylphenol (PTBP) at T1 and T2.

The GC-MS spectra of the biofield treated PTBP at T1, T2, T3, and T4 (Figures 2 and 3) disclosed the molecular ion peak $[M^+]$ at m/z 150 at R_t of 12.46, 12.42, 12.44, and 12.46 min, respectively. So, R_t of the biofield treated PTBP was close to the control sample. There was similar pattern of fragmentation observed in the biofield treated PTBP as compared with the control sample.

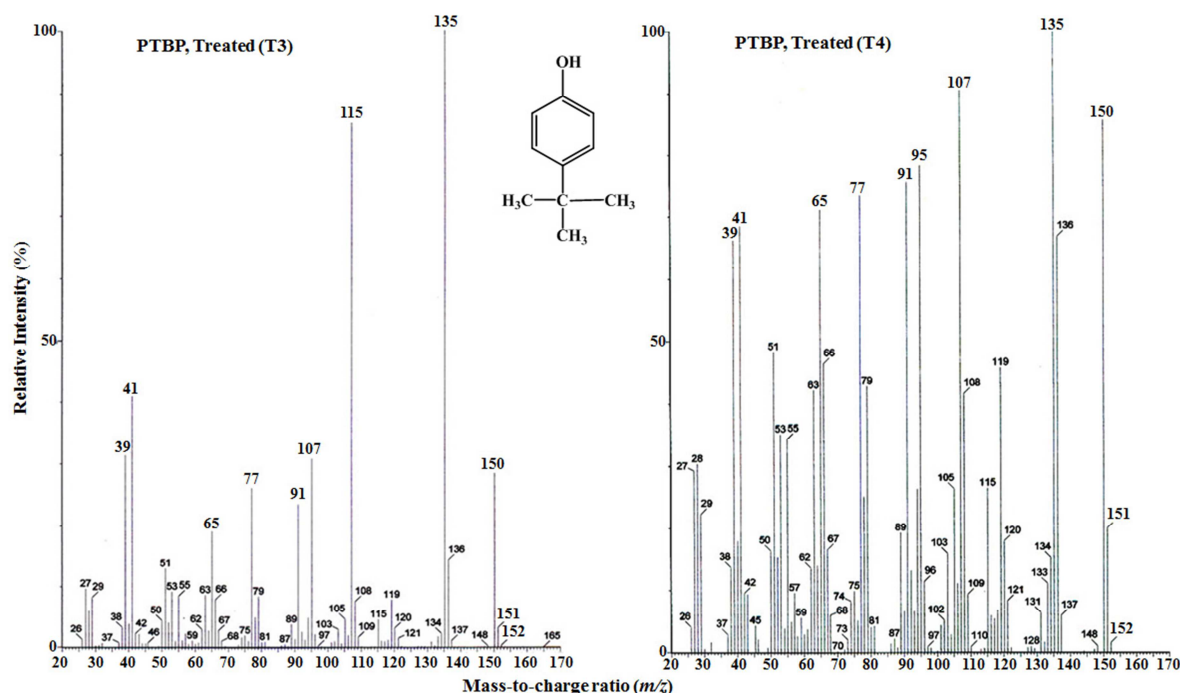


Figure 3. GC-MS spectra of the biofield energy treated *p*-tertiary butylphenol (PTBP) at T3 and T4.

The relative intensities of the parent molecule at m/z 150 and other fragmented ions at m/z 135, 107, 95, 91, 77, 65, 41, and 39 are presented in the Table 2. *tert*-Butylbenzene ion $(C_{10}H_{15})^+$ peak at m/z 135 that was derived from the degradation of the parent molecule exhibited 100% relative intensity (base peak). From the Table 2, it has been noticed that the relative intensities of the parent molecule along with its fragmented ions in the biofield treated PTBP were significantly changed as compared to the control PTBP.

Table 2. Relative intensities of the corresponding m/z of the parent molecule (PTBP) and its fragmented ions.

Mass of the peaks m/z	Relative intensity of the peak (%)				
	Control PTBP	Biofield energy treated PTBP			
		T1	T2	T3	T4
150	62.49	43.09	45.26	28.78	85.70
135	100	100	100	100	100
107	91.47	84.99	88.02	85.22	90.51
95	66.37	41.76	40.04	30.96	78.37
91	50.75	31.19	28.68	23.55	75.59
77	49.66	31.89	30.63	26.10	73.46
65	37.08	24.48	23.51	19.13	71.53
41	61.32	44.91	41.74	40.98	68.52
39	51.73	34.64	30.82	31.45	66.35

T1, T2, T3, and T4: Biofield energy treated sample analyzed at different time intervals.

3.2. Analysis of Isotopic Abundance Ratio

The molecular formula of PTBP is $C_{10}H_{14}O$ and the molecular ion $[M^+]$ peak for the control PTBP showed 62.49% relative intensity. P_{M+1} and P_{M+2} can be calculated theoretically according to the method described in the materials and method. The theoretical calculation for P_{M+1} is provided as follows:

$$P(^{13}C) = [(10 \times 1.1\%) \times 62.49\% \text{ (the actual size of the } M^+ \text{ peak)}] / 100\% = 6.87\%$$

$$P(^2H) = [(14 \times 0.015\%) \times 62.49\%] / 100\% = 0.13\%$$

$$P(^{17}O) = [(1 \times 0.04\%) \times 62.49\%] / 100\% = 0.03\%$$

P_{M+1} i.e. ^{13}C , 2H , and ^{17}O contributions from $(C_{10}H_{14}O)^+$ to m/z 151 = 7.03%

From the above calculation, it has been found that ^{13}C has major contribution to m/z 151.

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

$$P(^{18}O) = [(1 \times 0.20\%) \times 62.49\%] / 100\% = 0.13\%$$

So, P_{M+2} i.e. ^{18}O contribution from $(C_{10}H_{14}O)^+$ to m/z 152 = 0.13%.

P_M , P_{M+1} , P_{M+2} for the control and biofield energy treated PTBP at m/z 150, 151 and 152, respectively were accomplished 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 3.

Table 3. Isotopic abundance analysis results of the control and biofield energy treated *p*-tertiary butylphenol (PTBP).

Parameter	Control PTBP	Biofield energy treated PTBP			
		T1	T2	T3	T4
P_M at m/z 150 (%)	62.49	43.09	45.26	28.78	85.70
P_{M+1} at m/z 151 (%)	6.64	4.39	4.89	3.17	20.05
P_{M+1}/P_M	0.1063	0.1019	0.1080	0.1101	0.2340
% Change of isotopic abundance ratio (P_{M+1}/P_M)		-4.14	1.60	3.57	120.13
P_{M+2} at m/z 152 (%)	0.49	0.34	0.35	0.23	1.49
P_{M+2}/P_M	0.0078	0.0079	0.0077	0.0080	0.0174
% Change of isotopic abundance ratio (P_{M+2}/P_M)		1.28	-1.28	2.56	123.08

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.

The experimental values (Table 3) for the control sample and the above calculated theoretical values suggest that ^{13}C and ^{18}O might have major contributions from $(C_{10}H_{14}O)^+$ to m/z 151 and 152, respectively. The percentage change of the isotopic abundance ratios (P_{M+1}/P_M and P_{M+2}/P_M) in the biofield treated PTBP with respect to the control PTBP is presented in Table 3 and Figure 4. The percentage in the isotopic abundance ratio of P_{M+1}/P_M was increased in the biofield treated PTBP at T2, T3 and T4 by 1.60%, 3.57%, and 120.13%, respectively while it was decreased by 4.14%

in the treated sample at T1 with respect to the control PTBP. Consequently, the isotopic abundance ratio of P_{M+2}/P_M was enhanced in the biofield treated PTBP at T1, T3, and T4 by 1.28%, 2.56%, and 123.08%, respectively with respect to the control sample. On the other hand, it was decreased in the treated sample at T2 by 1.28% as compared to the control PTBP. Concisely, ^{13}C , 2H , and ^{17}O contributions from $(C_{10}H_{14}O)^+$ to m/z 151 and ^{18}O contribution from $(C_{10}H_{14}O)^+$ to m/z 152 in the biofield treated PTBP were significantly altered with respect to the control sample.



Figure 4. Percent change of the isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in the biofield energy treated *p*-tertiary butylphenol (PTBP) as compared to the control sample.

Figure 4 indicated an opposite trend in percentage change of the isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in the biofield treated PTBP at T1 and T2 and no significant change was observed with the respect of the control sample. But after certain day's storage, there was a gradual increasing trend found at T3 and T4 with respect to the time and T4 showed the highest alteration in the isotopic abundance ratio. Hence, the biofield energy treatment showed time dependent effect on the isotopic abundance ratio in PTBP.

Neutrinos are the charge less particles, but most probable carrier of hidden mass in the Universe and produced by the Sun, Cosmic rays, etc. They can travel through large distances in the matter without being affected by the electromagnetic forces and induce the fission reactions within a heavy nuclei. Thus, neutrinos can affect the natural abundance of isotopes of the element leading to the variation in the isotopic composition of the materials [46-48]. Literature proposed that "Mr. Trivedi's superconsciousness energy might be in the form of neutrinos changing mass into energy and *vice versa*" [49]. Thus, it can be postulated that Mr. Trivedi's unique biofield energy treatment might have the capability for introduction of the neutrino flux into both of the living and nonliving substances that might interact with protons and neutrons in the nucleus. This interaction might change the neutron to proton ratio in the nucleus that might be responsible for changing the behavior at atomic and molecular level. Based on this hypothesis, it is assumed 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 PTBP might be intervention of the neutrino fluence through the biofield energy treatment.

The variation of the isotopic abundance ratio of the molecule significantly affects the vibrational energy of the compound without affecting the electronic, translational, and rotational energies of the molecule. The relation between the vibrational energy and the reduced mass (μ) for a diatomic molecule is expressed as below [17, 51]:

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

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

f = force constant

μ = 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 PTBP molecule and their effect on the vibrational energy are provided in the Table 4. There is no effect on the reduced mass due to the alteration of the isotopic abundance ratios of $^{13}\text{C}/^{12}\text{C}$ for C-H bond and $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ for O-H bond (data not shown). Consequently, the variation in the isotopic abundance ratios of $^{13}\text{C}/^{12}\text{C}$ for C-O, $^2\text{H}/^1\text{H}$ for C-H and O-H bonds and $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ for C-O bond has significant effect on the ground state vibrational energy of the molecule due to the higher reduced mass (μ) as shown in the Table 4 resulting the isotope effects of the molecule. The isotopic abundance ratio analysis in PTBP revealed that the isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M were significantly increased in the biofield treated PTBP at T4 whereas at T1, T2 and T3, it was altered as compared to the control sample.

Table 4. Possible isotopic bond and their effect in the vibrational energy in *p*-tertiary butylphenol (PTBP) molecule.

Entry No.	Probable isotopic bond	Isotope type	Reduced mass (μ)	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	$^2\text{H}-^{12}\text{C}$	Heavier	1.04	Smaller
5	$^{12}\text{C}-^{16}\text{O}$	Lighter	6.86	Higher
6	$^{13}\text{C}-^{16}\text{O}$	Heavier	7.17	Smaller
7	$^{12}\text{C}-^{17}\text{O}$	Heavier	7.03	Smaller
8	$^{12}\text{C}-^{18}\text{O}$	Heavier	7.20	Smaller
9	$^{16}\text{O}-^1\text{H}$	Lighter	0.94	Higher
10	$^{16}\text{O}-^2\text{H}$	Heavier	1.78	Smaller

So, biofield treated PTBP might show diverse physical and chemical properties like lower diffusion velocity, mobility, evaporation, higher binding energy with respect to the control sample. The change in the isotopic abundance ratio can affect the thermal decomposition of the molecule [51, 52]. The alteration of a chemical reaction due to the variation in the isotopic abundance ratio of one of the atoms in the reactants is called as kinetic isotope effect (KIE). KIE is a potential tool to study the reaction mechanism, to stabilize the transition state of the rate-determining step of the reaction and for understanding the enzymatic transition state and all aspects of enzyme mechanisms that is supportive for designing enormously effective and specific inhibitors [17, 50, 53, 54]. The variation in the natural isotopic composition of the molecules happens due to the numerous reasons like radiogenic nuclides, interaction between cosmic rays and terrestrial matter, extraterrestrial materials, anthropogenic effects, etc. [17]. The current results infer that the biofield treatment might be an economical viable and alternative approach for the alteration of the natural isotopic abundance ratio of the compounds. Briefly, the physicochemical and thermal properties, rate of the reaction, selectivity and

4. Conclusions

Abbreviations

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