Determination of Isotopic Abundance of ^{13}C/^{12}C or ^{2}H/^{1}H and ^{18}O/^{16}O in Biofield Energy Treated 1-Chloro-3-Nitrobenzene (3-CNB) Using Gas Chromatography-Mass Spectrometry

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Abstract: 1-Chloro-3-nitrobenzene (3-CNB) is an aromatic halo-amine compound used as chemical intermediate for the production of several fine chemicals like pharmaceuticals, dyes, agricultural chemicals, etc. The stable isotope ratio analysis has drawn attention in numerous fields such as agricultural, food authenticity, biochemistry, etc. The objective of the current research was to investigate the impact of the biofield energy treatment on the isotopic abundance ratios of P_{M+1}/P_M, P_{M+2}/P_M and P_{M+3}/P_M in 3-CNB using gas chromatography - mass spectrometry (GC-MS). The sample, 3-CNB was divided into two parts - one part was denoted as control and another part was referred as biofield energy treated sample that was treated with biofield energy (The Trivedi Effect®). T1, T2, T3, and T4 were represented to different time interval analysis of the biofield treated 3-CNB. The GC-MS spectra of the both control and biofield treated 3-CNB indicated the presence of molecular ion peak [M$^+$] at *m/z* 157 (calculated 156.99 for $C_6H_4ClNO_2$) along with same pattern of fragmentation. The relative intensities of the parent molecule and other fragmented ions of the biofield treated 3-CNB were improved as compared to the control 3-CNB. The percentage change of the isotopic abundance ratio of P_{M+1}/P_M was significantly increased in the biofield treated 3-CNB at T1, T2 and T3 by 11.62, 18.50, and 29.82%, respectively with respect to the control 3-CNB. Accordingly, the isotopic abundance ratio of P_{M+2}/P_M in the biofield treated 3-CNB at T2 and T3 was significantly improved by 15.22 and 35.09%, respectively as compared to the control sample. The isotopic abundance ratios of P_{M+1}/P_M and P_{M+2}/P_M in the biofield treated 3-CNB at T1 and T4 were changed as compared to the control sample. The percentage change of the isotopic abundance ratio of P_{M+3}/P_M was enhanced in the biofield treated 3-CNB at T1, T2, T3, and T4 by 4.67, 18.69, 31.31 and 6.08%, respectively as compared to the control 3-CNB. The isotopic abundance ratios of P_{M+1}/P_M, P_{M+2}/P_M and P_{M+3}/P_M in the biofield treated 3-CNB changed with the time. So, the biofield energy treated 3-CNB might exhibit the altered isotope effects such as altered physicochemical and thermal properties, binding energy, and the rate of the chemical reaction as compared to the control sample. The biofield energy treated 3-CNB might assist in designing for the synthesis of pharmaceuticals, agricultural chemicals, dyes, corrosion inhibitors and other several useful industrial chemicals.

Keywords: Biofield Energy Treatment, The Trivedi Effect®, 1-Chloro-3-Nitrobenzene, Gas Chromatography - Mass Spectrometry, Isotopic Abundance Ratio, Isotope Effects, Kinetic Isotope Effect

1. Introduction

Chloronitrobenzenes (CNBs) are aromatic halo-amines and basically derivatives of monochlorobenzenes containing nitro group in different positions with respect to the chloro group. 1-Chloro-3-nitrobenzene or also commonly known as 3-chloronitrobenzene (3-CNB) as shown in the Figure 1 is one of the isomeric forms of chloronitrobenzene. It is a pale yellow crystalline solid having a molecular formula $C_6H_4ClNO_2$ and molecular weight of 157.55. Chloronitrobenzenes are widely used in the pharmaceutical and chemical industries as an intermediates for the production of pharmaceuticals, corrosion inhibitors, azo and sulfur dyes, herbicides, pigments, agricultural chemicals, rubber chemicals, photo chemicals, insecticides, and gasoline additives [1-6]. 3-Chloroaniline (Orange GC Base), a dye intermediate can be produced by reduction of 3-CNB. Pentachloronitrobenzene which is used as fungicide can be prepared by the exhaustive chlorination of 3-CNB [4-6].
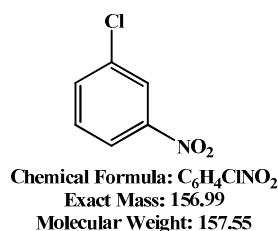

Chemical Formula: $C_6H_4ClNO_2$
Exact Mass: 156.99
Molecular Weight: 157.55

Figure 1. Structure of 1-chloro-3-nitrobenzene (3-CNB).

Analysis of natural abundance variations in the stable isotopes include ^{2}H, ^{13}C, ^{15}N, ^{18}O, ^{34}S, ^{37}Cl, etc. is a potential method for the measurement of the flow of materials and energy both within and among organisms. This is known as Stable Isotope Ratio Analysis (SIRA). This method is universally applied in agricultural, food authenticity, biochemistry, metabolism, medical research, environmental pollution, archaeology, etc. [7-10]. Isotope effects *i.e.* tiny 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 have an important role in thermal motion, molecular spectra, chemical reactions (reaction rate and bond strength), physicochemical properties, chemical equilibria, etc. [11-15]. SIRA can also be used 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 [8]. Among of the other technique like infrared spectroscopy, nuclear magnetic resonance spectroscopy, and neutron activation analysis, mass spectrometry (MS) technique such as GC-MS is widely used for isotope ratio measurement at low micromolar concentration levels with sufficient precision. But when the molecules have molar isotope enrichments at below 0.1%, specialized instruments, such as isotope ratio mass spectrometer (IRMS), multiple-collector inductively coupled plasma mass spectrometry are usually

used for the measurement of the ratio of natural isotopic abundances in the molecule [8, 9, 14, 16]. 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 [17-21].

Biofield is a dynamic electromagnetic field existing in surrounds of the human body that carries information for regulating the organism. Literature demonstrated that healing practitioner has the capability to harness the energy from the earth or environment, the "universal energy field" and can be transmitted the biofield energy into any living or non-living object (s) around the Globe in a useful way. This process is known as biofield energy treatment [22, 23]. Mr. Trivedi is one of the distinguished healing practitioners and has the astonishingly ability to transform the characteristic properties of several organic compounds [24-26], pharmaceuticals [27, 28], nutraceuticals [29], metals and ceramic in materials science [30, 31], culture medium [32, 33] and improve the overall productivity of crops [34, 35] as well as to modulate the efficacy of the various living cells [36-38]. Literature demonstrated that biofield energy treatment has the remarkable capability for alteration of the isotopic abundance ratio in the organic compounds [39-42]. Spectroscopic and thermal analysis of 3-CNB inferred that the physicochemical, structural and thermal properties of 3-CNB, such as crystallite size, vaporization temperature and thermal stability were significantly changed due to the biofield energy treatment. Finally, it was suggested that these altered properties might affect the reaction kinetics when it is used as intermediate [6]. Hence, it is hypothesized that alteration of the physicochemical, structural and thermal properties of biofield treated 3-CNB might have a correlation with the changes on the isotopic abundance ratio in biofield treated 3-CNB. So, isotopic abundance ratio analysis of the both control and biofield treated 3-CNB using GC-MS was performed to investigate the influence of the biofield energy treatment on the isotopic abundance ratios of P_{M+1}/P_M, P_{M+2}/P_M and P_{M+3}/P_M in 3-CNB.

2. Materials and Methods

2.1. Chemicals and Reagents

3-CNB was procured from Loba Chemie Pvt. Ltd., India. All the other chemicals used in this experiment were analytical grade purchased from local vendors.

2.2. Biofield Energy Treatment

The sample 3-CNB was divided into two parts: one was referred as control where no treatment was provided. The other part of the sample which denoted as biofield energy treated sample 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 product in a sealed pack under laboratory conditions for 5 minutes without touching the sample. After

treatment, control and the biofield treated samples were preserved at standard laboratory condition and analyzed by GC-MS. The biofield treated 3-CNB was characterized in different time intervals denoted as T1, T2, T3, and T4 in order to understand the impact of the biofield energy treatment on isotopic abundance ratio with respect to the time.

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 was conducted on a silica capillary column furnished with a quadrupole detector with pre-filter. 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: $\pm$ 0.1 m/z mass accuracy over 48 hours. The analytes were identified by retention time and by a comparison of the mass spectra of identified substances with references [42].

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

The isotopic abundances of the elements are basically categorized 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 [12, 20, 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 several kind of literature and presented in the Table 1 [8, 12, 13, 43, 44].

Based on the findings from the literature [12, 13, 18-21], the following method was used for calculating the isotopic abundance ratio in the current study:

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

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

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 have *A elements* (for *e.g.* ^{12}C, ^{1}H, ^{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

= (no. of ^{13}C x 1.1%) + (no. of ^{15}N x 0.40%) + (no. of ^{2}H x 0.015%) + (no. of ^{17}O x 0.04%)

i.e. the probability to have *A + 1 elements* (for *e.g.* ^{13}C, ^{2}H, ^{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

= (no. of ^{18}O x 0.20%) + (no. of ^{37}Cl x 32.50%)

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

P_{M+3} represents the relative peak intensity of the isotopic molecular ion [(M+3)$^+$] expressed in percentage

i.e. the probability to have the different possible combinations of ^{18}O and ^{37}Cl with ^{13}C, ^{2}H and ^{15}N contributions to the mass of isotopic molecular ion [(M+3)$^+$]

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$_{Treated}$ − IAR$_{Control}$)/ IAR$_{Control}$) x 100],

Where, IAR$_{Treated}$ = isotopic abundance ratio in the treated sample and IAR$_{Control}$ = 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 3-CNB are presented in the Figures 2-4. The GC-MS spectrum of the control 3-CNB (Figure 2) exhibited the presence of molecular ion peak [M$^+$] at m/z 157 (calculated 156.99 for $C_6H_4ClNO_2$) along with four major fragmented peaks in lower m/z region at the retention time (R$_t$) of 11.61 min. This fragmentation pattern of CNB was well matched with the literature [45]. The fragmented peaks at m/z 111, 99, 75 and 50 might be due to $C_6H_4Cl^+$, $C_6H_{11}O$, $C_6H_3^+$, and $C_4H_2^{4+}$ ions, respectively as shown in Figure 2.
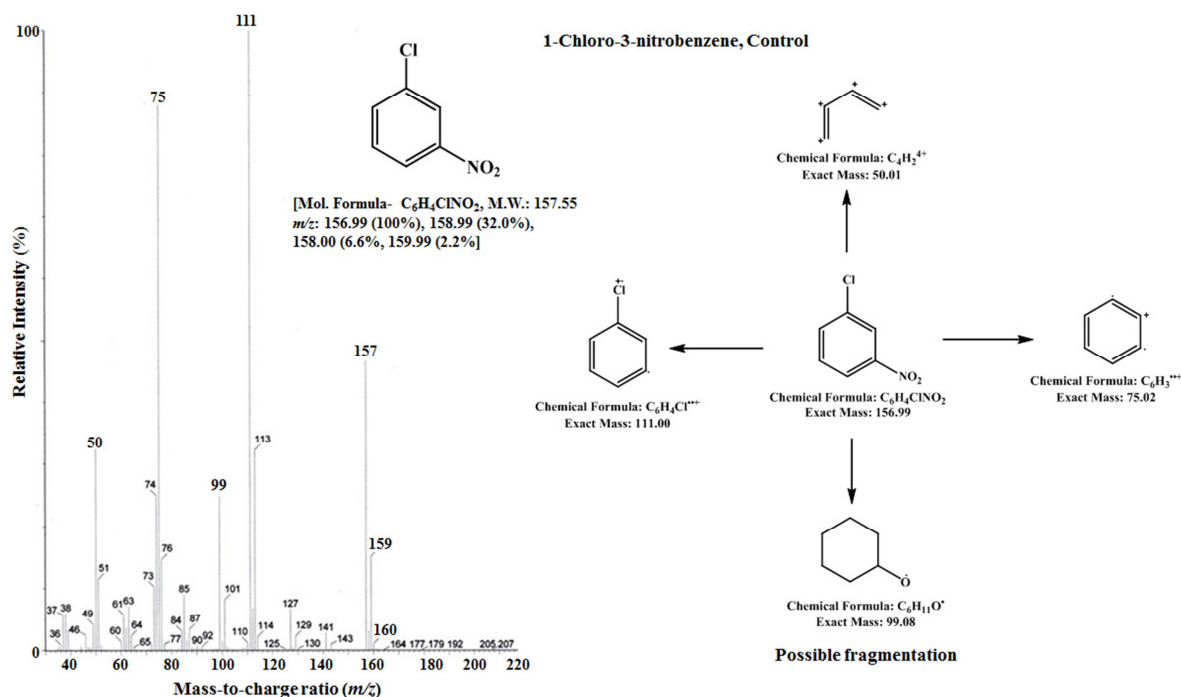
Figure 2. *GC-MS spectrum and possible fragmentation of the control sample of 1-chloro-3-nitrobenzene (3-CNB).*

The GC-MS spectra of the biofield treated 3-CNB at T1, T2, T3, and T4 as shown in Figures 3 and 4 exhibited molecular ion peak [M$^+$] at *m/z* 157 at R$_t$ of 11.58, 11.64, 11.62, and 11.62 min, respectively. The biofield treated 3-CNB showed similar R$_t$ and the same pattern of fragmentation as observed in the control sample. The relative peak intensities of the parent molecule and its major fragmented ions of the control and biofield treated 3-CNB are presented in the Table 2. It clearly indicated that the fragmented ion peak at *m/z* 111 was due to chlorobenzenes ion (C$_6$H$_4$Cl)$^+$, which showed 100% relative intensity (base peak). Table 2 also displayed that the relative intensities of the parent molecule at *m/z* 157 and other fragmented ions at *m/z* 99, 75, and 50 of the biofield treated 3-CNB were significantly changed as compared with the control 3-CNB.
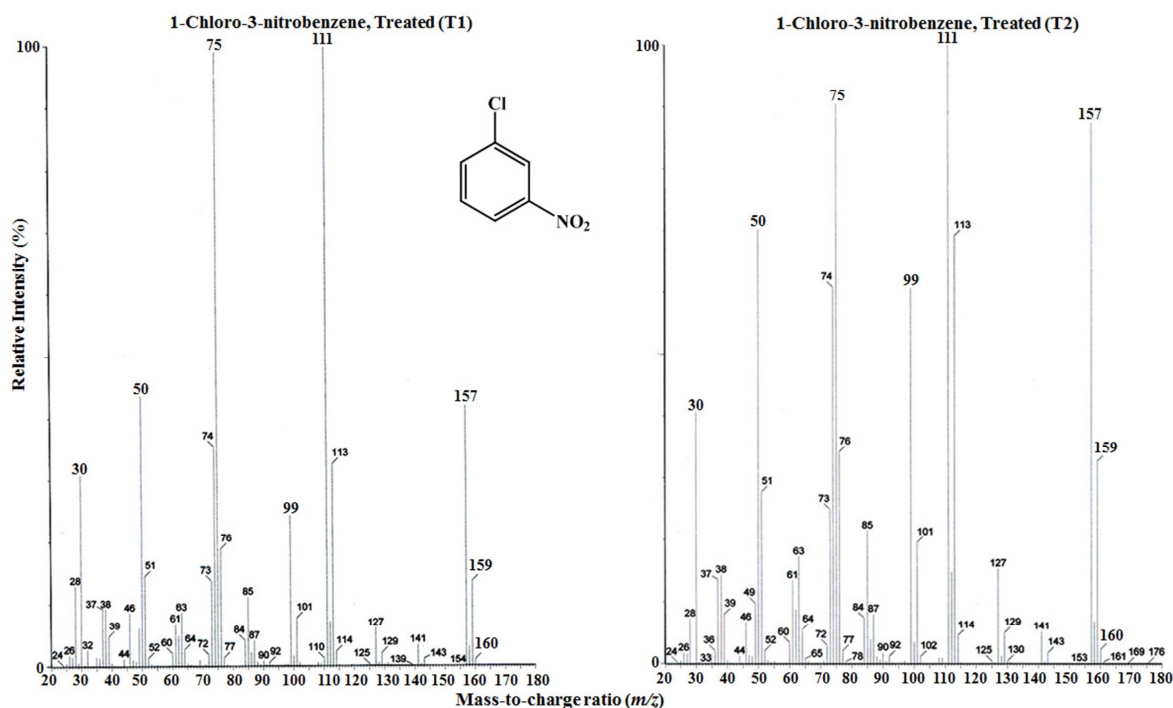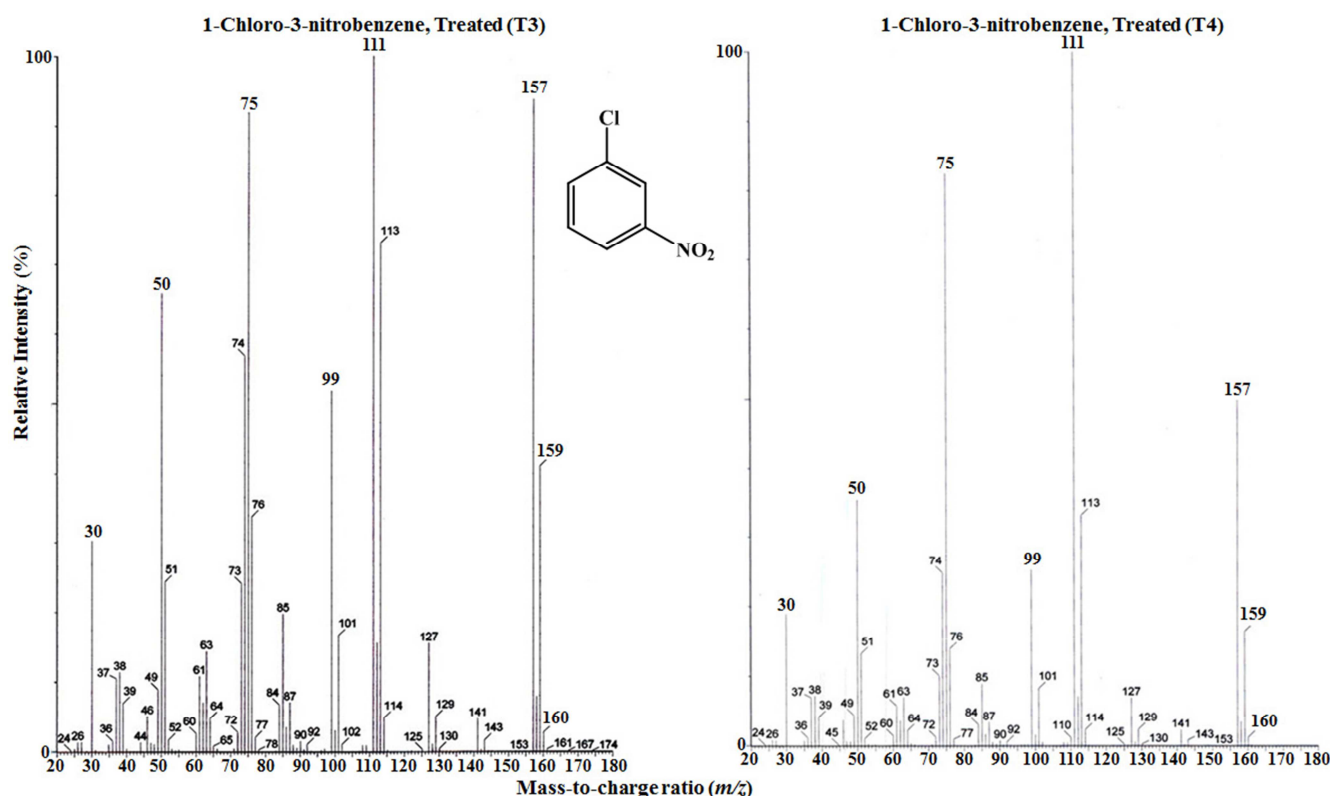

Figure 3. *GC-MS spectra of the biofield energy treated 1-chloro-3-nitrobenzene (3-CNB) at T1 and T2.*

Table 2. *Relative intensities of the corresponding m/z of the parent molecule (3-CNB) and its fragmented ions.*

| m/z | Relative intensity of the peak (%) | | | | |
| | Control 3- CNB | Biofield Energy Treated 3-CNB | | | |
		T1	T2	T3	T4
157	46.63	41.93	87.48	93.79	49.89
111	100	100	100	100	100
99	24.88	24.38	60.73	51.90	25.38
75	87.91	99.15	90.48	92.01	82.49
50	32.32	43.55	70.22	65.89	35.31

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

Figure 4. *GC-MS spectra of the biofield energy treated samples of 1-chloro-3-nitrobenzene (3-CNB) at T3 and T4.*

3.2. Analysis of Isotopic Abundance Ratio

3-CNB has the molecular formula of $C_6H_4ClNO_2$ and the molecular ion [M$^+$] peak for the control 3-CNB showed 46.63% 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). The theoretical calculation for P_{M+1} is provided as follows:

P (^{13}C) = [(6 x 1.1%) x 46.63% (the actual size of the M$^+$ peak)] / 100% = 3.08%

P (^{2}H) = [(4 x 0.015%) x 46.63%] / 100%= 0.03%

P (^{15}N) = [(1 x 0.40%) x 46.63%] / 100%= 0.19%

P (^{17}O) = [(2 x 0.04%) x 46.63%] / 100% = 0.04%

P_{M+1} *i.e.* ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_4ClNO_2)^+$ to m/z 158 = 3.34%

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) = [(2 x 0.20%) x 46.63%] / 100% = 0.19%

P (^{37}Cl) = [(1 x 32.50%) x 46.63%] / 100% = 15.15%

So, P_{M+2} *i.e.* ^{18}O and ^{37}Cl contributions from $(C_6H_4ClNO_2)^+$ to m/z 159 = 15.34%.
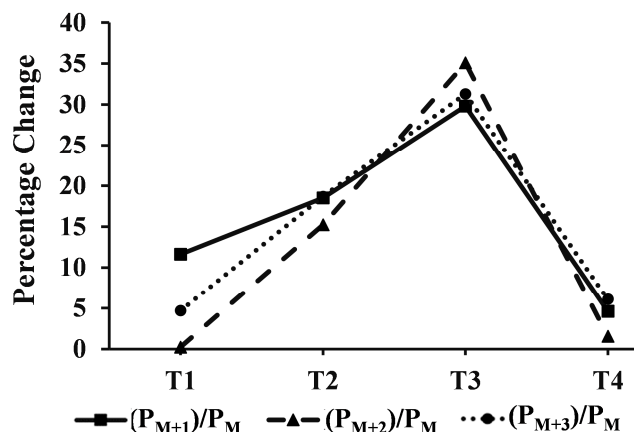
P_M, P_{M+1}, P_{M+2} for the control and biofield energy treated 3-CNB at m/z 157, 158 and 159, respectively were accomplished from the observed relative peak intensities of [M$^+$], [(M+1)$^+$], and [(M+2)$^+$] peaks in the GC-MS spectra, respectively and are presented in the Table 3.

Table 3. *Isotopic abundance analysis result of the control and biofield energy treated 1-chloro-3-nitrobenzene (3-CNB).*

Parameter	Control 3-CNB	Biofield Energy Treated 3-CNB			
		T1	T2	T3	T4
P_M at *m/z* 157 (%)	46.63	41.93	87.48	93.79	49.89
P_{M+1} at *m/z* 158 (%)	3.05	3.06	6.78	7.96	3.41
P_{M+1}/P_M	0.0654	0.0730	0.0775	0.0849	0.0684
% Change of isotopic abundance ratio (P_{M+1}/P_M)		11.62	18.50	29.82	4.59
P_{M+2} at *m/z* 159 (%)	15.11	13.62	32.66	41.05	16.42
P_{M+2}/P_M	0.3240	0.3248	0.3733	0.4377	0.3291
% Change of isotopic abundance ratio (P_{M+2}/P_M)		0.25	15.22	35.09	1.57
P_{M+3} at *m/z* 160 (%)	1.00	0.94	2.22	2.64	1.13
P_{M+3}/P_M	0.0214	0.0224	0.0254	0.0281	0.0227
% Change of isotopic abundance ratio (P_{M+3}/P_M)		4.67	18.69	31.31	6.08

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 $[(M+2)^+]$; P_{M+3} = the relative peak intensity of the isotopic molecular ion $[(M+3)^+]$; and M = mass of the parent molecule.

The experimental values as shown in the Table 3 are well accorded with the calculated theoretical values and it indicated that ^{13}C and ^{37}Cl might have major contributions from $(C_6H_4ClNO_2)^+$ to m/z 158 and 159, respectively. Beside these, an intense peak at m/z 160 $[(M+3)^+]$ that can be denoted as P_{M+3} was found in the GC-MS spectra of the both control and biofield treated 3-CNB. It is assumed that P_{M+3} might be resultant of the different possible combinations of ^{18}O and ^{37}Cl with ^{13}C, ^{2}H and ^{15}N for e.g. P $(^{37}Cl^{13}C)$, P$(^{15}N^{18}O)$, P$(^{18}O^{13}C)$, etc. The percentage change of the isotopic abundance ratios (P_{M+1}/PM, P_{M+2}/PM and P_{M+3}/P_M) in the biofield treated 3-CNB with respect to the control 3-CNB is shown in Table 3 and Figure 5. The isotopic abundance ratio of P_{M+1}/PM in the biofield treated 3-CNB at T1, T2, T3 and T4 was increased by 11.62, 18.50, 29.82, and 4.59%, respectively with respect to the control 3-CNB. Consequently, the percentage change of the isotopic abundance ratio of P_{M+2}/P_M was enhanced in the biofield treated 3-CNB at T1, T2, T3, and T4 by 0.25, 15.22, 35.09, and 1.57%, respectively with respect to the control sample. Similarly, the isotopic abundance ratio of P_{M+3}/P_M was improved in the biofield treated 3-CNB at T1, T2, T3, and T4 by 4.67, 18.69, 31.31 and 6.08%, respectively as compared to the control 3-CNB. Thus, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_4ClNO_2)^+$ to *m/z* 158, ^{37}Cl and ^{18}O contributions from $(C_6H_4ClNO_2)^+$ to *m/z* 159 and the different possible combinations of ^{18}O and ^{37}Cl with ^{13}C, ^{2}H and ^{15}N contributions from $(C_6H_4ClNO_2)^+$ to *m/z* 160 in the biofield treated 3-CNB were significantly increased gradually with respect to the time (T1 to T3) and was found to be highest at T3 as shown in the Figure 5. Amazingly, when biofield treated 3-CNB was kept for long time in the laboratory condition *i.e.* T4, the isotopic abundance ratio in biofield treated 3-CNB was decreased from T3. So, the biofield energy treatment exhibited time dependent effect on the isotopic abundance ratio in 3-CNB.

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

Neutrinos are the most possible carrier of the hidden mass in the nature. These electrically neutral particles that are part of all living systems blast through the space and can pass through large distances in the matter without being affected the electromagnetic force. Literature suggested that the neutrinos coming from the Sun have a potential effect on the isotopic composition of the materials through inducing the fission reactions within a heavy nuclei (*i.e.* the nucleosynthesis of various elements) [46-48]. Trillions of neutrinos are at any time passing through the human body without affecting it. The biofield energy can freely flow between human and environment that leads to the endless movement or matter of energy [49-51]. It has been reported that biofield energy might have effect on the variations of isotopic composition in water molecule [23]. It can be postulated that Mr. Trivedi's unique biofield energy treatment might have the capability for introduction of the neutrino fluence into the both of the living and nonliving substances that might responsible for modifying the behavior at atomic and molecular level. The neutrinos have the ability to interact

with protons and neutrons in the nucleus that might be responsible for the alteration of the neutron to proton ratio in the nucleus. Based on this hypothesis, it is assumed that the possible reason for the alteration of the isotopic abundance ratios (P_{M+1}/P_M, P_{M+2}/P_M and P_{M+3}/P_M) in the biofield treated 3-CNB might be due to the intervention of a neutrino flux through biofield energy treatment.

The energy of a compound comprises of the amount of the electronic, vibration, rotational and translation energies. The alteration of the isotopic abundance ratio *i.e.* isotopic composition of the molecule does not disturb electronic, translational, and rotational energies of the molecule, but significantly changes the vibrational energy [14, 15]. The relation between the vibrational energy and the reduced mass (μ) for a diatomic molecule is expressed as below [14, 15]:

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

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

f = force constant

$$\mu = \text{reduced mass} = \frac{m_a m_b}{m_a + m_b},$$

Where m_a and m_b are the masses of the constituent atoms.

The possible isotopic bond formation in the CNB molecule and their effect on the vibrational energy of 3-CNB are shown in the Table 4. From the Table 4, it has been found that the alteration of the isotopic abundance ratio of $^{13}C/^{12}C$ for C-C, C-Cl, and C-N bonds has much more effect on the vibrational energy of the molecule than changes in the isotopic abundance ratio of $^{37}Cl/^{35}Cl$ and $^{15}N/^{14}N$. Similarly, the changes of the isotopic abundance ratios of $^2H/^1H$ for C-H and $^{15}N/^{14}N$, and for $^{18}O/^{16}O$ for N-O bond have much more 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 4.

Table 4. Possible isotopic bond and their effect in the vibrational energy in 1-chloro-3-nitrobenzene (3-CNB) molecule.

Entry No.	Probable isotopic bond	Isotope type	Reduced mass (μ)	Zero point vibrational energy (E_0)
1	^{12}C-^{12}C	Lighter	6.00	Higher
2	^{13}C-^{12}C	Heavier	6.26	Smaller
3	^1H-^{12}C	Lighter	0.92	Higher
4	^1H-^{13}C	Heavier	0.93	Smaller
5	^2H-^{12}C	Heavier	1.04	Smaller
6	^{12}C-^{35}Cl	Lighter	8.94	Higher
7	^{13}C-^{35}Cl	Heavier	9.48	Smaller
8	^{12}C-^{37}Cl	Heavier	9.06	Smaller
9	^{12}C-^{14}N	Lighter	6.46	Higher
10	^{12}C-^{15}N	Heavier	6.67	Smaller
11	^{13}C-^{14}N	Heavier	6.74	Smaller
12	^{14}N-^{16}O	Lighter	7.47	Higher
13	^{15}N-^{16}O	Heavier	7.74	Smaller
14	^{14}N-^{17}O	Heavier	7.68	Smaller
15	^{14}N-^{18}O	Heavier	7.88	Smaller

The isotopic abundance ratio analysis in 3-CNB clearly revealed that the isotopic abundance ratios of P_{M+1}/P_M, P_{M+2}/P_M and P_{M+3}/P_M in the biofield treated 3-CNB were higher than the control 3-CNB. Hence, biofield treated 3-CNB might exhibit altered isotope effects such as lower diffusion velocity, mobility, evaporation and reaction rate, higher binding energy [11] with respect to the control sample. Isotope effects have a positive role in the thermal decomposition of the molecules [52-54]. 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 3-CNB might have altered physicochemical and thermal properties as well as different reaction kinetic than control 3-CNB. Hence, the current results concluded that the increased isotopic abundance ratio in biofield energy treated 3-CNB might be responsible for alteration in vaporization rate and thermal stability of the biofield treated 3-CNB that was well supported with our previous findings [15]. The alteration in the isotopic abundance ratio of one of the atoms in the reactants causes changes in the rate of a chemical reaction that is known as kinetic isotope effect (KIE). KIE is a very powerful technique 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 helpful for designing enzyme inhibitors [14, 15, 55, 56]. Thus, biofield treated 3-CNB might have altered physicochemical and thermal properties, different rate of the reaction, selectivity and binding energy.

4. Conclusions

The present study concluded that biofield energy treatment had potential impact on the isotopic abundance ratios of P_{M+1}/P_M, P_{M+2}/P_M, and P_{M+3}/P_M in 3-CNB that might lead to alteration of the physicochemical and thermal properties. The GC-MS spectra of the both control and biofield treated 3-CNB specified the presence of molecular ion peak $[M^+]$ at m/z 157 (calculated 156.99 for $C_6H_4ClNO_2$) along with nearly similar fragmentation pattern. In addition, the relative intensities of the parent molecule and other fragmented ions of the biofield treated 3-CNB were altered with respect to the control 3-CNB. The isotopic abundance ratio of P_{M+1}/P_M in the biofield treated 3-CNB at T1, T2, T3 and T4 was increased by 11.62, 18.50, 29.82, and 4.59%, respectively with respect to the control 3-CNB. Consequently, the percentage change of the isotopic abundance ratio of P_{M+2}/P_M was increased in the biofield treated 3-CNB at T1, T2, T3, and T4 by 0.25, 15.22, 35.09, and 1.57%, respectively with respect to the control sample. Similarly, the percentage of the isotopic abundance ratio of P_{M+3}/P_M was improved in the biofield treated 3-CNB at T1, T2, T3, and T4 by 4.67, 18.69, 31.31 and 6.08%, respectively with respect to the control 3-CNB. In brief, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_4ClNO_2)^+$ to m/z 158, ^{37}Cl and ^{18}O contributions from $(C_6H_4ClNO_2)^+$ to m/z 159 and the different possible combinations of ^{18}O and ^{37}Cl with ^{13}C, ^{2}H and ^{15}N contributions from $(C_6H_4ClNO_2)^+$ to m/z 160 in the biofield treated 3-CNB were significantly increased particularly at T2 and T3 and was found that biofield energy treatment has time dependent effect on it. The biofield energy treated 3-CNB might display the different isotope effects due to the increased isotopic abundance ratio with respect to the control sample. Hence, the biofield treated 3-CNB might have the altered physicochemical and thermal properties and the rate of the chemical reaction as compared to the control sample. The biofield energy treated 3-CNB might play an important role in designing the synthesis of pharmaceuticals, agricultural chemicals, dyes, corrosion inhibitors and other several useful industrial chemicals.

Abbreviations

A: Element; 3-CNB: 1-Chloro-3-nitrobenzene; 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 $[(M+2)^+]$; P_{M+3}: The relative peak intensity of isotopic molecular ion $[(M+3)^+]$; R_t: Retention time.

Acknowledgements

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