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Quantitative Determination of Isotopic Abundance Ratio of ^{13}C , ^2H , and ^{18}O in Biofield Energy Treated Ortho and Meta Toluic Acid Isomers

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Abstract: O-Toluic acid (OTA) and m-toluic acid (MTA) are two isomers of toluic acid that act as an important organic intermediates, mostly used in medicines and pesticides. The aim of the study was to evaluate the impact of biofield energy treatment on isotopic abundance ratios of $^2\text{H}/^1\text{H}$, $^{13}\text{C}/^{12}\text{C}$, (PM+1)/PM and $^{18}\text{O}/^{16}\text{O}$, (PM+2)/PM, in toluic acid isomers using gas chromatography-mass spectrometry (GC-MS). The OTA and MTA samples were divided into two parts: control and treated. The control sample remained as untreated, while the treated sample was further divided into four groups as T1, T2, T3, and T4. The treated group was subjected to biofield energy treatment. The GC-MS spectra of both the isomers showed five m/z peaks due to the molecular ion peak and fragmented peaks of toluic acid derivatives. The isotopic abundance ratio of (PM+1)/PM and (PM+2)/PM were calculated for both the isomers and found significant alteration in the treated isomers. The isotopic abundance ratio of (PM+1)/PM in treated samples of OTA was decreased and then slightly increased upto 2.37% in T2, where the (PM+2)/PM in treated OTA, significantly decreased by 55.3% in T3 sample. Similarly, in case of MTA, the isotopic abundance ratio of (PM+1)/PM in the treated sample showed a slight increase the (PM+2)/PM was decreased by 11.95% in T2 as compared to their respective control. GC-MS data suggests that the biofield energy treatment on toluic acid isomers had significantly altered the isotopic abundance of ^2H , ^{13}C , and ^{18}O in OTA and MTA as compared to the control.

Keywords: Biofield Energy Treatment, o-Toluic Acid, m-Toluic Acid, Gas Chromatography-Mass Spectrometry

1. Introduction

The distribution of contaminant sources of any molecule on a native or global scale can be understood by determining the isotopic abundance ratio, and characterization of elementary reaction mechanisms [1]. The natural abundance or any kinetic process that leads to the local depletion or enhancement of isotopes in organic molecules can be successfully determined using gas chromatography-mass spectrometry (GC-MS) [2]. Moreover, the rate of chemical reaction may vary with the mass of the nucleus with different isotopic substitutions, which slightly affect the partitioning of energy within the molecules. These deviations from perfect chemical equivalence are termed as isotope effects.

The isotopic abundance ratio is commonly reported in terms of atom percent and determined by high resolution mass spectrometry (HRMS spectrometry) [3].

Two toluic acid derivatives, o-toluic acid (OTA) and m-toluic acid (MTA) were taken for mass spectroscopy study. Both of the toluic acid derivatives are widely used as a chemical intermediate to synthesis the insect repellent in the chemical industry, as plastic stabilizer in polymer industry and as a starting material in pharmaceutical products [4, 5]. MTA is used specifically as starting material in the production of 3-carboxybenzaldehyde, 3-methylbenzophenone, 3-benzoylphenylacetic acid, N,N-diethyl-3-methylbenzamide, etc. [6]. They are mostly found in crude oil and artificially synthesized from toluidines and oxidation of ortho and meta xylenes [7]. Besides, toluic acid isomers are biodegradable

products and its bioaccumulation potential is very low as compared to their homologs. This chemical is stable to hydrolysis in water in abiotic conditions, however, indirect photo-oxidation may have occurred via hydroxy radicals in the atmosphere. If xylene and toluene were taken with contaminated food they are metabolized mainly to methylbenzoic and benzoic acid respectively, which eventually conjugated with glycine to produce methylhippuric and hippuric acid, then excreted in the urine [8].

The photophysical stability as well as chemical reactivity of toluic acid isomers is the primary concern and could be altered by an alternative approach: Mr. Trivedi's biofield energy treatment. The properties could be enhanced by utilizing The Trivedi Effect®, a unique biofield energy treatment by Mr. Trivedi which is already known to alter the physical and structural properties of various living and non-living substances [9,10]. The National Center for Complementary and Alternative Medicine (NCCAM) has recommended the use of biofield therapy as a part of complementary and alternative medical therapies (CAM) in the healthcare sector [11]. CAM includes numerous energy-healing therapies, in which the biofield therapy is a form of putative energy medicine that is being widely used worldwide to improve the overall health of human beings. Humans have the ability to harness energy from the environment/universe and can be transmitted to any object (living or non-living) around the globe. This process is called biofield energy treatment. The impact of the Trivedi Effect® has been studied in various research fields like microbiology, biotechnology, and agricultural research [9,10,12-14]. Based on the outstanding results achieved by the biofield energy treatments on ceramics, microbiology, and agricultural research, an attempt was made to evaluate the effect of biofield energy treatment on the isotopic abundance ratio of (PM+1)/PM and (PM+2)/PM in OTA and MTA.

2. Experimental

2.1. Materials and Methods

Both, o-toluic acid (OTA) and m-toluic acid (MTA) were procured from S D Fine Chemicals Pvt. Limited, India. Each of the samples was distributed into two parts, where one part of each sample was referred as control sample and the other part was considered as treated. The treated sample was further divided into four groups (*i.e.* T1, T2, T3, and T4) and handed over to Trivedi for biofield energy treatment under standard laboratory conditions. Trivedi provided the treatment through his energy transmission process to the treated group without touching the sample. The control and treated samples were characterized using gas chromatography-mass spectrometry (GC-MS).

2.2. GC-MS Spectroscopy

GC-MS analysis was performed on Perkin Elmer(USA)/auto system XL with Turbo mass, having detection limit up to 1 picogram. The GC-MS spectra were

obtained in the form of % abundance vs. mass to charge ratio (m/z). The isotopic abundance ratio $2\text{H}/1\text{H}$ or $^{13}\text{C}/^{12}\text{C}$ (PM+1)/PM and $^{18}\text{O}/^{16}\text{O}$ (PM+2)/PM was expressed by its deviation in treated samples as compared to the control one. The percentage change in isotopic ratio (PM+1)/PM and (PM+2)/PM were calculated with the following formula:

$$\text{Percent change in isotopic abundance ratio} = \frac{R_{\text{Treated}} - R_{\text{control}}}{R_{\text{control}}} \times 100$$

Where, R_{Treated} and R_{Control} are the ratios of intensity at (PM+1) to PM in mass spectra of treated and control samples, respectively.

3. Results and Discussion

3.1. GC-MS Study of OTA

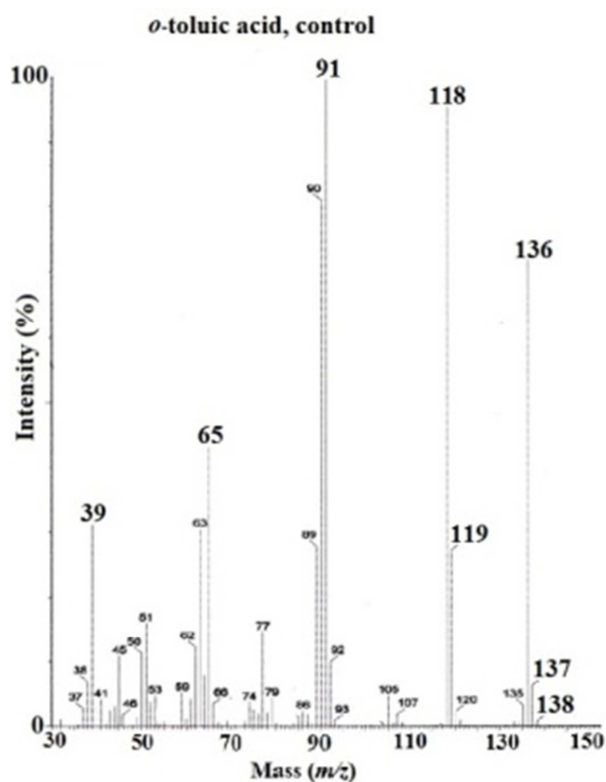


Figure 1. GC-MS spectrum of control o-toluic acid sample.

The GC-MS spectra of control and treated (T1, T2, T3, and T4) samples are presented in Figure 1 and 2, respectively. Mass spectra of OTA is well matched with the reported literature [15]. MS spectra showed five peaks including molecular ion peak (PM) for both the control and treated samples of OTA. Five major peaks at m/z 136, 118, 91, 65, and 39 were observed that corresponded to the following ions respectively: $\text{C}_8\text{H}_8\text{O}_2^+$, $\text{C}_8\text{H}_6\text{O}^+$, C_7H_7^+ , C_5H_5^+ , and C_3H_3^+ ions. Peak at m/z 136 was the molecular ion peak and the other peaks observed in both, control and all treated OTA samples were due to the fragmentation of OTA inside mass spectrum.

The base peak at m/z 91 was observed due to breaking of *o*-toluic acid to stable toluene molecule that showed the highest intensity in the mass spectrum. Further, m/z 118, 65, and 39 peaks were observed due to first reduction of the molecular ion to aldehyde, which again fragmented to give

cyclopentene and propyne respectively. The intensity ratio and calculated percentage isotopic abundance ratio of (PM+1)/PM and (PM+2)/PM is presented in the Table 1. Moreover, the isotopic abundance ratio (PM+1)/PM and (PM+2)/PM in control and treated OTA was plotted in Figure 3.

Table 1. GC-MS isotopic abundance analysis result of *o*-toluic acid.

Peak Intensity	Control	Treated				
		T1	T2	T3	T4	
m/z of PM	71.9	69.8	55.63	72.9	56.28	
m/z of (PM+1)	6.3	5.77	4.99	6.37	5.03	
(PM+1)/ PM	0.087	0.082	0.089	0.087	0.089	
Percent change as compared to the control		-5.65	2.37	-0.27	2.00	
m/z of (PM+2)	0.64	0.51	0.44	0.29	0.48	
(PM+2)/ PM	0.0089	0.0073	0.0079	0.0039	0.0085	
Percent change as compared to the control		-17.91	-11.14	-55.3	-4.18	

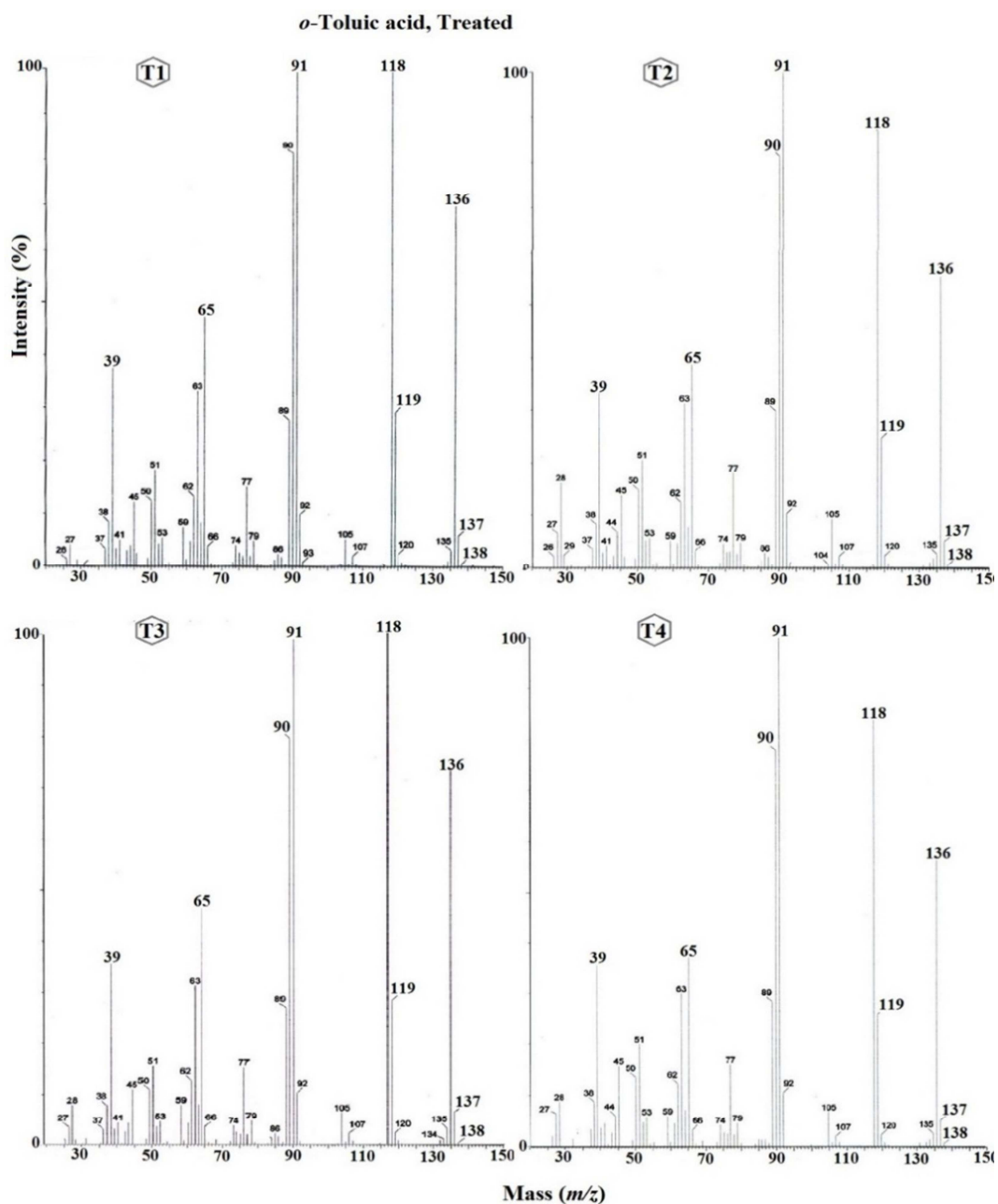


Figure 2. GC-MS spectra of treated *o*-toluic acid samples T1, T2, T3, and T4.

It was clearly seen from the Table 1 and Figure 3, that, the isotopic abundance ratio of (PM+1)/PM of OTA sample was decreased in T1 (5.65%) and T3 (0.27%), but increased slightly in T2 (2.37%) and T4 (2%) after biofield energy treatment as compared to the control. However, the isotopic ratio of (PM+2)/PM in treated OTA sample was decreased significantly in all the four treated samples (T1=17.91%, T2=11.14%, T3=55.3%, and T4=4.18%). The slight alteration in the isotopic abundance ratio of (PM+1)/PM may have nominal effect on the bond energies and reactivity of the molecules of treated samples. However, the decreased isotopic abundance ratio of (PM+2)/PM may reduce the number of higher isotope (^{18}O) in the molecule. It may directly affect the bond strength of the C-O and O-H bonds. The decreased isotopic abundance ratio of (PM+2)/PM in the treated OTA sample may decrease μ (effective mass) and binding energy in the $-\text{COOH}$ group in the molecule with lighter isotopes, and this may result in enhance reactivity of the molecule [16].

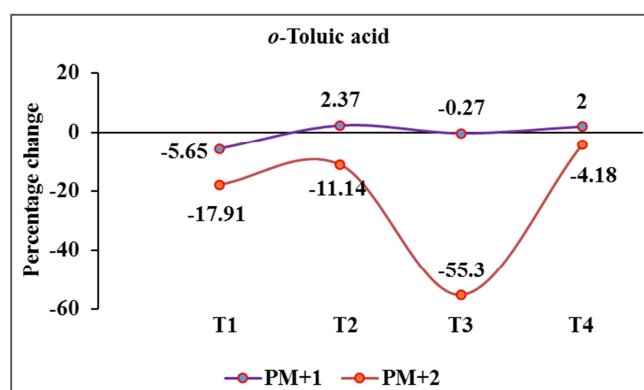


Figure 3. Percentage change in the isotopic abundance (PM+1)/PM and (PM+2)/PM of *o*-toluic acid under biofield energy treatment as compared to the control.

3.2. GC-MS Study of MTA

Mass spectra of both control and treated samples were shown in Figure 4 and 5, respectively. The molecular ion peak was observed at m/z 136 in mass spectra of both control and treated samples. The calculated relative intensity ratio of (PM+1)/PM, (PM+2)/PM and percentage abundance ratios are given in Table 2. Total five major peaks were observed in the mass spectra of both control and treated spectra of MTA at m/z 136, 118, 91, 65, and 39, corresponded to the following ions respectively: $\text{C}_8\text{H}_8\text{O}_2^+$, $\text{C}_8\text{H}_6\text{O}^+$, C_7H_7^+ , C_5H_5^+ , and C_3H_3^+ ions. Both OTA and MTA exhibited similar fragmentation pattern with the base peak at m/z 91 due to the formation of toluene in the mass spectra and molecular ion peak at m/z 136. The isotopic abundance ratio of (PM+1)/PM and (PM+2)/PM of control and treated MTA was calculated and presented in Figure 6. The isotopic abundance ratio of (PM+1)/PM of treated MTA was increased upto 4.18% (T1=1.43%, T2=2.48%, T3=2.9%, and T4=4.18%) as compared to the control. However, the isotope abundance ratio of (PM+2)/PM was decreased upto 11.95% (T1= 6.88%, T2= 11.95%, T3= 10.96% and T4=5.69%). The mass spectra

of MTA is well supported with the reported literature [17].

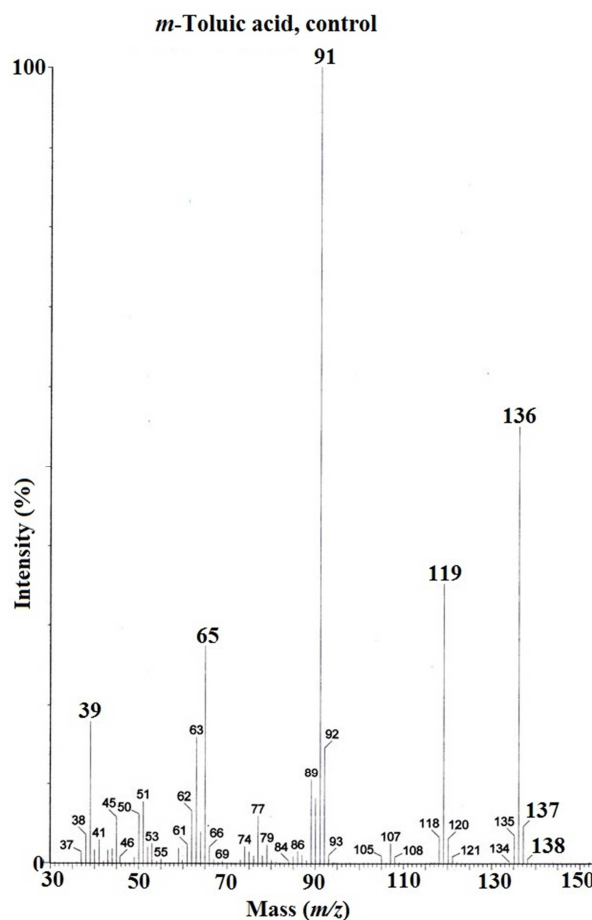


Figure 4. GC-MS spectrum of control sample of *m*-toluic acid.

In a chemical bond, if the lighter isotopes were substituted by heavier isotopes then the effective mass (μ) of the particular bond is increased and subsequently binding energy was increased [16]. The reverse may also happen if the heavier isotopes, part of the chemical bonding were substituted by lighter isotopes in a molecule. Thus, the increased isotopic abundance ratio of (PM+1)/PM in OTA and MTA might increase the effective mass and binding energy after biofield energy treatment that may enhance the chemical stability of toluic acid derivatives. On the contrary, the decreased isotopic abundance ratio of (PM+2)/PM in treated OTA and MTA might reduce the effective mass of the particular bond thus decreasing the binding energy. In toluic acid derivatives, only ^{18}O contributes to the alteration in (PM+2)/PM ratio [18]. Therefore, the decrease in isotopic abundance ratio of (PM+2)/PM may decrease the effective mass of the C-O and O-H bonds that may consequently increase the reactivity of the functional group. Further, the reactivity of the aromatic ring might be decreased with increased isotopic abundance ratio of (PM+1)/PM and the effective mass.

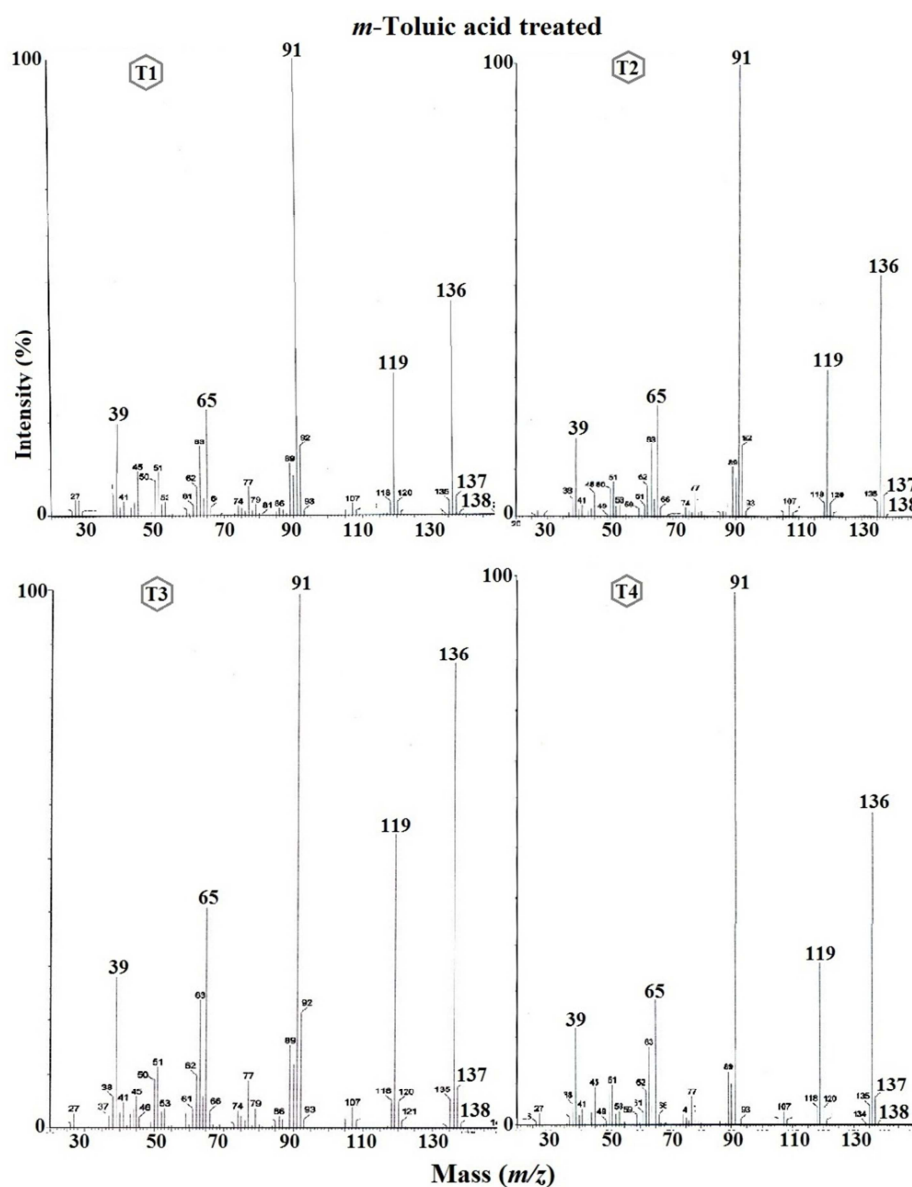


Figure 5. GC-MS spectra of treated samples of m-toluic acid (T1, T2, T3, and T4).

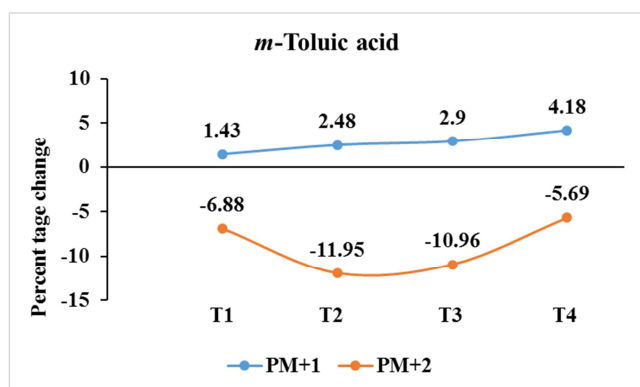


Figure 6. Percentage change in the isotopic abundance of (PM+1)/PM and (PM+2)/PM of m-toluic acid under biofield energy treatment as compared to the control.

Table 2. GC-MS isotopic abundance analysis result of m-toluic acid.

Peak Intensity	Control	Treated			
		T1	T2	T3	T4
m/z of PM	55.35	45.82	52.39	86.77	58.69
m/z of (PM+1)	4.68	3.93	4.54	7.55	5.17
(PM+1)/ PM	0.084	0.085	0.086	0.087	0.088
Percent change as compared to the control		1.43	2.48	2.9	4.18
m/z of (PM+2)	0.48	0.37	0.4	0.67	0.48
(PM+2)/ PM	0.0086	0.0080	0.0076	0.0077	0.0081
Percent change as compared to the control		-6.88	-11.95	-10.96	-5.69

Some probable bonds that might present in the biofield treated molecules such as ^{12}C - ^{12}C , ^1H - ^{12}C , ^{13}C - ^{12}C , ^2H - ^{12}C , ^1H - ^{13}C , ^{12}C - ^{18}O , ^1H - ^{18}O , ^{13}C - ^{16}O , and ^2H - ^{16}O . Effective mass

is calculated and presented in Table 3. The result showed that μ of normal ^{12}C - ^{12}C ($\mu=6$), ^1H - ^{12}C ($\mu=0.923$), and ^1H - ^{16}O ($\mu=0.941$) bonds were increased in case of heavier isotopes (*i.e.* ^{13}C - $^{12}\text{C}=6.26$, ^2H - $^{12}\text{C}=1.71$, and ^2H - $^{16}\text{O}=1.77$) respectively. After biofield treatment, bond strength, stability, and binding energy of the aromatic ring of OTA and MTA molecules might be increased due to the higher effective mass (μ), these parameters might be decreased in carboxylic acid group after biofield energy treatment.

The increased reactivity of the functional group and decreased reactivity of the ring part may increase the utility of the toluic acid derivatives as good starting material for pesticides and as a plasticizer in polymer industry. Further, it will enhance the degradation kinetics in the environment while contaminated from the industrial waste.

Table 3. Possible isotopic bonds in OTA and MTA.

Isotopes Bond	Isotope type	Reduced mass (mAmB / (mA + mB))
^{12}C - ^{12}C	Lighter	6.000
^{13}C - ^{12}C	Heavier	6.260
^1H - ^{12}C	Lighter	0.923
^1H - ^{13}C	Heavier	0.929
^2H - ^{12}C	Heavier	1.710
^1H - ^{18}O	Heavier	0.940
^2H - ^{16}O	Heavier	1.770
^{18}O - ^{12}C	Heavier	7.200
^{16}O - ^{13}C	Heavier	7.170

Where, mA- mass of atom A, mB- mass of atom B, here A may be C or H and so on.

Most interestingly, when methylbenzoic acid, toluene, and xylenes are taken internally, they are all converted to hippuric acid (derivative of benzoic acid) by reaction with the amino acid, glycine then passed through kidneys and eventually removed from the body with urine [19]. The increased reactivity of carboxylic group after biofield energy treatment may enhance this metabolic process to remove toluic acid derivatives from the body.

4. Conclusions

In summary, toluic acid isomers were studied with GC-MS under the influence of biofield energy treatment and observed a significant change in isotope abundance of $^2\text{H}/^1\text{H}$, $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ as compared to the control sample. The alteration in isotopic abundance ratios have significant impact on bond energies and chemical reactivity of the molecules. The smaller enhancement in the isotopic abundance ratio of (PM+1)/PM in OTA and MTA, the reactivity may be reduced slightly at the aromatic ring by slight increase in the effective mass (μ) and the binding energy of the treated sample. However, significant depletion in heavier isotopic abundance ratio, (PM+2)/PM, may increase the reactivity of the $-\text{COOH}$ group after biofield energy treatment. From the above observations, it can be concluded that the enhancement of heavier isotopes in the aromatic ring may decrease the reactivity of the aromatic ring but decreased heavier isotopes may increase the reactivity of the functional group of toluic acid isomers, consequently enabling them to be used as an intermediate in the organic synthesis. It

will also provide quicker biodegradation pathway in the environment while leaching.

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Abbreviations

PM- primary molecule, (PM+1)- isotopic molecule either for ^{13}C or ^2H , and (PM+2)- is the isotopic molecule for ^{18}O .

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