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Mass Spectrometry Analysis of Isotopic Abundance of ^{13}C, ^{2}H, or ^{15}N in Biofield Energy Treated Aminopyridine Derivatives

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Abstract: 2-Aminopyridine (2-AP) and 2,6-diaminopyridine (2,6-DAP) are two derivatives of aminopyridines that act as an important organic intermediates, mostly used in medicines, dyes and organic sensors. The aim of the study was to evaluate the impact of biofield energy treatment on isotopic abundance ratios of $^{2}H/^{1}H$, $^{13}C/^{12}C$, or $^{15}N/^{14}N$, in aminopyridine derivatives using gas chromatography-mass spectrometry (GC-MS). The 2-AP and 2,6-DAP 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 Mr. Trivedi's biofield energy treatment. The GC-MS spectra of 2-AP and 2,6-DAP showed five and six *m/z* peaks respectively due to the molecular ion peak and fragmented peaks of aminopyridine derivatives. The isotopic abundance ratio of $^{2}H/^{1}H$, $^{13}C/^{12}C$, or $^{15}N/^{14}N$ were calculated for both the derivatives and significant alteration was found in the treated samples as compared to the respective control. The isotopic abundance ratio of $^{2}H/^{1}H$, $^{13}C/^{12}C$, or $^{15}N/^{14}N$ in treated samples of 2-AP was decreased by 55.83% in T1 and significantly increased by 202.26% in T4. However, in case of 2,6-DAP, the isotopic abundance ratio of $^{2}H/^{1}H$, $^{13}C/^{12}C$, and $^{15}N/^{14}N$, in the treated sample showed a significant increase (up to 370.54% in T3) with respect to the control. GC-MS data suggested that the biofield energy treatment on aminopyridine derivatives had significantly altered the isotopic abundance of ^{2}H, ^{13}C, or ^{15}N in the treated 2-AP and 2,6-DAP as compared to the control.

Keywords: Biofield Energy Treatment, 2-Aminopyridine, 2,6-Diaminopyridine, Gas Chromatography-Mass Spectrometry

1. Introduction

Most of the elements occurred in nature as a mixture of isotopes. The relative abundances of isotopes is different at different places on the earth and remained constant for years. The distribution of contaminant sources of any molecule on a native or global scale can be understood by determining the isotopic abundance ratio [1]. 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]. 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]. For example, ^{13}C

$$\text{Atom percent } ^{13}C = [^{13}C/(^{12}C+^{13}C)]\times 100 \quad (1)$$

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 [4].

The aminopyridines, selected for this study are heterocyclic pharmacophores for many bioactive small organic molecules [5]. The growing interest of these molecules pharmacologically due to its rare tendency to be oxidized like aniline derivatives [6]. Aminopyridines avoid such problems due to its reduced oxidation potential, thus make it a safer

alternatives for drug design. Additionally, they are advantageous because they have exhibited strong absorption and emission spectra and thus useful as fluorescent tags while investigating the activities on a target [7].

Aminopyridines are used as a drug for symptomatic treatment of multiple sclerosis by blocking potassium channels and prolonging action potentials [8, 9]. It has been used as intermediate for the synthesis of pharmaceutical agents such as piroxicam sulfapyridine, tenoxicam, and tripelennamine [6, 10]. Besides, diaminopyridines are mostly used for synthesis of dyes, cosmetics, drugs and explosives [11, 12]. It is also used as intermediate for the synthesis of epoxy curing agents, polyamides, and preparation of analgesic phenazo- pyridine hydrochloride [13]. Additionally both the aminopyridine derivatives were successfully utilized as organic fluorescence sensors for the detection of metal cation [14].

The isotopic abundances of ratios of $^{2}H/^{1}H$, $^{13}C/^{12}C$, or $^{15}N/^{14}N$, could be locally altered by kinetically driven chemical reactions. There is an alternative and well-known approach, Mr. Trivedi's biofield energy treatment also known as The Trivedi Effect®, that can be applied on aminopyridine derivatives to undergo the isotopic changes. The physicochemical properties of various molecules and crystals were already altered by utilizing Mr. Trivedi's biofield energy treatment [15-17]. The National Center for Complementary and Alternative Medicine (NCCAM) has recommended the use of energy therapy as a part of Complementary and alternative medical therapies (CAM) in the healthcare sector [18]. CAM includes numerous energy-healing therapies, in which the biofield therapy is a form of putative energy therapy 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 that can be transmitted to any objects around.

Based on the previous results achieved by the biofield energy treatments by Mr. Trivedi in various fields, biofield energy treated 2-AP and 2,6-DAP were taken for mass spectroscopy studies to evaluate the isotopic abundance ratio of $^{2}H/^{1}H$, $^{13}C/^{12}C$, or $^{15}N/^{14}N$ (PM+1)/PM, [where PM is the primary molecule and (PM+1) is isotopic molecule].

2. Experimental

2.1. Materials

Both, 2-aminopyridine (2-AP) and 2,6-diaminopyridine (2,6-DAP) were procured from S. D. Fine Chemicals Pvt. Limited, India.

2.2. Method

Each of the 2-AP and 2,6-DAP sample was distributed into two parts, where one part was referred as control and the other part was considered as treated sample. The treated sample was further divided into four groups (*i.e.* T1, T2, T3, and T4) and handed over to Mr. Trivedi for biofield energy treatment under standard laboratory conditions. Mr. Trivedi provided the treatment through his energy transmission process to the treated groups without touching the sample. The control and treated samples were characterized using gas chromatography-mass spectrometry (GC-MS).

2.3. GC-MS Spectroscopy

The gas chromatography-mass spectroscopy (GC-MS) analysis was performed on Perkin Elmer/auto system XL with Turbo mass, USA, 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 (PM+1)/PM was expressed by its deviation in treated samples as compared to the control. The percentage change in isotopic ratio (PM+1)/PM was calculated from the following formula:

$$\text{Percent change in isotopic abundance ratio} = \frac{R_{Treated} - R_{control}}{R_{control}} \times 100 \quad (2)$$

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 2-AP

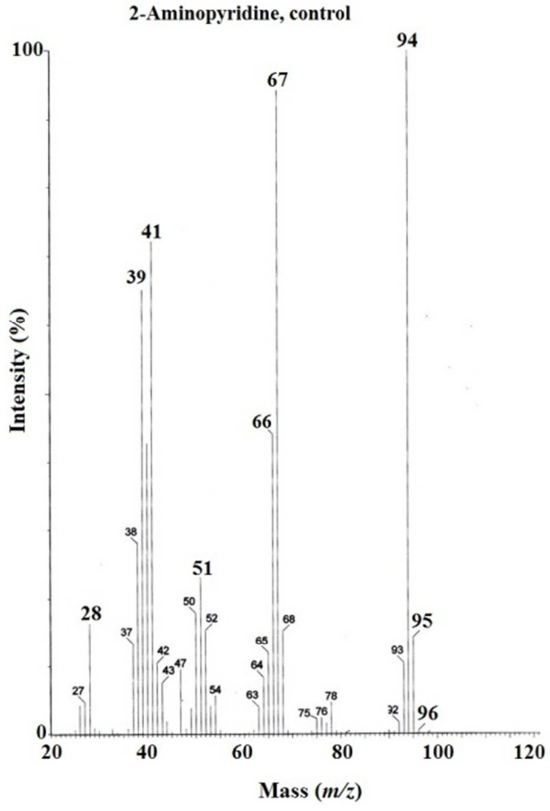

Figure 1. *GC-MS spectrum of control 2-aminopyridine.*

The GC-MS spectra of control and treated (T1, T2, T3, and T4) samples are presented in Fig. 1 and 2, respectively.

Mass spectrum of 2-AP is well matched with the reported literature [19]. MS spectra showed five peaks including molecular ion peak for both the control and treated samples of 2-AP. Five major peaks at *m/z*=94, 67, 51, 41, and 28 were observed that corresponded to the following ions respectively: $C_5H_6N_2^+$, $C_4H_5N^+$, $C_4H_3^+$, $C_3H_5^+$, and $C_2H_4^+$ ions. The molecular ion peak is the base peak in both control and treated samples at *m/z*=94. The other four peaks were observed in both, control and all treated 2-AP samples due to the fragmentation of molecular ion inside mass spectrum. Further, *m/z*=67, 51, 41, and 28 peaks were observed might be due to first reduction of the molecular ion to pyrrole, which again fragmented to give buten-3-yne, propene and ethane, respectively.

The intensity ratio and calculated percentage isotopic abundance ratio of all three elements presented in Table 1. Moreover, the isotopic abundance ratio (PM+1)/PM in control and treated 2-AP was plotted in Fig. 3.

It was clearly seen from the Table 1 and Fig. 3, that, the isotopic abundance ratio of (PM+1)/PM of 2-AP sample was decreased in T1 (55.83%) and T2 (25.12%), but increased significantly in T3 (117.19%) and T4 (202.26%) after biofield energy treatment as compared to the control. The decrease in the isotopic abundance ratio of (PM+1)/PM in T1 and T2 may have nominal effect on the bond energies and reactivity of the molecules of treated samples. However, the increased isotopic abundance ratio of (PM+1)/PM in T3 and T4 may increase the number of higher isotopes (PM+1) in the molecule. It may directly affect the bond strength of the C-H, N-H and N-C bonds. The increased isotopic abundance ratio of (PM+1)/PM in the treated (T3 and T4) sample may increase μ (effective mass) and binding energy in the 2-AP molecule with heavier isotopes, and this may result in enhance binding energy and stability of the molecule [20].

Table 1. *GC-MS isotopic abundance analysis result of 2-aminopyridine.*

Peak Intensity	Control	Treated			
		T1	T2	T3	T4
m/z of PM	100	100	100	100	100
m/z of (PM+1)	14.13	6.24	10.58	30.69	42.71
(PM+1)/ PM	0.141	0.062	0.105	0.306	0.427
Percent change		-55.83	-25.12	117.19	202.26

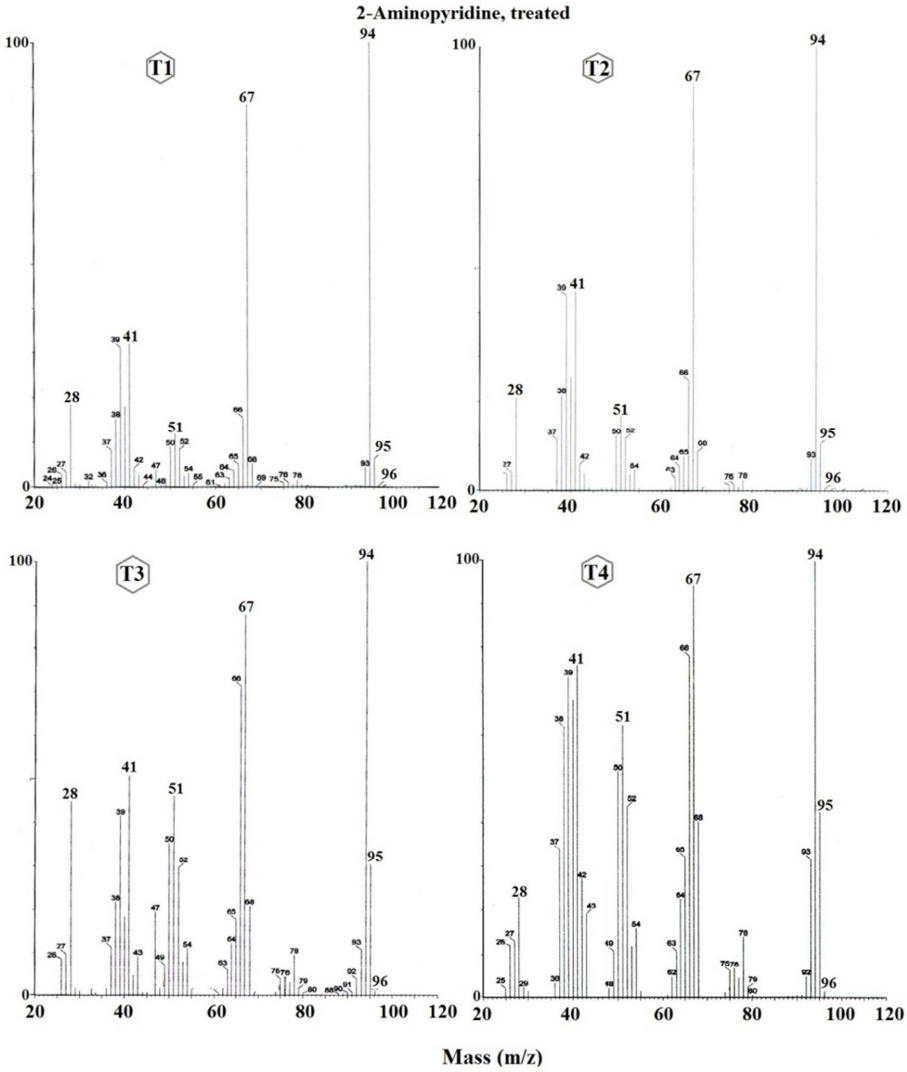

Figure 2. *GC-MS spectra of treated 2-aminopyridine samples (T1, T2, T3, and T4).*

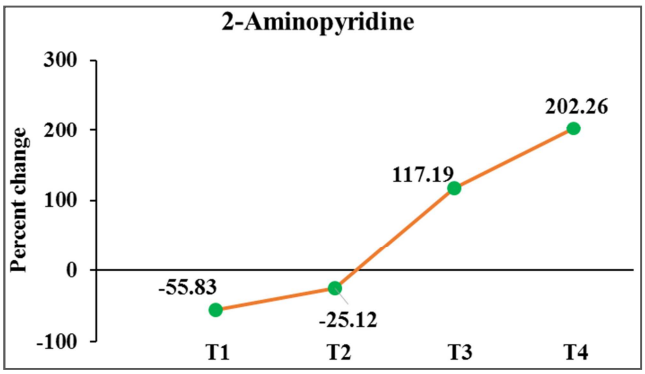

Figure 3. *Percent change in the isotopic abundance (PM+1)/PM of 2-aminopyridine after biofield energy treatment as compared to the control.*

3.2. GC-MS Study of 2,6-DAP

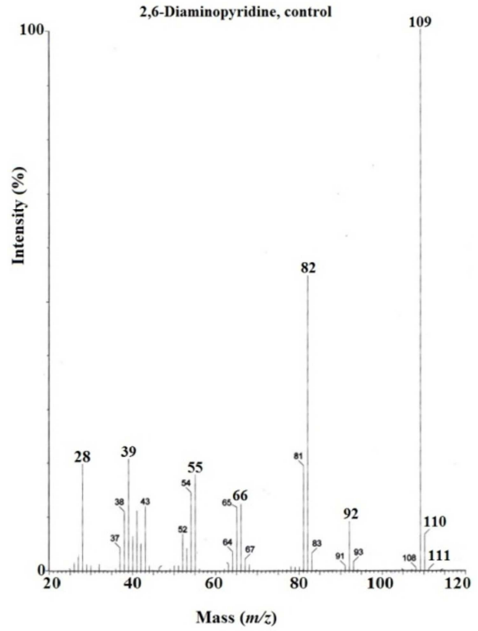

Figure 4. *GC-MS spectrum of control sample of 2,6-diaminopyridine.*

Mass spectra of both control and treated samples were shown in Fig. 4 and 5, respectively. The molecular ion peak was observed at *m/z*=109 in mass spectra of both control and treated samples. The calculated relative intensity ratio of (PM+1)/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 2,6-DAP at *m/z*=109, 82, 66, 55, 39, and 28, corresponded to the following ions respectively: $C_5H_7N_3^+$ (PM), $C_5H_8N^+$ (1,2-dihydropyridine), $C_4H_4N^+$ (pyrrole), $C_4H_7^+$ (but-1-ene), $C_3H_3^+$ (but-1-ene) and $C_2H_4^+$(ethene) ions. In both the molecules 2-AP and 2,6-DAP, molecular ion peak was observed as a base peak. However, they exhibited different fragmentation pattern due to the structural and reactivity differences in them. The isotopic abundance ratio of (PM+1)/PM of control and treated 2,6-DAP was calculated and presented in Fig. 6. The isotopic abundance ratio of (PM+1)/PM of treated 2,6-DAP was increased upto 370.54% in T3 (T1=7.30%, T2=40.24%, and T4=12.82%) as compared to the control. The mass spectrum of control 2,6-DAP is well supported with the reported literature [21].

If the lighter isotopes were substituted by heavier isotopes then the effective mass (μ) of the particular bond is increased and subsequently binding energy will be increased [20]. 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 2-AP and 2,6-DAP might increase the effective mass and binding energy after biofield energy treatment that may enhance the chemical stability of aminopyridine derivatives. On the contrary, the slight decrease in isotopic abundance ratio of (PM+1)/PM in treated T1 and T2 in 2-AP might reduce the effective mass of the particular bond and binding energy will be decreased. Further, the increased (PM+1)/PM will increase the effective mass which may decrease the reactivity of the aminopyridine derivatives.

Table 2. *GC-MS isotopic abundance analysis result of 2,6-diaminopyridine.*

Peak Intensity	Control	Treated			
		T1	T2	T3	T4
m/z of PM	100	100	100	99.8	100
m/z of (PM+1)	6.71	7.2	9.41	31.51	7.57
(PM+1)/PM	0.067	0.072	0.094	0.315	0.075
Percent change		7.30	40.24	370.54	12.82

Table 3. *Possible isotopic bonds in 2-aminopyridine and 2,6-diaminopyridine.*

Isotopes Bond	Isotope type	Reduced mass ($m_Am_B/(m_A+m_B)$
^{12}C-^{12}C	Lighter	6.000
^{13}C-^{12}C	Heavier	6.260
^{1}H-^{12}C	Lighter	0.923
^{1}H-^{13}C	Heavier	0.929
^{2}H-^{12}C	Heavier	1.710
^{1}H-^{15}N	Heavier	0.940
^{2}H-^{14}N	Heavier	1.750
^{15}N-^{12}C	Heavier	7.200
^{14}N-^{13}C	heavier	6.500
^{15}N-^{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.

Effective mass of some probable isotopic bonds were calculated and presented in Table 3. The result showed that μ of normal ^{12}C-^{12}C (μ=6), and ^{1}H-^{12}C (μ=0.923) bonds were increased in case of heavier isotopes (*i.e.* ^{13}C-^{12}C=6.26, and ^{2}H-^{12}C=1.71), respectively. After biofield treatment, bond strength, stability, and binding energy of the aromatic ring of 2-AP and 2,6-DAP molecules might be increased due to the higher effective mass (μ) after biofield energy treatment [22].

The decreased reactivity of the aminopyridine derivatives may increase the stability of the aminopyridine based products in pharmaceutical industry, by reducing the degradation kinetics in the finished products after production.

Furthermore, the organic molecules used for fluorescence sensors are exposed to UV radiation continuously for a longer period of time. Photo stability is a great concern in order to use them successfully as sensors without degradation. After biofield treatment the stability may enhance due to the presence of higher isotopes an enable them to expose the materials under UV radiation for longer period [14].

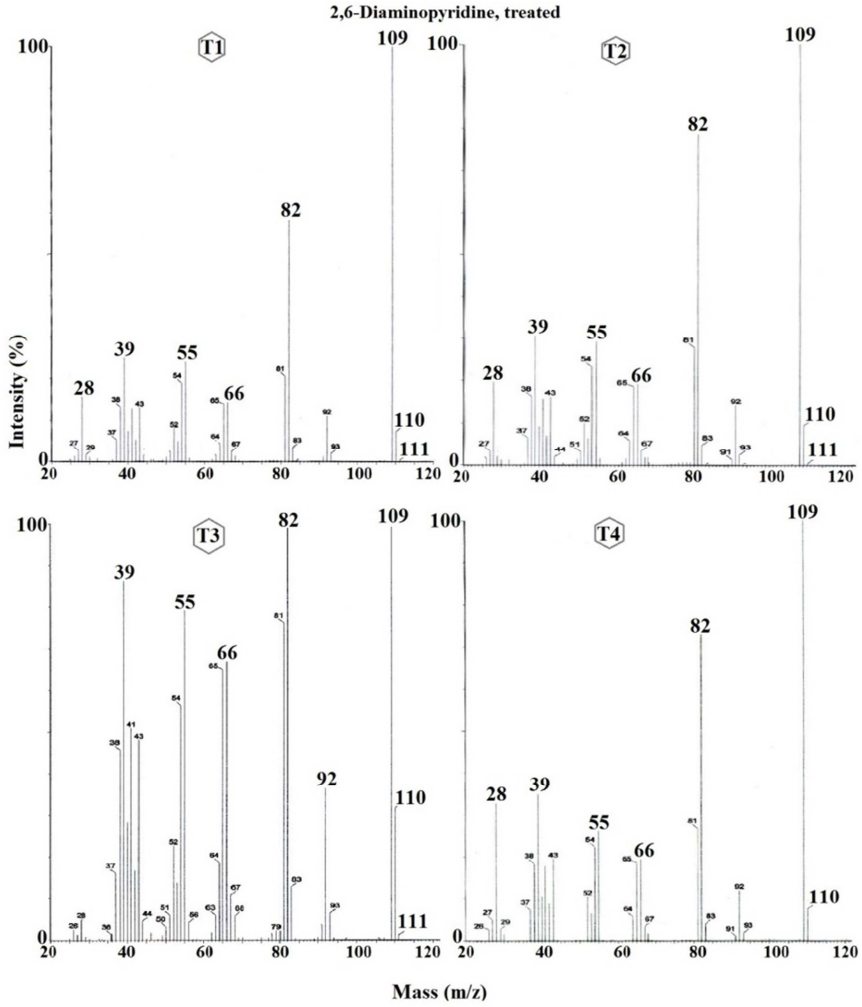

Figure 5. *GC-MS spectra of treated samples of 2,6-diaminopyridine (T1, T2, T3, and T4).*

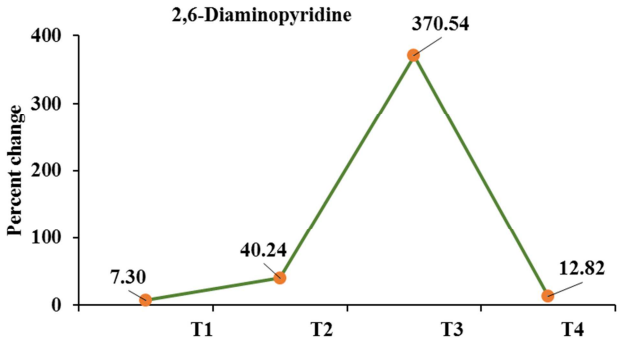

Figure 6. *Percent change in the isotopic abundance of (PM+1)/PM of 2,6-diaminopyridine after biofield energy treatment as compared to the control.*

4. Conclusions

In summary, aminopyridine derivatives, 2-AP and 2,6-DAP were studied with GC-MS under the influence of biofield energy treatment and observed a significant change in isotope abundance of $^{2}H/^{1}H$, $^{13}C/^{12}C$ or $^{15}N/^{14}N$ as compared to the respective control samples. The percent change in isotope abundance ratio of (PM+1)/PM was increased upto 202.26% in 2-AP (T4), while the isotopic abundance ratio was increased significantly upto 370.54 in treated 2,6-DAP (T3) sample. The changes in isotopic abundance ratios have significant impact on bond energies and chemical reactivity of the molecules. Due to the enhancement in the isotopic abundance ratio of (PM+1)/PM in 2-AP and 2,6-DAP, the reactivity may be reduced significantly by increase in the effective mass (μ) and the binding energy of the treated sample. It can be concluded from the above observations that the enhancement of heavier isotopes in the aromatic ring as well as the functional groups may decrease the reactivity of the aromatic ring and the functional groups of aminopyridine derivatives, consequently enabling their utility as pharmacophore in the pharmaceutical industry and as an active material in fluorescence sensors.

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