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# Isotopic Abundance Ratio Analysis of the Consciousness Energy Healing Treated Berberine Chloride Using LC-MS and GC-MS Spectrometry

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## Abstract

Berberine is a benzylisoquinoline alkaloid found in many medicinal plants such as Berberis vulgaris, Mahonia aquifolium, etc. Although it has enormous therapeutic potential, the bioavailability is very poor (<1%) due to its low solubility and poor intestinal absorption. This study was performed to investigate the impact of the Trivedi Effect® on the structural properties and the isotopic abundance ratio of berberine chloride using LC-MS and GC-MS spectroscopy. Berberine chloride sample was divided into two parts, one part of berberine chloride was considered as a control sample (received no Biofield Energy Treatment), while the second part was treated with the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a famous Biofield Energy Healer, Alice Branton, called a treated sample. The LC-MS spectra of both the samples at retention time ($R_t$) ~2.1 minutes exhibited the mass of the molecular ion peak at *m/z* 336.3 $[M]^+$ (calculated for $C_{20}H_{18}NO_4^+$, 336.12). The LC-MS based isotopic abundance ratio of $P_{M+1}/P_M$ in the treated berberine was significantly increased by 24.41% compared with the control sample. Thus, $^{13}C$, $^{2}H$, $^{15}N$, and $^{17}O$ contributions from $(C_{20}H_{18}NO_4)^+$ to *m/z* 337 in the treated sample was significantly increased compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of $P_{M+1}/P_M$ in the treated berberine was significantly increased by 12.97% compared with the control sample. Hence, $^{13}C$, $^{2}H$, $^{15}N$, and $^{17}O$ contributions from $(C_{20}H_{16}NO_4)^{3+}$ to *m/z* 335 in the treated sample were significantly increased compared with the control sample. But, the isotopic abundance ratio of $P_{M+2}/P_M$ in the treated berberine chloride was significantly decreased by 38.59% compared with the control sample. Hence, 18O contribution from $(C_{20}H_{16}NO_4)^{3+}$ to *m/z* 336 in the treated sample was significantly decreased compared with the control sample. The isotopic abundance ratios of $P_{M+1}/P_M$ and $P_{M+2}/P_M$ in the treated berberine chloride were significantly altered compared to the control sample. The altered isotopic abundance ratios ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$ or $^{18}O/^{16}O$) would influence the intra-atomic bond strength, its physical stability, and alter the rate of reactions in the body. The changes in isotopic abundance might be due to changes in nuclei, possibly through the interference of neutrino particles *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. The new form of berberine chloride would be more efficacious novel pharmaceutical formulations that might offer better solubility, bioavailability and therapeutic response against diarrhoea, gastroenteritis, bacterial, fungal and other microbial infections, cancer, arrhythmia, diabetes, hyperlipidemia, inflammation in the body, etc.

**Keywords:** Berberine chloride; The Trivedi Effect®; Biofield Energy; Consciousness Energy Healing Treatment; LC-MS, GC-MS

**Abbre*via*tions:** NCCIH: National Centers of Complementary and Integrative Health; CAM: Complementary and Alternative Medicine; MS: Mass spectrometry; GC-MS: Gas Chromatography-Mass Spectrometry; LC-MS: Liquid Chromatography-Mass Spectrometry; TIC: Total Ion Chromatograms.

## Introduction

Berberine is a quaternary ammonium salt form of benzylisoquinoline alkaloid found in many medicinal plants such as Berberis vulgaris, Mahonia aquifolium, Coptis chinensis, Hydrastis canadensis, etc [1,2]. Traditionally it has been used for centuries for many applications [3]. Berberine used as a natural dye with a very high colour index of 75160 [1,4]. Many researchers suggested that berberine can treat diarrhoea, gastroenteritis, bacterial, fungal, and other microbial infections [5-7]. Some of the recent investigations have also shown that it may have applications for treating cancer, arrhythmia, diabetes, hyperlipidemia, and inflammation [8-12]. There is some evidence that berberine may have anti-aging and better anti-glycaemic (insulin sensitizer) properties [13]. Because of the multiple biological activities with less toxicity, and low cost, berberine has recently gained a great interest in the treatment of human diseases [14-17]. Although, berberine has the massive therapeutic potential as a drug molecule, due to its poor bioavailability (less than 1%) made it challenging to develop it as a clinical candidate. The less bioavailability of the berberine is due to its low solubility, poor membrane permeability, poor intestinal absorption, and rapid biotransformation also account for the low plasma concentrations [18,19].

Several research is going on for the improvement of bioavailability of berberine [20]. In this scenario, the Biofield Energy Healing Treatment (the Trivedi Effect®) has the considerable impact on particle size, surface area, thermal behaviour, along with bioavailability of the pharmaceutical/nutraceutical compounds [21-24]. The Trivedi Effect® is a natural and scientifically proved phenomenon in which an individual can harness this inherently intelligent energy and transmit it anywhere on the planet *via* the possible mediation of neutrinos [25]. Biofield Energy is an electromagnetic field that exists around the human body [26]. Use of energy medicine and healing has been studied and reported with a significant outcome [27,28]. The biofield is generated from the movements of charged particles in the body, i.e., ions, cells, blood/lymph flow, brain functions, and heart function [24]. Biofield Energy Healers can harness the energy from the Universe and can transmit into any living and non-living object(s), this process is called Biofield Energy Healing Treatment [29,30]. The National Centers of Complementary and Integrative Health (NCCIH) has recognized and accepted Biofield Energy Healing as a Complementary and Alternative Medicine (CAM) health care approach along with the other therapies, medicines, and practices such as Ayurveda, yoga, Qi Gong, Tai Chi, hypnotherapy, Reiki, etc. [31]. These CAM therapies have been accepted by most of the U.S.A. population [32]. Similarly, the Trivedi Effect®-Consciousness Energy Healing Treatment also reported with significant impact on the characteristic properties of the several living and non-living object(s), i.e., organic compounds [33], metals and ceramic [34,35], crops [36], microbes [37], etc.

The stable isotope ratio analysis has various applications in different scientific fields for the understanding of isotope effects resulting from the variation of the isotopic composition of the molecule [38,39]. Isotope ratio analysis can be performed by using the conventional mass spectrometry (MS) techniques, i.e., gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) in low micromolar concentration with sufficient precision [39,40]. The Consciousness Energy Healing Treatment has also found altering the isotopic abundance ratio of the chemical compounds [41,42]. The Trivedi Effect®-Biofield Energy Healing Treatment could be an economical approach for designing better pharmaceutical compounds. Therefore, in this study, special attention was taken to improve the quality of berberine chloride. Hence, LC-MS and GC-MS were used in this study to characterize the structural properties and evaluate the isotopic abundance ratio analysis of $P_{M+1}/P_M$ ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) and $P_{M+2}/P_M$ ($^{18}O/^{16}O$) in the Consciousness Energy Healing Treated berberine chloride compared to the control sample.

## Materials and Methods

### Chemicals and Reagents

The test sample berberine chloride hydrate (98.1% HPLC) powder was purchased from Tokyo Chemical Industry Co. Ltd., Japan. Other chemicals, i.e., formic acid (Merck), methanol (Advent), and purified water (Evoqua) were of analytical grade purchased in India.

### Consciousness Energy Healing Treatment Strategies

The test sample berberine chloride powder was divided into two parts. One part of the test sample was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely under standard laboratory conditions for 3 minutes and known as the Biofield Energy Treated sample. The treatment was provided through the healer's unique energy transmission process by the famous Biofield Energy Healer, Alice Branton (USA) to the test sample berberine chloride. Another part of the test sample was called as the control sample, which was treated with a "sham" healer who did not

have any knowledge about the Biofield Energy Treatment. After all, the Biofield Energy Treated and untreated berberine chloride samples were kept in sealed conditions and characterized using LC-MS and GC-MS analytical techniques.

## Characterization

**Liquid chromatography-mass spectrometry (lc-ms) analysis and calculation of isotopic abundance ratio:** The LC-MS analysis of the berberine chloride was carried out with the help of LC-MS ThermoFisher Scientific, the USA, equipped with an ion trap detector and a triple-stage quadrupole mass spectrometer. The column used here was a reversed phase Thermo Scientific Synchronis C18 (Length-250 mm X ID 4.6 mm X 5 micron), maintained at 35˚C. The diluent used for the sample preparation was acetonitrile and methanol. 5 µL of berberine chloride solution was injected, and the analyte was eluted using acetonitrile + 0.1% formic acid (50:50) pumped at a constant flow rate of 1 mL/min. Chromatographic separation was achieved using gradient condition and the total run time was 10 min. Peaks were monitored at 210 nm using the PDA detector. The mass spectrometric analysis was performed under +ve ESI mode. The total ion chromatogram, peak area% and mass spectrum of the individual peak which was appeared in LC along with the full scan were recorded. The natural abundance of each isotope (C, H, N, and O) can be predicted from the comparison of the height of the isotope peak with respect to the base peak. The values of the natural isotopic abundance of the common elements are obtained from the literature [39,43-45]. The LC-MS based isotopic abundance ratios ($P_{M+1}/P_M$) for the control and Biofield Energy Treated berberine chloride was calculated using equation 1.

% Change in isotopic abundance ratio = [($IAR_{Treated}$ – $IAR_{Control}$)/ $IAR_{Control}$) x 100] (1)

Where $IAR_{Treated}$ = isotopic abundance ratio in the treated sample and $IAR_{Control}$ = isotopic abundance ratio in the control sample.

**Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:** GC-MS of the berberine chloride were analyzed with the help of Perkin Elmer Gas chromatograph equipped with a PE-5MS (30M x 250 micros x 0.250 microns) capillary column and coupled to a single quadrupole mass detector was operated with electron impact (EI) ionization in positive ion mode. The oven temperature was programmed from 75°C (5 min hold) to 280°C (14.5 min hold) @ 10°C /min (total run time 40 min). The sample was prepared taking 50 mg of the berberine chloride in 2.5 ml methanol as a diluent. The identification of analyte was done by GC retention times and by a comparison of the mass spectra of samples. The GC-MS based isotopic abundance ratios ($P_{M+1}/P_M$ and $P_{M+2}/P_M$) for the control and Biofield Energy Treated berberine chloride was calculated using equation 1.

## Results and Discussion

### Liquid Chromatography-Mass Spectrometry (LC-MS)

The total ion chromatograms (TIC) and mass spectra of both the samples of berberine chloride are shown in Figures 1 and 2, respectively. Berberine chloride showed the single major chromatographic peak at retention time ($R_t$) of ~2 minutes in the TIC of both the case (Figure 1).

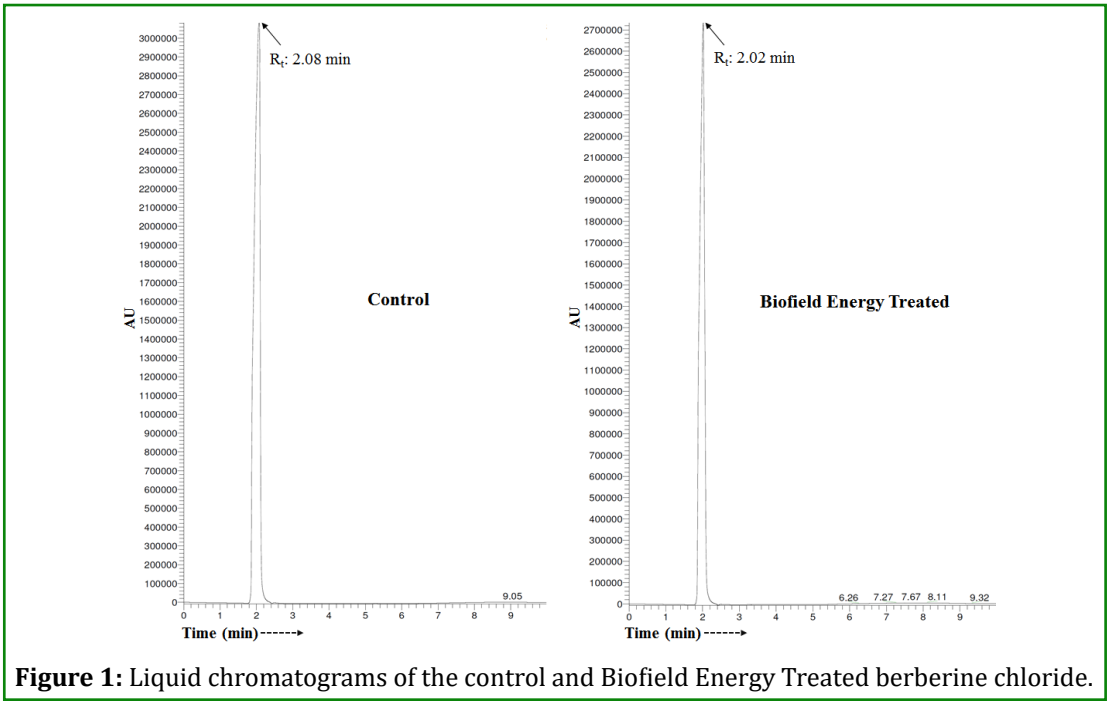


**Figure 1:** Liquid chromatograms of the control and Biofield Energy Treated berberine chloride.

This indicated that the polarity of both the samples remained the same. Similarly, the mass spectra of berberine chloride exhibited the mass of the molecular ion peak at *m/z* 336.3 $[M]^+$ (calculated for $C_{20}H_{18}NO_4^+$, 336.12) along with other fragmentation peaks at *m/z* 321.33 ($C_{19}H_{15}NO_4^{2+}$), *m/z* 278.08 ($C_{18}H_{16}NO_2^{3+}$), *m/z* 192.08 ($C_{14}H_{10}N_3^+$) in both the control and Biofield Energy Treated samples (Figures 2 and 3). The mass spectra of berberine chloride show the molecular peak $[M]^+$ at *m/z* 336 in the mass spectrum in +ve ion mode [46].

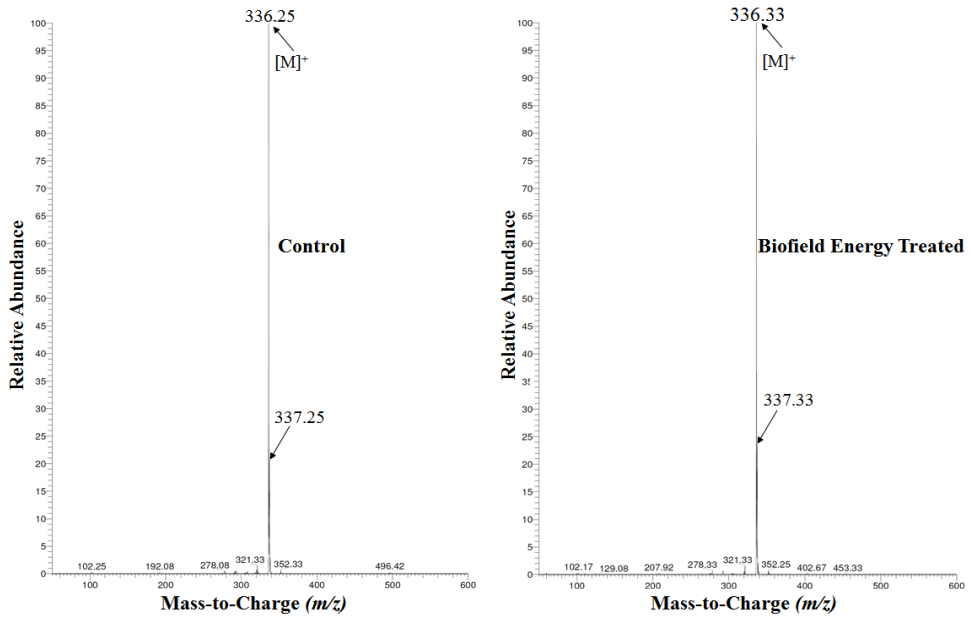


**Figure 2:** Mass spectra of the control and Biofield Energy Treated berberine chloride at $R_t$ ~2.1 minutes.

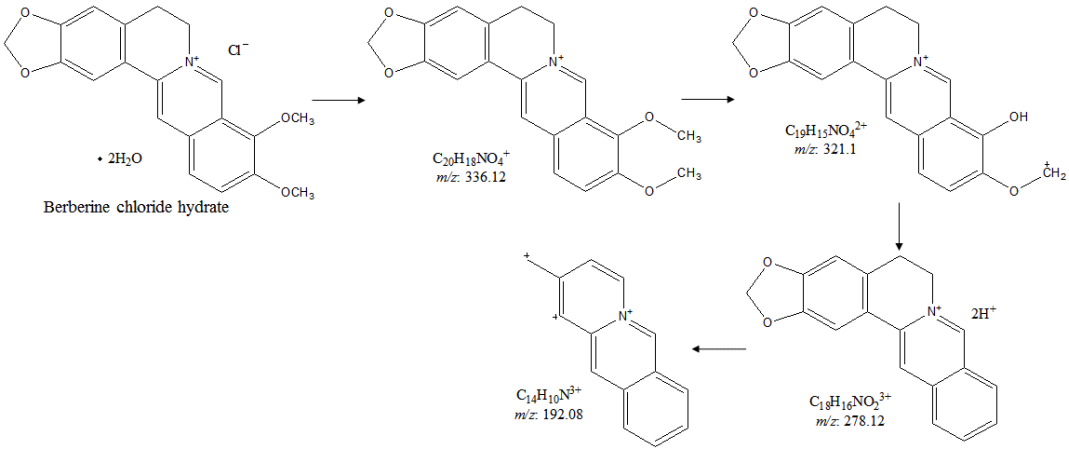


**Figure 3:** Proposed fragmentation pattern of berberine chloride.

The LC-MS spectra of both the samples of berberine chloride showed the mass of the molecular ion peak at *m/z* 336.3 $[M]^+$ (calculated for $C_{20}H_{18}NO_4^+$, 336.12) with 100% relative intensity. The theoretical calculation of $P_{M+1}$ for berberine chloride was presented as below:

P ($^{13}C$) = [(20 x 1.1%) x 100% (the actual size of the $M^+$ peak)] / 100% = 22%

P ($^{2}H$) = [(18 x 0.015%) x 100%] / 100%= 0.27%

P ($^{15}N$) = [(1 x 0.4%) x 100%] / 100% = 0.4%

P ($^{17}O$) = [(4 x 0.04%) x 100%] / 100% = 0.16%

$P_{M+1}$, i.e. $^{13}C$, $^{2}H$, $^{15}N$, and $^{17}O$ contributions from $(C_{20}H_{18}NO_4)^+$ to *m/z* 337 = 22.83%

From the above calculation, it has been found that $^{13}C$, and $^{15}N$ have the major contribution to *m/z* 337. The $P_{M+1}$ theoritical value of the berberine was close to the experimental value

(Table 1). The isotopic abundance ratio analysis $P_M$ and $P_{M+1}$ for berberine chloride near *m/z* 336 and 337 of the control and Biofield Energy Treated samples, which were obtained from the observed relative peak intensities of $[M^+]$ and $[(M+1)^+]$ peaks, respectively in the mass spectra (Table 1). The isotopic abundance ratio ($P_{M+1}/P_M$) in the Biofield Energy Treated berberine chloride was significantly increased by 24.41% compared with the control sample (Table 1). Therefore, it was concluded that the $^{13}C$, $^{2}H$, $^{15}N$, and $^{17}O$ contributions from $(C_{20}H_{18}NO_4)^+$ to *m/z* 337 in the Biofield Energy Treated sample were significantly increased compared to the control sample.

| Parameter | Control sample | Biofield Energy Treated sample |
|---|---|---|
| $P_M$ at *m/z* 336 (%) | 100 | 100 |
| $P_{M+1}$ at *m/z* 337 (%) | 17.82 | 22.17 |
| $P_{M+1}/P_M$ | 0.18 | 0.22 |
| % Change of isotopic abundance ratio ($P_{M+1}/P_M$) with respect to the control sample | | 24.41 |

$P_M$: the relative peak intensity of the parent molecular ion $[M^+]$; $P_{M+1}$: the relative peak intensity of the isotopic molecular ion $[(M+1)^+]$, M: mass of the parent molecule.

**Table 1:** LC-MS based isotopic abundance analysis results in Biofield Energy Treated berberine chloride compared to the control sample.

## Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The control and Biofield Energy Treated berberine chloride showed the presence of a sharp chromatographic peak at $R_t$ of ~22 min in the GC-MS chromatograms (Figures 4 and 5). The parent molecular ion peak of berberine chloride at *m/z* 334 $[M]^+$ (calculated for $C_{20}H_{16}NO_4^{3+}$, 334.11) was observed in the control sample and Biofield Energy Treated sample, along with the lower mass fragment ion peaks (Figures 4 and 5) (Table 2).

The mass spectra of both the control and Biofield Energy Treated berberine chloride showed the molecular ion peak $[M]^+$ at *m/z* 334 $[M]^+$ (calculated for $C_{20}H_{16}NO_4^{3+}$, 334.11). The theoretical calculation of $P_{M+1}$ and $P_{M+2}$ for berberine chloride was presented as below:

P ($^{13}C$) = [(20 x 1.1%) x 74.03% (the actual size of the $M^+$ peak)] / 100% = 16.29%

P ($^{2}H$) = [(16 x 0.015%) x 74.03%] / 100%= 0.18%

P ($^{15}N$) = [(1 x 0.4%) x 74.03%] / 100% = 0.3%

P ($^{17}O$) = [(4 x 0.04%) x 74.03%] / 100% = 0.12%

$P_{M+1}$, i.e. $^{13}C$, $^{2}H$, $^{15}N$, and $^{17}O$ contributions from $(C_{20}H_{16}NO_4)^{3+}$ to *m/z* 335 = 16.89%

Similarly,

P ($^{18}O$) = [(4 x 0.2%) x 74.03%] / 100% = 0.12%

$P_{M+2}$, i.e. $^{18}O$ contributions from $(C_{20}H_{16}NO_4)^{3+}$ to *m/z* 336 = 0.12%

From the above calculation, it has been found that $^{13}C$, $^{15}N$, and $^{18}O$ have major contribution to *m/z* 335 and 336.

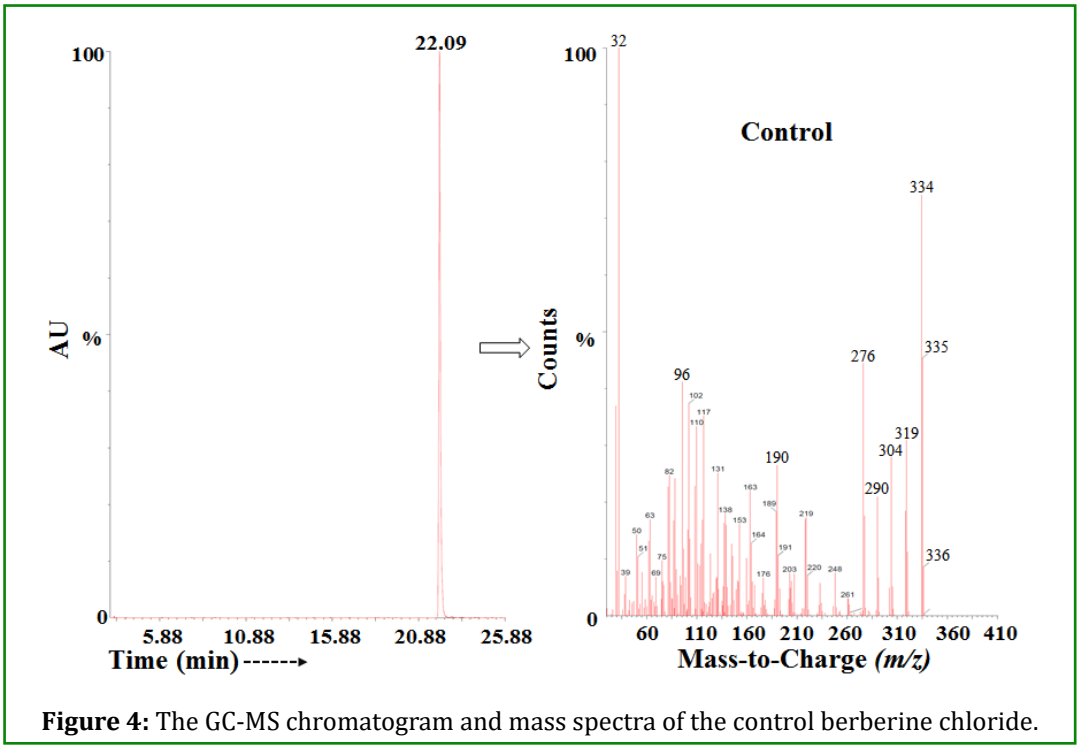


**Figure 4:** The GC-MS chromatogram and mass spectra of the control berberine chloride.

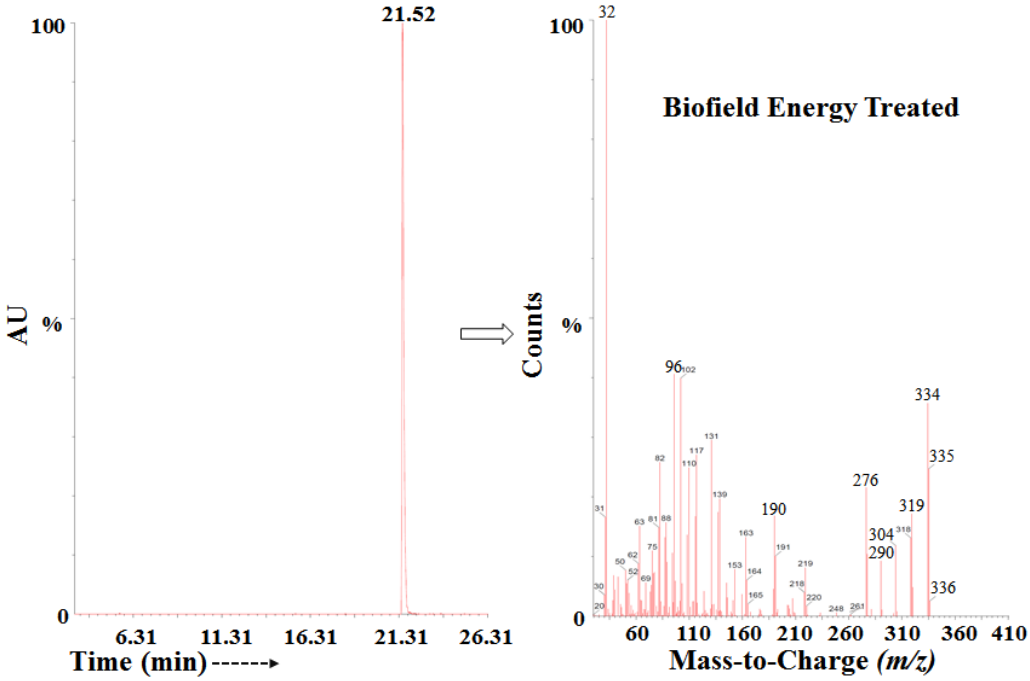


**Figure 5:** The GC-MS chromatogram and mass spectra of the Biofield Energy Treated berberine chloride.

| Parameter | Control sample | Biofield Energy Treated sample |
|---|---|---|
| $P_M$ at *m/z* 334 (%) | 74.03 | 35.67 |
| $P_{M+1}$ at *m/z* 335 (%) | 45.34 | 24.68 |
| $P_{M+1}/P_M$ | 0.61 | 0.69 |
| % Change of isotopic abundance ratio ($P_{M+1}/P_M$) with respect to the control sample | | 12.97 |
| $P_{M+2}$ at *m/z* 336 (%) | 8.55 | 2.53 |
| $P_{M+2}/P_M$ | 0.12 | 0.07 |
| % Change of isotopic abundance ratio ($P_{M+2}/P_M$) with respect to the control sample | | -38.59 |

$P_M$: 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 $[(M+2)^+]$; M: mass of the parent molecule.

**Table 2:** GC-MS based isotopic abundance analysis results of Biofield Energy Treated berberine chloride compared to the control samples.

The GC-MS based isotopic abundance ratio analysis of the Biofield Energy Treated berberine chloride were calculated compared to the control sample. $P_M$, $P_{M+1}$ and $P_{M+2}$ for berberine chloride near *m/z* 334, 335, and 336, respectively of the control and Biofield Energy Treated samples, which were obtained from the observed relative peak intensities of $[M^+]$, $[(M+1)^+]$, and $[(M+2)^+]$ peaks, respectively in the mass spectra and are calculated in Table 2. The isotopic abundance ratio of $P_{M+1}/P_M$ in the Biofield Energy Treated berberine chloride was significantly increased by 12.97% compared with the control sample (Table 2). Hence, $^{13}C$, $^{2}H$, $^{15}N$, and $^{17}O$ contributions from $(C_{20}H_{16}NO_4)^{3+}$ to *m/z* 335 in the Biofield Energy Treated sample were significantly increased compared with the control sample. But, the isotopic abundance ratio of $P_{M+2}/P_M$ in the Biofield Energy Treated berberine chloride was significantly decreased by 38.59% compared with the control sample (Table 2). Hence, $^{18}O$ contribution from $(C_{20}H_{16}NO_4)^{3+}$ to *m/z* 336 in the Biofield Energy Treated sample was significantly decreased compared with the control sample.

LC-MS and GC-MS study confirmed the structure of berberine. The isotopic abundance ratios of $P_{M+1}/P_M$ ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) and $P_{M+2}/P_M$ ($^{18}O/^{16}O$) in the Biofield

Energy Treated berberine chloride were significantly altered compared to the control sample.

It can be assumed that the changes in isotopic abundance could be due to changes in nuclei, possibly through the interference of neutrino particles *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. A neutrino is an elementary particle which interacts only *via* the weak subatomic force and gravity [47]. The neutrinos change identities, and it is only possible if the neutrinos possess mass and have the ability to interchange their phase from one phase to another internally. Therefore, this particle has the ability to interact with protons and neutrons in the nucleus, which specified a close relation between neutrino and the isotope formation [25,39,40]. The altered isotopic composition in the molecular level of the Biofield Energy Treated berberine chloride might have altered the neutron to proton ratio in the nucleus. The altered isotopic abundance ratios ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$ or $^{18}O/^{16}O$) would influence the intra-atomic bond strength, its physical stability, and alter the rate of reactions in the body [48]. The overall results concluded that the Trivedi Effect®-Consciousness Energy Healing Treatment might create a new form of berberine chloride which would show better for the prevention and treatment of various diseases diarrhea, gastroenteritis, bacterial, fungal and other microbial infections. It would also help the treatment of cancer, arrhythmia, diabetes, hyperlipidemia, and inflammation in the body more effectively.

## Conclusion

The Trivedi Effect®-Consciousness Energy Healing Treatment showed a significant impact on the isotopic abundance ratios and mass peak intensities of berberine chloride. The LC-MS spectra of both the control and Biofield Energy Treated samples at retention time ($R_t$) ~2.1 minutes exhibited the mass of the molecular ion peak at *m/z* 336.3 $[M]^+$. The LC-MS based isotopic abundance ratio of $P_{M+1}/P_M$ in the Biofield Energy Treated berberine chloride was significantly increased by 24.41% compared with the control sample. Thus, $^{13}C$, $^{2}H$, $^{15}N$, and $^{17}O$ contributions from $(C_{20}H_{18}NO_4)^+$ to *m/z* 337 in the Biofield Energy Treated sample were significantly increased compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of $P_{M+1}/P_M$ in the Biofield Energy Treated berberine chloride was significantly increased by 12.97% compared with the control sample. Hence, $^{13}C$, $^{2}H$, $^{15}N$, and $^{17}O$ contributions from $(C_{20}H_{16}NO_4)^{3+}$ to *m/z* 335 in the Biofield Energy Treated sample were significantly increased compared with the control sample. But, the isotopic abundance ratio of $P_{M+2}/P_M$ in the Biofield Energy Treated berberine chloride was significantly decreased by 38.59% compared with the control sample. Hence, $^{18}O$ contribution from $(C_{20}H_{16}NO_4)^{3+}$ to *m/z* 336 in the Biofield Energy Treated sample was significantly decreased compared with the control sample. The isotopic abundance ratios of $P_{M+1}/P_M$ and $P_{M+2}/P_M$ in the Biofield Energy Treated berberine chloride were significantly altered compared to the control sample. The altered isotopic abundance ratios ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$ or $^{18}O/^{16}O$) would influence the intra-atomic bond strength, its physical stability, and alter the rate of reactions in the body. It can be assumed that the changes in isotopic abundance could be due to changes in nuclei possibly through the interference of neutrino particles *via* the Trivedi Effect®-Consciousness Energy Healing Treatment. The new form of berberine chloride would be more efficacious pharmaceutical formulations that might offer better solubility, dissolution, absorption, bioavailability and therapeutic response against diarrhoea, gastroenteritis, bacterial, fungal and other microbial infections, cancer, arrhythmia, diabetes, hyperlipidemia, inflammation in the body, etc.

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