ISSN: 2644-1217

(9)

**DOI:** 10.32474/OAICAM.2021.03.000152

**Research Article** 

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

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Received: 

February 05, 2021

Published: 

March 03, 2021

#### **Abstract**

Berberine is an alkaloid class of drug which has enormous therapeutic potential, but the bioavailability is very poor (<1%) due to its low solubility and poor intestinal absorption. In this study, the influence of the Trivedi Effect® on the structural properties and the isotopic abundance ratio of berberine chloride was evaluated using LC-MS and GC-MS analytical techniques. Berberine chloride sample was divided into control and treated parts. Only the treated part was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a well-known Biofield Energy Healer, Mahendra Kumar Trivedi. The LC-MS spectra of both the samples observed at retention time 2.0 minutes and the molecular ion peak at m/z 336.25 [M] in the mass spectra. The peak area of the treated berberine was significantly increased by 4.42% compared to the control sample. The LC-MS based isotopic abundance ratio of  $P_{M+1}/P_M$  in the treated berberine was significantly increased by 34.4% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of  $P_{M+1}/P_{M}$  in the treated berberine was very increased by 1547.15% compared with the control sample. Thus, 13C, 2H, 15N, and 17O contributions from  $(C_{20}H_{18}NO_4)$ + to m/z 337 in the treated sample were significantly increased compared with the control sample. The isotopic abundance ratio of  $P_{M+1}/P_M$  ( $^2H/^1H$  or  $^{13}C/^{12}C$  or  $^{15}N/^{14}N$  or  $^{17}O/^{16}O$ ) in the treated berberine was significantly altered compared to the control sample. The increased isotopic abundance might occur due to the  $interference\ of\ neutrino\ particles\ \textit{via}\ the\ Trivedi\ Effect^{\scriptsize @}-Consciousness\ Energy\ Healing\ Treatment.$  The increased isotopic abundance ratio of the Consciousness Energy Healing Treated berberine chloride may increase the chemical bond strength, stability, solubility, and bioavailability in the body. The new form of berberine chloride would be more efficacious novel pharmaceutical formulations against diarrhoea, gastroenteritis, bacterial and fungal infections, cancer, diabetes, arrhythmia, inflammation, hyperlipidemia, etc.

Keywords: Berberine Chloride; Biofield Energy; Consciousness Energy Healing Treatment; The Trivedi Effect®; Isotopic Abundance

## Introduction

Berberine is an alkaloid class of drug, extracted from the plant sources, i.e., *Mahonia aquifolium, Hydrastis Canadensis, Coptis chinensis, Berberis vulgaris, etc.* [1,2]. Mainly it has been used as a natural dye from the old age. It is also used for the treatment of diarrhoea, gastroenteritis, cancer, arrhythmia, diabetes, bacterial, fungal, and other microbial infections, hyperlipidemia, inflammation, etc. [3-13]. Similarly, berberine also has antiglycaemic and anti-aging properties [14]. Many biological activities of berberine with less toxicity and low cost made it a great interesting candidate for the treatment of human diseases [15-18]. The poor

bioavailability of the berberine is due to its low solubility, poor intestinal absorption, and rapid biotransformation also account for the low plasma concentrations [19,20]. The bioavailability of the berberine is very poor (<1%), which is the big challenge to develop it as a clinical candidate.

The improvement of bioavailability is a challenging task, but many types of research are going on for the improvement in berberine [21]. The Trivedi Effect®-Consciousness Energy Healing Treatment proved to have a significant impact on the particle size, surface area, crystal properties, thermal behaviour, along with bioavailability of

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the pharmaceutical/nutraceutical compounds [22-25]. The Trivedi Effect® is a natural and only scientifically proven phenomenon in which a Biofield Energy expert can harness this energy from the Universe and transfer it anywhere on the planet via the possible mediation of neutrinos [26]. The Biofield is an electromagnetic field that exists around the human body generated by continues moment of the charged particles and movements in the body, i.e., particles, ions, cells, blood/lymph flow, brain functions, and heart function [25,27]. The use of energy therapy has been studied and reported with significant outcomes in different disease conditions [28,29]. The mechanism involved in the Biofield Energy Healing Treatment process is the energy harness from the universe by the healer and transfer the energy to any living and non-living object(s) for the wellness [30,31]. The National Center of Complementary and Integrative Health (NCCIH) has recognized and accepted Energy Therapy as a Complementary and Alternative Medicine (CAM) health care approach along with the other therapies, medicines, and practices, i.e., Ayurveda, Chinese herb and traditional medicine, homeopathy, Qi Gong, Tai Chi, yoga, Reiki, hypnotherapy, etc. [32]. These CAM therapies have been accepted by most of the USA people [33]. The Trivedi Effect®also reported having a significant impact on the characteristic properties of the several living and non-living object(s), i.e., organic compounds, ceramic, metals, cancer cell lines, crops, microbes [34-39] etc.

The Trivedi Effect®-Consciousness Energy Healing Treatment also has the isotopic abundance ratio analysis [34, 40, 41]. The analysis of stable isotope ratio has various applications for the understanding of isotope effects resulting from the variation of the isotopic composition of the molecule [34, 42]. Isotope ratio analysis generally performed by using the conventional mass spectrometry techniques, *i.e.*, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), which can detect the low micromolar concentration with sufficient precision [42, 43]. Thus, in this study, the LC-MS and GC-MS analytical techniques were used to characterize the structural properties and evaluate the isotopic abundance ratio in the Treated berberine chloride compared with the control sample.

#### **Materials and Methods**

#### **Chemicals and Reagents**

The test compound berberine chloride hydrate (98.1% HPLC) was purchased from Tokyo Chemical Industry Co. Ltd., Japan, and other HPLC grade reagents like acetonitrile and formic acid were purchased from Merk, India.

## **ConsciousnessEnergy Healing Treatment Strategies**

The test sample berberine chloride was divided into two parts and termed as control and treated sample based on the type of treatment. One part of the berberine chloride was received the Trivedi Effect®-Consciousness Energy Healing Treatment under standard laboratory conditions by the renowned Biofield Energy Healer, Mahendra Kumar Trivedi, USA, known as the Biofield Energy Treated sample for 3 minutes. The other part of the sample did not receive the Biofield Energy Treatment but was treated with a "sham" healer who did not have any knowledge about the Biofield Energy Treatment. After the treatment, both the samples were kept in sealed conditions and characterized using LC-MS and GC-MS analytical techniques.

#### Characterization

# Liquid Chromatography-Mass Spectrometry (LC-MS) Analysisand Calculation of Isotopic Abundance Ratio

The LC-MS analysis of both the sample was performed with the help of LC-MS ThermoFisher Scientific, the USA, connected with an ion trap detector and triple-stage quadrupole mass spectrometer. A reversed phase Thermo Scientific Synchronis C18 (Length-250 mm X ID 4.6 mm X 5 micron) column was used (35°C). Acetonitrile and methanol were used as diluent for the sample preparation. 5  $\mu L$  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 in gradient condition (total run time 10 min). Peaks were monitored at 210 nm using the PDA detector. The mass spectrometric analysis was performed in +ve ESI mode.

The natural abundance of each isotope (C,H,N, and 0) 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 [40,44-46]. The LC-MS based isotopic abundance ratio  $(P_{M+1}/P_M)$  for the control and treated berberine was calculated using equation (1).

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

 $\label{eq:Where IAR} Where IAR_{\text{Treated}} = isotopic abundance \ ratio \ in the \ treated \ sample \\ and \ IAR_{\text{Control}} = isotopic \ abundance \ ratio \ in the \ control \ sample.$ 

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

GC-MS of both the samples was analyzed with the help of Perkin Elmer GC 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 (+ve mode). The oven temperature was 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 change in the GC-MS based isotopic abundance ratio  $(P_{\rm M+1}/P_{\rm M})$  of the treated berberine chloride was calculated compared to the control sample using equation (1).

## **Results and Discussion**

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

The chromatograms of both the samples of berberine chloride showed a single major chromatographic peak at retention time (R<sub>.</sub>) of ~2 minutes (Figure 1). The peak area of the Biofield Energy Treated berberine was significantly increased by 4.42% compared to the control sample. This indicated that the solubility of treated

berberine was significantly increased, which may improve the bioavailability of berberine compared to the control sample. The mass spectra of berberine exhibited the mass of the molecular ion peak at m/z 336.3 [M]<sup>+</sup> (calculated for  $C_{20}H_{18}NO_4+$ , 336.12) along with other fragmentation peak at 321 (C<sub>19</sub>H<sub>15</sub>NO<sub>4</sub><sup>2+</sup>) in both the spectra (Figures 2&3). The mass spectra of berberine show the molecular peak [M] $^+$  at m/z 336.3 in the mass spectrum [47].

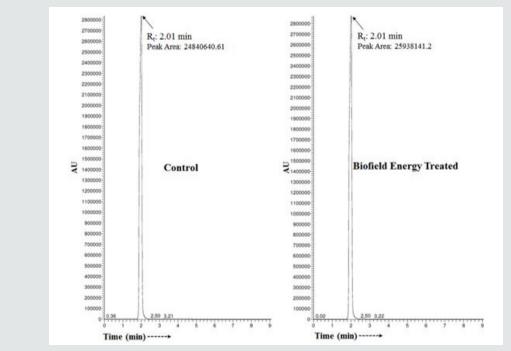


Figure 1: Liquid chromatograms of the control and Biofield Energy Treated berberine.

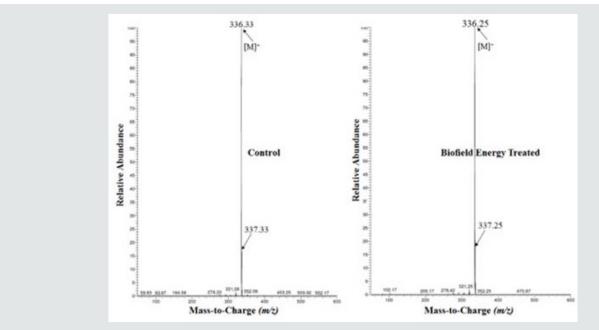


Figure 2: Mass spectra of the control and treated berberine at R,~2 minutes.

Figure 3: Proposed fragmentation pattern of berberine.

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

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

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

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

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

 $P_{M+1}$  i.e. <sup>13</sup>C, <sup>2</sup>H, <sup>15</sup>N, and <sup>17</sup>O contributions from  $(C_{20}H_{18}NO_4)^+$  to

m/z 337 = 22.83%

From the above calculation, it has been found that  $^{13}\text{C}$ , and  $^{15}\text{N}$  have the major contribution to m/z 337. Based on the LC-MS data, the isotopic abundance ratio analysis  $P_{\text{M}}$  and  $P_{\text{M+1}}$  near m/z 336 [M\*] and 337 [(M+1)\*] of the control and treated berberine samples were evaluated (Table 1). The isotopic abundance ratio ( $P_{\text{M+1}}/P_{\text{M}}$ ) in the treated berberine was significantly increased by 34.4% compared with the control sample (Table 1). Thus, it was concluded that the  $^{13}\text{C}$ ,  $^{2}\text{H}$ ,  $^{15}\text{N}$ , and  $^{17}\text{O}$  contributions from ( $C_{20}H_{18}\text{NO}_{4}$ )\* to m/z 337 in the Biofield Energy Treated berberine was significantly increased compared to the control sample.

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

| Parameter   | Control Sample | Biofield Energy Treated Sample |
|---|----------------|--------------------------------|
| P <sub>M</sub> at <i>m/z</i> 336 (%)  | 100.00         | 100.00                         |
| $P_{M+1}$ at $m/z$ 337 (%)  | 15.03          | 20.2                           |
| $P_{M+1}/P_{M}$   | 0.15           | 0.20                           |
| % Change of isotopic abundance ratio $(P_{M+1}/P_M)$ with respect to the control sample |                | 34.40                          |

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

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

The control and treated berberine showed a sharp chromatographic peak at  $R_{\rm t}$  21.5 minutes in the chromatograms

(Figures 4&5). The parent molecular ion peak of berberine at m/z 334 [M]\* (calculated for  $\rm C_{20}H_{16}NO_4^{~3+}$ , 334.11) was observed in both the samples, along with the lower mass fragment ion peaks (Figures 4&5).

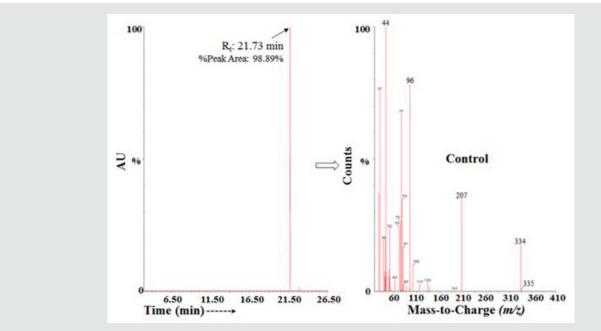


Figure 4: The GC-MS chromatogram and mass spectra of the control berberine.

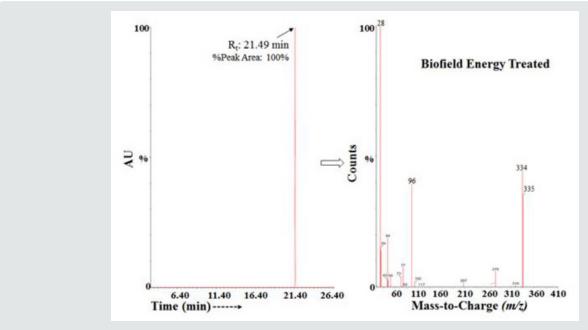


Figure 5: The GC-MS chromatogram and mass spectra of the Biofield Energy Treated berberine.

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

 $P(^{13}C) = [(20 \times 1.1\%) \times 17.63\% \text{ (the actual size of the M}^+ \text{ peak)}]$  / 100% = 3.88%

 $P(^{2}H) = [(16 \times 0.015\%) \times 17.63\%] / 100\% = 0.04\%$ 

 $P(^{15}N) = [(1 \times 0.4\%) \times 17.63\%] / 100\% = 0.07\%$ 

 $P(^{17}O) = [(4 \times 0.04\%) \times 17.63\%] / 100\% = 0.03\%$ 

 $\rm P_{M+1,}$   $i.e.^{13}C,~^2H,~^{15}N,~and^{17}O$  contributions from  $\rm (C_{20}H_{16}NO_4)^{3+}$  to m/z~335=4.02%

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

The GC-MS based isotopic abundance ratio analysis of the treated berberine was calculated compared to the control sample.  $P_{M'}$  and  $P_{M+1}$  for berberine near m/z 334 [M+] and 335 [(M+1)+],

respectively, of both the samples were calculated (Table 2). The isotopic abundance ratio of  $P_{\tiny M+1}/P_{\tiny M}$  in the treated berberine was very significantly increased by 1547.15% 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 treated sample were significantly increased compared with the control sample.

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

| Parameter   | Control Sample | Biofield Energy<br>Treated Sample |
|---|----------------|-----------------------------------|
| P <sub>M</sub> at m/z 334 (%)   | 17.63          | 44.40                             |
| P <sub>M+1</sub> at m/z 335 (%)   | 0.85           | 35.26                             |
| $P_{M+1}/P_{M}$   | 0.05           | 0.79                              |
| % Change of isotopic abundance ratio $(P_{M+1}/P_{M})$ with respect to the control sample |                | 1547.15                           |

 $P_{M}$ : the relative peak intensity of the parent molecular ion [M<sup>+</sup>];  $P_{M+1}$ : the relative peak intensity of the isotopic molecular ion [(M+1)<sup>+</sup>]; M: mass of the parent molecule.

The structure of berberine was confirmed by chromatographic and spectroscopic study. The isotopic abundance ratio of  $P_{M+1}/P_{M}$  $(^{2}H/^{1}H \text{ or }^{13}C/^{12}C \text{ or }^{15}N/^{14}N \text{ or }^{17}O/^{16}O)$  in the treated berberine was significantly altered compared to the control sample. The altered isotopic composition in the treated berberine might be due to the altered neutron to proton ratio in the nucleus *via* the interference of neutrino particles done by the Trivedi Effect®-Consciousness Energy Healing Treatment. Neutrino is an elementary particle that interacts via the weak subatomic force and gravity [48]. The neutrinos can change identities which specified a close relation between neutrino and the isotope formation [26 40,41]. The isotopic abundance ratio  ${}^2H/{}^1H$  or  ${}^{13}C/{}^{12}C$  or  ${}^{15}N/{}^{14}N$  or  ${}^{17}O/{}^{16}O$ would highly influence the atomic bond vibration and strength of the Biofield Energy Treated berberine chloride [49]. The increased isotopic abundance ratio  $(P_{M+1}/P_M)$  of the Consciousness Energy Healing Treated berberine chloride may significantly increase the chemical bond strength, improve its stability, solubility, and bioavailability in the body. The Trivedi Effect®-Consciousness Energy Healing Treatment might create a new form of berberine chloride, which would be better for the prevention and treatment of diarrhea, gastroenteritis, bacterial, fungal, and other microbial infections. It would be helpful for the treatment of cancer, arrhythmia, diabetes, hyperlipidemia, and inflammation also.

#### Conclusion

The Consciousness Energy Healing Treatment showed a significant impact on the isotopic abundance ratio of berberine chloride. The LC-MS spectra of both the samples observed at retention time 2.0 minutes and the molecular ion peak at m/z 336.25 [M] $^+$  in the mass spectra. The peak area of the Biofield

Energy Treated berberine was significantly increased by 4.42% compared to the control sample. The LC-MS based isotopic abundance ratio of  $P_{M+1}/P_{M}$  in the Biofield Energy Treated berberine was significantly increased by 34.4% compared with the control sample. Similarly, the GC-MS based isotopic abundance ratio of P<sub>M+1</sub>/P<sub>M</sub> in the Biofield Energy Treated berberine was very increased by 1547.15% compared with the control sample. Thus, 13C, 2H, 15N, and <sup>17</sup>O contributions to m/z 337 in the Biofield Energy Treated sample were significantly increased compared with the control sample. The isotopic abundance ratio of P<sub>M+1</sub>/P<sub>M</sub> (<sup>2</sup>H/<sup>1</sup>H or <sup>13</sup>C/<sup>12</sup>C or  $^{15}N/^{14}N$  or  $^{17}O/^{16}O)$  in the Biofield Energy Treated berberine was significantly altered compared to the control sample. The increased isotopic abundance might occur due to the interference of neutrino particles via the Trivedi Effect®-Consciousness Energy Healing Treatment. The increased isotopic abundance ratio of the Consciousness Energy Healing Treated berberine chloride may increase the chemical bond strength, stability, solubility, and bioavailability in the body. The new form of berberine chloride would be more efficacious novel pharmaceutical formulations against diarrhoea, gastroenteritis, bacterial and fungal infections, cancer, diabetes, arrhythmia, inflammation, hyperlipidemia, etc.

## Acknowledgements

The authors are grateful to Sophisticated Instrumentation Centre for Applied Research & Testing (SICART) India, Trivedi Science, Trivedi Global, Inc., and Trivedi Master Wellness for their assistance and support during this work.

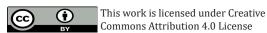
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DOI: 10.32474/OAJCAM.2021.03.000152



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