Journal of Pharmacy and Pharmaceutics

OMMEGA Publishers
Answering Intelligence

ISSN: 2377-1313
OPEN ACCESS

Research article

DOI: 10.15436/2377-1313.20.3025

The LC-MS and GC-MS Based Isotopic Abundance Ratio Analysis of the Biofield Energy Treated Metronidazole

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Abstract

Metronidazole is a well-known antibacterial and antiprotozoal antibiotic. In this study, the impact of the Trivedi Effect®-Consciousness Energy Healing Treatment on the structural properties and the isotopic abundance ratio of metronidazole were evaluated using LC-MS and GC-MS spectroscopy. The test sample (metronidazole) was divided into two control and treated parts. Only the treated sample received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely by a renowned Biofield Energy Healer, Dahryn Trivedi. The LC-MS spectra of both the samples of metronidazole at the retention time 2.61 minutes exhibited the mass of the protonated molecular ion peak at *m/z* 172 $[M+H]^+$, but the peak area of the treated metronidazole was significantly decreased by 13.1% compared to the control sample. The LC-MS and GC-MS based isotopic abundance ratios of P_{M+1}/P_M in the treated metronidazole was significantly increased by 46.43% and 13.52%, respectively compared with the control sample. Thus, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_{10}N_3O_3)^+$ to *m/z* 173 in the treated sample were significantly increased compared with the control sample. The increased isotopic composition in the molecular level of the treated metronidazole might be due to the alteration in neutron to proton ratio in the nucleus possibly through the interference of neutrino *via* the Trivedi Effect®. The isotopic abundance ratios $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{17}O/^{16}O$, and $^{15}N/^{14}N$ would highly influence the atomic bond vibration of treated metronidazole. The increased isotopic abundance ratio of the treated metronidazole would stronger the chemical bond and increase the stability in the body. The treated metronidazole would be very useful to design more efficacious pharmaceutical formulations that might offer better therapeutic response against bacterial vaginosis, giardiasis, trichomoniasis, pseudomembranous colitis, pelvic inflammatory disease;liver, skin, brain, and respiratory tract infections; aspiration pneumonia, rosacea, intra-abdominal infections, lung abscess, fungating wounds, periodontitis, amoebiasis, oral infections, etc.

Keywords: Metronidazole, Biofield Energy, The Trivedi Effect®, Consciousness Energy Healing Treatment, LC-MS, GC-MS.

Introduction

Over 50 years, metronidazole is used clinically as anantimicrobial antibiotic. Metronidazole inhibits the microorganism by means of disturbing the DNA of microbial cells. It has a little effect on human cells or aerobic bacteria[1,2]. It is used in the treatment of bacterial vaginosis, giardiasis, trichomoniasis, pseudomembranous colitis, pelvic inflammatory disease, brain, and respiratory tract infection, aspiration pneumonia, rosacea, fungating wounds, intra-abdominal infections, lung abscess, periodontitis, amoebiasis, and oral infections[2-5]. It is also used to treat *Giardia* in animals[2-6]. The common side effects linked with the metronidazole therapy are nausea, vomiting, dizziness, headache, diarrhoea, weight loss, abdominal pain, metallic taste in the mouth, thrombophlebitis, hypersensitivity reactions, stomatitis, central nervous system toxicity, paraesthesia, *etc*.[2,7]. Metronidazole is available in the form of metronidazole benzoate in the liquid suspension as it is bitter. It has high oral bioavailability. It is also delivered in the form of a tablet, capsule, and intravenous injection[7-9]. It is hazardous to the skin and eye on inhalation and ingestion. The solubility profile of metronidazole is very poor. It is very slightly soluble in water, alcohol, chloroform, dilute acid, and dimethylformamide[10,11].

Received date: January 20, 2021
Accepted date: March 15, 2021
Published date: March 19, 2021

Citation: Trivedi, D., et al. The LC-MS and GC-MS Based Isotopic Abundance Ratio Analysis of the Biofield Energy Treated Metronidazole (2021) J pharma pharmaceutics 7(1): 1-6.

The physicochemical properties of the pharmaceutical ingredients havean essential role in its dissolution, absorption, and bioavailability in the biological system[12]. In this scenario, it was found that the Trivedi Effect®-Consciousness Energy Healing Treatment has a significant impact on the physicochemical propertiesof pharmaceutical and nutraceutical compounds[13-17]. The Trivedi Effect® is a scientifically proven natural phenomenon in which anindividual can harness this energy from the Universe and transfer it anywhere on the planet *via* the possible mediation of neutrinos[18]. The "Biofield Energy" the electromagnetic energy field generated by the continuous movement of the electrically charged particles (ions, cells, *etc*.) inside the body. The Biofield Energy Healing specialists have the ability to harness the energy from the environment or the "Universal Energy Field" and can transmit into any living and non-living object(s) and this method of transfer is called Biofield Energy Healing Treatment[19-21]. The Biofield based Energy Therapies have been reported with significant outcomes against various disease[22]. The National Center of Complementary and Integrative Health has accepted Biofield Energy Healing treatment as a Complementary and Alternative Medicine health care approach in addition to other therapies, medicines, yoga, Qi Gong, Tai Chi, Reiki, hypnotherapy, *etc*. [23,24]. These type therapies have been accepted by most of the U.S.A. people[24]. The Trivedi Effect®-Biofield Energy Healing Treatment has also been proved scientifically with outstanding performance in the fields of materials science[25,26], chemical science[27,28], agricultural science[29,30], microbiology[31,32], cancer research[33,34], *etc*. The Trivedi Effect®-Biofield Energy Healing Treatment can be an economical approach to resolve the problems associated with metronidazole and its pharmaceuticals formulations. The stable isotope ratio analysis has various applications in different scientific fields for understanding the isotope effects resulting from the variation of the isotopic composition of the molecule[35,36]. In this study, the isotope ratio analysis can be performed by using the conventional mass spectrometry (MS) techniques such as gas chromatography - mass spectrometry (GC-MS) and liquid chromatography - mass spectrometry (LC-MS). The LC-MS and GC-MS can detect the isotope composition in low micromolar concentration with sufficient precision[35,37]. Thus, LC-MS and GC-MS based isotopic abundance ratio analysis of P_{M+1}/P_M ($^2H/^1H$ or $^{13}C/^{12}C$ or $^{17}O/^{16}O$ or $^{15}N/^{14}N$) and P_{M+2}/P_M ($^{18}O/^{16}O$) in the metronidazole was evaluated.

Materials and Methods

Chemicals and Reagents

The test sample metronidazole was purchased from Tokyo Chemical Industry Co., Ltd., Japan and other chemicals used in the experiments were purchased in India.

Consciousness Energy Healing Treatment Strategies

The test sample metronidazole powder was divided into two parts and termed as control and treated sample. The control metronidazole powder did not receive the Biofield Energy Treatment, but treated with a "sham" healer, who did not have any knowledge about the Trivedi Effect®. However, the treated metronidazole was received the Trivedi Effect®-Consciousness Energy Healing Treatment remotely for 3 minutes by the renowned Biofield Energy Healer, Dahryn Trivedi, USA. After the treatment both the samples of metronidazole 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 samples of metronidazole was carried out with the help of LC-MS Thermo Fisher Scientific, the USA equipped with anion trap detector connected with a triple-stage quadrupole mass spectrometer. The column used here was a reversed phase Thermo Scientific Synchronis C18 (Length-250 mm XID 4.6 mm X 5 micron), maintained at 25ºC. 10 µL of metronidazole solution (methanol used as diluent) was injected and the analyte was eluted using 5% 10 mM ammonium formate (pH 3.5 with formic acid) (mobile phase A; 5%) and acetonitrile (mobile phase B; 95%) 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 300 nm using the PDA detector. The mass spectrometric analysis was performed under +ve ESI mode.

The experimental abundance of each isotope (C, O, H, and N) 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[36,38-40]. The LC-MS based isotopic abundance ratios (P_{M+1}/P_M) for the control and Biofield Energy Treated metronidazole was calculated using equation 1.

$$\% \text{ change in isotopic abundance ratio} = [(IAR_{Treated} - IAR_{Control})/IAR_{Control})] \times 100 \quad (1)$$

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 both the samples of metronidazole were analyzed with the help of Perkin Elmer Gas chromatograph equipped with a PE-5MS (30M x250 microsx0.250 microns) capillary column and coupled to a single quadrupole mass detector was operated with electron impact (EI) ionization in positive mode. The oven temperature was programmed from 75°C (5 min hold) to 280ºC (14 min hold) @ 10°C /min (total run time 40 min). The sample was prepared taking 60 mg of the metronidazole is in 3 mL acetonitrile as a diluent. Mass spectra were scanned from *m/z* 20 to 400. 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) for the control and Biofield Energy Treated metronidazole were calculated using equation 1.

Results and Discussion

LiquidChromatography-Mass Spectrometry (LC-MS)

The LC-MS chromatograms of both the metronidazole showed the single largest chromatographic peak at the retention time (R_t) of 2.62 minutes (Figure 1). But, the results indicated that the peak area of the Biofield Energy Treated metronidazole was significantly decreased by 13.1% compared to the control sample.

This result indicated that the solubility of the Biofield Energy Treated metronidazole was reduced compared to the control sample. The previous study also showed that the Biofield Energy Treated metronidazole increased the crystallite sizes, particle sizes, and thermal stability compared with the control sample[41]. The reduced surface area of the treated metronidazole may reduce the solubilty profile compared to the control sample, which was supported by the peak area results.

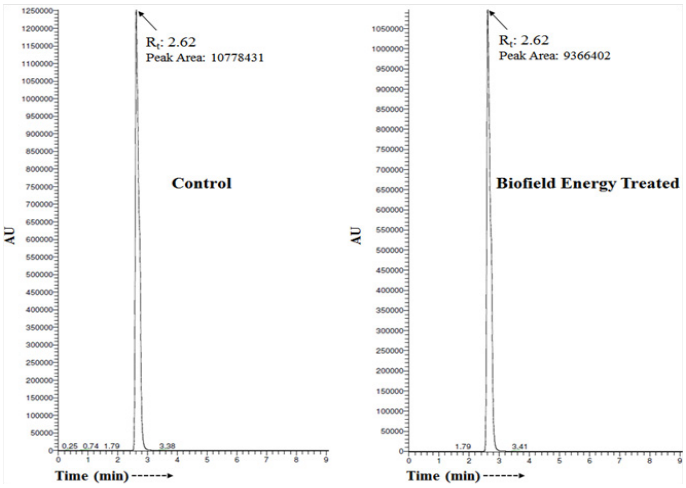

Figure 1: Liquid chromatograms of the control and Biofield Energy Treated metronidazole.

Metronidazole shows the molecular mass peak $[M]^+$ at *m/z* 171 MS spectrum in positive ion mode[42]. The mass spectra of both the samples of metronidazole (Figure 2) exhibited the mass of the protonated molecular ion peak at *m/z* 172 $[M+H]^+$ (calculated for $C_6H_{10}N_3O_3^+$, 172.07) (Figure 3).

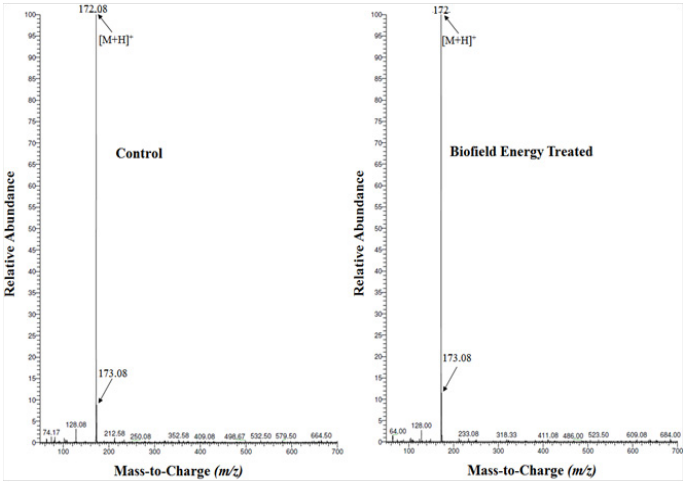

Figure 2: Mass spectra of the control and Biofield Energy Treatedmetronidazole at R_t 2.62 minutes.

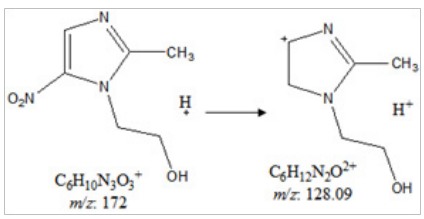

Figure 3: Proposed fragmentation pattern of metronidazole.

The LC-ESI-MS spectra of both the metronidazole showed the mass of the molecular ion peak at *m/z* 172 $[M+H]^+$ (calculated for $C_6H_{10}N_3O_3^+$, 172.07) with relative intensity of 100%. The theoretical calculation of P_{M+1} for metronidazole was presented as below:

$P(^{13}C)$ = [(6 x 1.1%) x 100% (the actual size of the M+ peak)] / 100% = 6.6%

$P(^{2}H)$ = [(10 x 0.015%) x 100%] / 100%= 0.15%

$P(^{15}N)$ = [(3 x 0.4%) x 100%] / 100% = 1.2%

$P(^{17}O)$ = [(3 x 0.04%) x 100%] / 100% = 0.12%

P_{M+1}, i.e. ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_{10}N_3O_3)^+$ to *m/z* 173= 8.07%

From the above calculation, it has been found that ^{13}C and ^{15}N have major contribution to *m/z* 173.

The LC-MS based isotopic abundance ratio of the Biofield Energy Treated metronidazole was calculated compared to the control sample. The P_M and P_{M+1} for metronidazole near *m/z* 172 and 173, respectively were obtained from the observed relative peak intensities of $[M^+]$ and $[(M+1)^+]$ peaks, respectively in both the spectra (Table 1). The percentage change of the isotopic abundance ratio (P_{M+1}/P_M) in the treated metronidazole (0.08) was significantly increased by 46.43% compared with the control sample (0.06). Thus, it can be concluded that the ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_{10}N_3O_3)^+$ to *m/z* 173 in the treated metronidazole were significantly increased compared to the control sample.

Table 1: LC-MS based isotopic abundance analysis results of metronidazole in the treated sample compared to the control sample.

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 172 (%)	100	100
P_{M+1} at *m/z* 173 (%)	5.75	8.42
P_{M+1}/P_M	0.06	0.08
% Change of isotopic abundance ratio (P_{M+1}/P_M) compared to the control sample		46.43

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.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC chromatograms of both the samples showed the presence of a single chromatographic peak (Figures 4 and 5). The retention times of both the samples were close to each other (16.4 minutes). The parent molecular peak of metronidazole at *m/z* 171 $[M]^+$ (calculated for $C_6H_9N_3O_3^+$, 171.06) along with the fragment ion peaks near *m/z* 154, 124, 96, and 81 were proposed corresponded to the molecular formula $C_6H_8N_3O_2^+$, $C_6H_9N_2O^+$, $C_5H_8N_2^+$, and $C_4H_5N_2^+$, respectively (Figures 4 & 5). The mass peak intensities influence the isotopic abundance ratio, which

was well supported by the LC-MS data.

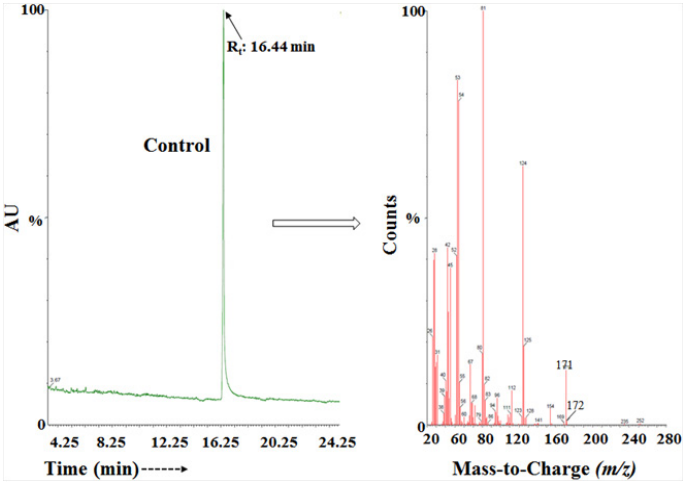

Figure 4: The GC-MS chromatogram and mass spectra of the control metronidazole.

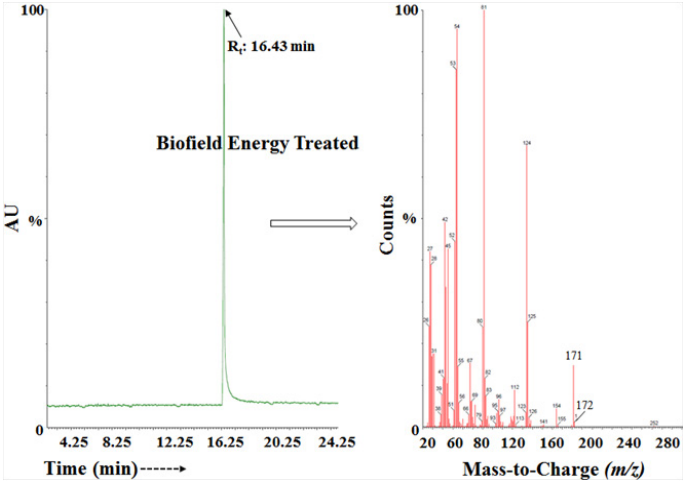

Figure 5: The GC-MS chromatogram and mass spectra of the Biofield Energy Treated metronidazole.

The GC-MS spectra of both the samples of metronidazole showed the mass of the molecular ion peak $[M]^+$ at *m/z* 171 (calculated for $C_6H_9N_3O_3^+$, 171.06). The theoretical calculation of P_{M+1} for metronidazole was presented as below:

$P(^{13}C) = [(6 \times 1.1\%) \times 13.22\%$ (the actual size of the M+ peak)] / 100% = 0.87%

$P(^{2}H) = [(9 \times 0.015\%) \times 13.22\%] / 100\% = 0.018\%$

$P(^{15}N) = [(3 \times 0.4\%) \times 13.22\%] / 100\% = 0.16\%$

$P(^{17}O) = [(3 \times 0.04\%) \times 13.22\%] / 100\% = 0.016\%$

P_{M+1}, i.e. ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_9N_3O_3)^+$ to *m/z* 172 = 1.06%

From the above calculation, it has been found that ^{13}C and ^{15}N have major contribution to *m/z* 172. The calculated isotopic abundances (1.06%) were close to the experimentally observed value (0.92%).

The GC-MS based isotopic abundance ratio was calculated. The P_M and P_{M+1} for metronidazole near *m/z* 171 and 172, respectively were obtained from the observed relative peak intensities of $[M^+]$ and $[(M+1)^+]$, respectively in the mass spectra (Table 2). The isotopic abundance ratio of P_{M+1}/P_M in the Biofield Energy Treated metronidazole was significantly increased by 13.52% compared with the control sample (Table 2). Hence, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_9N_3O_3)^+$ to *m/z* 172 in the Biofield Energy Treated metronidazole were significantly increased compared with the control sample.

Table 2: GC-MS based isotopic abundance analysis results of metronidazole in control and Biofield Energy Treated samples.

Parameter	Control sample	Biofield Energy Treated sample
P_M at *m/z* 171 (%)	13.22	14.81
P_{M+1} at *m/z* 172 (%)	0.92	1.17
P_{M+1}/P_M	0.07	0.08
% Change of isotopic abundance ratio (P_{M+1}/P_M) with respect to the control sample		13.52

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.

The LC-MS and GC-MS based isotopic abundance ratio of P_{M+1}/P_M ($^{2}H/^{1}H$ or $^{13}C/^{12}C$ or $^{15}N/^{14}N$ or $^{17}O/^{16}O$) in the Biofield Energy Treated metronidazole was significantly increased compared to the control sample. The natural abundance and relative proportion of the stable isotopes significantly affected by the environment, climate, *etc*.[43]. The increased isotopic composition in the molecular level of the Consciousness Energy Healing Treated metronidazole might be due to the alteration in neutron to proton ratio in the nucleus possibly through the interference of neutrino particles *via* the Trivedi Effect®. The neutrinos have the ability to interact with protons and neutrons in the nucleus, which indicated a close relation between neutrino and the isotope formation[18,36,37]. The isotopic abundance ratios $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{17}O/^{16}O$, and $^{15}N/^{14}N$ would highly influence the atomic bond vibration of treated metronidazole. The increased isotopic abundance ratio of the Consciousness Energy Healing Treated metronidazole would stronger the chemical bond and increase the stability in the body[43-44]. The new form of metronidazole (Biofield Energy Treated) would be very useful to design more efficacious pharmaceutical formulations that might offer better therapeutic response against bacterial and protozoal infection in the vagina (bacterial vaginosis), stomach (giardiasis, trichomoniasis, pseudomembranous colitis), joints (pelvic inflammatory disease), liver, skin, brain, and respiratory tract, aspiration pneumonia, rosacea, intra-abdominal infections, lung abscess, fungating wounds, periodontitis, amoebiasis, oral infections, *etc*.

Conclusions

The Trivedi Effect®-Consciousness Energy Healing Treatment showed a significant impact on the isotopic abundance ratios and mass peak intensities of metronidazole. The LC-MS spectra of both the samples of metronidazole at the retention time

2.61 minutes exhibited the mass of the protonated molecular ion peak at *m/z* 172 $[M+H]^+$, but the peak area of the Biofield Energy Treated metronidazole was significantly decreased by 13.1% compared to the control sample. The LC-MS and GC-MS based isotopic abundance ratios of P_{M+1}/P_M in the Biofield Energy Treated metronidazole was significantly increased by 46.43% and 13.52%, respectively compared with the control sample. Thus, ^{13}C, ^{2}H, ^{15}N, and ^{17}O contributions from $(C_6H_{10}N_3O_3)^+$ to *m/z* 173 in the Biofield Energy Treated sample were significantly increased compared with the control sample. The increased isotopic composition in the molecular level of the Consciousness Energy Healing Treated metronidazole might be due to the alteration in neutron to proton ratio in the nucleus possibly through the interference of neutrino *via* the Trivedi Effect®. The isotopic abundance ratios $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{17}O/^{16}O$, and $^{15}N/^{14}N$ would highly influence the atomic bond vibration of the Biofield Energy Treated metronidazole. The increased isotopic abundance ratio of the Biofield Energy Treated metronidazole would stronger the chemical bond and increase the stability in the body. The Biofield Energy Treated metronidazole would be very useful to design more efficacious pharmaceutical formulations that might offer better therapeutic response against bacterial and protozoal infection in the vagina (bacterial vaginosis), stomach (giardiasis, trichomoniasis, pseudomembranous colitis), joints (pelvic inflammatory disease), liver, skin, brain, and respiratory tract, aspiration pneumonia, rosacea, intra-abdominal infections, lung abscess, fungating wounds, periodontitis, amoebiasis, oral infections, *etc*.

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

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