Nat. Volatiles & Essent. Oils, 2021; 8(4): 16558-16571

NVEO
Natural Volatiles &
Essential Oils

Simultaneous Determination Of Carbamazepine, Benzodiazepine In Perfluorodecalin By HPLC And GC-MS Techniques

Sangeeta Verma[1,*] , Ashutosh Singh[2] , Priti Singh[2] , Neena Srivastava[3] and Abhishek Singh[4]

[1]Department of Chemistry, Shri Jai Narain Misra Post Graduate College, Lucknow- 226001, India.

[2]Department of Chemistry, K.S. Saket P.G. College, Ayodhya-224001, India.

[3]Department of Chemistry, Mahila Vidyalaya Degree College, Lucknow-226018, India.

[4]Department of Chemistry, U.P. College, Varanasi-221002, India.

ABSTRACT

Lennox-Gastaut Syndrome (LGS) is commonly characterized by a triad of features including multiple seizure types, intellectual disability or regression. The long-term prognosis for LGS is generally poor due to uncontrolled seizures. During the time of such abnormal vibrations, both the seizures and the lungs suffer a lack in oxygen content to a considerable extent. These result in prolonged vibrations and loss nervous control. As a neuro-lung protective strategy, a novel attempt has been made to enrich both seizures and lungs with oxygen content through the support of Perfluorodecalin (an excellent oxygen carrier) $C_{10}F_{18}$ (PFD) along with an enhancement in the antiepileptic activity by the two chosen antiepileptic drugs (AEDs) Carbamazepine (CBZ) and Benzodiazepine (BDZ). High Performance Liquid Chromatography (HPLC), Gas Chromatography – Mass Spectrometry (GC-MS) and Energy dispersive X-ray Spectroscopy (EDS) studies on the emulsion produced by the sonication of both CBZ+PFD, BDZ+PFD has been made to explore the possibility of multiple number of fluorine atoms to be physically present though not as complete organized chemical reaction. The enrichment of oxygen content and the antiepileptic activity is expected to be taken care by both PFD and the chosen drug respectively.

Keywords: Antiepileptic drugs; Seizures; Perfluorodecalin; Oxygen carrier; High Performance Liquid Chromatography; Gas Chromatography – Mass Spectrometry.

1. INTRODUCTION

Lennox-Gastaut type of syndrome is complex epilepsy occurring due to abnormality in seizures. Lennox-Gastaut type of seizures that affects the Central Nervous System (CNS), are facing drug administration problems. Control of seizures is difficult [1-5]. An unexpected disharmony in seizures results in epilepsy.

Drugs that are most preferred and prescribed for such kind of disease causes adverse side effects. Further any new drug for that matter faces such problems and their effectiveness on the affected seizures is still an unsolved problem [6-12].

Though the valid reasons for the abnormal behavior of seizures, at any particular time, are still a mystery, there seems to be an in adequacy in the supply of oxygen content to both the seizures and lungs. Patients affected by such problems, happens to find it difficult to breath freely, tends to lose the nervous balance. Seizures, at the time of abnormal vibrations, too lack in oxygen content and hence the abnormal vibrations prolongs for a longer period of time [13-17].

In the present work, as a Neuro-Lung protective strategy, a novel attempt has been made through the phenomenon of oxygen enrichment to both seizures (brain) and lungs simultaneously with the support of Perfluorodecalin $C_{10}F_{18}$ (PFD). At the same time the antiepileptic activity is expected to enhance through the chosen antiepileptic drugs (AEDs), Carbamazepine (CBZ) and Benzodiazepine (BDZ). Though Valproate, Topiramate and Lamotrigine are to be first line drugs, CBZ and BDZ are chosen for this study because of their simple chemical structure **Figures 1(a), 1(b)** respectively and could be more adoptive to accommodate such fluorinated compounds.

To achieve this, either one has to come out with a novel drug containing Perfluorodecalin (PFD) and the suitable Antiepileptic Drugs (AEDs), both as single organized structure (in the form of a single crystal) or try to convert both the drug and the Perfluorodecalin (PFD) into an emulsified mixture and determine simultaneously their predominant contents through Chromatographic and EDS analysis. David S.Hage et al.,[18-19] have made quite a good number of studies using chromatographic analysis, to prove it to be a powerful technique for such works.

The above first idea is purely optional because, PFD is absolutely neutral by structure **Figure 1 (c)**. It is quite difficult to break any of its bonds and to make it reactive with either CBZ or BDZ. Though the neutral structure of PFD is not so conducive for perfect chemical reactions, the basic reason for choosing $C_{10}F_{18}$ is due to the fact that it is an excellent oxygen carrier and chemically it is highly electronegative. The role of PFD in Cardiopulmonary by- pass, lung ventilation, ventilation fluids, cell-culture supplement, diagnostic imaging agents, drug formulation and targeting agents were extensively proved [20-25]. Hence the choice of Perfluorodecalin is thus justified.

Addition of fluorine atom to any drug not only helps to enrich the oxygen content at the active site but also a) reduces the toxicity of the drug b) increases the solubility c) reduces the melting point d) more adoptive structure and so on [26-27]. Apart from this capacity, addition of one or more number of fluorine atoms in any basic structure could drastically enhance its biological activity.

In spite of all these applications their perfect neutral chemical structure is not supportive for any chemical reaction with any compounds. The only effective way to use them is to convert them into an emulsion. There are decent numbers of studies substantiating the excellent oxygen carrying capacity of PFD in emulsified form [28-35]. Based on the above said facts the second idea is more vulnerable.

2. EXPERIMENTAL

2.1. Materials and Methods

Carbamazepine (CBZ), Benzodiazepine (BDZ) and Perfluorodecalin (PFD) used were generic 99% pure from sigma-Aldrich. They were utilized as received without any further purification. HPLC grade Acetonitrile and Deionized water obtained from Merck scientific Inc was used for HPLC analysis.

The study involves three steps. In the first step, the emulsion of 10 mL of perfluorodecalin (PFD) were prepared by sonication using a probe sonicator, PCI model KS-250F. The sonication was performed with 5 KHz sweep frequency for about two minutes, in continuous phases and duty cycle at 40%, keeping the tube immersed in dry-ice cage to avoid the over-heating of the sample during sonication process. Surfactants were deliberately avoided. The emulsion is then mixed with 10 mg of CBZ and BDZ exclusively and sonicated for another one minute with above mentioned specifications. The two sets of emulsion were preserved at -15^0C in a cryostat for ten days. To create thermal agitation in the mixture the sample is removed from the cryostat and placed at room temperature at every two days interval.

In the second step, a part of the CBZ+PFD, BDZ+PFD emulsion were exclusively diluted. The remaining part is allowed to undergo slow evaporation. Once they form powder it is dried using nitrogen incubator and separately dried at nearly 100^0C for about a minute to get complete dry powder. In the third step, the diluted samples were subjected to HPLC and GC-MS analysis. The obtained powder has been subjected to EDS analysis. The interaction of Perfluorodecalin (PFD) and the drugs were investigated using HPLC, GC-MS and EDS studies.

2.2. Apparatus

Chromatographic Analysis was performed by Shimadzu make LC-2010 RP-HPLC –LC-2010 HT module instrument. The chromatographic system consisted of an isocratic, gradient pump and UV100 absorbance detector. Chromatographic Data were collected and processed using a Lab solution LC-solution. The GC-MS analysis was examined by GC-MS – Bruker CP-8410 GC with SCION detector has been used. The EDS analysis was performed in TESCAN microscope at the operating voltage of 10-20 kV. The images were processed with VEGA 3 software system and then probed.

2.3. Chromatographic and GC-MS Procedures

Chromatographic separation was performed using X-terra C18G, 250mmx4.6mm, 5µm column. The mobile phases were composed of Acetonitrile, Deionized water in the ratio of 40:60 v/v. In order to make the dissolution more sensitive and accurate, the mobile phase is filtered through 0.22µm membrane filter paper and degassed by sonication for 15minutes. Samples were injected at a constant flow rate of 1 mL/min for 15 minutes. The column was maintained at 25^0C and 20µL of samples were injected onto the column. The U-V wavelength set for CBZ at 215nm and BDZ at 240 nm.

GC-MS is a powerful qualitative technique for the determination of mass. The retention time was set to 20 minutes. The column was set to BR-5ms, 15mx0.25mm and the ID DF = 0.25, 5% Phenyl and 95% Dimethyl Polysiloxan. The injection volume was 1 µL and the ionization was Electron impact ionization with the ionization-energy to be 70eV.

3. RESULTS AND DISCUSSION

3.1. HPLC Chromatogram Analysis

Optimization of separation condition for CBZ+PFD, BDZ+PFD emulsion was carried out in HPLC system. The specifications were mentioned in the previous section. Since we have only two components to be separated, we choose gradient elution. **Figures 2, 3** represent the chromatograms of CBZ+PFD and BDZ+PFD emulsion respectively. The chromatogram exhibits a single sharp tall peak at a retention time of 2.837 for CBZ+PFD **(Figure.2)**. The retention time of CBZ is generally above 6 [19, 36-38], whereas for retention time of PFD is 1.78. The interesting fact is that, PFD is insoluble in any of the solvent which are widely used for HPLC studies. The sharp tall peak at Retention Time (R.T) is 2.837 almost lies between the R.T of antiepileptic drugs, Nitrazepam is 2.74 and Chlorobenzodiazepine is 3.72 [39, 40]. This is an indication of the halogen like physical presence of adequate fluorine content along with the drug content which is the most anticipated result.

In the case of BDZ+PFD, we do with the same conditions of optimization. In this case too a single sharp tall peak is observed at Retention Time (R.T) is 1.432 **(Figure.3)**. This again lies between the R.T of antiepileptic drugs Chlordiazepoxide (1.32) and Bromazepem (1.76) [39], which is close to that of PFD, indicating the physical presence of halogen like substance specifically fluorine in this case.

3.2. Gas Chromatography – Mass Spectrometry (GC-MS) Analysis:

Figure 4 and 5 represents the Gas chromatogram of CBZ+PFD and BDZ+PFD emulsion respectively.

Figure 6 and 7 represents the Mass-Spectrogram of CBZ+PFD and BDZ+PFD emulsion respectively. The ultimate aim of this study is to identify at least the physical presence of adequate Fluorine content along with the drug. Since HPLC is not much suitable for PFD, GC-MS study has been made. Interestingly, the drug part is identified by the Gas-chromatography and the PFD (Fluorine) part identified by Mass-spectrograph.

The Gas Chromatogram (GC) peak identified at 11.7minutes for both CBZ+PFD and BDZ+PFD mixtures **(Figure 4, 5)** [41, 42] were pertaining to drug. There exist multiple peaks in the case of BDZ. This may be due to the fact that BDZ shows much involvement in substituting more of its Hydrogen by Fluorine.

The Mass Spectrogram (MS) peak observed at nearly 150 is the fragment of $C_{10}F_{18}$ (C_3F_6) as shown in **Figures 6 and 7**. This supports the adequate presence of fluorine content which is the most expected result. Though the completed organised chemical reaction is much difficult, the co-existence of both PFD and the drugs are very much evident.

3.3. Energy dispersive X-ray Spectroscopy (EDS) Analysis

HPLC and GC-MS result are much complementing with each other, the first is more drug sensitive and the second is more PFD sensitive. EDS spectrums of CBZ+PFD and BDZ+PFD emulsion were reported in **Figure 8 and 9** respectively. To doubly confirm the physical presence of both the drug and PFD together we perform EDS. The report is very much enthusiastic and highly encouraging (**Figures. 8, 9**). There is a distinct indication of a) excess Carbon content (both drug and PFD) b) Nitrogen and Oxygen of the drug and c) the presence of Fluorine in CBZ+PFD and BDZ+PFD mixtures.

From these analyses, the co-existence of excess fluorine and drug ensure tremendous oxygen uptake by both the anatomical system and the drug thus protecting Neuro-Lung. This is in turn supports the Neuro-Lung protective strategy.

4. CONCLUSION

The expectation of both Perfluorodecalin along with any of the Antiepileptic drugs as a single organized crystal with a definite structure is farfetched. This is because of the basic fact that Perfluorodecalin possess a highly neutral structure. But its high electro negativity helps to co-exist with any chosen antiepileptic drug. Its physical presence along with the drug is unto itself a great support for the result to be achieved. That is, incorporation of one or more fluorine atoms into a compound or to be physically present along with the compound can have a dramatic effect on its chemical and physical properties, and fluorinated molecules are of considerable significance in a delivering more oxygen content to affected parts of the body and also to the lungs. This intention is well established by the above said results.

The difficulty in identifying a complete chemical activity is may be due to the fact that PFD is a perfect non-polar liquid, whereas our drug is a polar solid. Hence an attempt to come out with a neuro-lung protective strategy, that involves enrichment of both seizures and lungs with oxygen content through the support of perfluorodecalin (an excellent oxygen carrier) $C_{10}F_{18}$ (PFD) along with an enhancement in the antiepileptic activity should complement the control of prolonged an harmonic vibrations specifically in Lennox-Gas taut type of seizures.

REFERENCES

1. A.P. Aldenkamp, M. De Krom, R.Rejis, Newer antiepileptic drugs and cognitive issues, Epilepsia, 44: 21-29 (2003).

2. S. C. Tomp, J. W.Weber, A.P.Aldenkamp, J.Arends, I.Vander Linden, L.Diepman, Relative Influence of Epileptic Seizures and of Epilepsy Syndrome on Cognitive Function, Journal of Child Neurology, 18:407-412 (2003).

3. M.Feely, Drug treatment of epilepsy, British Medical Journal, 318: 106-109 (1999).

4. P. J. W. McKee, I. Percy-Robb, M.J. Brodie, Therapeutic drug monitoring improves seizure control and reduces anti-convulsant side effects in patients with refractory epilepsy, Seizure, 1(4): 275-279 (1992).

5. J. Vermeulen, A.P.Aldenkamp, Cognitive side-effects of chronic antiepileptic drug treatment; A review of 25 years of research, Epilepsy Research, 22: 65-95 (1995).

6. K. J. Meador, Networks Cognition and Epilepsy, Neurology, 77: 930-932 (2011).

7. P. C. Ross, J. O. Terence, J .K. Christine, R. M. Lachain, J. H. Rodney, A. M. Michael, C. B. Stephen, H. K. Andrew , Mark J.Cook, Temporal Lobe Epilepsy, Journal of Neurosurgery, 103(4): 768-769 (2005).
8. Norman Delanty, Jacqueline French, Treatment of Lennox-Gastaut Syndrome, CNS Drugs, 10(3): 181-188 (1998).

9. Aspasia Michoulas, Dr Kevin Farrell, Medical management of Lennox-Gastaut syndrome, CNS Drugs, 24 (5): 363-374 (2010).

10. Raman Sankar, $GABA_A$ Receptor Physiology and Its Relationship to the Mechanism of Action of the 1, 5-Benzodiazepine Clobazam, CNS Drugs, 26(3): 229-244 (2012).

11. Eyal Sobol , Meir Bialer , Boris Yagen, Tetramethylcyclopropyl Analogue of a Leading Antiepileptic Drug, Valproic Acid. Synthesis and Evaluation of Anticonvulsant Activity of Its Amide Derivatives, J. Medicinal Chemistry, 47 (17):4316-4326 (2004).

12. Lenkov DN, Volnova AB, Pope AR, Tsytsarev V. Advantages and limitations of brain imaging methods in the research of absence epilepsy in humans and animal models. J Neurosci. Methods. 30; 212(2):195-202, (2013).

13. Merce Falip, Xavier Salas-Pulg, Carlos Cara, Causes of CNS Inflammation and Potential Targets for Anticonvulsants, CNS Drugs, 27: 611-623 (2013).

14. V. M. Jerome, Arun Angelo Patil, Stimulation of the Nervous System for the Management of Seizures, CNS Drugs, 17(2): 101-115 (2003).

15. L. Brunbech, A.Sabers, Effect of Antiepileptic Drugs on Cognitive Function in individuals with epilepsy, Drugs, 62: 593-604 (2002).

16. Giangennaro Coppola, Treatment of Partial Seizures in Childhood, CNS Drugs, 18(3): 133-156 (2004).

17. Emilio Perucca, The Pharmacology of New Antiepileptic Drugs, CNS Drugs, 25(11): 909-912 (2011).

18. D.S. Hage, High-performance affinity chromatography: a powerful tool for studying serum protein binding, Journal of chromatography B, 768: 3-30 (2002).

19. Hee Seung Kim, and David S. Hage, Chromatographic analysis of carbamazepine binding to human serum albumin, Journal of chromatography B, 816: 57-66 (2005).

20. K C Lowe, Perfluorinated blood substitutes and artificial oxygen carriers, Blood Rev., 13:171-184 (1999).

21. Riess, J.G., Understanding the Fundamentals of Perfluorocarbons and Perfluorocarbon Emulsions Relevant to In Vivo Oxygen Delivery, Artif.Cells Blood Substit. Immobil. Biotechmoil.., 33: 47-63 (2005).

22. Belta, R., Blin, J.L., Stebe, M.J., Solubilization of Various Fluorocarbons in a Fluorinated Surfactant/Water System: Relation with the Design of Porous Materials, Journal of Physical chemistry B, 110: 23547-23556 (2006) .

23. Marsh JN, Senpan A, Hu G, Scott MJ, Gaffney PJ, Wickline SA, Lanza GM, Fibrin-targeted perfluorocarbon nanoparticles for targeted thrombolysis, Nanomedicine (Lond), 2(4): 533-43 (2007).

24. Jon N. Marsh, Kathryn C. Partlow, Dana R. Abendschein, Michael J. Scott, Gregory M. Lanza, and Samuel A. Wickline, Molecular Imaging with Targeted Perfluorocarbon Nanoparticles: Quantification of the Concentration Dependence of Contrast Enhancement for Binding to Sparse Cellular Epitopes, Ultrasound Med Biol., 33(6): 950-958 (2007).

25. Trung D Tran, Shelton D Caruthers, Michael Hughes, John N Marsh, Tillmann Cyrus, Patrick M Winter, Anne M Neubauer, Samuel A Wickline, Gregory M Lanza, Clinical applications of

perfluorocarbon nanoparticles for molecular imaging and targeted therapeutics., International Journal of Nanomedicine, 2(4): 515-26 (2007).

26. V. Natchimuthu, J. Amoros, S. Ravi, Fluorination of an Antiepileptic Drug: A self-supporting transporter by oxygen enrichment mechanism, Journal of Chemical Neuro- anatomy, 72: 8–15 (2016).

27. V. Natchimuthu, Srinivas Bandaru, Anuraj Nayarisseri, S. Ravi, Design, synthesis and computational evaluation of a novel intermediate salt of N-cyclohexyl-N-(cyclohexyl carbamoyl)-4-(trifluoromethyl) benzamide as potential potassium channel blocker in epileptic paroxysmal seizures, Computational Biology and Chemistry, 64: 64–73 (2016).

28. A. M. A. Dias, J. C. Pamies , J.A. P. Coutinho, I. M. Marrucho , L. F. J. Vega, SAFT Modeling of the Solubility of Gases in Perfluoroalkanes, Journal Physical Chemistry B, 108: 1450-1457 (2004).

29. A. M. A. Dias, M. G. Freire, J.A. P. Coutinho, I. M. Marrucho, Solubility of oxygen in liquid perfluorocarbons, Fluid Phase Equilibria, 222: 325-330 (2004).

30. C.Varescon, A. Manfredi, M.Le Blanc, J.G. Riess, Deviation from molecular diffusion aging model in fluorocarbon emulsions stabilized by perfluoroalkylated surfactants, Journal of Colloid and Interface Science 137:373-379 (1990).

31. A. M. A. Dias, A. I. Caco, J. A. P. Coutinho , M.M. Pineiro, L. F. J. Vega, M. F. Costa Gomes , I. M. Marrucho, Thermodynamic properties of perfluoro-n-octane, Fluid Phase Equilibria, 225: 39-47 (2004).

32. S. Ravi, J.Amoros, K. Arockia Jayalatha, Effective Method of Characterizing Specific Liquid Fluorocarbon Interactions Using Ultrasound, Journal of Physical Chemistry B, 112: 6420-6425 (2008).

33. Christopher A. Fraker, Armando J.Mendez, Cherie L.Stabler, Complementary Methods for the Determination of Dissolved Oxygen Content in Perfluorocarbon Emulsions and Other Solutions, Journal of Physical chemistry B, 115: 10547-10552 (2011).

34. M. G. Freire, A. M. A. Dias, A. Z. C. Maria, J. A. P. Coutinho, I. M. Marrucho, Aging mechanisms of perfluorocarbon emulsions using image analysis, Journal of Colloid and Interface Science, 286:224-232 (2005).

35. M. G. Freire, A. M. A. Dias, A. Z. C. Maria, J. A. P. Coutinho, I. M. Marrucho, Enzymatic method for determining oxygen solubility in perfluorocarbon emulsions, Fluid Phase Equilibria, 231: 109-113 (2005).

36. Chun-Lai Ma, Zheng Jiao, Yang Jie, Xiao-Jin Shi, Isocratic Reversed-Phase HPLC for Simultaneous Separation and Determination of Seven Antiepileptic Drugs and Two of their Active Metabolites in Human Plasma, Chromatographia 65: 267-275 (2007)

37. K.M. Patil, and S.L. Bodhankar, Simultaneous determination of lamotrigine, phenobarbitone, carbamazepine and phenytoin in human serum by high-performance liquid chromatography, Journal of Pharmaceutical and Biomedical Analysis, 39(1–2):181-186 (2005).

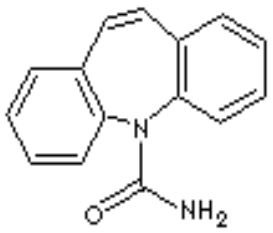
38. M. Meyler, M. T. Kelly, M. R. Smyth, New method for the determination of four antiepileptic drugs in human plasma by high performance liquid chromatography, Chromatographia 36(1): 27-32 (1993)

39. Květa Kalíková, Martina Riesová, Richard Chudoba, Martin G. Schmid, and Eva Tesařová, Separation and Quantification of 1, 4-benzodiazepines: HPLC versus CZE, Croat. Chem. Acta, 84 (3):367-373 (2011).

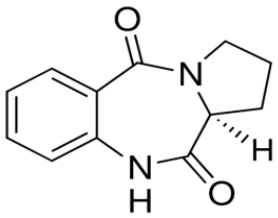
40. McClean S, O'Kane E, Hills J, Smyth W F., Determination of 1,4-benzodiazepines and their metabolites by capillary electrophoresis and high-performance liquid chromatography using ultraviolet and electrospray ionisation mass spectrometry, Journal of Chromatography A, 838(1-2):273-291 (1999).

41. Ioannis I. Papoutsis', Sotirios A. Athanaselis, Panagiota D. Nikolaou, Constantinos M. Pistos,Chara A.Spiliopoulou and Constantinos P. Maravelias, Development and validation of an EI–GC–MS method for the determination of benzodiazepine drugs and their metabolites in blood: Applications in clinical and forensic toxicology, Journal of Pharmaceutical and Biomedical Analysis, 52(4): 609-614 (2010).

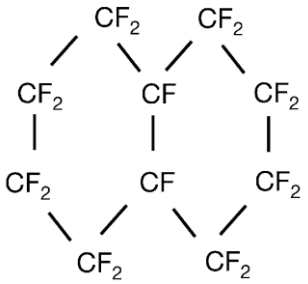
42. Michel Audran, Marie Pierre Krafft , Jacques De Ceaurriz, Jean-Charles Maturin, Marie-Thérèse Sicart, Bénédicte Marion, Guillaume Bougard and Françoise Bressolle., Determination of perfluorodecalin and perfluoro-N-methylcyclohexylpiperidine in rat blood by gas chromatography–mass spectrometry, Journal of Chromatography B, 745(2):333-343 (2000).



a) Carbamazepine



b) Benzodiazepine



c) Perfluorodecalin

Figure 1. (a-c): Chemical structure of a) Carbamazepine b) Benzodiazepine c) Perfluorodecalin

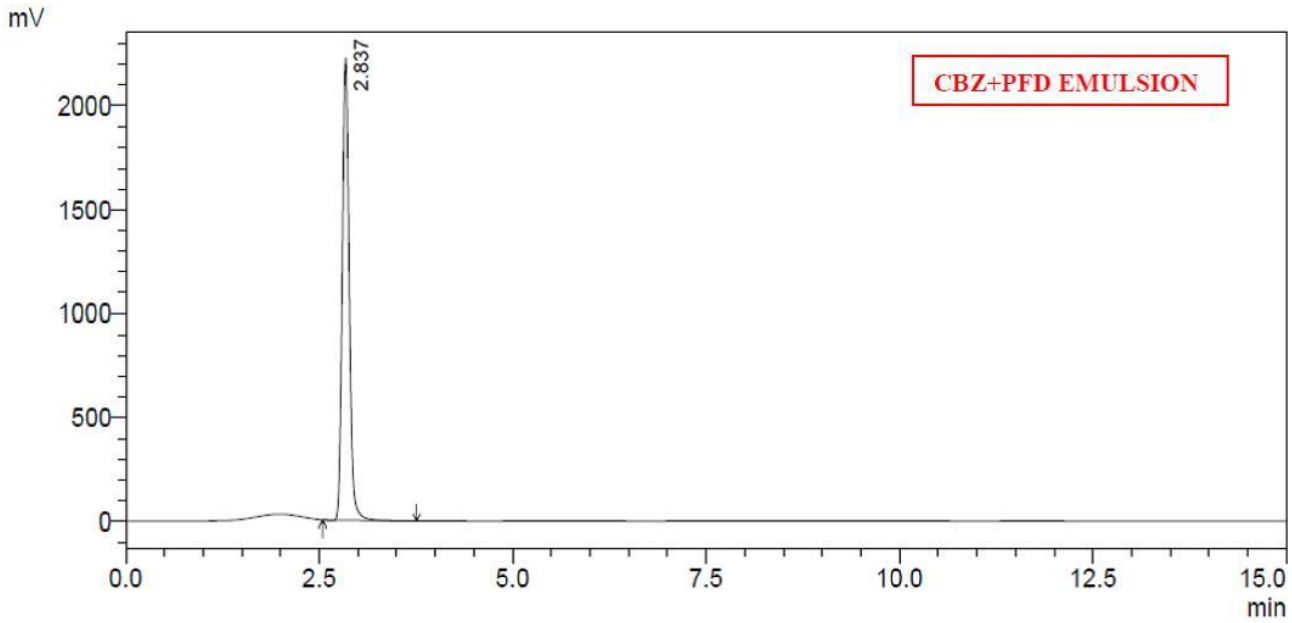


Figure 2: Typical Chromatography of CBZ+PFD Emulsion

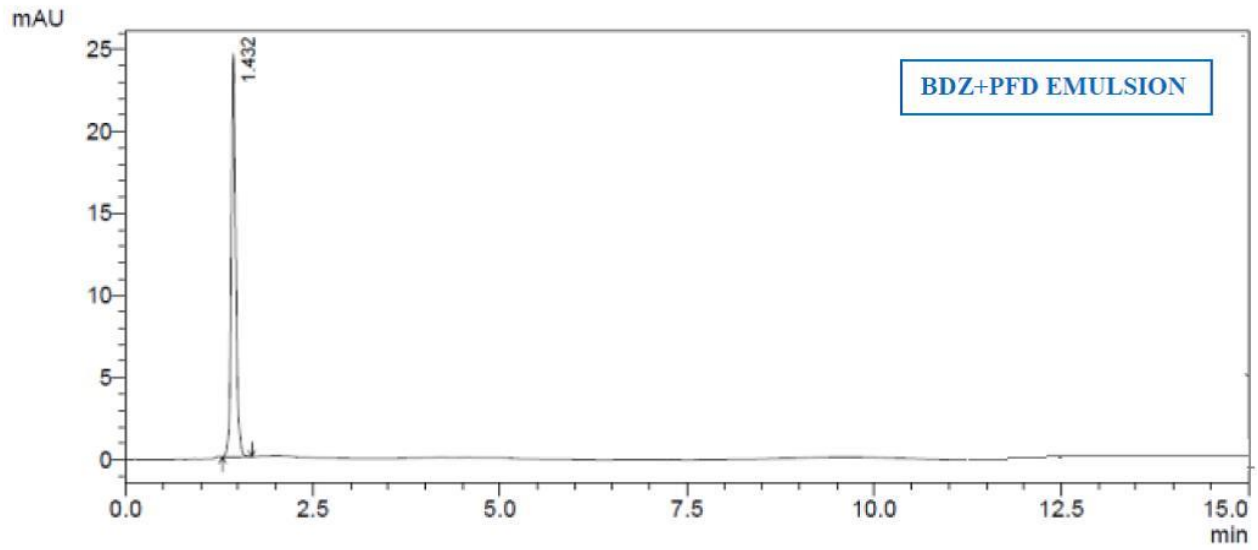


Figure 3: Typical Chromatography of BDZ +PFD Emulsion

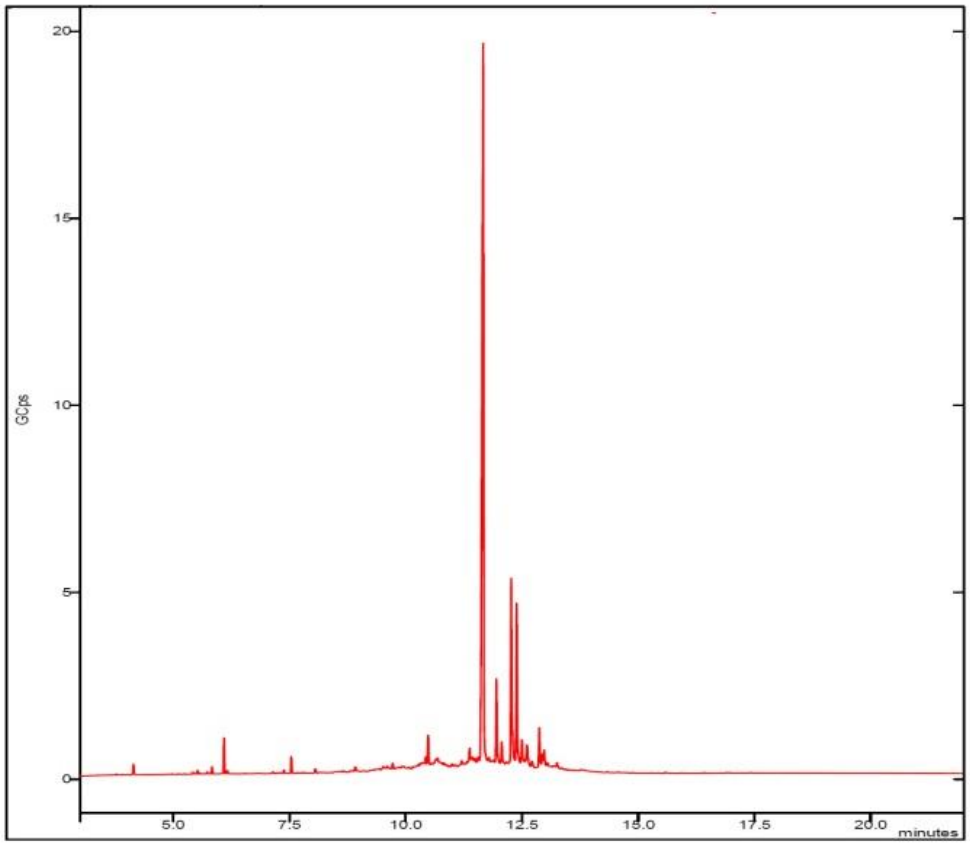


Figure 4: Gas Chromatogram of CBZ+PFD Emulsion

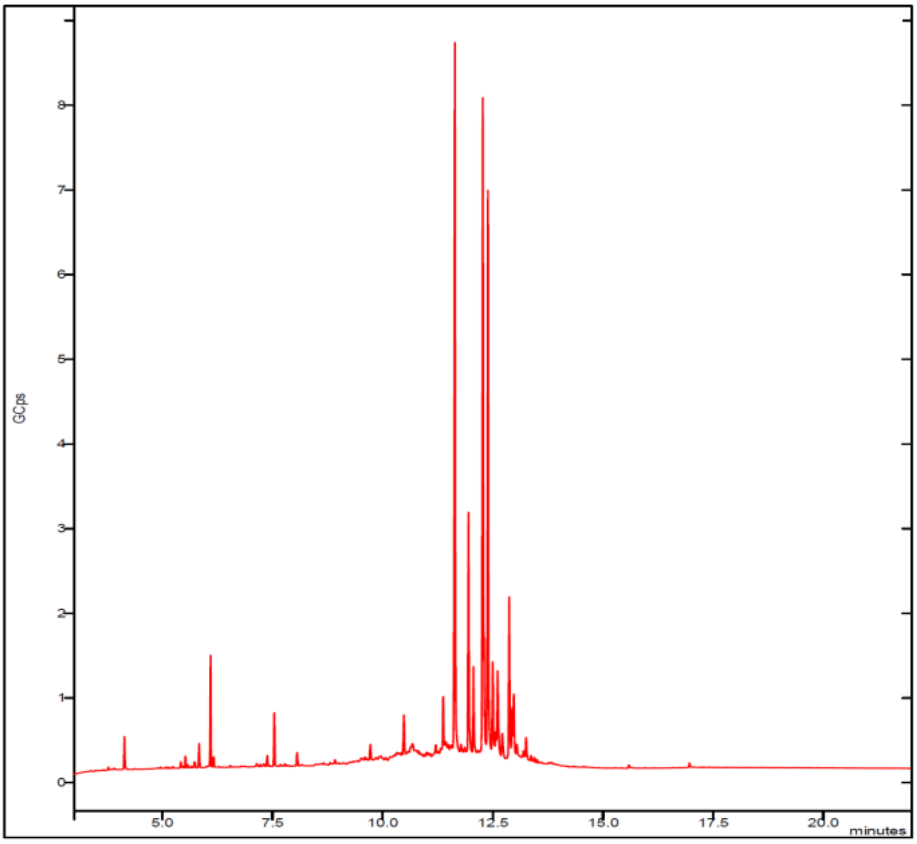


Figure 5: Gas Chromatogram of BDZ+PFD Emulsion

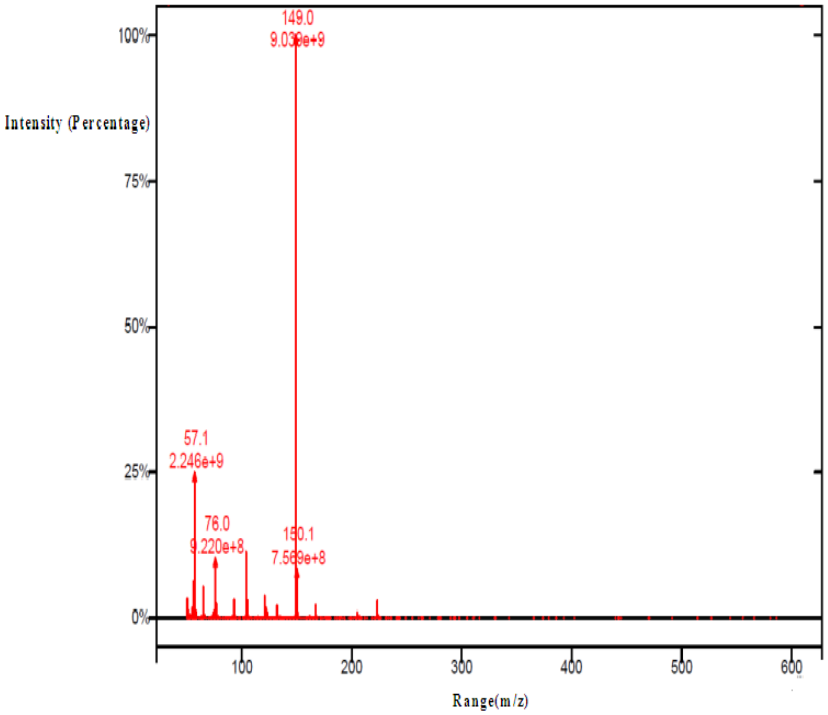


Figure 6: Mass-Spectrogram of CBZ+PFD Emulsion

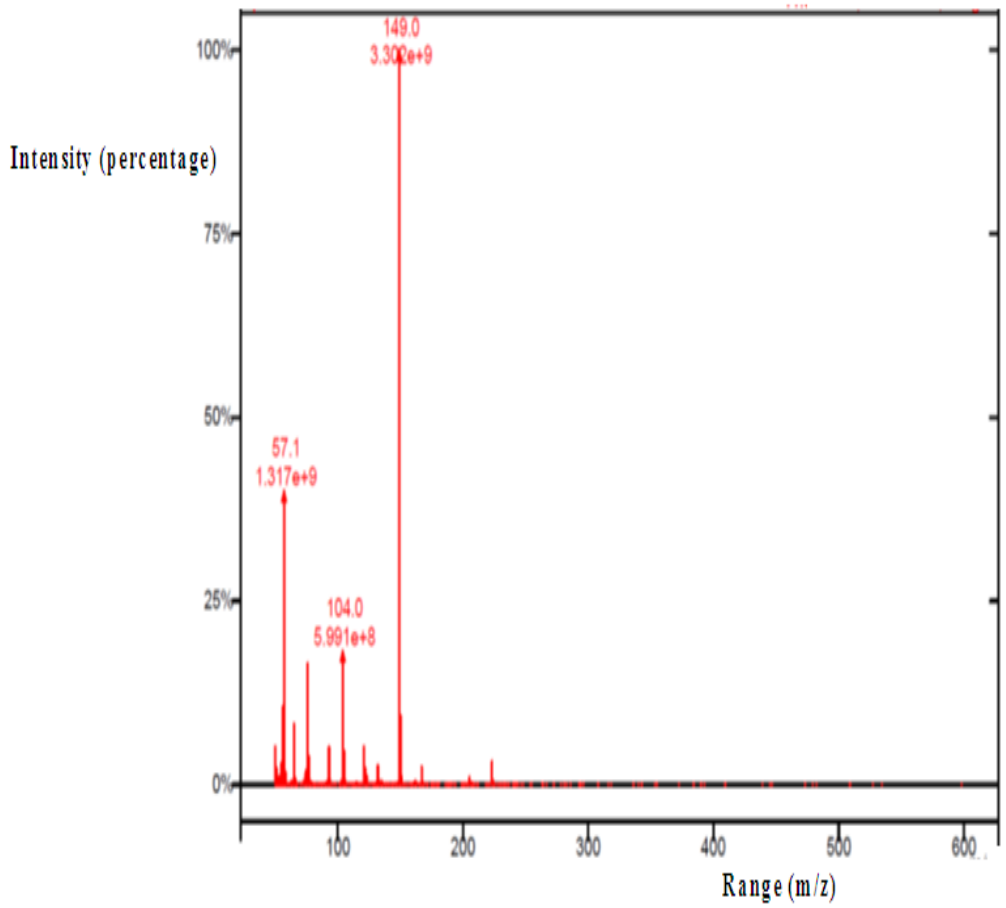


Figure 7: Mass-Spectrogram of BDZ+PFD Emulsion

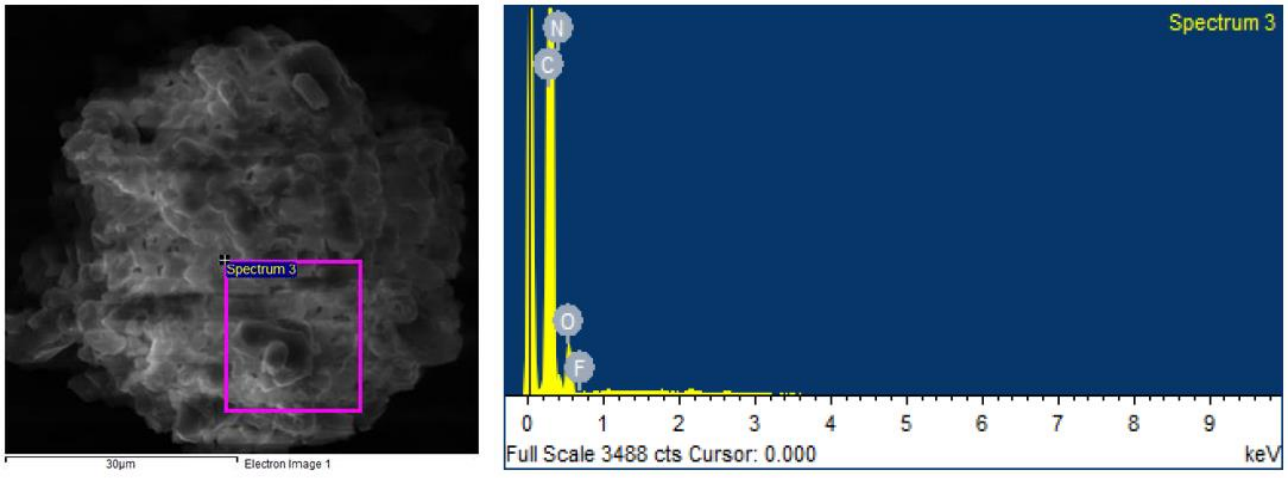


Figure 8: EDS spectrum of CBZ+PFD Emulsion

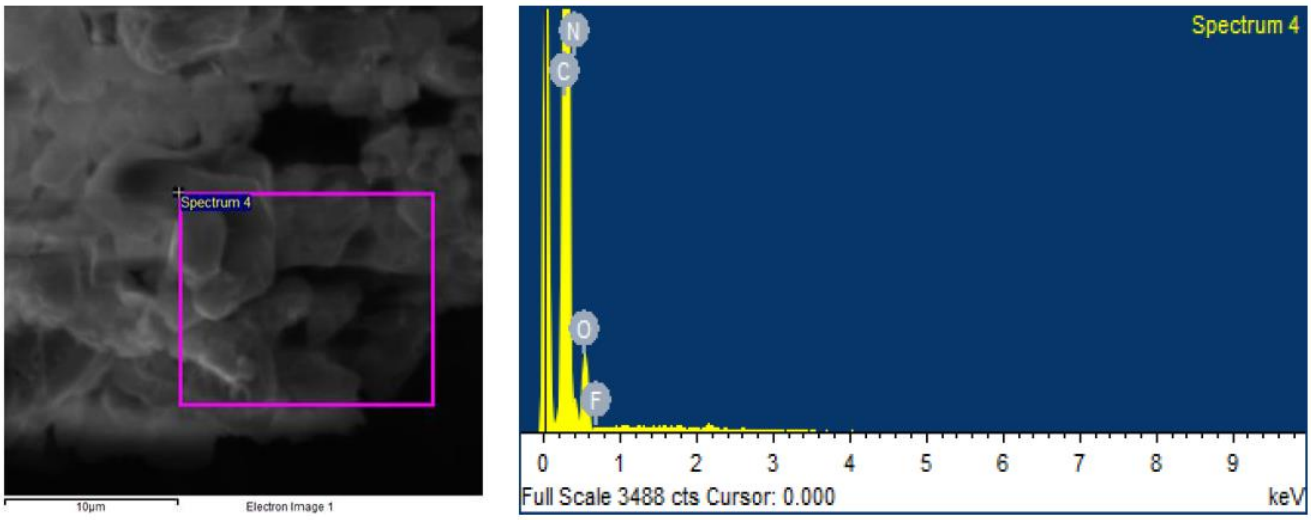


Figure 9: EDS spectrum of BDZ+PFD Emulsion