(RESEARCH ARTICLE)

Detection and mass spectral characterization of carbofuran and its degradation product

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Abstract

Carbofuran is an N-methyl carbamate pesticide that is extensively used to control insects and nematodes on a range of farming crops because of its wide-ranging biological activity & relatively low persistence when compared to other pesticides. It is highly toxic to birds, mammals, fish and wildlife due to its anticholinesterase activity. In humans, Carbofuran is associated with endocrine disrupting activity, reproductive disorders, cytotoxic and genotoxic abnormalities. In biological and environmental conditions Carbofuran tend to undergo degradation. The screening, identification and characterization of the Carbofuran and its degradation product in biological samples are an important task in the area of forensic toxicology. In a case study Carbofuran and its degradation product Carbofuran phenol were detected and identified by Gas Chromatography Mass Spectrometry. The present study deals with the successful detection & characterization of the Carbofuran and its degradation product under positive ion Electron Ionization (EI) mass spectrometry. The EI mass spectra of the Carbofuran and its degradation product showed the stable molecular ion [M$^{+\bullet}$] peaks in addition to the other diagnostic fragment ions that enable unambiguous identification of Carbofuran and its Carbofuran phenol. The general fragmentation patterns of these two compounds are discussed in detail. Carbofuran showed an ion at m/z 164 as the base peak corresponds to loss of Methylisocyanato group (CH$_3$NCO) from the [M$^{+\bullet}$] ion, which was formed by the Mclafferty rearrangement. Carbofuran phenol showed a more stable Molecular ion [M$^{+\bullet}$] peak compared to Carbofuran and showed a major peak at m/z 149 corresponds to the loss of methyl group from the [M$^{+\bullet}$] ion. The detection and identification of these compounds will be an invaluable support during investigation and in conviction of criminal cases in courts of law in Carbofuran poisoning cases.

Keywords: Carbofuran; Degradation; GC-MS; Mclafferty rearrangement; Carbofuran phenol

1. Introduction

Carbofuran is an organocarbamate insecticide, which is extensively used to control insects and nematodes on a range of farming crops because of its wide-ranging biological activity and relatively low persistence when compared to other Organochlorine pesticides [1]. Carbofuran technical or chemical name is 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, and its CAS number is 1563-66-2. The widespread use of pesticides/insecticides for farming and non-farming purposes causing ill health effect on living organisms and also responsible for the environmental contamination. Different poisons show different symptoms on human being. Carbofuran poisoning shows the symptoms such as nausea, vomiting, abdominal cramps, sweating, diarrhoea, excessive salivation, weakness, imbalance, blurred vision, breathing difficulty, increased blood pressure, and incontinence. At high doses, death may result from failure of the respiratory system [2, 3]. Nowadays, many household insecticides consist of carbamates and pyrethroids and thus poisoning involving them are on the rise, mostly among children. Poisoning due to other insecticides is also possible among individuals who are occupationally exposed. Owing to easy availability, pesticides such as organophosphates

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and carbamates have always been extremely popular in India for the purpose of accidental, suicidal and or homicidal poisoning [4, 5]. It is marketed under the trade name Furadan in India by FMC Corporation [6].

In cases of poisoning, the persons will be taken to the hospital and undergone treatment where sometimes the ingested poison gets degraded/metabolized very quickly and may not be present in its original form. So the identification of the degradation products (DPs) of the ingested poison conform the prior presence of its parent compounds. Hence it's very important to identify the degradation products of poisoning chemicals. Carbofuran can undergo degradation in biological, chemical and environmental conditions [2, 3 & 7]. The identification and characterization of the Carbofuran and its DPs play a very important role in forensic toxicology, which will be an invaluable support to the investigation agencies and in conviction of criminal cases in courts of law. Gas Chromatography Mass Spectrometry (GC-MS) combined with electron ionization technique has been well established for the detection, identification and characterization of various toxic chemicals, insecticides and their degradation products [8, 9 & 10]. In a case study, Carbofuran and its DP Carbofuran phenol were detected in the exhibit. The current study deals with the detail mass spectral study of Carbofuran and its DP by GC-MS under electron ionization conditions. The two compounds were well separated, identified and characterized by GC-MS.

2. Material and methods

2.1 Chemicals

The solvents and the chemicals used were purchased from Sd-Fine chemicals (Mumbai, India).Analytical grade solvents were obtained from E-Merck (Mumbai, India) and used for GC-MS analysis.

2.2 Gas Chromatography Mass spectrometry

The EI mass spectra were recorded on the Shimadzu Nexis GC-2030 equipped with a model GC-MS-QP2020 NX mass selective detector. A capillary column SH-Rxi-5Sil MS (length, 30m; film thickness, 0.25μm; i.d., 0.25mm) was used, and the column oven was programmed initially from 90 ℃ with 0.7 min hold up time with 35 ℃/min ramp up to 240 and then 8 ℃/min upto 290 and then 25 ℃/min to the final temperature 300 ℃ hold for 7 min (Total run time 18.64 min). The GC interface temperature was maintained at 280ºC. Helium was used as the carrier gas at a flow rate of 1ml/min. The typical EI-MS conditions were: electron energy 70eV; ion source temperature, 150 ℃; inlet temperature, 150 ℃; interface temperature, 260 ℃; quadrupole analyzer, 150 ℃. The mass spectrometer was scanned from a mass range of m/z 30-600.

2.3 Sample Preparations

The test sample was shaken by adding an equal amount of anhydrous sodium sulphate and transferred into a conical flask. The extraction of the sample carried out by adding 50 ml of n-Hexane to the sample taken in a flask and shaken vigorously. The contents were filtered and again extracted twice with 25 ml of n-Hexane and filtered. The filtered n-Hexane portions were combined taken into a separating funnel and shaken with 15 ml, 10 ml and 10 ml portions of acetonitrile which was previously saturated with n-Hexane. The acetonitrile layers were mixed, diluted with water and extracted thrice with 25ml portions of n-Hexane. The hexane layers were combined and concentrated to 2ml and used for the analysis.

3. Results and discussion

The aim of present study was to detect and characterize Carbofuran and its DP by GC-MS analysis. The extracted sample subjected for GC-MS analysis. The GC-MS total ion chromatogram (TIC) of the sample was shown in Figure 1. A total of two peaks were observed in the TIC of the test sample and identified as Carbofuran and its Carbofuran phenol.

The molecular structures of the Carbofuran and its Carbofuran phenol were shown in Figure 2 and these two compounds were well separated and each compound was eluted as a single peak in the GC-MS analyses. The GC retention times (RT) of Carbofuran and Carbofuran phenol were 5.68 min and 4.02 min respectively.
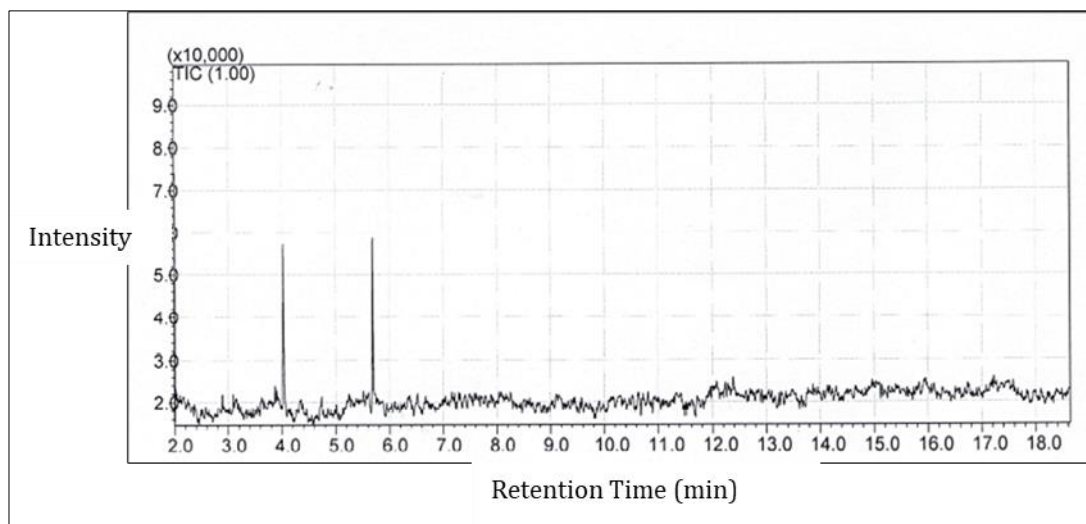
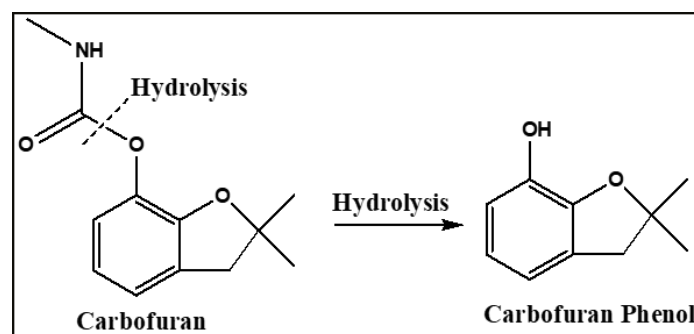**Figure 1** GC-MS total ion chromatogram (TIC) of the extracted sample

Figure 2 Possible Degradation pathway of Carbofuran

3.1   Mass spectral characterization of Carbofuran and its DP

Under EI conditions, Carbofuran and its DP were showed molecular ion ([M]$^{+\bullet}$) peak but Carbofuran phenol showed more abundant [M]$^{+\bullet}$ ion compared to Carbofuran. The EI mass spectra of the Carbofuran and its DP showed structure related fragment ions that enable unambiguous identification of these compounds (Figure 3 and 5).

3.2   GC-EI-MS analysis of Carbofuran Phenol

Carbofuran phenol could be formed by the hydrolysis of Carbofuran. Carbofuran phenol showed more abundant [M]$^{+\bullet}$ ion at m/z 164 under positive ion EI-MS conditions. The GC-EI mass spectrum of peak at RT 4.02 min shown in Figure 3.

The EI mass spectrum shown a major product ion at m/z 149 which corresponds to the loss of methyl radical and this ion confirm the presence of a methyl group in Carbofuran. The spectrum also showed an ion at m/z 131 corresponds to the loss of water molecule from m/z 149 and further loss of ethylene group results in the formation of other major ion at m/z 103. The spectrum also showed an ion at m/z 147 corresponds to the loss of hydroxyl radical from [M]$^{+\bullet}$ ion, which confirms the presence of –OH group in the molecule. In addition to this, Carbofuran phenol showed other ions at m/z 123 and m/z 122 which corresponds to the loss of C_3H_5 and C_3H_6 group respectively from the [M]$^{+\bullet}$ ion. The spectrum also showed an ion at m/z 107 corresponds to the loss of C_3H_5O moiety from [M]$^{+\bullet}$ ion or loss of C_2H_2O moiety from m/z 149 ion. In addition to these, the spectrum also showed other fragment ions with m/z 91 (loss of OH from m/z 108), 77 (loss of CH_2 from m/z 91), m/z 65 (loss of C_2H_2 from m/z 91), m/z 51 (loss of C_2H_2 from m/z 77), m/z 39 (loss of C_2H_2 from m/z 65) were also formed and these ions confirms the presence of aryl group in the molecule. All these proposed fragmentation pathways were depicted in Figure 4.
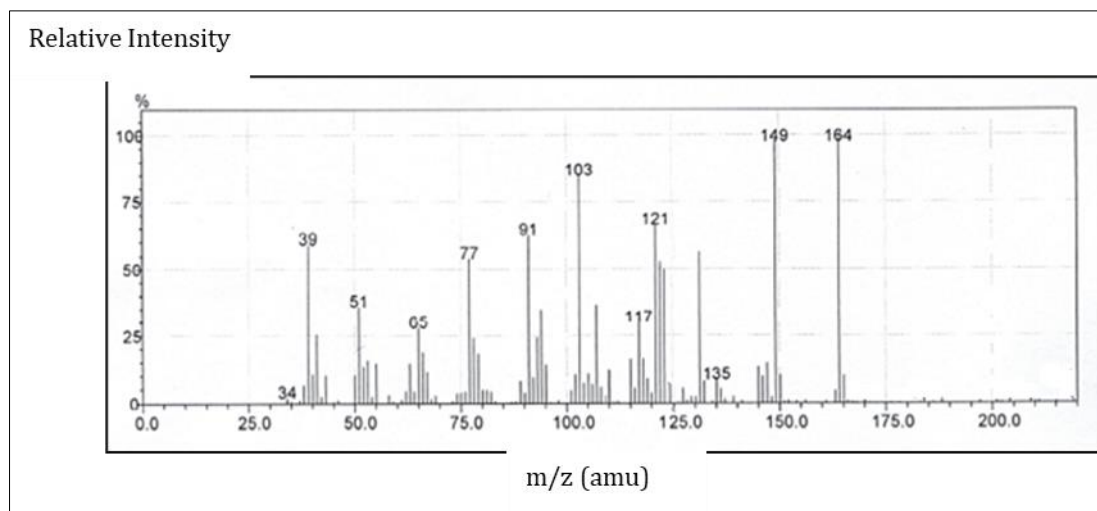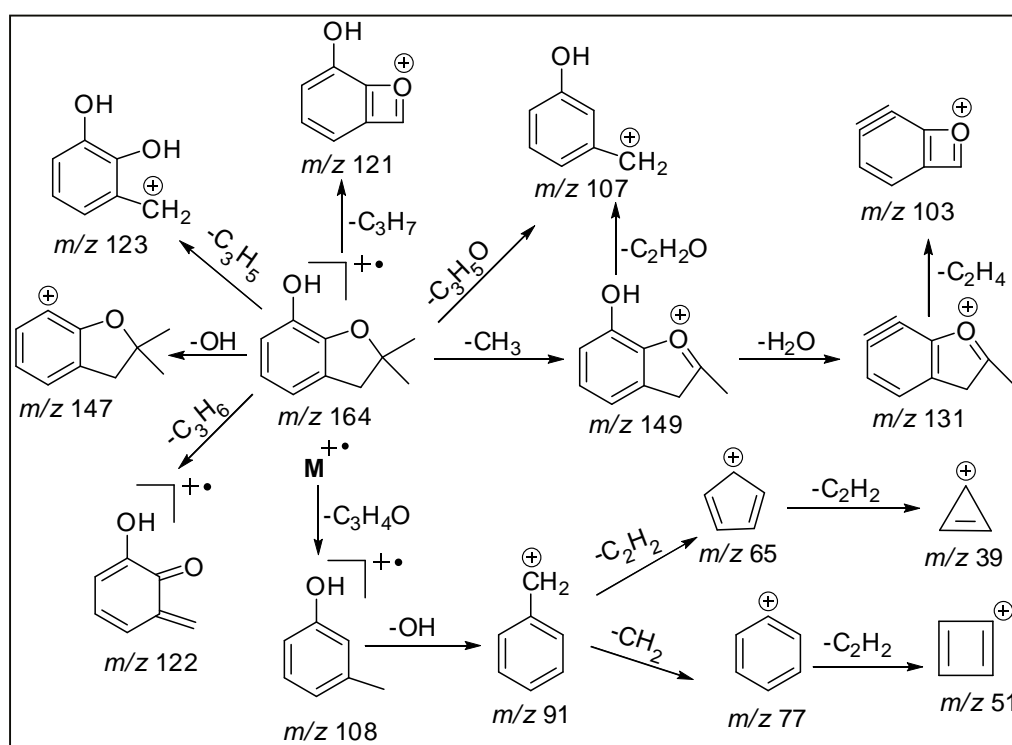
Figure 3 GC-EI-MS spectrum of Carbofuran phenol

Figure 4 GC-EI-MS proposed fragmentation pattern of Carbofuran phenol

3.3   GC-EI-MS analysis of Carbofuran

The GC-EI mass spectrum of the peak at RT 5.68 min shown in Figure 5. Carbofuran showed low abundant [M]$^{+\bullet}$ ion at m/z 221 under positive ion EI-MS conditions.

The spectrum of [M]$^{+\bullet}$ ion shown a major product ion at m/z 164 (Carbofuran phenol ion) which corresponds to the loss of CH_3NCO group, i.e. the characteristic Mclafferty fragmentation (γ hydrogen transfer to hetero atom followed α, β bond homolytic cleavage) confirm the presence of the N-methyl carbamate group in the molecule [11]. The spectrum also showed the ions at m/z 147 (loss of –OH group from m/z 164) and m/z 149 (of –CH_3 group from m/z 164) and these ions confirms the presence of hydroxyl group and methyl group in the molecule. Carbofuran showed other ions at m/z 123 ([M- C_3H_5]$^+$) m/z 122 ([M- C_3H_6]$^{+\bullet}$) and m/z 107 ([M- C_3H_5O]$^{+\bullet}$). In addition to these, the spectrum also showed other aryl group characteristic fragment ions at m/z 91, m/z 77, m/z 65, m/z 51 and m/z 39. All these proposed fragmentation pathways were depicted in Figure 6.
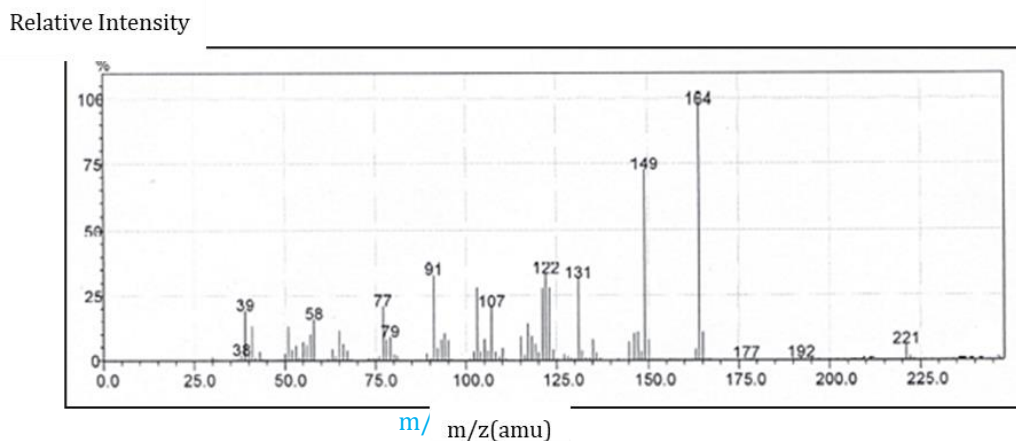
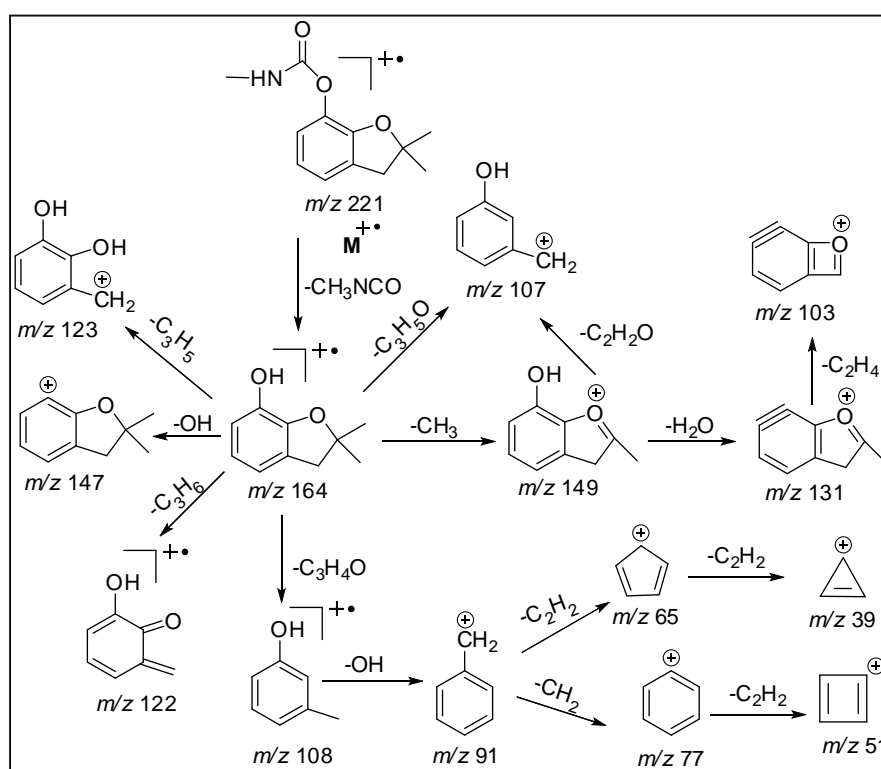**Figure 5** GC-EI-MS spectrum of Carbofuran

Figure 6 GC-EI-MS proposed fragmentation pattern of Carbofuran

List of Abbreviations

- GC-MS:
- Gas Chromatography Mass Spectrometry,
- EI: Electron Ionization,
- DP: Degradation product,
- TIC: Total ion chromatogram,
- [M$^{+\bullet}$]: Molecular ion.

4. Conclusion

Many poisoning cases were occurred due to the ingestion of carbamate insecticides either intentionally or accidentally. Carbofuran is widely used in paddy fields and it is one of the most toxic pesticides still in use. Carbofuran insecticide was prone to undergo degradation in various types of matrices. The detection of DPs in suspected samples confirm the

prior presence of its parent compounds. Therefore, the detection, identification and characterization of the DPs play a very important role in the area of forensic toxicology. In a case study, we have detected Carbofuran and its DP Carbofuran phenol by GC-MS. Here we also studied the mass spectral fragmentation pattern of Carbofuran and its DP and the detailed fragmentation pathways were proposed. Under electron ionization conditions the mass spectra of Carbofuran and its DP showed the molecular ion [M$^{+\bullet}$] in addition to the other diagnostic fragment ions that enable unambiguous identification of these compounds. The current study is very much useful in the detection and identification of Carbofuran and its DP and it will be an invaluable support during investigation and in conviction of criminal cases in courts of law in the cases of Carbofuran poisoning.

Compliance with ethical standards

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