Original Communication

Detection of Ethanol and Propoxur from whole Blood Samples by Gas Chromatography: A Case Study

Rakhi Agarwal,[a1] M. S. Dahiya[b1]

Abstract

Propoxur, carbamate compound is a non-systematic insecticide, classified as highly toxic substance to humans. It shows depletion in whole blood cholinesterase activity. Present work includes a case study on the detection (qualitative) of propoxur from whole blood samples of 21 victims. The victims were administered alcohol (local brand), which wasaccidentally contaminated with propoxur (baygon). Three patients were died during treatment while one death was observed after two days of exposure. Gas Chromatography- Head Space (GC-HS) analysis has confirmed the presence of ethanol in all 21 blood samples. Propoxur was found in all samples, analyzed by Gas Chromatography with nitrogen phosphorus detector (GC-NPD).

Key Words: Ethanol, Propoxur, Gas Chromatography

Introduction

Ethanol, also known ethyl alcohol, is a psychoactive drug which is volatile, flammable and colorless liquid. It is used as recreational drugs and generally found in alcoholic beverages. Ethanol is miscible with water and thus found in paints, tinctures, markers, and personal care products such as perfumes and deodorants. It is a byproduct of the metabolic process of yeast. As such, it is present in any yeast habitat. Ethanol can commonly be found in overripe fruit.[1]Chemically it is a clear, colorless liquid with a characteristic, agreeable odor. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste. Ethanol, CH_3CH_2OH, is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group(–OH), bonded to a carbon atom.

Ethanol is the principal psychoactive constituent in alcoholic beverages, and having depressant effects on the central nervous system. It has a complex mode of action and affects multiple systems in the brain, the most notable one being its agonistic action on the GABA receptors.[2,3] Prolonged heavy consumption of alcohol can cause significant permanent damage to the brain and other organs. As such, ethanol is a nutrient. However, the final product its breakdown inside the body is acetaldehyde.[4] which more toxic than ethanol. Ethanol itself is not a carcinogen,[5] but the metabolic product of ethanol i.e. acetaldehyde, is toxic, mutagenic and carcinogenic. Ethanol within the human body is converted into acetaldehyde by alcohol dehydrogenase and then into the acetyl in acetyl CoA by acetaldehyde dehydrogenase.[6]

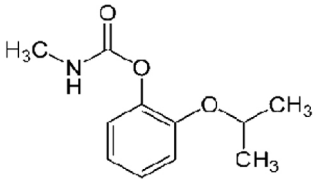
Nausea, vomiting and intoxication are symptoms of ingestion. Long-term use by ingestion can result in serious liver damage.[7]Due to ethanol toxicity, the gastrointestinal, urinary, cardiovascular, and nervous systems are affected strongly. Ethanol easily crosses membranes like water and is totally absorbed from stomach / upper small intestines. Absorption is slower from

[a]Assistant Professor, Forensic Chemistry and Toxicology Laboratory, [b] Director, [1]Institute of Forensic Science, Gujarat Forensic Sciences University, Sector 09, Gandhinagar, Gujarat, India
Correspondence:Dr. (Mrs.) Rakhi Agarwal
Email: rakhi.gfsu@gmail.com, rakhi.agarwal@gfsu.edu.in
Mobile**:** 079 23977146

the more muscular stomach (~ 25%) than from the small intestines (~75%).[8] Ethanol is sometimes used to treat poisoning by other, more toxic alcohols, in particular methanol and ethylene glycol.[9]

The amount of ethanol in the body is typically quantified by blood alcohol content (BAC), which is here taken as weight of ethanol per unit volume of blood. Small doses of ethanol, in general, produce euphoria and relaxation; people experiencing these symptoms tend to become talkative and less inhibited, and may exhibit poor judgment. At higher dosages (blood alcohol concentration [BAC] > 1 g/L), ethanol acts as a central nervous systemdepressant, producing at progressively higher dosages, impaired sensory and motor function, slowed cognition, stupefaction, unconsciousness, and possible death.[8]

Propoxur (Baygon) is a carbamateinsecticide is also used as molluscicide and also in pest control for other domestic animals, *Anopheles* mosquitoes, ants, gypsy moths, and other agricultural pests.Propoxur is highly toxic to many bird species, but its toxicity varies by the species. It is moderately slightly toxic to fish and other aquatic species. Propoxur is highly toxic to honeybees.[10]



IUPAC Name: 2-isopropoxyphenyl *N*-methylcarbamate

Carbamate insecticides block the production and action of cholinesterase, an essential nervous system enzyme. These materials quickly paralyze the nervous systems of insects, gaining them a reputation of having a rapid "knockdown" effect.[11] Carbamatecompounds are generally excreted rapidly and do not accumulate in mammalian tissue. If exposure stops, cholinesterase inhibition reverses rapidly.

Propoxur is an *N*-methyl carbamate insecticide which is quite soluble in water, extremely soluble in polar organic solvents but a little soluble in non-polar organic solvents. It is hydrolysed rapidly at pH 9 while very slowly at pH 4 and slowly at pH 7. Propoxur is having mammalian toxicity as it is rapidly metabolized and does not accumulate in tissues. However, there is no evidence that propoxur is carcinogenic, teratogenic or embryotoxic (post-implantation loss occurred only at doses above the level at which maternal toxicity occurred).[11]

Propoxur is known to be as highly toxic to humans. The propoxur's cholinesterase-inhibiting effect is short-term and reversible.[12] Animal experimentations also showed some cellular changes in the liver.[13] Symptoms of propoxur poisoning include nausea, vomiting, abdominal cramps, sweating, diarrhea, excessive salivation, weakness, imbalance, blurring of vision, breathing difficulty, increased blood pressure or 'hypertension' and lack of control of urine or feces release (incontinence). In propoxur exposure the death may be due to respiratory system failure.[14,15] Complete recovery from an acute poisoning by propoxur, with no long-term health effects, is also reported, if exposure ceases and the victim has time to reform their normal level of cholinesterase and to recover from s ymptoms.[16,17] In non-fatal cases, the illness generally lasts less than 24 hours.[18]

Propoxur is broken down and excreted rapidly in urine.[19,20] In humans given a single oral dose of 92.2 mg of Baygon, 38 percent of the dose was excreted in urine over the next 24 hours, with most of it excreted in the first 8 to 10 hours.[21] The postmortem examination of a human adult who died six hours after eating an unknown quantity of unden (a pesticide containing propoxur), revealed swelling of the brain with excess amount of fluid, bloated lungs, and increased blood in the capillaries of internal organs.[22]

In some areas, the homemade liquor is prepared to get the recreational effects. Sometimes,

during preparation of the liquor is contaminated with substances accidently, which can be more toxic than the liquor. One incident was happened in which, the 21 victims are admitted to the hospital with ill effect including of nausea, vomiting, abdominal cramps, sweating, diarrheaetc after the consumption of the homemade liquor. Treatment had started in the hospital and the blood samples of all 21 victims sent to the Forensic Laboratory for the detection of the toxic chemicals.

Materials and Methods

Chemicals and Materials

Methanol was procured from Merck, India. Diethyl ether from Fisher Scientific, India and n-propanol from Ranbaxy, New Delhi were used for the experimental work. Ethanol was obtained from Baroda Chemical Industries Ltd, Gujarat under license and it was 99.5% v/v pure. All chemicals used for the extraction and analysis were highly purified and of analytical grade. Dry baygon powder was procured from Bayer Company, Bombay and the standard for the same obtained as a gift from Directorate of Forensic Science Laboratory, Gandhinagar.

Detection of Ethanol or methanol in blood by gas chromatography head space analysis

All 21 samples were analyzed for the presence of ethanol and methanol by gas chromatography. A control blood sample spiked with ethanol and methanol was also analyzed. A volume of 0.5 ml of blood and 0.5 ml of internal standard (0.2% w/v solution of n-propanol in distilled water) was taken into the glass vial and placed into the turntable of head space sampler, thermostated at 70?C for 20 minutes. Vapours of vial content, 1 ml is injected into the column and analyzed with the gas chromatography instrument (Perkin-Elmer Gas Chromatography). The sensitivity of the instrument for ethanol and methanol detection is 1mg/100 ml sample (w/v). The conditions for the analysis are as follows:

- Column: 1/8" OD S.S. column 2 meter, P/W 3.8% halcomid M- 18 on chromosorb W (HP), Mesh range 100-120.
- Detector: Flame ionization detector
- Carrier Gas: Nitrogen 30 Lbf/in2
- Temperatures: Injection= 75?C, Transfer= 80?C, Column= 70?C, FID= 150?C, Sample= 70?C
- Programme: Thermostate time: 20 minute, Injection time: 0.05 minute, Pressurization time: 0.1 minute, Cycle time: 4 minute

Extraction of propoxur from blood and its Detection by Gas Chromatography

A volume of blood was taken in separating funnel and then extracted with diethyl ether solvent (1:3) three times. Organic layer was collected and evaporated till dryness to get concentrated residue. Residue was dissolved in diethyl ether to prepare a solution for GC injection. The blank blood sample was also performed with the same method for reference analysis. The standard of propoxur is dissolved in diethyl ether. Analysis with dried baygon powder is also performed by the preparation of air dried residue of baygon and then dissolved it into diethyl ether. All samples, standard, dry baygon powder and a control blood sample (as blank) were analyzed by gas chromatographyinstrument (Perkin-Elmer Gas Chromatography). The sensitivity

- Column Temperatures: Initial temperature= 100?C with 2 minute hold time, finally reached to 250 with 10?C/minute rate
- Injection Temperature= 150?C

Results and discussion

Presence of ethanol in blood samples

The control sample of blood spiked with ethanol and methanol was used for GC-HS analysis having internal standard (figure 1). GC chromatogram shows three peaks in figure 1, i.e. peak 1 for ethanol at Rt 0.96 minute, peak 2 for

methanol at Rt 1.22 minute and peak 3 for internal standard at Rt 2.21 minute. The GC-HS analysis of all 21 blood samples

of the instrument for propoxur detection is 1ppm. Conditions used for the analysis are as below:

- Column: Elite 1, Capillary column, 30 meter length, 0.32 mm i.d., thickness: 1.00 µm
- Detector: Nitrogen Phosphorus detector
- Carrier Gas: Nitrogen with flow rate 1ml/ minute

indicates the presence of ethanol in all the blood samples (figure 2) with Rt= 0.95 minute with a peak of internal standard. The peak for methanol is absent in all 21 blood samples, which shows the absence of methanol in blood. Thus from the findings it is clear that the homemade liquor was ethanol only and it was not contaminated with methanol. The sensitivity of the instrument with the method used for extraction is 1 mg/100 ml of blood and correlation coefficient [r/r^2] during calibration is 0.99; however the quantitation was not done in this work as this is only qualitative study.

Figure 1: Control Blood sample spiked with ethanol and methanol

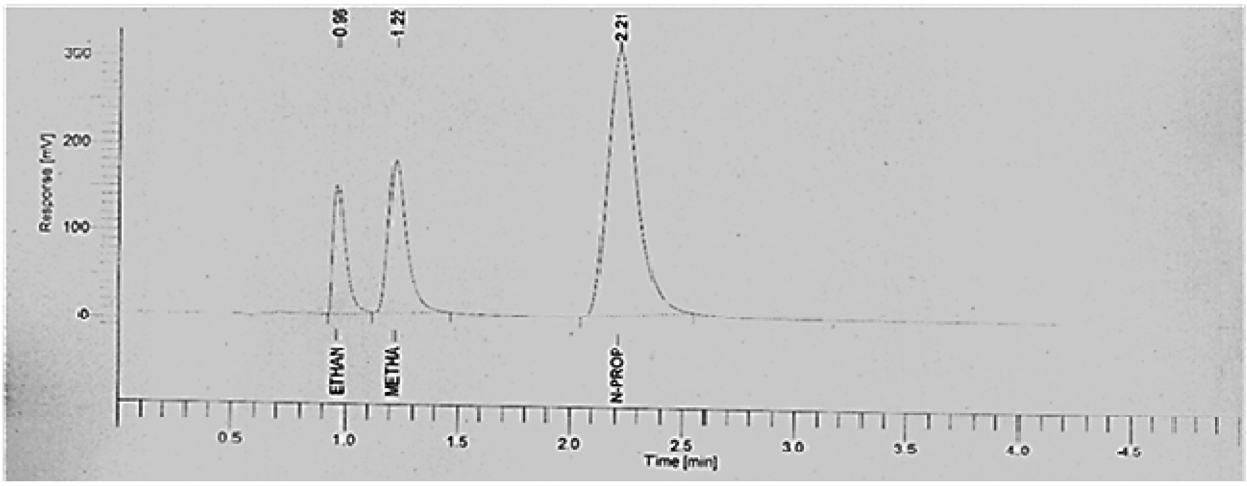


Figure 2: Blood sample (Gas Chromatogram of Blood showing presence of ethanol)

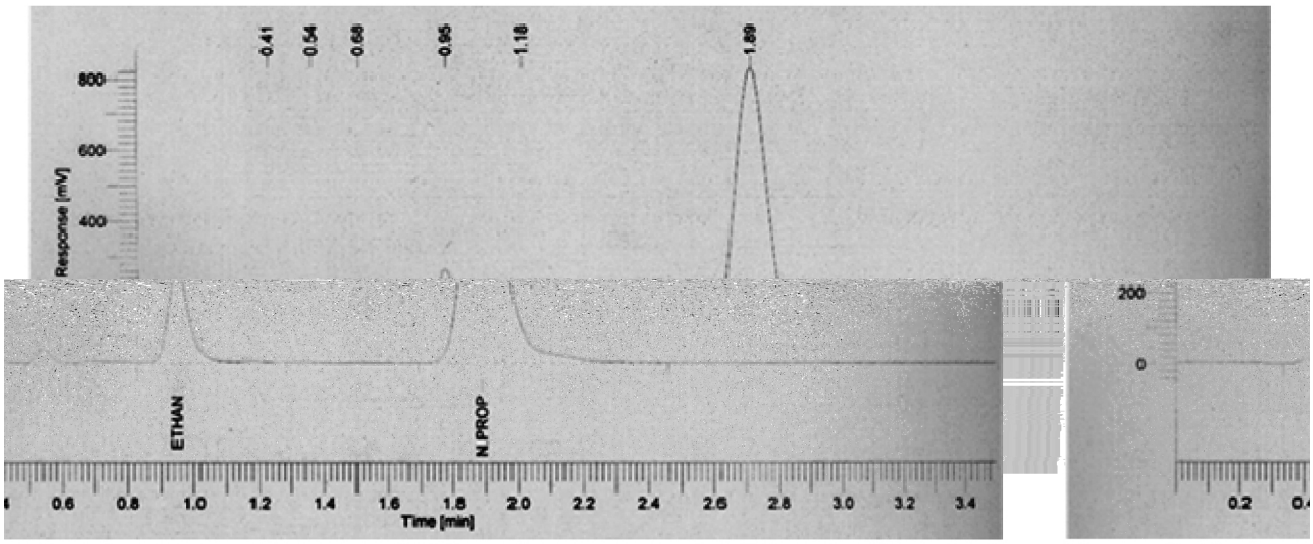
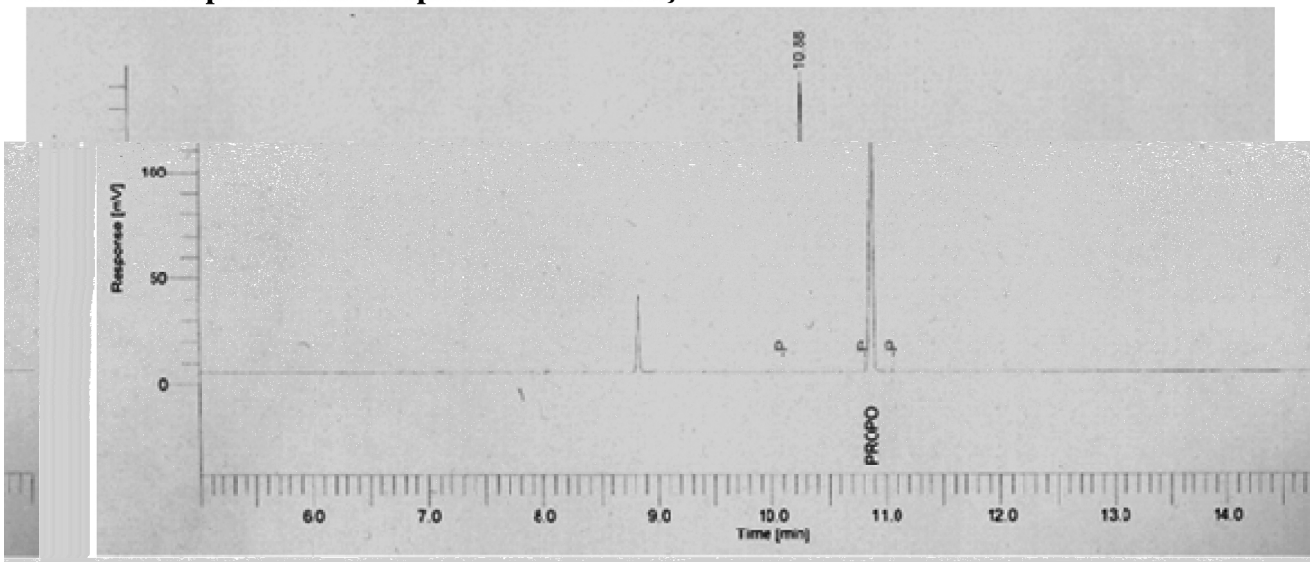


Figure 3: Propoxur Standard (Pure compound) {Gas chromatograph of Standard analysis indicates the presence of Propoxur at Rt=10.88}



sis Figure 4: Dry powder of Baygon {Gas chromatograph of dry powder of Baygon analy indicates the presence of Propoxur at Rt=10.85}

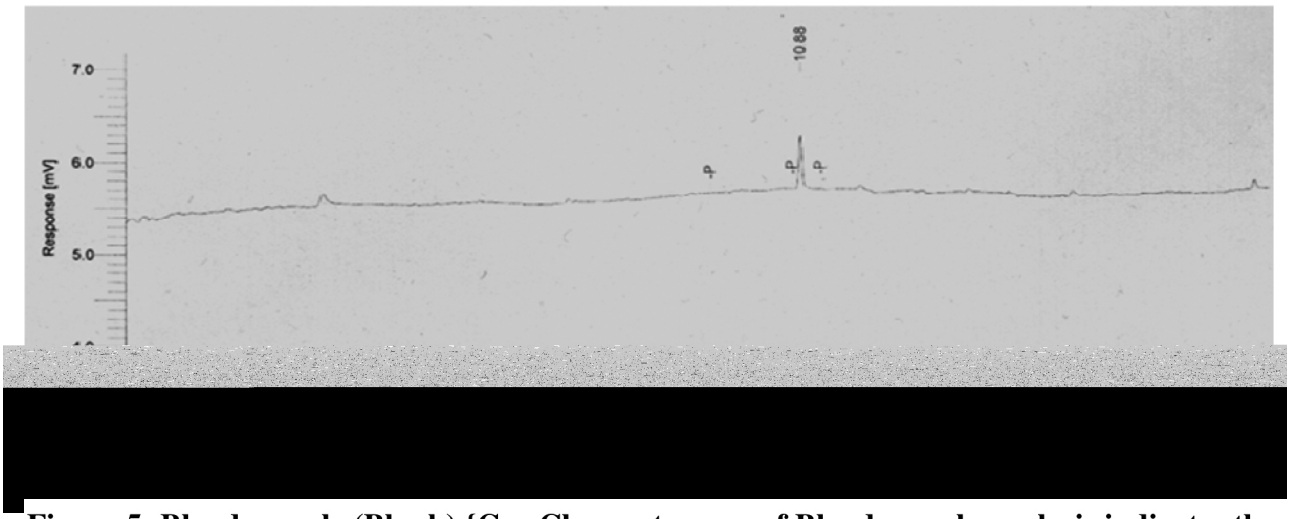
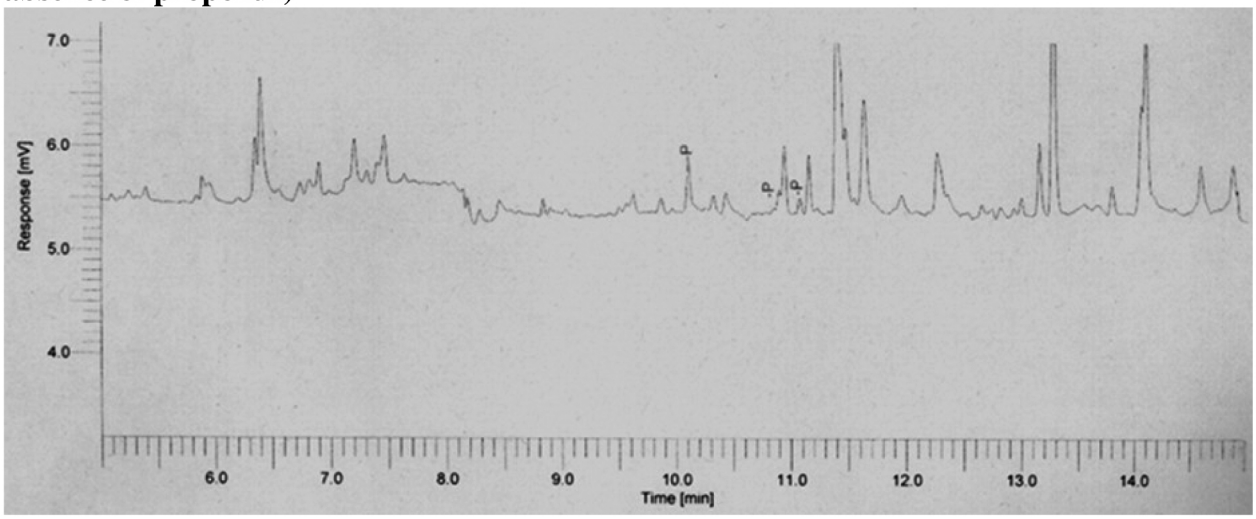


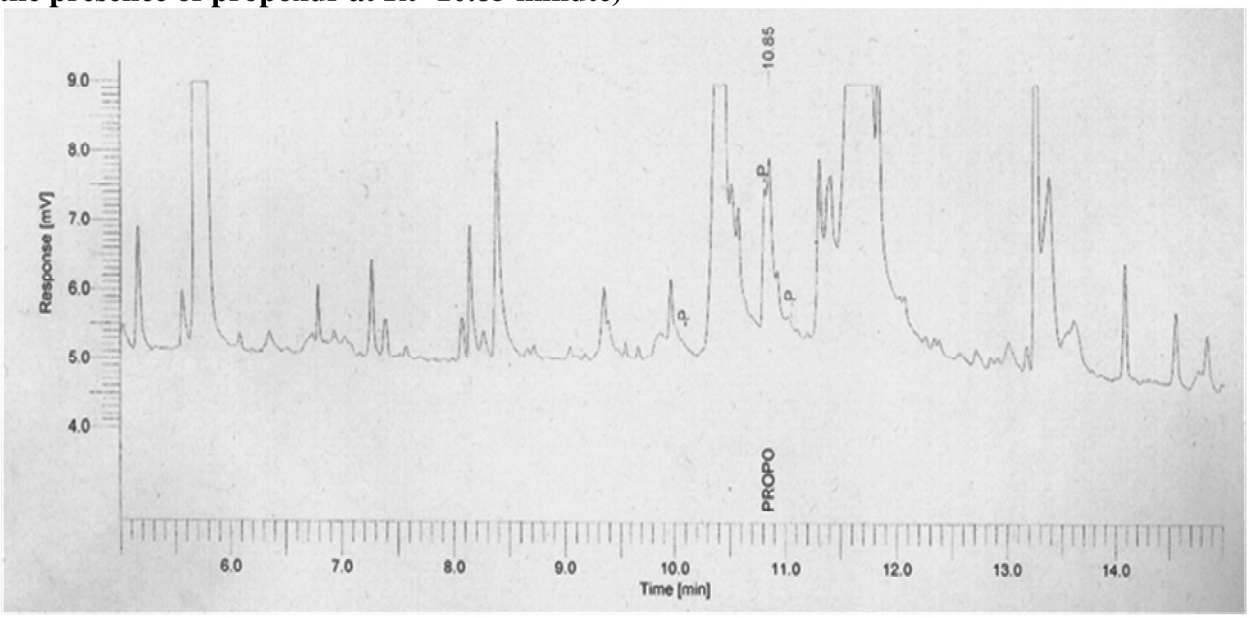
Figure 5: Blood sample (Blank) {Gas Chromatogram of Blood sample analysis indicates the absence of propoxur)



Presence of propoxur in blood samples

Under the mentioned experimental conditions, the pure propoxur compound was run with GC-NPD and its give a sharp peak at Rt 10.88 minute (figure 3). The air dried powder shows a peak at Rt 10.85 minute (figure 4), which was short in compare to standard peak, that is due to the concentration of the compound injected to the column and also may be due to the impurity present with powder. The extract of the blank blood sample was also injected and analyzed with GC, so many minute peaks were obtained due to complex matrices of blood but there was no peak corresponding to the propoxur peak (figure 5). The blood samples of victims show the distinct peak at Rt 10.85 minute and confirm the presence of propoxur in all blood samples (figure 6).

Figure 6: Blood sample from case (Gas Chromatogram of Blood sample analysis indicates the presence of propoxur at Rt=10.85 minute)



The sensitivity of the instrument with the method used for extraction is 1 ppm. The quantitative study was not conducted. The detection of propoxur is possible with chromatographic (liquid and gas chromatography) and electrophoretic techniques and also with enzyme linked immunosorbent assay (ELISA) and biosensors.[23] Even the spectroscopic analysis can also be performed with its metabolites to find out the presence of propoxur in biological tissues.[24] However, in forensic cases the important point is to give the accurate results in due course of time and for that the present extraction method followed by the mentioned GC-NPD conditions is best for quick and reliable qualitative analysis to identify the presence of propoxur in the case samples, those were from the biological origin.

The case had come into the notice due to the mass illness reported at the same time among people, who consumed homemade liquor (ethanol) from the same place. They all were admitted with the health problems. The symptoms observed almost in all the patients are general but similar to the propoxur toxicity.[25] Mechanism of action/toxicity for carbamates includes reversible cholinesterase inhibition (carbamoyl-acetylcholin esterase [AChE] complex dissociates much more easily and quickly than OP-AChE complex).[25-27] In humans studies were conducted to evaluate the health risks from potential acute exposures to propoxur and the results shows that the Lowest-Observed-Effect-Level (LOEL) in humans for cholinergic signs (stomach discomfort, blurred vision, moderate facial redness and sweating) was 0.36

mg/kg, and NOEL was 0.2 mg/kg. In acute exposures to propoxur, carbamylation of cholinesterase produces accumulation of acetylcholine, resulting in both muscarinic (diarrhea, urination, miosis, bradycardia, bronchorrhea, emesis, lacrimation, sweating) and nicotinic (fasciculations, weakness, paralysis) effects.[28]

Three male subjects were given single oral doses of 50 mg propoxur. By 8 hours, approximately 27.4% of orally administered propoxur was excreted as 2- isopropoxyphenol in the urine[29] and by 24 hours, the percentage of the dose of propoxur recovered in urine as 2-isopropoxyphenol had increased from 27.4% to 29.7%. In another case of attempted suicide by ingesting a "large" amount of propoxur by an individual, the urine was analyzed for the presence of metabolites; some metabolites (depropylation, O-hydrolysis, N-demethylation and ring hydroxylation at the 5 position) were present in free and conjugated forms, others only as conjugates. Two additional compounds, alpha-methyl-benzyl urea and 2-isopropoxyphenol, were present in very low concentrations.[30]

In the present case study, the treatment provided to the patients was symptomatic and as it is reported that propoxur can be eliminated from the body in due course of time[15], total 18 patients had recovered. However, due to health condition of the deceased and amount of consumed liquor by them, those three deaths had occurred within 24-48 hours of the consumption of the liquor. The fatal and specific sign-symptoms for carbamate pesticides are CNS depression with coma, seizures, hypotonicity in serious toxic exposures.[26]

Both Gas Chromatography- Head Space (GC-HS) analysis and Gas Chromatography with nitrogen phosphorus detector (GC-NPD) revealed the presence of propoxur and ethanol, respectively in all 21 blood samples. Thus it is clear that the liquor was contaminated with the propoxur, a carbamate and those three deaths might be due to propoxur toxicity.

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