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Review Article

Invention of Propoxur Identification Method as a Cause of Human Death Using Necrophage Insects

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Abstract - *Cases of unnatural death, when the cause was unknown, were often followed by an autopsy to determine the cause of death. However, sometimes the victim's family refused the autopsy for various reasons, leading to many cases remaining unresolved. Deaths caused by illegal drugs, such as propoxur, could be analyzed using forensic entomology, which used necrophagous insect larvae to analyze the time of death when the body's internal organs had been degraded, and no usable blood or urine remained. The study aimed to analyze the levels of propoxur [2-isopropoxyphenyl N-methyl carbamate] as a cause of death by using necrophage insects. Maggot samples were extracted and analyzed using GC-MS, including those cultivated on fresh beef as a blank and meat mixed with propoxur as the test sample. The linearity, LOD, and LOQ were ascertained during the procedure. The concentration of propoxur in the sample was found to be 62.759 ppm, with a peak area of 259,904 and a retention time of 4.107 minutes. The linear regression equation y = 10906x – 424556 was used to determine linearity, and the R2 value was 0.9913. Furthermore, it was discovered that the LOD and LOQ values were, respectively, 13.48 mg/L and 44.96 mg/L. The study concluded that, in forensic applications, the simpler and more effective autopsy approach might be replaced by the forensic entomo-toxicology method employing maggots.*

Keywords - *Forensic entomology, GCMS, Manggot, Medicine, and Propoxur.*

1. Introduction

Toxicology in forensic entomology focuses on two primary areas: (i) identifying medicines and/or toxins in necrophagous insects and (ii) examining the potential impacts of these substances on their developmental processes [5]. Drug- and toxin-related deaths have become increasingly common, particularly in rural regions. These cases often result in the discovery of decomposed bodies [6], where the organs may no longer be identifiable and are unsuitable for toxicity analysis. During this time, the larvae of necrophagous insects accumulate drugs and/or toxins by consuming the degraded body. However, their ability to recover the substances depends on the body's condition and the chemical properties of the drug or toxin itself. Despite several attempts to connect medication and toxin concentrations in insect specimens to human tissues [7], challenges remain.

The survival time of insects on a corpse helps determine the time of death in forensic cases [8]. For instance, if eggs and larvae are present, the body has been dead for less than 48 hours. If the larvae develop into pupae, this suggests the body has been decomposing for approximately 5 to 6 days, while the emergence of adult flies typically occurs after about 11 days [9]. The increasing number of deaths has led many researchers to use necrophagous insects as analytical media. This study utilized necrophagous insects to analyze toxin content through Liquid-Liquid Extraction (LLE) with validation using gas chromatography-mass spectrometry (GC-MS). This method was chosen for its efficiency, availability of materials, and affordability.

2. Theory

2.1. Post-Mortem Interval

The post-mortem interval, commonly referred to as PMI, is the estimated duration of time that has elapsed since a person or animal has died. The results obtained from this post-mortem interval are key to death investigations, helping to minimize charges against dead suspects [10]. PMI is also used as evidence of death in forensic entomology [11, 12]. It enables the identification of a person's bodily parts or tissues using insects discovered at the incident scene [13, 14]. Factors determining this post-mortem interval include chemical, physical, weather, and insect-related elements [15]. The two components of PMI are extrinsic and intrinsic. Extrinsic factors include aspects such as humidity, temperature, precipitation, and insects, whereas intrinsic factors relate to the state of the organism itself [16]. Since temperature is the main factor influencing insect development, this link has been

measured for a species, and a specimen's age may be determined by its thermal history and rate of development [17]. Medical examiners can accurately determine the Post-Mortem Interval (PMI) within 72 hours after death using pathological alterations such as rigor mortis, algor mortis, and livor mortis [7].

2.2. Propoxur

Agricultural chemicals encompass not only pesticides [18], such as insecticides, germicides, herbicides, and rodenticides designed to safeguard plants but also fertilizers and growth regulators used in agricultural production [19]. In many developing countries, poisoning incidents frequently involve these agricultural chemicals and toxins. Due to their widespread use, easy accessibility, and unregulated sale, pesticides are primarily to blame [20, 21]. Suicide through self-intoxication with herbicides or insecticides dominated as much as 89 percent, mostly involving organophosphate compounds (85 percent) and paraquat (15 percent) [22].

The carbamate insecticide Propoxur (2-Isoproxy-phenyl-N-methylcarbamate) (Baygon®) exhibits anticholinesterase properties. Food or plant contamination or inhalation of a spray may cause carbamate poisoning. It is one of the most popular household ex-insecticides because of its broad spectrum of effectiveness [23]. Propoxur can be used as a concentrated emulsifier, powder absorber, and external building dust repellent. Propoxur can also be used alone or mixed with other insecticides [24]. Extremely severe conditions can result in death, typically starting with respiratory failure brought on by the interaction of metabolic and neuro damage effects. Regarding propoxur, in vivo, hydrolysis of complex carbamate cholinesterase happens spontaneously. This process is typically indicated by clinical symptoms that subside within 24 hours [25]. Figure 1 depicts the propoxur structure.

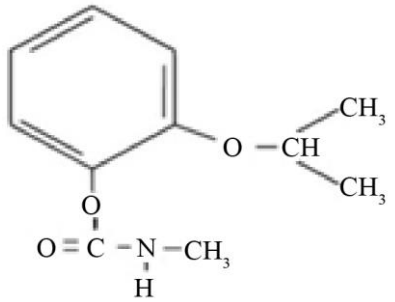

Fig. 1 Propoxur structure [2 (1-methylethoxy) phenol methyl carbamate]

Propoxur was easily absorbed in the intestines of mice (almost 100 percent), hamsters, and cows. Propoxur content of less than one percent was found in stools. Intestinal absorption in humans was tested in the laboratory with similar stages to those observed in the animals; 95 percent of the dosage was absorbed in 48 hours according to the elimination principle in the urine of laboratory animals and humans. Propoxur only built up in the kidneys during elimination and did not accumulate in many other soft tissues in animals. The amount of propoxur in cow's milk was less than 0.1 percent. Propoxur's clinical effects could take up to six months to manifest, depending on the victim's age, the self-protection equipment used, the exposure route, and the dosage [26].

Propoxur's primary biological activity was the enzyme cholinesterase (ChE) carbamylation, which inhibited it. The body contained a family of enzymes called ChE, which hydrolyzed choline esters. By quickly hydrolyzing the neurotransmitter acetylcholine, acetylcholinesterase (AChE) played a role in the nervous system's ability to catch impulses in all synapses, including neuromuscular connections. Because AChE inhibition resulted in increased nerve stimulation, acetylcholine accumulated in the synaptic gap. Cholinergic neurons in the central and peripheral nervous systems consequently become paralyzed or inhibited [27]. When an inhibitor was present, acetylcholinesterase was rapidly blocked, eventually preventing it from breaking down acetylcholine into choline and acetic acid [28]. The metabolite of propoxur is shown in Figure 2.

Humans acutely exposed to propoxur through the gastrointestinal tract exhibited minor cholinergic symptoms such as dizziness, nausea, vomiting, sweating, and tachycardia, along with an inhibition of the cholinesterase enzyme in red blood cells; these effects were only transient. Long-term exposure inhibited the levels of the enzyme cholinesterase, causing symptoms of headaches, nausea, and vomiting in humans. Animal tests also showed signs of weight loss, effects on the liver and bladder, and slight increases in neuropathy. Propoxur was categorized as a class II toxic by oral contact and a class III poison by skin contact. A lethal dose of fifty, or LD50, is the quantity of chemical that kills half (50 percent) of the laboratory animals. Rats' LD50 propoxur ranged from 50 mg/kg to 110 mg/kg ·̈·30̈, whilst mice's ranged from 40 mg/kg to 110 mg/kg. Males aged 12 months exhibited an LD50 of above 800 mg/kg. Propoxur poisoning in mice resulted in altered organ weight and/or brain and learning at concentrations lower than cholinesterase inhibitory concentrations.

2.3. Forensic Entomology

A study of insects in forensics was known as forensic entomology. This study aimed to assist law enforcement agencies in criminal cases [31]. In forensic entomology, entomological evidence is used to estimate the post-mortem interval (PMI), or the amount of time that has elapsed since a person's death [32]. The period between the colonization of carnivorous insects and the insects' ultimate development was known as the post-mortem interval [33]. The use of PMI was applied when a body was found that was no longer recognizable. The time of death was determined using PMI when it had exceeded 72 hours. In the 1990s, Wang et al.

conducted research in China on four and five common species of sarcosaprophagous flies. The findings showed that, in addition to the number of spiracle gaps and body length changes, the presence, thickness, color, and sclerotization area of the posterior peritrema might be utilized to determine larval age [34]. This held true for estimating changes in body length, larval age, and morphological characteristics (such as the posterior spiracles, cephalopharyngeal skeleton, and larval cuticle). A number of experts have been employing beetles to steadily broaden their research in recent years. The reason was that beetles could be used to expand the range of PMI estimation [35]. The age of immature stages was estimated using morphological observations, cuticular hydrocarbons [36], soluble proteins in hemolymph, and differential gene expression. Significant changes in cutaneous hydrocarbons with weathering time were observed in the fly puparium, suggesting their possible application for PMI. The use of insects' regular and unsurprising life cycle as a key chronology for calculating the amount of time that has passed since an incident was detailed by Catts and Goff in 1992 [37]. In 2006, Gomes and Zuben reported that French physician Bergeret had solved the first forensic case with entomological evidence. According to Benecke's 2005 remark, the study of forensic entomology originated in France and spread to Canada, the United States, and Europe [38].

Although entomology is a long and time-consuming area of forensics, it can be useful in circumstances when criminal activity is odd and inexplicable. Legal investigators depended more on forensic entomology data than ever before due to more scientific advancements, barcoding and DNA typing to identify insects [39]. Based on the data above, insects in forensic entomology were considered accurate because the life cycle of insects on the victim's body was considered capable of estimating the time of death. This study used a more efficient, dynamic, and non-invasive method that did not damage other tissues, so the victim's body remained intact for further examination.

Insect research in forensic entomology using GC-MS also allowed for detecting substances other than poisons, such as drugs and other chemicals. Necrophagous larvae could accumulate drugs in the body that had rotted when insects consumed the body, even with the condition of the body part exposed and the type of drug consumed. However, the recovery did not guarantee that all levels of the drug could be restored [6]. The use of larvae was expected to increase the level of accuracy in death investigations so that the results of the analysis could provide information in revealing death cases.

3. Materials and Methods

3.1. Growth of Maggot

An experimental study was carried out in the lab to ascertain the outcomes of the concentration of propoxur in maggot samples. Beaker glasses, residues, pinsetters, glass bottles, analytical balances, 250 mL flasks, 50 mL flasks, 10 mL flasks, drip pipettes, mixer bars, spatulas, porcelain cups, Erlenmeyers, 100 µL micropipettes, centrifuges, sonicators, splitting tubes, stations and clamps, loops, clamps, and an Agilent Technologies 5890N Gas Chromatograph with an Agilent 1909 capillary column (1j-41 3HP-5 30.0 m × 0.25 mm × 0.25 µm) using helium (He) gas and an Agilent Technologies 5973-inert mass spectrometer were among the tools used in this investigation. The materials used in the study included commercial fertilizers of the brand Poksindo containing 50% propoxur, methanol, carbosulfan (ISTD), acetone, dichloromethane (CH_2Cl_2), aqua, ethyl acetate, n-hexane, and acetonitrile. During the experiment, 1 kg of fresh beef blank was divided into large doughnut-shaped portions. It was inserted into the residue to prevent the maggot from emerging, and wheat flour was applied to the meat's perimeter. The residue was then covered with a perforated triple cover to protect it from sunlight and rain. It was left for approximately five days in a damp place until maggots were obtained. The maggots collected were used as blanks and transferred to the sample.

3.2. Transfer of Manggot to Sample

A half kilogram (0.5 kg) of fresh beef was prepared to treat the propoxur maggot sample. Then, 0.5 g of propoxur was dissolved in aqua and mixed with the meat. The maggot was transferred from the previously grown meat, and wheat flour was placed around it. The setup was left for approximately three days in a moist place, away from sunlight and rain. The maggot was then collected using pinsetters. Both the blank and treated maggot samples were collected and preserved by refrigeration until further analysis.

3.3. Manufacture of Standard Solution

A total of 0.25 g of standard propoxur was weighed into a 250 mL flask, and acetone was added up to the exact limit mark. The solution was homogenized and further diluted to 100 ppm and 250 ppm. The standard solution was prepared for injection into the GC-MS using 1 µL per sample.

3.4. Maggot Extraction with Propoxur Content

The extraction process used the Liquid-Liquid Extraction (LLE) approach based on the Ameno model [40]. Two grams of maggot larvae specimens were combined with five milliliters of acetonitrile, homogenized, and centrifuged for five minutes at 3000 rpm in order to clear the solution. The extraction procedure was carried out twice after the resultant deposits were gathered. The resultant acetonitrile solution was mixed with 40 mL of a 2% NaCl solution and 13 mL of a 1:1 v/v mixture of n-hexane and ethyl acetate in a 125 mL separating funnel. After the coat was allowed to dry at room temperature, 200 µL of methanol was used to dissolve any remaining solution. The prepared sample was then introduced into a GC-MS using 1 µL [41]. It was noted that larvae

weighing less than 1 g might pose limitations for accurately detecting propoxur concentrations.

3.5. Extraction of Maggot Blank

Empty larvae, which were stored in the refrigerator as controls and not exposed to samples, were used. Frozen larvae were treated with dichloromethane and homogenized using ultrasound for the initial washing stage. One gram of the dried larva was weighed and heated after the mixture was dried. The volatilized material was then injected into the GC-MS after 2 mL of methanol was added [42].

3.6. Analysis of Gas Chromatography and Mass Spectroscopy

Standard solutions, maggot blank extracts, and maggot extract samples were analyzed using GC-MS using an HP5MS column (30 m length, 250 μm diameter, and 0.25 μm film thickness) and a stationary phase composed of 5% diphenyl and 95% methyl polysiloxane. The analysis employed a 10 μL autosampler syringe and helium gas as a carrier at high speed. An Agilent 7683 automated injector and an Agilent 5975 selective mass detector were included with the Agilent 7890A GC (Agilent Technologies, Shanghai, China). The carrier gas pressure was set at 100 kPa, and the injector temperature was set at 300°C. The column temperature was increased to 240°C at a rate of 3°C per minute after one minute at 80°C, and it was kept there for five minutes. The temperature was eventually raised to 280°C and maintained there for five minutes at a rate of 10°C per minute. 1.46 mL/min was the column's flow rate [43].

4. Results and Discussion

4.1. Growth Sampel of Maggot

Maggots were obtained from fresh meat kept in a dark, moist environment for approximately five days. Flour was put around the meat to keep the maggots from fleeing the container. In accordance with the lethal dosage of propoxur (>250 mg), 1 kg of fresh meat was prepared, and 0.5g of [2-isopropoxyphenyl N-methyl carbamate] was crushed in aqua to test the growth of the sample maggot in the meat exposed to propoxur. The maggot grew on meat mixed with samples so flies could be hung and consume the samples containing meat, exposing the maggot to the substances. The only way the drugs could be identified in the larvae was by the way they ate the tissue that contained the drug. The exposed maggot was then collected and placed into a cooling machine to deactivate it, and the sample was kept stable. Freezing was considered a better preservation method than using alcohol. Table 2 shows the observation of maggot proliferation from fresh beef samples. Maggots were chosen because they are the larvae of flies that are immune to extreme environments, such as garbage sites that contain alcohol, salt, acid, and ammonia. Forensic entomology found that maggots were the most suitable specimens to substitute in drug toxicology analyses. The time it took for a Black Soldier Fly (BSF) egg to hatch, from when it was first placed in the hatching medium to the maximum hatching period, was called the hatching time. No BSF eggs were found after two days; the eggs only hatched on the third day. The hatching began on the 3rd day and peaked on the 6th day, at which time the hatching observation was stopped, and the remaining eggs were discarded. The air around them needed to have a humidity level of 30 to 40% to hatch the eggs. The egg-hatching process took about three to four days.

Maggots could decompose organic matter quite effectively and had a great appetite. The remains of livestock, food, vegetables, and all other organic matter were food for the maggots. In addition, due to their ability to survive in harsh conditions, maggots could cooperate with other microbes to dispose of organic garbage. Inappropriate chemicals, poor nutritional food quality, humidity, and non-optimum temperatures were some of the conditions that could hinder maggot growth. BSF stopped eating during the pre-pupa phase and used its body fat reserves as an energy source until it transformed into a fly. BSFs typically sought areas with less light and drier conditions during the prepupa phase. After transforming into a prepupa, the prepupa would emerge from a wet and damp environment. Growth conditions, media, and nutritional value of the material used for rearing maggots were factors that influenced maggot cultivation. Furthermore, adding microorganisms to the media could lead to the release of higher levels of organic material from bacterial decomposition, which increased the amount of food available and promoted maggot growth. Maggots consumed garbage and could be found in decaying organic matter. High protein and carbohydrate ingredients met the nutritional needs of the larvae, ensuring healthy growth.

4.2. Extracting Maggot

Samples were extracted to enable processes that obtain (separate) active components from a sample using selective solvents through existing procedures. The extraction principle was that the solvent would enter the cells and dissolve the active compounds inside. This caused a concentration difference between the dissolved compound both inside and outside the cell. Liquid-liquid extraction techniques were used in this process. When two non-mixable solvent phases were used for liquid-liquid extraction with a separator funnel, some chemical components were soluble in the first phase and others in the second phase. After combining the two phases containing the dispersed material, the mixture was humidified to form two separate layers of liquid phase. At this point, the chemical component was separated into two phases based on a determined concentration ratio according to its polarity.

The extraction procedure was based on the principle of "like dissolves like," which states that polar substances will dissolve in other polar compounds while non-polar substances will dissolve in other non-polar compounds. In the initial stage of the extraction process, two grams of the sample were added

to acetonitrile, homogenized, and centrifuged at 3000 rpm for five minutes to purify the solution. These deposits were created following two extractions. A centrifuge is a machine that separates substances based on their mass using centrifugal force. The resultant acetonitrile solution was combined with 40 milliliters of 2% NaCl solution and 12.5 milliliters of a 1:1 v/v n-hexane and ethyl acetate mixture in a 250 milliliter separating funnel. Organic solvents break down organic molecules, polar solvents break down polar compounds, and non-polar solvents break down non-polar chemicals. Methanol and acetonitrile are polar solvents, n-hexane is non-polar, and ethyl acetate is semi-polar. Semi-polar chemicals are attracted to both polar and non-polar substances; polar compounds dissolve in other polar compounds, while non-polar compounds dissolve in non-polar substances. The formed layer of n-hexane/ethyl acetate was dried at room temperature. The residual solution was then dissolved by adding 200 µl of methanol. Methanol was used as a solvent because it could destroy cell walls and cause cell components to be dissolved. The next step in the drying process was to prepare an analytical sample for GC-MS analysis. The analyte was mixed with methanol to volatilize after drying and then injected into the GC-MS.

4.3. Validation of the GC-MS

Method validation was a confirmation action that verified that the analytical method used was in accordance with the intended purpose. Before conducting the analysis, the method validation procedure was crucial because it was expected to produce high-quality and accurate data. One measurement could be made at various concentrations to determine linearity. The least squares method was then used to analyze the collected data to calculate the slope, intercepts, and correlation coefficients. The linearity determination was done using a calibration curve to measure the correlation between the concentration value and the area value. A good correlation value was close to one, which meant the test results showed a good relationship between the signal and the gas chromatography detector. This study tested linearity using a raw solution with concentration variations of 50, 100, 125, 150, and 175 ppm. This was in line with findings that used different concentration ranges from 10 to 100 ppm, with calibration curves that described the relationship between concentration (ppm) and area width, made from concentration variations. Table 1 displays the outcomes. The equation y = ax + b was then used to create a calibration curve between concentration (ppm) and area width. The slope or inclination of the line represented a, while the intercept or line of the y-axis represented b. Consequently, the determination coefficient that explained the linear relationship between the x- and y-axes was R^2, as shown in Figure 2.

Table 1. Data of concentration and peak area of propoxur

Concentration (X) ppm	Peak area (Y)
50	162970
100	629578
125	871053
150	1244575
175	1512442

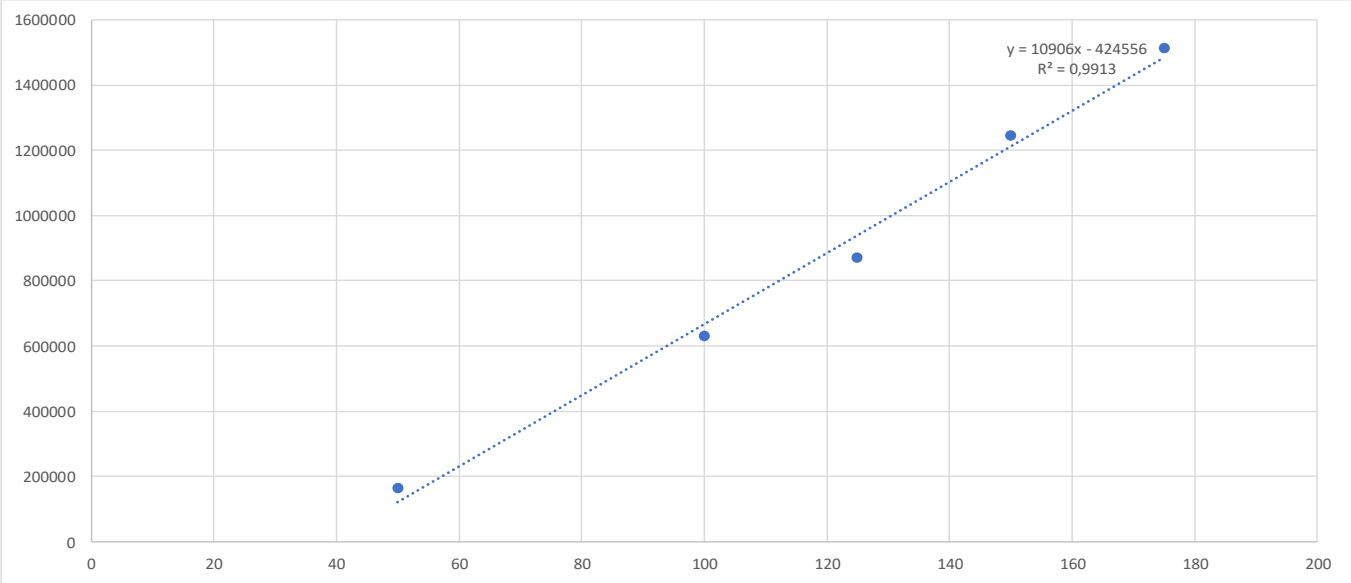

Fig. 2 Propoxur calibration curve

Based on the calibration curve, the relationship between the concentration variable and the linear area width, with an R^2 value of 0.9913, was very close to one. The method's strong linearity was indicated by the correlation coefficient value, which was close to one. This defined the method's capacity to provide a response to variations in analyte concentration within a specific range in linearity validation. The higher the analyte concentration, the better the signal produced by the instrument. The linearity of propoxur was determined using the linear regression equation y = 10906x – 424556, with an

R^2 value of 0.9913, based on the study's findings. The resulting slope was 10906, which indicated that the sensitivity of the test method was good. A good test method demonstrated high sensitivity, marked by higher slope values. Because the x and y values were comparatively straight, a positive slope indicated a positive association between concentration and absorption, implying that if the x-value was high, the y-value would likewise be high.

The results from the obtained curves indicated a directly proportional linear relationship between the area width and concentration. From the existing curve equation, the concentration of propoxur in the sample maggot analyzed by GC-MS could be determined. In this case, Y was the area of the sample, and X was the concentration to be determined. The sample area width value of 259904 resulted in a propoxur concentration in the analyzed sample of 62.759 ppm, or 0.0062759%, as shown on the calibration curve in Figure 2.

The linear regression equation's output can be used to calculate propoxur's Limit of Detection (LOD) and Limit of Quantification (LOQ). By measuring the blank response and calculating the standard deviation from the average analysis results, the LOD and LOQ values are obtained. The LOD represents the lowest detectable concentration of an analyte (active ingredient) in a sample that still provides a meaningful result when compared to a blank.

The lowest quantity of an analyte (active ingredient) in a sample that can be accurately measured while still fulfilling the necessary precision requirements is known as the LOQ. The LOQ functioned as a parameter in the analysis. The average sample value plus three times the sample's standard deviation (SD) served as the indicator for the limit of detection. The LOQ size was usually expressed by the mean value of the sample plus ten times the SD. Another way to determine the detection and quantification limits was by determining the signal-to-noise (S/N) ratio. Figure 3 presents the LOD and LOQ measurements.

Concentration (X) ppm	Peak area (Y)	Y'	Y-Y'	$(Y-Y')^2$
Blank	0	0	0	0
50	162970	120744	42226	1783035076
100	629578	666044	-36466	1329769156
125	871053	938694	-67641	4575304881
150	1244575	1211344	33231	1104299361
175	1512442	1483994	28448	809288704
Total				9601697178
Total $(Y-Y')^2/n-1$				2400424295
SD=SQRT dikali $(Y-Y')^2/n-1$				48994,1251
LOD = 3 x SD/b				13,48833397
LOQ = 10 x SD/b				44,96111324

Fig. 3 Validation method results

Based on the data in Table 2, the LOD value was found to be 13.48 ppm, and the LOQ value was 44.96 ppm. The LOD value indicated that the minimum limit of 2-isopropoxyphenyl N-methyl carbamate that could be analyzed in the sample was 13.48 ppm, while the LOQ indicated a value above 44.96 ppm that could still be quantified accurately.

Diamond electrodes were used to detect propoxur in earlier research; the LOD and LOQ values were 0.50 and 1.63 µmol/L, respectively. Further investigation into the analysis of pesticide residues in fruit also yielded LOD and LOQ values of 0.36 ng/mL and 0.011 ng/mL, respectively, using HCl. Other studies identified propoxur at a concentration of 0.017 µg/L, with LOD and LOQ values of 0.30 µg/L and 0.56 µg/L, respectively. However, the study's findings indicated that the lowest detectable propoxur [2-isopropoxyphenyl N-methyl carbamate] concentration limit (LOD) was 13.48 mg/L. The minimum concentration limits of propoxur [isopropyl-proxyphenyl-methyl carbamate] were found to meet the accuracy and precision of the LOQ, which was 44.96 mg/L.

4.4. Analysis of the Sample with the GC-MS

The maggot sample was subsequently extracted and analyzed using a GC-type instrument, with a phase length and diameter of 25 µm and a column phase of 50 µm. The injector temperature was set to 300°C, with helium used as the carrier gas at a pressure of 100 kPa. The column was initially held at 80°C for one minute, then the temperature was increased to 240°C at a rate of 3°C per minute and held for five minutes. Subsequently, the temperature was gradually raised to 280°C at a rate of 10°C per minute and maintained for an additional five minutes, with a flow rate of 1.46 mL/min. The GC-MS data is shown in Figure 4.

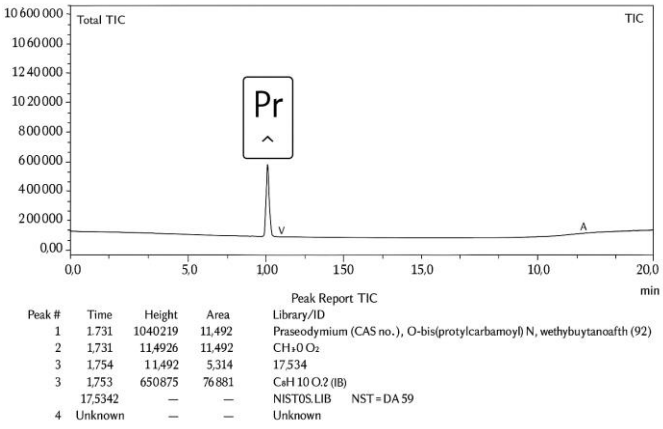

Fig. 4 Gas-Mass Spectrometry propoxur chromatography results

Propoxur was detected in samples from maggots with an area of 259,904 and a retention duration of 4.107 minutes, according to the analysis of gas-mass spectrometry chromatography. A study that used GC-MS to analyze propoxur in Sarcophaga sp. larvae revealed that the drug was eliminated at an LD50 of 100 mg/kg BW^2 and a retention

period of 4.280 minutes. A separate study analyzing propoxur in a blood sample from a Wistar rat using GC-MS found that propoxur was detected with a retention time between 4.6 and 4.7 minutes.

The concentration of propoxur was 57.876 ppm at the fourth hour, 37.565 ppm at the sixteenth hour, and 31.427 ppm at the thirty-second hour. The time differences in analyzing these propoxur samples were attributed to several factors, including differences in column type, stationary phase conditions, and the type of solvent used.

Table 2 presents the observation results of maggot growth on fresh meat samples over five days.On the 1st day**,** the meat still looked fresh, with no signs of decomposition, but flies were already present. By the 2nd day**,** the meat began to change in color and appearance, becoming dull, with a strong rotten smell and the presence of fly eggs. On the 3rd day, the meat turned black and bloody, releasing a corpse-like odor. Small white and black maggots appeared, and the degradation process was marked by a blurred surface as the maggots fed on the meat, causing it to become blackened. By the 4th day, the maggots had consumed a significant portion of the meat, and the corpse-like smell intensified. The maggots had grown to about 1 cm in size, and the meat showed further signs of breakdown. On the 5th day**,** the number and size of the maggots increased. As they were nearing the pupal stage to eventually become flies, they could be harvested. However, some maggots had finished feeding, became full, and died.

Table 2. Maggot observation results from fresh meat samples

Growth	Observation	Description
1st day	The color and shape of the meat still looked fresh.	The meat sample hadn't broken down yet, but the flies were already present.
2nd day	The color and shape of the meat slowly changed to a dull appearance, with a bloody and rotten smell that began to sting.	The meat sample had already produced a strong rotten smell, and fly eggs were visible.
3rd day	The color of the meat turned black and bloody, and it emitted a rotten, corpse-like smell.	Small white and black maggots appeared on the meat, and the degradation process was marked by a blurred surface of the meat, which was eaten by the maggots, turning it blackened.
4th day	The meat samples had been significantly consumed by the maggots, and the corpse-like smell intensified.	The maggots had grown to about 1 cm in size, and the meat began to break down as the maggots continued feeding.
5th day	The number of maggots increased, and their size grew.	Since the maggots were about to enter the pupal stage to eventually become flies, they could be harvested on the fifth day. However, some maggots had passed through their feeding phase, becoming full and dying.

5. Conclusion

The research findings indicate that Gas Chromatography-Mass Spectrometry (GC-MS) is a suitable method for detecting the chemical [2-isopropoxyphenyl N-methyl carbamate], commonly known as propoxur, in maggot samples. The GC-MS analysis was carried out using an HP5MS column (30 m in length, 250 µm diameter, 0.25 µm film thickness) with a stationary phase mixture of 95% methylpolysiloxane and 5% diphenyl, revealing a propoxur concentration of 62.759 ppm, or 0.0062759%. The analysis was conducted with a helium carrier gas at a flow rate of 62.759 ppm, an area of 259,904, and a retention time of 4.107 minutes. The results showed a) linearity indicated by an R2 value of 0.9913 and b) the Limit of Quantification (LOQ) and Limit of Detection (LOD) values were 44.96 mg/L and 13.48 mg/L, respectively. This maggot-based entomo-toxicology method has the potential to replace traditional autopsy techniques, offering a more efficient and direct tool for forensic investigations.

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