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GC–MS/MS analysis of chlorpyrifos in forensic samples with varied survival time

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Abstract

Organophosphorus pesticides are extensively used in the agricultural sector to kill insects, worms, and other pests. Many people may be poisoned by chlorpyrifos either accidentally or intentionally, including accidental, suicidal, and homicidal poisoning cases in India. The effect of chlorpyrifos on human health depends on factors such as the time, amount and frequency of exposure, the individual's health, and certain environmental conditions. The main objective of this investigation is to identify the post-mortem biological sample that shows the longest detection window, enabling precise chlorpyrifos detection in cases of acute poisoning with varying survival durations. Our research focuses on the detection and distribution of chlorpyrifos in cases of acute poisoning using a simple liquid–liquid extraction and GC–MS/MS analysis. We validated the method, which proved to be effective and reliable. Upon examining various organs, we detected the presence of chlorpyrifos in the stomach tissue, liver tissue, kidney tissue, and blood samples of individuals who consumed chlorpyrifos and passed away immediately, as well as in those who survived for the first 3 days following ingestion. Analysing urine, blood, and liver tissue from individuals who survived for 3 days provided more precise results compared to stomach tissue. Additionally, urine samples played a crucial role in detecting chlorpyrifos in individuals who survived for 4 and 5 days. A blood sample is the most suitable post-mortem biological sample for detecting chlorpyrifos in individuals who survived for a duration of 2 to 4 days. This finding highlights the significance of analysing urine as a valuable sample type, particularly in determining the presence of chlorpyrifos in cases where individuals have survived for a long period of time before their demise. The experimental data and information provided in this study will serve as a valuable resource for forensic toxicologists.

Keywords Post-mortem samples · Suicidal cases · Chlorpyrifos distribution · Acute poisoning · GC–MS/MS · Survival duration

Introduction

Chlorpyrifos (CPF), a chlorinated organophosphorus insecticide (OPI), is a significant cause of poisoning related fatalities in both developing and developed countries, particularly in India [1]. It is widely used to protect crops and control pests in non-agricultural settings. CPF exposure has been linked to neurological impairments in individuals of all age groups, including children and adults [2–4]. Prenatal exposure to CPF is particularly concerning, as it has been associated with various negative outcomes, such as decreased birth weight, impaired IQ, attention disorders, and delayed motor development. CPF's mechanism of action involves blocking the enzyme acetylcholinesterase (AChE), leading to an accumulation of acetylcholine, neurotoxicity, and potentially fatal symptoms [5]. The onset of CPF poisoning symptoms can occur shortly after exposure and may last for days or weeks [5]. The neurotoxic effects of CPF extend to humans, pets, and other animals, similar to its impact on targeted pests. Pure CPF is a white or colourless crystal with a slightly skunky odour, like rotten eggs or garlic. Chemically, it is a non-volatile substance derived from triphosphoric acid with the IUPAC name O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate. Structurally, CPF consists of chlorine groups attached to the pyridine moiety at positions 3, 5, and 6, and a diethyl thiophosphoric group at position 2 (Fig. 1).

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Acute exposure to CPF can lead to paralysis, seizures, loss of consciousness, and even death. Upon ingestion, CPF is quickly absorbed from the intestine into the bloodstream, distributing rapidly throughout the body's organs, including the brain. CPF's biological half-life in the blood is up to 24 h, and in adipose tissue, it is about 60 h. CPF's active metabolites, like 3, 5, 6-trichloro-2-pyridinol (TCP) and chlorpyrifos-oxon (CPO), exhibit better persistence and are equally toxic, causing severe health complications [6, 7]. The presence of a chlorine group in CPF's chemical structure enhances its lipid solubility and prolongs its half-life, gradually reducing acetylcholinesterase (AChE) levels compared to other organophosphorus pesticides [5]. The commonly found metabolites are 3, 5, 6-trichloro-2-pyridinol, and diethyl phosphorus. These metabolites are excreted with an average elimination half-time of 6 ± 2 h in the initial phase and 80 ± 25 h in the slower elimination phase [8, 9].

Testing biological fluids or tissues for the identification of poisons is a complex process requiring sophisticated instrumentation and trained analysts. The toxicology division of a forensic laboratory typically employs various methods to detect insecticides. These methods include chemical spot tests, thin-layer chromatography (TLC), and high-performance thin-layer chromatography (HPTLC) [10]. Routine use of GC–MS and LC–MS in laboratories can be challenging due to the requirement for highly purified extracts and the high cost and maintenance associated with these instruments. However, HPTLC is a specific and confirmatory test that can be used effectively. Therefore, it is necessary to select a biological specimen that contains the highest concentration of CPF even after different survival time durations, as the LOD (limit of detection) and LOQ (limit of quantification) for HPTLC are relatively high, at 0.5 µg/g and 1.5 µg/g, respectively. This selection of the specimen will vary depending on the survival time [11].

According to the study carried out by the department of forensic medicine and toxicology at Gauhati Medical College, laboratory tests confirmed poisoning in 41.8% of cases; at the same time, 33.7% of cases tested negative for poisonous substances in laboratory tests conducted, and in many cases, even the autopsies that had given positive sign had

shown negative results, which is indeed contradictory. These instances should be considered serious problem, and they require solemn concern to address the issues associated with negative results after conducting an autopsy [12]. Toxicology test results were sometimes negative, even when the initial history of acute poisoning was positive. This discrepancy may be attributed to factors such as the duration of survival or the submission of unsuitable samples. Therefore, understanding the levels of CPF retained in body tissues and fluids after consumption or exposure becomes crucial. The kinetics and determination of poison levels in post-mortem tissues and fluids after varied survival durations in toxicokinetic have gained enormous attention in the forensic fraternity. Concentrations of toxic substances may vary according to the sampling site and the interval between consumption of poison and death. As a consequence, the primary goal of this study is to determine the CPF levels in forensically relevant samples, such as viscera and bodily fluids, in acute CPF poisoning with varying survival times.

Materials and methods

Chemicals

HPLC grade acetonitrile (MeCN), petroleum ether (PE), and ethyl acetate (EA) were procured from Merck, India. LCMS grade methanol was purchased from J.T. Baker, India. Chlorpyrifos and dimethoate was procured from Sigma-Aldrich, India, and Florisil (Florisil 60–100 mesh) was from NP Chem, India. All other reagents used were of analytical grade.

Sample preparation

We categorised male individuals who passed away as a result of acute CPF poisoning into six groups based on their survival duration. These groups ranged from immediate death (occurring within 3 h of poison consumption) to those who survived up to 5 days. Each group includes ten deceased individuals ($n = 10$) who survived for approximately the same duration. All of the above cases were selected based on case history and hospital records. The age, survival duration, and time between death and post-mortem examination are shown in Table 1. All deceased individuals in this study were stored at -10 °C until autopsy. Post-mortem examinations of all cadavers showed no external injuries. Internal examinations also revealed that there were no internal organ injuries or diseases. The autopsied tissue samples and body fluids, including the stomach, liver, kidney, blood, and urine, were collected in sterile glass containers with sodium chloride added as a preservative. These samples were stored at -20 °C [13, 14].
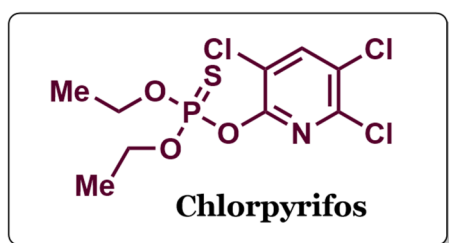

Fig. 1 Structure of chlorpyrifos: O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate

Extraction of chlorpyrifos from post-mortem samples

The extraction of CPF from various organ tissues, such as stomach tissue, liver tissue, and kidney tissue was performed according to an optimised extraction method developed from a previously described method [15]. Ten grams of tissues were homogenised in 5 mL of 0.1 M phosphate-sodium buffer (pH 7.0). Following the tissue homogenate preparation, 20 mL of petroleum ether and ethyl acetate mixture (PE: EA) in a volume ratio of 1:0.5 (v/v) was added to the homogenate. The homogenate was kept in a rota shaker (Remi-rotary shaker, India) overnight at 37 °C and 100 rpm. Next, the organic layer was collected, and the residue was subjected to re-extraction with 20 mL of PE: EA (1:0.5 v/v). The collected extract from each sample was evaporated to dryness using a nitrogen evaporator (Eppendorf Concentrator Plus, Germany), and the residues were reconstituted with 5 mL of methanol separately and subjected to partial purification using Agilent Bond Elut C-18 solid-phase extraction (SPE) cartridges in vacuum manifolds (Perkin Elmer, France). The purified extract was collected and dried, and then the residues obtained were redissolved in 10 mL of methanol and vortexed for complete solubilisation. If the concentration of CPF exceeded the calibration range, the aforementioned extract was diluted to bring it within the calibration curve. Subsequently, a 1 μL aliquot of the diluted sample was used for GC–MS/MS analysis. The dilution factor was taken into account and incorporated into the LabSolutions software to determine the precise concentration of CPF in the sample.

A blood sample was subjected to CPF extraction by taking 10 mL of the blood sample and vortexing it for 5 min with 5 mL of acetonitrile. To this mixture, 20 mL of PE: EA (1:0.5 v/v) was added and vortexed for 5 min. The mixture was then centrifuged at 3000 rpm for 10 min, resulting in phase separation. The organic layer was carefully separated, and the extraction process was repeated twice using 10 mL of PE: EA (1:0.5 v/v). The collected extracts from the repetitions were pooled and subjected to purification using solid-phase extraction (SPE).

A urine sample was subjected to CPF extraction by taking 10 mL of the urine sample and vortexing it for 5 min with 20 mL of a mixture of PE: EA (1:0.5 v/v). The mixture was then centrifuged at 3000 rpm for 10 min, resulting in phase separation. The organic layer was carefully separated and passed through anhydrous sodium sulphate to remove any residual water content. This extraction process was repeated twice using 10 mL of the PE: EA mixture. The collected extracts from the repetitions were combined and subjected to purification using solid-phase extraction (SPE).

Method validation

A standard stock solution of CPF was prepared in methanol with a concentration of 1 μg/mL and stored at 4 °C. Working standard solutions of 0.5 μg/mL were obtained through dilution. Known concentrations of CPF (0.01–0.1 μg/mL) and an internal standard (dimethoate) were added to control tissue, blood, and urine samples, followed by extraction, purification, and GC–MS/MS analysis, following the same procedure as described for poisoned deceased samples. The calibration curve was prepared with five concentrations (0.01–0.1 μg/mL) in triplicate. LOD, LOQ, linearity, intra- and inter-day accuracy, precision, and recovery were estimated. LOD and LOQ were determined by analysing control tissue samples spiked with known CPF concentrations. Extraction recovery was analysed by spiking control tissue, blood, and urine samples with low (0.01 μg/mL) and high (0.1 μg/mL) CPF concentrations in five replicates. Intra-day precision was determined by spiking control tissue with low and high CPF concentrations in 10 replicates. Inter-day precision was assessed over five different days at intervals of 10 days. All assessments were followed by the same extraction procedure as described previously.

GC–MS/MS instrumentation

The purified extracts were analysed using a gas chromatography-tandem mass spectrometry system (Triple-quad GCMS-TQ8040 NX, Shimadzu, Japan) equipped with an automatic sampler and electron ionization. A high-resolution DB-5 Sil MS capillary column separated the compounds. Helium gas (99.999%) was used as a carrier

Table 1 Details of deceased individuals showing age, varying survival period, and time duration between death and post-mortem examination

Group no.	No. of samples (*n*)	Age of deceased (years)	Range of survival duration after poisoning (hours)	Time duration between death and post-mortem examination (hours)
Day 0	10	25 ± 3	0–3	9 ± 6
Day 1	10	25 ± 3	2–22	9 ± 5
Day 2	10	25 ± 3	25–46	9 ± 6
Day 3	10	26 ± 4	52–71	10 ± 6
Day 4	10	25 ± 3	76–95	9 ± 6
Day 5	10	26 ± 3	98–119	9 ± 5

gas at a 0.84 mL/min flow rate. The oven temperature was programmed, starting at 100 °C and ramping up to 280 °C. The injection port temperature was 280 °C. Split ratio 5 was set on the injector port. The total running time was 26.33 min, and the mass detection range was 50–500 amu.

CPF was confirmed using full scan mode, and quantification was performed in multiple reaction monitoring (MRM) mode. MRM method and matrix-matched calibration curves (0.01–0.1 µg/mL) were constructed. The operation conditions of the mass spectrometer were as follows: electron impact ionisation (70 eV) in MRM; emission current, 50 µA; and ion source temperature and interface temperature were set to 250 °C and 280 °C, respectively. For CPF, the most abundant and characteristic mass fragment for quantitation and two others for confirmation were chosen. The total ion chromatogram (TIC) was used to determine the mass (m/z) of CPF, and the corresponding quantifier (target) ion-197 > 168.90, the qualifier (reference) ion-199 > 170.90, and retention time were recorded. The MS/MS detector demonstrated sensitivity at ppb levels. For higher CPF concentrations (day 1–day 3 after exposure), appropriate dilutions were made to prevent overloading the detector while ensuring accurate measurements. GCMS Solutions software processed the data, providing precise CPF concentrations. Statistical analysis was conducted using Prism 9 software. Data are expressed as the mean ± standard deviation (SD) from independent experiments. All experimental analyses were compared with one-way analyses of variance (ANOVAs). Differences were considered significant at $p < 0.05$.

Results

The analysis of CPF and dimethoate (IS) in post-mortem samples using a DB-5 Sil MS capillary column, with IS at 14.84 min and CPF eluting at 19.10 min. The chromatograms of a blank, a standard CPF, and a representative tissue extract were analysed in scan mode (Fig. 2). CPF was detected and quantified using m/z values from the mass spectral chromatograms. In scan mode, the quantifier ion (197 > 168.90) and qualifier ion (199 > 170.90) were used to identify and quantify CPF (Fig. 3). The calibration curve showed linearity ($R^2 = 0.998$) between substance concentrations (Y) and peak area ratios (X). The LOD for CPF was 0.001 µg/mL, and the LOQ was 0.01 µg/mL. In the estimation of intra-day precision at low CPF concentrations (0.01 µg/mL), the obtained precision values were 7.33% (tissue), 6.11% (blood), and 3.21% (urine). When higher concentrations were considered (0.1 µg/mL), the intra-day precision measurements were 4.13% (tissue), 3.71% (blood), and 3.68% (urine). In the estimation of inter-day precision at low CPF concentrations (0.01 µg/mL), the obtained precision values were 12.90% (tissue), 10.02% (blood), and

9.97% (urine). When higher concentrations were considered (0.1 µg/mL), the inter-day precision measurements were 5.87% (tissue), 2.66% (blood), and 2.61% (urine). Recoveries in low concentration (0.01 µg/mL) ranged between 61 and 83% in tissues, 74 and 94% in blood, and 74 and 96% in urine. In high concentration (0.1 µg/mL), recoveries ranged between 81 and 94% in tissues, 89 and 96% in blood, and 93 and 99% in urine. The analysis method accurately detected and quantified CPF in various sample matrices, providing precise and reproducible results.

We investigated the distribution of CPF in the tissues and body fluids of male individuals who died from acute oral CPF poisoning. Post-mortem samples from deceased individuals with different survival times were analysed using GCMS Solutions software for high speed and throughput.

The concentration of CPF in different tissues and body fluids of acute CPF poisoned individuals with varying survival periods are shown in Fig. 4. In cases of immediate death (day 0), CPF was most prominently detected in stomach tissue (898 ± 89 µg/g), followed by the liver (435 ± 66 µg/g), kidney (401 ± 41 µg/g), blood (186 ± 14 µg/mL), and least in urine (77 ± 3 µg/mL), as shown in Fig. 4. When comparing CPF concentrations in stomach tissue among individuals with different survival durations, those who died immediately after consuming CPF exhibited significantly higher levels of CPF compared to those who survived for 1 to 5 days following CPF consumption. However, CPF concentrations in the stomach tissue of individuals who survived for 2 days (114 ± 25 µg/g) and 3 days (156 ± 27 µg/g) were found to be statistically insignificant. Notably, the CPF concentration in the stomach tissue of individuals who survived for 1 day (381 ± 25 µg/g) showed a 2.3-fold decrease compared to those who experienced immediate death (898 ± 89 µg/g) following CPF consumption. Moreover, the CPF concentration in the stomach tissues of individuals who survived for 2 days (114 ± 35 µg/g) was 3.3 times lower than that in individuals who survived for just 1 day (381 ± 25 µg/g). Additionally, the CPF concentration (Fig. 4a) showed a rapid decline in the stomach tissues of individuals who survived for 4 and 5 days (21 ± 12 µg/g and 7 ± 13 µg/g, respectively).

The parent compound CPF was readily detectable in the liver tissue of individuals who died immediately after CPF consumption (436 ± 67 µg/g) as well as in those who survived up to 3 days after CPF ingestion (436 ± 22 µg/g). In comparison to the liver tissue of individuals who died immediately after CPF consumption (436 ± 67 µg/g), those who survived for 1 day exhibited a 1.12-fold higher concentration (492 ± 36 µg/g). Conversely, there was a 1.5 and 1.4-fold decrease in CPF concentration in individuals who survived for 2 (309 ± 77 µg/g) and 3 (436 ± 220 µg/g) days, respectively, which was statistically not significant. Notably, we observed a sharp decline in CPF concentrations, with
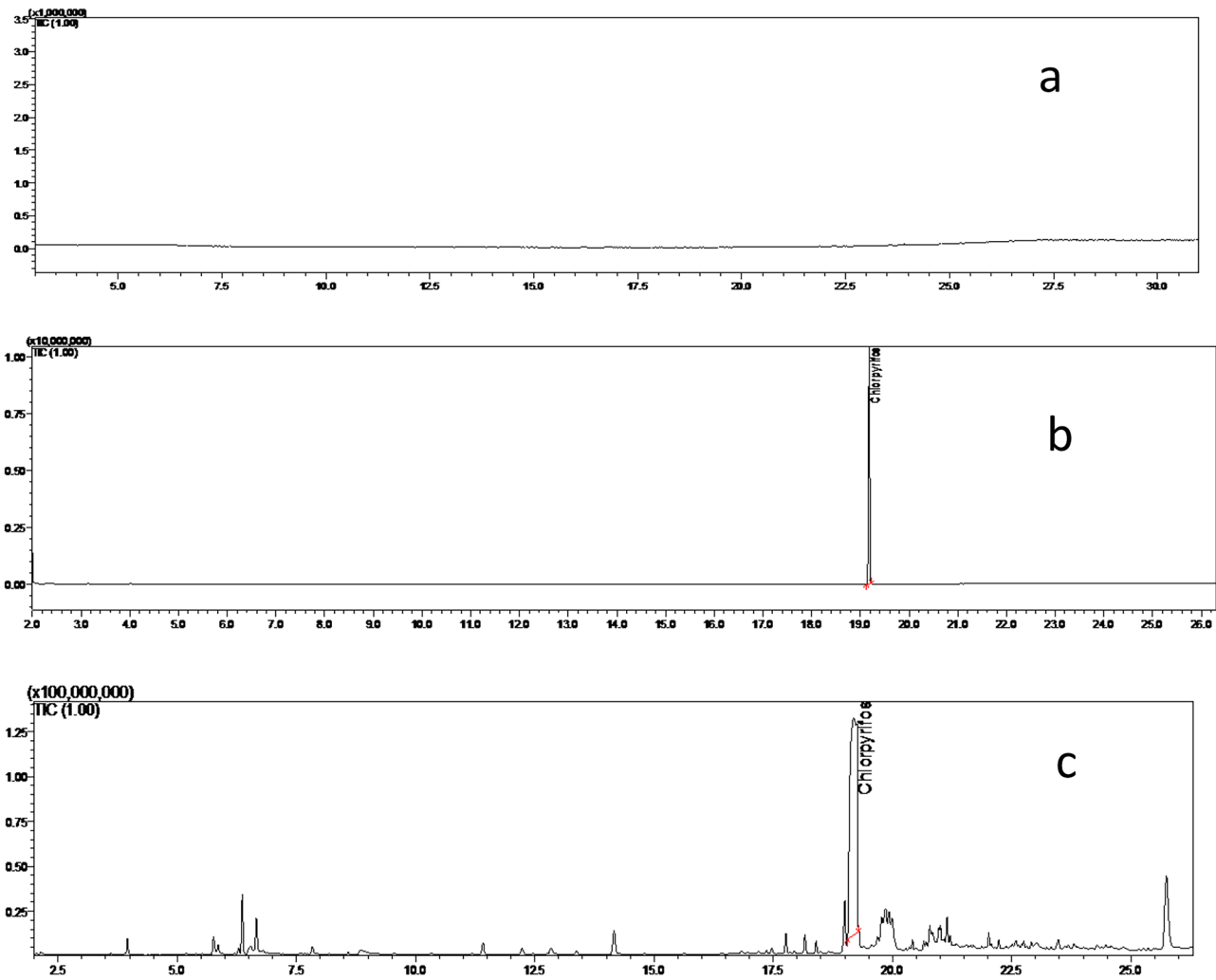
Fig. 2 The results of a GC–MS scan analysis of **a** blank, **b** chlorpyrifos standard show a peak at RT 19.10 min, and **c** representative tissue extract show peak of CPF at RT 19.10 min along with metabolite of CPF, cholesterol, etc.

a 7.9-fold decrease in the liver tissue of individuals who survived for 4 days and a 27.3-fold decrease for those who survived for 5 days (Fig. 4b).

As CPF and its metabolites are primarily excreted through the kidneys, in individuals who died immediately after consuming CPF, the concentration of CPF in kidney tissue (401 ± 41 μg/g) was similar to that in liver tissue (436 ± 67 μg/g). The highest CPF concentration was observed in the kidney tissues (473 ± 124 μg/g) of individuals who survived for 3 days. However, in individuals who survived for 4 and 5 days, the CPF concentration in kidney tissues (14 ± 15 μg/g) experienced a significant reduction, dropping by 12 and 34-fold when compared to the kidney tissues of individuals who survived for 3 days (Fig. 4c).

The concentration of CPF in the blood displays significant variation based on survival time. Individuals who died immediately after consuming CPF had a lower CPF concentration in their blood (186 ± 14 μg/mL) compared to those who survived for 1 day (445 ± 33 μg/mL). There was a slight decrease in CPF concentration in blood among individuals who survived for 2 days (335 ± 73 μg/mL), followed by a marginal increase in blood of those who survived for 3 days (474 ± 107 μg/mL) (Fig. 4d).The concentration of CPF in the blood samples of individuals who survived for 4 days was ninefold lower (48 ± 22 μg/mL) than in the blood samples of individuals who survived for 3 days, and it was notably very low (18 ± 15 μg/mL) in the blood samples of individuals who survived for 5 days.

Statistically significant concentrations of CPF were observed in the urine samples of individuals who survived for 3 days (940 ± 167 μg/mL). Nevertheless, CPF was detectable in forensically measurable concentrations in urine samples across the entire range, from immediate death (day 0) to individuals who survived for 3 days. It is worth noting
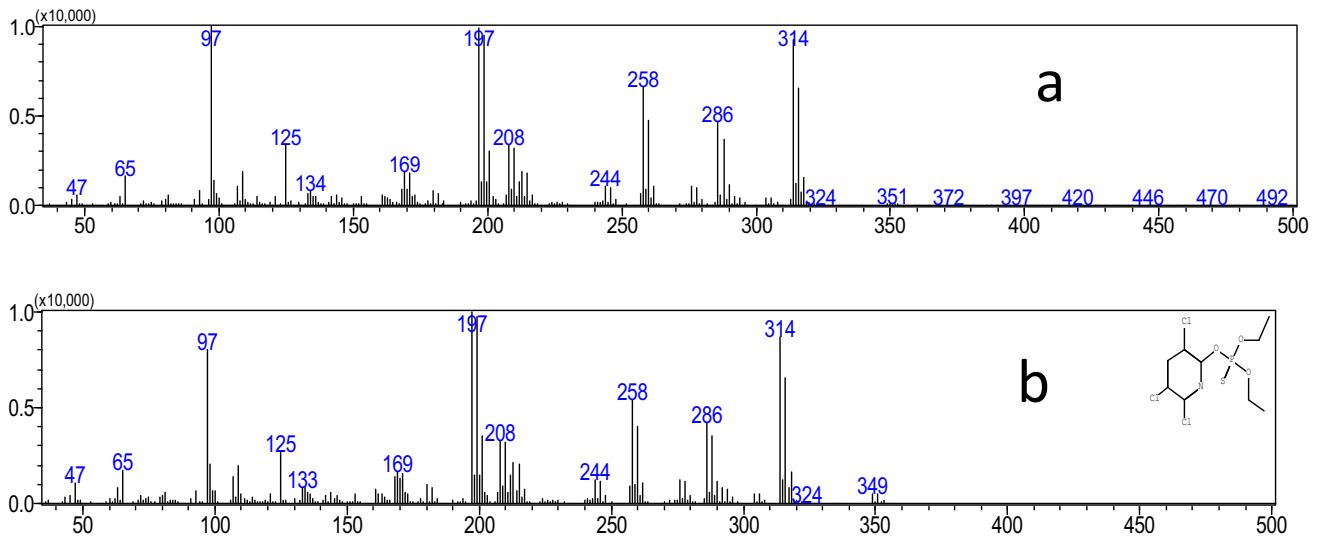
Fig. 3 Mass spectrum of **a** chlorpyrifos standard with mass fragments 97,197, 258 in comparison with **b** NIST Library
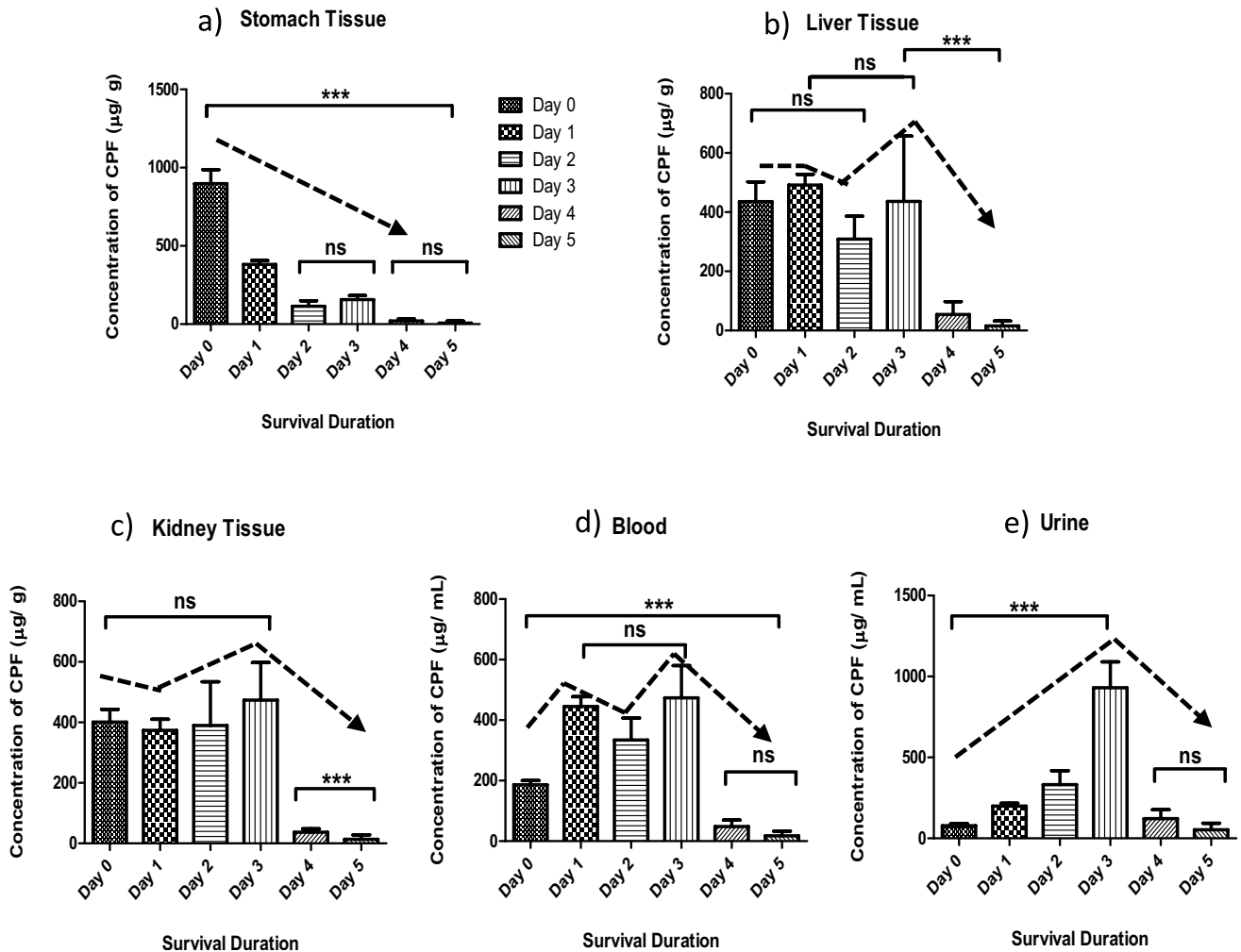

Fig. 4 Graph showing relation between concentration of chlorpyrifos in **a** stomach, **b** liver, **c** kidney, **d** blood, and **e** urine under different survival durations from immediate death (day 0) to 5 days of survival period ($p < 0.001$; ns, non-significant; and ***significant)
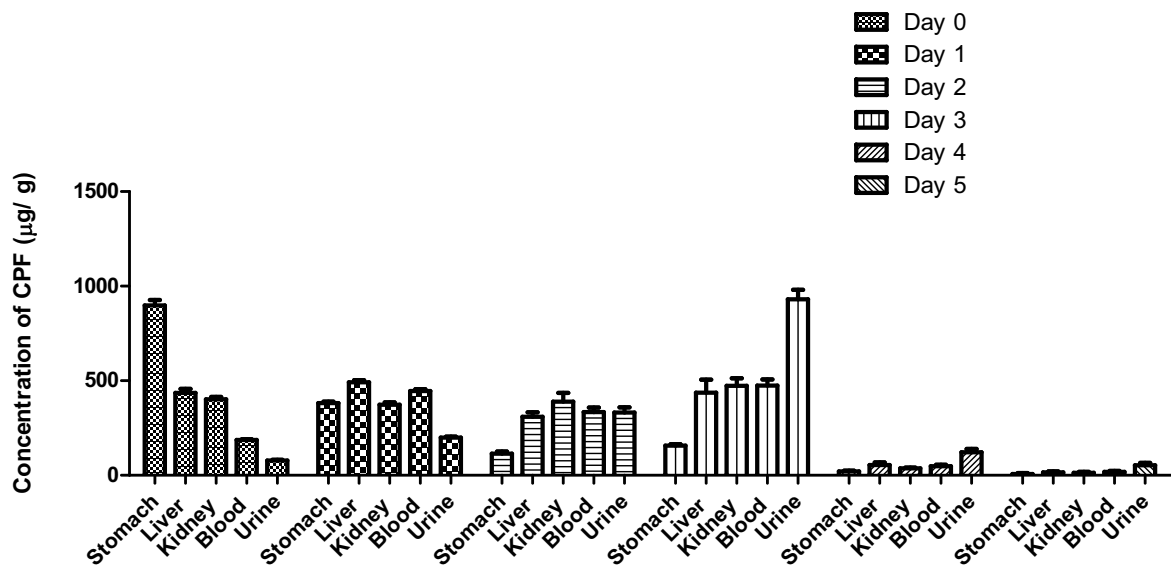
Fig. 5 Comparing the concentration of chlorpyrifos in stomach tissue, liver tissue, kidney tissue, blood, and urine samples with different survival durations from immediate death (day 0) to day 5 survival period

that urine samples exhibited the highest CPF concentration (53 ± 40 μg/mL) compared to all other forensically relevant tissues and fluids from individuals who survived for 5 days (Fig. 4e) [16]. The comprehensive comparison of CPF levels in forensically significant tissues and body fluids across different survival times, ranging from immediate death (day 0) to a 5-day survival period, is visually depicted in Fig. 5.

Discussion

Our pioneering investigation studied appropriate post-mortem tissues and body fluids for detecting CPF pesticide in acute poisoning cases with varying survival durations. We used GC–MS/MS techniques and simple solvent extraction procedures. A total of 60 cases were included in this study, with 10 cases being analysed within each category, from immediate death to 5 days survival period after acute CPF poisoning. All deceased individuals were within the age range of 20 to 32 years, known for higher suicidal tendencies and metabolic activity. The average duration between death and post-mortem examination was approximately 9 h, and all cadavers were preserved at −10 °C. To ensure statistical reliability, we meticulously analysed five distinct post-mortem biological samples from ten deceased ($n = 10$) in each category, guaranteeing credible and valid results.

The method showed low precision values for both intra-day and inter-day analyses, indicating high repeatability and reproducibility. Recovery percentages for CPF in various sample matrices (tissues, blood, and urine) were within reasonable ranges, indicating accurate detection and quantification of both low and high concentrations. To ensure accurate

quantification, a dilution strategy was employed for samples with very high analyte concentrations, reducing CPF levels to fall within the calibration curve range (0.01–0.1 μg/mL). The dilution factor depended on the initial CPF concentration and desired range. A small volume (1 μL) of the diluted sample was injected into the GC–MS/MS for analysis. The dilution factor is incorporated in GCMS Solutions, which calculates the total concentration of CPF for precise analysis.

In individuals who died within 3 h, CPF was found in high concentrations in the stomach tissue, indicating recent consumption of poison before death (Fig. 5) [15, 17, 18]. CPF was detected in the blood, liver, kidney, and urine, indicating ongoing metabolic and elimination processes [19]. However, detecting CPF in the stomach tissue becomes challenging after 4 or 5 days of survival, leading to potential false negatives (Fig. 4a). Methods like HPTLC, TLC, and colour tests are less effective beyond 3 days of treatment due to their limited detection capabilities [11]. Instead, analysing urine, blood, and liver tissue provides more accurate results for detecting CPF in deceased individuals who survived for 2 and 3 days [19]. In liver tissue, CPF was reliably detectable in individuals who died immediately (day 0) and those who survived up to 3 days. Beyond this period, detecting CPF in the liver becomes challenging, and the reason for the decline remains unknown (Fig. 4b). A plausible explanation could be liver tissue saturation with CPF in the deceased who survived for 2 and 3 days, leading to limited capacity to accumulate more CPF and subsequent elimination through urine. The kidneys play a vital role in CPF excretion, with similar CPF concentrations in deceased who died within 3 h and till 2 days of survival period, followed by a decline in deceased who survived 3 days (Fig. 4c). This decline could

be due to CPF elimination through urine. CPF is transported in the body through the bloodstream, with varying concentrations over different survival durations (Fig. 4d). Detecting CPF in blood samples after 5 days of survival may be negligible. Urine is a significant excretory route for CPF removal, with a high concentration of CPF excreted within 3 days of exposure [8]. CPF concentration in urine is higher than in other body tissues/fluids, making it a valuable sample for accurate detection of CPF in the deceased who survived for 4 and 5 days (Fig. 5).

Certain limitations in this study are inevitable due to the utilization of real human samples. Firstly, the exact amount of poison intake was unknown as we focused on cases of acute poisoning in individuals who attempted suicide. Secondly, the duration of treatment and survival of the deceased were assumed based on police records and the available hospital records of the patients. Additionally, the distribution and detection of CPF in various tissues and fluids can be influenced by factors such as the unknown quantity of poison consumed, the individual's metabolic rate, and the extent of treatment received. Working with human samples introduces inherent challenges and constraints that affect the scope and interpretation of the research findings. To address some of the limitations, we have employed a large number of samples in each category ($n = 10$), which increases the reliability of our results. By doing so, we aim to provide valuable insights for medical officers and the forensic toxicologists' community, helping them choose the appropriate biological sample from the deceased to obtain reliable and accurate results. This information can greatly assist forensic toxicologists in formulating their examination outcomes.

Conclusion

Our research focused on the detection and distribution of CPF in cases of acute poisoning using simple liquid–liquid extraction and GC–MS/MS analysis. The method was validated and found effective and reliable. Careful selection of forensically significant biological samples enabled a comprehensive study on CPF detection. This research is highly relevant to forensic toxicologists, as they commonly handle the same set of biological samples in forensic laboratories.

In individuals who died immediately after consumption of CPF, stomach tissue showed extremely high CPF concentrations, indicating recent consumption before death. CPF was also detected in the blood, liver, kidney, and urine, indicating metabolism and elimination had begun. In cases with no medical treatment and death within 3 h, CPF was detectable in various tissues and body fluids. However, detecting CPF in the stomach tissue of individuals hospitalized and

survived for 4 or 5 days was challenging and could lead to false negatives. Analysing urine, blood, and liver tissue from deceased individual survived for 3 days provided more precise results compared to stomach tissue. When examining particular organs, it was evident that CPF could be easily identified in the liver tissue of individuals who consumed CPF and died either immediately or up to 3 days after consumption. However, beyond this timeframe, the detection of CPF varied and the results could be positive depending on the quantity of CPF ingested and the type of treatment administered. The CPF concentration in kidney tissues showed similarities between individuals who experienced immediate death and those who survived for 2 days. There was a slight increase in individuals who survived for 3 days, but a noticeable decline was observed in those who survived for 4 and 5 days, suggesting substantial elimination through urine.

In light of our findings, we can recommend that the blood sample is the most suitable post-mortem biological sample for detecting CPF in individuals who survived for a duration of 2 to 4 days. Additionally, urine sample played a crucial role in detecting CPF in individuals who survived for 4 and 5 days. In comparison to stomach, liver, kidney, and blood samples, the concentration of CPF was notably lower than in urine samples. Particularly in individuals survived for 4 and 5 days, the CPF concentration in urine was higher than that of all other tissues and body fluids. This emphasizes the importance of utilizing urine sample as a valuable sample for detection in cases where patients survived for 4 and 5 days.

Key points

1. Detecting poison in post-mortem tissues and fluids after varied survival periods in toxicokinetic is gaining traction among forensic toxicologists.
2. This innovative study investigates the most appropriate post-mortem tissue or body fluid for detecting chlorpyrifos pesticide in cases of acute poisoning with varying survival durations, utilizing GC–MS/MS techniques and simple solvent extraction procedures.
3. The research recommendations proposed in this study will contribute to the tissue- and time-targeted detection of chlorpyrifos and provide valuable insights to resolve conflicting experimental findings.
4. The experimental data and information provided here in this study will serve as a valuable resource for forensic toxicologists.

Declarations

Conflict of interest The authors declare no competing interests.

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