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Toxicological Findings in Death due to Ante-Mortem Brain Injuries: A Case Study

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Abstract

Background: Forensic toxicology offer valuable perception into the capability contribution of poison to outside-reason deaths. There is a lack of material that guides forensic researchers at the ethics that want to be taken into consideration while inspecting the contribution and presence of poison to those deaths. Herein, we record a case of twenty-two year old male died because of antemortem brain damage with greenish gray coloured semi-solid material determined in stomach. The correct mechanisms and reason of dying aren't sincerely recognized without the suitable toxicological finding due to the fact the inquiry and post-mortem are restrained to figuring out manner and exterior examination.

Methods: Chromatographic techniques like, thin layer chromatography and gas chromatography-mass spectrometry evaluation have been used in the present study which demonstrated as an powerful technique for addressing intoxication instances associated with ante-mortem accidents.

Results: In chemical evaluation, traces of organophosphate pesticide combination (Metrifonate, Dichlorvos and Ethion) have been determined in furnished specimen samples such as blood. It turned into recommended that the reason of dying will be pesticide combination poisoning.

Conclusion: The present study emphasizes at the significance of seriously examine toxicological findings by the use of chromatographic techniques in terms of the reason and way of dying.

Keywords: Forensic toxicology • Pesticide mixture • TLC • GC/MS

Abbreviations: UNODC: United Nations Office on Drugs and Crime; HPLC: High-Performance Liquid Chromatography; mL: Millilitre; TLC: Thin Layer Chromatography; cm: Centimeter; µl: Microlitre; UK: United Kingdom; v/v: Volume by Volume; GC/MS: Gas Chromatography–Mass Spectrometry; TQ: Triple Quadrupole; Rt: Retention Factor; UV: Ultraviolet; m/z: Mass/Charge; TIC: Total Ion Chromatogram; min: Minute; EPA: Environmental Protection Agency; Da: Dalton; °C: Degrees Celsius

Introduction

According to global study data 2019 on homicide available with the UN Office on Drugs and Crime (UNODC) organisation, about 464,000 people across the world were killed in homicides in 2017, which was increased from 395,542 in 1992. In 2017, 589,000 people lost their lives due to lethal violence. The annual rate of global violent deaths in 2017 was second highest in the period 2004 to 2017. This increase in the number of global violent deaths is primarily associated with an increase in homicides cases [1]. In India which is a developing county, the number of homicide fatalities cut down from 48,167 in 2000 to 42,678 in 2016. The overall homicide rate has decreased by 10 % over a period of 6 years till 2015 but the trend increased "evidently" in some northern states of India [2]. Around 33 % of respondents thought that most people killed through interpersonal violence died from sharp objects, but the actual share of these victims was found around 25 % of all violent related deaths in the country [3].

The forensic autopsy expert is able to recommend the investigative agency about the nature of the death in confirmed or suspected violence related cases. Medical autopsy remains the aptest tool of investigation used for observing the role of violence, if any [4]. Death due to any toxic substance called poisoning deaths, which account for more than 60% of all undecided deaths nationwide;

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plays a considerable role in any forensic investigation. It is an essential parameter of any criminal investigation to disclose whether or not the deceased was under the influence of any toxic chemical substances at the moment of a criminal offence. Postmortem toxicology testing is an important ancillary of the forensic autopsy. In line with many other forensic toxicological studies, chemical poisoning was the most common foremost cause of death. As a good practice by forensic experts, chemical test results are always interpreted in combination with autopsy findings and circumstantial evidences of scene of crime [5]. It is essential to examine case exhibits to correlate the cause of death in most violence related criminal cases, which is attributed to a variety of fatal outcomes. Forensic toxicology incorporates a number of chromatographic techniques to search of toxic chemical substances from variety of samples to aid the legal investigation and find out the actual cause of death. [6]. It is a challenging assignment for a toxicologist to separate and find out the suspected poison from biological matrices. In this paper, we report the importance of chromatographic techniques in a case related to the death of a young person after cerebral damage as per result of blunt force trauma to the head. But chromatographic analysis in post-mortem samples revealed the presence of three dissimilar pesticides. The literature survey shows other cases where a combination of pesticides was used with the purpose of homicide or suicide. The present case is the additional report of death using pesticide mixture having three toxic components by young person where cause of death given by autopsy expert is different. The study also describes the methodological improvements that can determine the qualitative estimation of substances in pesticide mixture analysis.

Case Presentation

A 22-year old unmarried male was declared brought dead in the emergency service of hospital. He had no preceding records of any poison ingestion.. The chief medical officer of hospital informed local police about the incidence. An investigation to found the cause of death was initiated. The dead body was sent to autopsy expert for postmortem. The medico-legal autopsy was conducted for autopsy findings. It advised that point of demise became 22 hours (approx.) earlier than post-mortem. In external examination, two reddish brown abrasion marks size 3.5 cm × 2 cm present on back of abdomen and 2 cm × 1 cm present on the front of left knee observed. In internal examination, effusion of blood present in the scalp layers over bilateral occipital region. A subdural hematoma layered present on both cerebral hemispheres except bilateral frontal lobes. No effusion of blood present in the neck tissue. Liver, lungs, gastric mucosa, spleen, bowels with each kidney have been discovered in a congested at some stage in postmortem. Autopsy expert observed 300 ml greenish-grey colored semi-solid material in stomach contents. The cause of death given by chief medical officer was cerebral damage as per result of blunt force trauma to the head. All injuries were ante-mortem in nature caused by blunt force, fresh prior to death. However, specimens of the stomach with contents, pieces of the liver, spleen, intestine and kidneys were preserved in sodium chloride preservative for rule out any concomitant poisoning. The blood sample was also sealed for toxicological evaluation. The specimens have been tested in Forensic Science Laboratory to understand the unknown deadly substance with the aid of using systematic toxicological screening.

Material and Methods

Chemicals and reagents

All organic solvents used have been of HPLC grade and acquired from Merck (Merck Life Science, Mumbai, India). All actual neat standards (>95% purity) have been received from the Sigma-Aldrich. Name of chemical substances and standards used during analysis are cited in Table 1.

Sample preparation

The systematic toxicological evaluation became achieved for unknown poisoning. The autopsy specimens (liver, spleen, kidney, small intestine, stomach with contents, and blood) have been tested for popularity of unknown poisons like insecticides, drugs, gaseous, volatile and metallic poisons. Liquid-liquid extraction manner became used for pesticide examination. Briefly, 10 mL of blood sample became de-proteinised with the aid of using the usage of five mL sodium tungastate (10% in water) accompanied with the aid of using addition of few drops of focused sulphuric acid and agitate for five min., then filtered to make clean solution. The filtrate became extracted with acetone the usage of setting apart funnel. The extracts have been dehydrated over anhydrous sodium sulphate.

Tissue samples (one hundred gm) have been taken and reduce into small portions. Samples have been homogenised the usage of a excessive pace mixer with acetone, positioned the samples undisturbed for the night time and subsequent day pour the solvent from the samples. Remove water contents from poured solvent with the aid of using passing from white solid sodium sulphate (anhydrous). The organic layer after filter became made air dry. The dried filtrates have been reconstituted in solvent acetone for increase pesticides residue study.

Chromatographic evaluation

TLC evaluation: Thin Layer Chromatography (TLC) became done as defined earlier [7]. Briefly, silica gel 60F254. TLC plate of thickness two hundred μm, 20 × 10 cm (Merck, Darmstadt, Germany) used for evaluation. 1 μl glass capillaries (Camlab, UK) used for applying samples as spots 1 cm from plate edge and about 2 cm apart. TLC improvement became done at R.T. in 20 × 10 cm horizontal chamber (Camag, Switzerland). Hexane: Acetone (70:30, v/v) mobile phase used for separation and then permitting the plate to dehydrated. The developed TLC plates have been visible first of all in UV light at 254 nm and after that spraying a number finding reagents. After spray, spots have been tested with the aid of using differentiate the Rf and spot coloration with reference standard.

GC-MS evaluation: Further, organic extracts have been tested for identity of poisonous chemical substances using a easy internally advanced Gas Chromatography–Mass Spectroscopy (GC-MS) technique. The technique became constantly used for pesticides evaluation in extraordinary crook cases. A bench top Shimadzu GC-MS-MS device made from gas chromatograph GC-2010 Plus interfaced to an upgraded TQ8040 triple quadrupole analyzer used for chromatographic evaluation. GC of the device became ready with a split/splitless injection port, and which became run in splitl mode

(ratio 5.0:1). Injector and MS transfer line temperatures have been each set at 280°C. Shimadzu

AOC-20i auto injector became used to inject 1 µl of every organic extract into the GC-MS with the aid of using AOC-20's auto sampler. All chromatographic separation became sporting *via* a capillary column Optima 5 MS–0.25 µm (30 m length, 0.25 mm inner diameter and 1.0 µm film thickness, Composition: 5% Diphenyl–95% Dimethylpolysiloxane). Helium (He) gas of excessive purity grade became used as a provider gas at a consistent flow rate of 1.46 mL/min.

Temperature of oven became first of all held for two min at 80°C, after which extended to 180°C (20°C/min), after which extended to 280°C (5°C/min) and held for nine min. The overall run time became 36 min. Mass Spectroscopy became operated withinside the Electron-Ionization (EI) mode with the electron strength set to 70 eV therefore protecting time of evaluation (36 min). It became tuned and calibrated earlier than sample injection. Scan technique became used to discover the presence of residue of pesticide in sample. Samples have been screened at cyclic test mode wherein mass/charge value monitored from 50 to 500 AMU in a duration of 0.8 sec. The acquisition, data processing and record generation have been completed with the aid of using making use of GC-MS real time evaluation and GC-MS Lab Solutions. The NIST library 11.2 readings have been as compared and designated spectra with Rt of the exhibits are tallied with the used standard. Samples have been analysed double.

Results

After spraying the all motioned reagents as according to Table 1, yellow and blue shade spots have been evolved in TLC plate at the vicinity in which Malathione and Dichlorvos have been separated respectively. Portions of stomach, intestine, liver, spleen kidney, and blood have been formerly analysed *via* way of means of TLC and Rf values have been tallied with Malathione and Dichlorvos standards. It became located that no any spot of sample extracts coincide with the spot of organo-chloro, carbamate and synthetic pyrethroids standards pesticide utilized in screening (*i.e.* Edosulfan, Propoxur and Pyrethrine). Chemical reagent used withinside the TLC evaluation and observations are summarised in the Table 1.

Type of poison (Pesticide)	Chemical reagent	Standard used for screening	Observation
Organo-phosphorus	Acidified palladium chloride solution	Malathione	Yellow color spot was observed in both sample and standard
Organo-phosphorus	4,4 Nitrobenzylidine pyridine followed by Tetraethylenepentamine	Dichlorvos	Blue color spot was observed in both sample and standard
Organo-chloro	Zinc chloride-diphenylamine solution-UV/ sunlight	Edosulfan	Parrot green color spot was observed in standard only
Carbamate	Fast Blue-B (O-Dianisidine)	Propoxur	Blue color spot was observed in standard only
Synthetic Pyrethroids	Silver Nitrate	Pyrethrine	Black color spot was observed in standard only

Table 1. Type of poison, chemical standard with reagent used in the TLC analysis and observations.

Compound	Retention time (min)	Diagnostic ions (m/z)	Top Peak
Metrifonate	6.927	47, 79, 109, 185,	109
Dichlorvos	6.94	47, 79, 109, 145, 185,	109
Ethion	18.56	45, 65, 97, 125, 153, 231, 261, 384	231

Table 2. The retention time, diagnostic ions and top peak ion for the identified pesticides.

Compound	Structural group	Molecular formula	Molecular weight (Da)	Melting point (°C)
Metrifonate	Organophosphorus	$C_4H_8Cl_3O_4P$	256	83
Dichlorvos	Organophosphorus	$C_4H_7Cl_2O_4P$	220	26
Ethion	Organophosphorus	$C_9H_{22}O_4P_2S_4$	384	−12.2

Table 3. Physicochemical properties of the identified pesticides.

All organic extracts have been tested *via* way of means of GC-MS following TLC test. Optimize tested situations have been utilized in biological samples for the qualitative evaluation of pesticide residue. The Total Ion Chromatogram (TIC) obtained for every sample in test mode. Retention time and mass spectra have been used to recognized peaks. Table 2 indicates the effects acquired from GC-MS instrumental evaluation of samples Table 3.

TIC peaks have been found at Rt 6.927, 6.940 and 18.560. Its diagnostic ions m/z summarised in Table-2, which have been closed to that of organophosphate pesticides, recognized as Metrifonate (Figure 1), Dichlorvos (Figure 2) and Ethion (Figure 3). Endogenous interference was not found throughout the quantitative evaluation of pesticide.

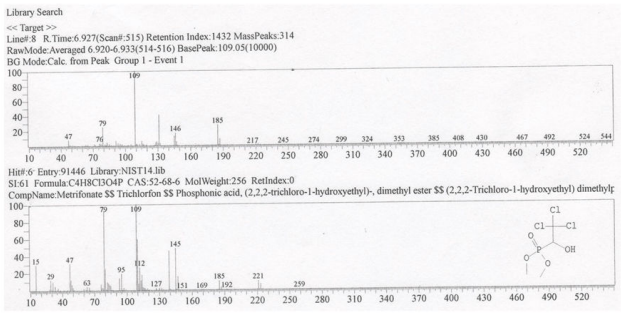

Figure 1. Mass spectra of Metrifonate peak at retention time 6.927 min

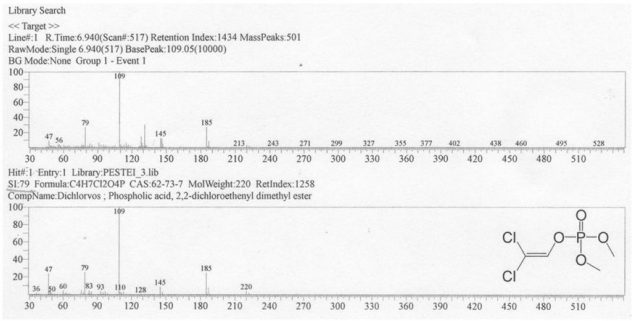

Figure 2. Mass spectra of Dichlorvos peak at retention time 6.940 min.

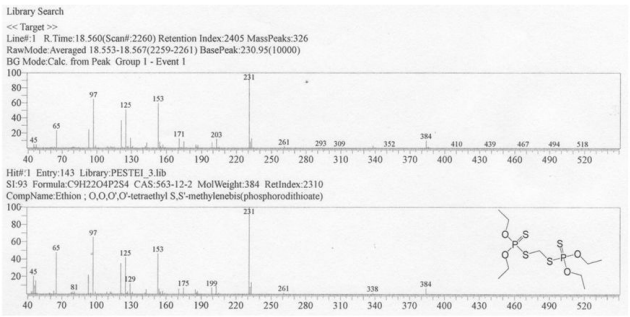

Figure 3. Mass spectra of Ethion peak at retention time 18.560 min.

Discussion

Forensic toxicology is the division of discipline dealing with the study of criminal cases. The analytical findings of forensic scientist play a primary position in crime research particularly in homicidal instances [7]. It involves the use of various chromatographic techniques for the analysis of a range of lethal substances from the biological samples together with blood, nails, hair and urine. High precision instruments and techniques use in forensic analysis depend upon nature of pattern and sort of poison. The variety of murder instances obtained in forensic Sciences Laboratories for toxicological evaluation is continuously rising [8]. Toxic compounds are primarily isolated from the preserved post-mortem samples in unknown poisoning case. It is a tough task for a forensic toxicologist to split and discover the suspected poison from biological matrices in homicidal cases.

According to the Environmental Protection Agency (EPA), any substance or aggregate of materials used for destroying, preventing, mitigating or repelling any pest is referred to as as pesticide. A pesticide combination is ready through blending 2 or extra insecticides and used with inside the shape of single solution [9]. Nowadays numerous complicated pesticide combos used on lots of plants because it has vast spectrum of activity. The poisonous capacity of mixtures of pesticides is usually excessive compared to exposure of agrochemical components alone [10]. In present study, a case regarding the dying of someone after cerebral harm as in keeping with end result of blunt pressure trauma to the head became reported. But chromatographic evaluation found out the presence of 3 extraordinary insecticides (Metrifonate, Dichlorvos and Ethion) in autopsy samples. All detected pesticides come under organophosphate category. Organophosphate compounds can control frame glucose homeostasis *via* severa pathways together with inhibition of paraoxonase, pancreatitis, physiological and oxidative stress, nitrosative stressinhibition of cholinesterase, interruption in the metabolism of liver tryptophan, and stimulation of the adrenal gland [11]. The blended impact of pesticide is the sum of the efficiency corrected amount of individual substance having comparable mechanism of action. Multiple organophosphate pesticide exposures on acetyl-choline esterase activity are including the inhibitory outcomes of individual compounds [12]. Table 3 supplied the physicochemical properties of recognized pesticides.

There are many study published where the pesticides combination became used with the aim of suicide or homicide. The current study is the additional report of death using pesticide mixture having three toxic components in young person where cause of death given by autopsy expert was cerebral damage as per result of blunt force trauma to the head. The presumed reasons of dying become observed to be incorrect in 28% of instances which indicates the significance of post-mortem in the occasion of unnatural deaths [13]. For identity of suspected structural group, thin layer chromatography is the maximum often used separation method. This study is attempted for detection of type of poison using this technique in different biological matrices including blood with the intention of presume which one of them gives good results. TLC is the preferable procedure in this study because, it is precise, requires easily available chemicals, less time consuming and cost effective [14]. This study proved to be a further literature which support TLC followed by GC-MS analysis as a consistently good analytical technique in suspected death cases. The study also describes the methodological improvements that can determine the qualitative estimation of Metrifonate and Dichlorvos in pesticide mixture analysis.

Metrifonate and its rearrangement product Dichlorvos differentiated inspite of same top peak (109) and diagnostic ions (47,79 and 185), except 145 present in case of Dichlorvos only. The mass difference between Metrifonate and Dichlorvos was found to be the same as that of hydrochloric acid (36 Da). Hydrochloric acid product was eluted from capillary column before the solvent (methanol), so was not detected in the TIC. We also concluded that

visceral part along with post-mortem blood sample well-matched for pesticide detection by using chromatographic techniques, even if the pesticide is used in slight amount and this study also support that suicidal poisoning with organophosphate chemical compounds has increased incidence and bears 4 to 30% mortality rate in developing country like India.

Globally, but particularly in low-income countries like India, pesticide ingestion is used as a means of self-harm and poisoning. Approximately 3 million pesticides acute poisoning cases reported worldwide every year. Out of 3 million cases, 1 million are accidental or occupational poisoning cases and the rest of these are suicide attempts. Chemical compounds having same modes of action can act together to produce a combination of effects are particularly larger than the effects of every mixture constituent applied individually. These effects can be expressed by dose and concentration summation [15]. Multiple observational researches have confirmed pesticide poisoning throughout India primarily based totally in healthcare settings, wherein fatality rates moving from approximately 5% to over 70% [16]. The tendency closer to the expanded advertising and marketing of pesticide is predicted to bring about an increase effect at the instances of combined pesticide toxicity. These combination compounds are characterized through excessive insecticidal efficiency and neuro-poisonous behaviour of those compounds in acute publicity cause excessive detrimental consequences in human beings and different mammals [17].

Conclusion

Forensic toxicological evaluation is great now no longer only for a criminal research wherein a foul play is believed it is further vital for figuring out suicides and dying because of accident and in violence associated cases.

Declarations

Ethics approval and consent to participate

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article

Competing Interests

Not applicable

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