



MODIFIED SOLID PHASE EXTRACTION OF ORGANOPHOSPHORUS PESTICIDES; REPORT OF TWO CASES OF SUICIDES

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ABSTRACT

Suicide is considered as the tenth cause of the death worldwide. Among different pesticides, organophosphorus pesticides (OPs) are the most widely used, but they are highly toxic, so there are increasing suicide reports about use of them. Considering the low rate of organophosphorus pesticides in the suicide reports, these two cases helped us to improve our customary methods of extraction. In this study, two cases of suicide related to a 25-year-old girl and 36-year-old man who have taken high doses of Diazinon. Both cases were dead on arrival to the hospital so no medical cases were done for them and they were brought to the Fars Legal Medicine Organization. They were suspected of using some toxins or drugs. Diazinon and Dithion were extracted using modified solid phase extraction method in the gastric content of both cases by GC-MS analysis. Modified solid phase extraction method is so helpful for toxicological analysis and detection of cause of the death in suicide, homicide or accidental died cases of organophosphorus poisoning referred to legal medicine organization.

KEY WORDS

Suicide; Organophosphate, Poisoning; Diazinon, Forensic Medicine

INTRODUCTION

Acute pesticide toxicity is extremely common in developing countries of the Asia-Pacific region, particularly in individuals with low education and poor regulatory frameworks [1]. The excessive and unregulated use of pesticides in agriculture and public health has caused severe environmental pollution and increased risks for human health [2, 3]. Among different pesticides, Organophosphorus pesticides (OPs) are the most widely used, but are highly toxic, so that new compounds with high potency and less toxicity are being developed continuously [1]. Nowadays, contact with organophosphorus pesticides is an important health problem [4]. Although rapidly metabolized, they are highly toxic to insects and mammals. Due to easy access to organophosphorus pesticides and their higher degree of toxicity, accidental poisonings and also suicides by using them are so frequent. So, it is one of the toxic materials causing human poisoning and death worldwide annually [5]. Diazinon (DZN) [DIA: O, O-diethyl-O-(2-

isopropyl-4-methyl-6-pyrimidinyl) phosphorothionate] is an organophosphorus (OP) insecticide, and is designed as an irreversible acetylcholine esterase inhibitor [6] and it is classified as moderately hazardous class-II OP insecticide. Diazinon is oxidized by microsomal enzymes to cholinesterase inhibiting metabolites such as diazoxon, hydroxy diazoxon and hydroxydiazinon [7]. New and reliable toxicology analytical methods are needed to detect and confirm the cause of death. Solid phase extraction method (SPE) beside analytical methods such as high performance liquid chromatography (HPLC) and Gas chromatography-mass spectroscopy (GC-MS) can be considered for such needs. Hereby, we report two cases of suicide with organophosphorus pesticides which with introducing new extracting method (SPE) of such toxic agents from the cadaver's samples.

Case report

Case 1: The decedent was a 25 years old girl who, as her mother said, had eaten an unknown liquid for suicide. She had a bachelor degree with no previous history of suicide attempts. Although tried to save her, but she died at arrival to the hospital and no medical action was useful. Because the hospital confirmed her death as aspiration due to unknown toxin or drug her body was delivered to the Fars legal medicine organization, and after autopsy, her blood, gastric content, liver and bile were sent to toxicology laboratory for further analysis. Autopsy findings were miosis, hyperemia in facial skin and conjunctiva, eyelid and genital bleeding, white foam in larynx and thorax, petechial hemorrhage in her lungs and 90 ml of dark green/brownish fluid in the stomach.

Case 2: The decedent was a 36 years old man who, according to his wife claim, had a bottle labeled as Diazinon beside him and found dead in his livestock. As there was no sign of conflicts, it was suspected as a suicide. He also had no previous suicidal attempts. Reported as poisoning with unknown toxin or drug, after autopsy in Fars Legal medicine organization, his blood, gastric content, liver and bile were analyzed in toxicology laboratory. Autopsy findings were miosis, facial hyperemia, conjunctiva and eyelid bleeding, petechial in lungs and 100 mL of dark greenish fluid in the stomach.

Analytical Methods

Analytical solvents were of HPLC grade and reagents were of analytical grade, Merck®. Reagents and

chemicals were from Sigma Chemical Co, St. Louis, MO, USA. Solid phase extraction (SPE) method was used to extract organophosphorus pesticides. The general SPE procedure is to load a solution onto the SPE phase, wash away undesired components, and then wash off the desired analytes with another solvent into a collection tube. This separation method uses the same type of stationary phases as are used in liquid chromatography columns. The stationary phase is contained in a glass or plastic column above a glass wool. After isolation of agents by SPE method, gas chromatography-mass spectroscopy (GC-MS) was used to detect OP materials in obtained biological samples. Although SPE method had been very effective in multiple drugs monitoring, but we were not satisfied with the results of pesticides extraction, so we decided to add a step of pretreatment of samples by saturated calcium chloride before pouring the sample onto the SPE cartridges to make this method feasible in pesticides extraction. In the first case, Hydroxy Diazinon was found in gastric content after SPE extraction followed by HPLC and confirmed with GC-MS analysis. In the bottle found nearby the cadaver Diazinon, Dithion and Hydroxy Diazinon were detected by GC-MS analysis. In case 2, after SPE extraction followed by GC-MS analysis of Diazinon, Dithion and Hydroxy Diazinon were found in gastric content. Finally the cause of death for both cases was reported poisoning with organophosphorus pesticide.

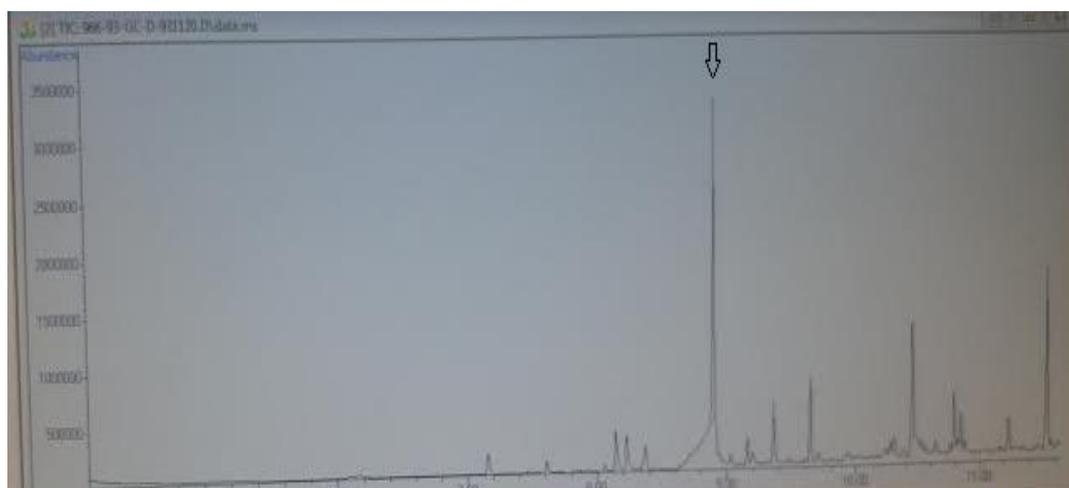


Figure 1- Detection of Dithion by GC-MS analysis

DISCUSSION

Lethality of suicidal organophosphorus poisoning has been evaluated in so many studies worldwide [8-11]. There are some reports of suicidal organophosphorus poisonings [12-14]. Forensic medicine administrations are responsible for definite detection of the cause of homicides, suicides or accidental deaths [15]. Thus, using reliable and sensitive methods especially for detection of poison ingestion deaths are so critical and important. Organophosphate (OP) pesticides are neurotoxic compounds that are widely used in agriculture. Classical methods for monitoring OP exposure comprise the measurement of intact OP, its metabolites or cholinesterase activity [16, 17]. Newly developed methods such as modified SPE method for extraction and analysis of the toxic materials and suicide cases is so helpful. Acidic, neutral and basic drugs are extracted from biological fluids or tissues using solid phase extraction (SPE) and are detected by HPLC or gas chromatography-mass spectrometry (GCMS) [15]. We modified this procedure for postmortem toxicological analyses in our forensic toxicology laboratory [11]. It is well known that Diazinon is quite stable in basic media, so clearly adding saturated calcium chloride will be beneficial. On the other hand adsorption of Diazinon can be attributed to the differences in the topological environment of OH groups on absorbent surfaces [18].

In both cases, Diazinon and Dithion as its impurities were found after SPE extraction followed by GC-MS analysis. Compared with liquid/liquid extraction method for pesticides in postmortem specimen, SPE general extraction by adding this simple pretreatment step has positive effects detection of definite cause of the death. So it can easily be considered as a new method of detection of organophosphorus compounds.

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Conflict of Interest: Non- Declared.

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