Analysis of Insecticides in Body Fluids: A Review

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Abstract

Analysis of insecticides in body fluids is the choice of research from very long time before to know the impact of its on living beings. The analytical methods of insecticides have been improved in last few years by advance and sophisticated techniques. The analysis of insecticides in urine and visceral samples are preferred among other body fluids as drugs and its metabolites found in urine are usually stable and are present in higher concentration as compared to other biological fluids. These are also detectable for a relatively longer period of time. Several techniques for the qualitative as well as quantitative analysis of insecticides are reviewed in this paper ranging from conventional chromatographic methods to the modern GC-MS methods, in order to suggest a better, efficient, fast and result oriented method that can be utilized in future work of analysis.

Keywords: Forensic Science; Insecticide; Extraction; GC-MS

Introduction

The principal objective of forensic and analytical toxicological methods of extraction, isolation and cleanup is to provide a suitable concentrate to which the chromatographic, spectroscopic and other instrumental methods of identification as well as chemical reactions can be applied. Before applying the methods of their detection and determination, it is necessary to effect separation of substances suspected or unknown from the biological material under investigation. This material can include blood, urine, gastric contents, tissue foodstuffs etc. Due to the differences in nature and chemical composition, and the presence of proteins and fat, each type of material presents an individual problem.

The method of approach to an analysis of biological material for the presence of drugs depends very much on the type of material provided. Urine is the most useful material that is available. Urine will contain not only the unchanged substance but also the metabolites and the concentration of these is generally much higher than blood. In case of a living person large quantities of urine are generally available which enables the toxicologist to make full investigations resulting into final identification. Urine also has one more advantage that it is usually protein free as a result of which the analyst does not face the problems in the analysis that appear in case of other materials like tissues, foodstuffs and putrefied articles.

Useful information can also be obtained from the examination of gastric contents. This has the advantage that only unchanged substances will be encountered, and the analysis will not be complicated by the presence of numerous and sometimes unknown metabolites. Gastric contents can be obtained from a cadaver or from living person admitted to hospital with symptoms of poisoning. The composition of gastric contents can show great variations depending upon the type and quantity of food present. The method of extraction is dependent on the nature of the gastric material.

Blood is also an important biological material. Some analysis prefers serum for the purpose of analysis, but there are limitations. Drug concentration in the blood may be very low, which necessitates the use of techniques that require expensive equipment such as spectrofluorometers. In addition, blood is not available frequently and freely in large quantities.

In the case of a dead person, the specimens received by the analyst will consist of soft tissues (e.g., liver, kidney, and brain), small quantities of urine and blood and some gastric and intestinal contents. Such materials always pose difficult problem for analysis.

To overcome these aforesaid difficulties, which are likely to be faced by the analyst, different approaches have been developed for extraction of different classes of poisons from biological materials.

A method proposed for the extraction of organic poisons from biological materials. Finely divided biological material is mixed with twice the volume of alcohol acidified with tartaric or oxalic acid heated to 75°C, cooled and filtered. The residue is washed with alcohol and the filtrate evaporated at 35°C. Insoluble matter which separates during evaporation is filtered and evaporation is continued as above at 35°C. The final residue obtained on completion of the evaporation is taken up with small quantity of water and filtered. The filtrate is made alkaline with sodium bicarbonate and shaken with four or five volumes of ether. The ether layer is separated and allowed to evaporate leaving behind the suspected drug or poison [1,2].

A procedure has been described which is applicable to the screening of large numbers of samples for anticholinesterase activity. The method is sufficiently sensitive to detect cholinesterase depression due to as little as 30 ppt paraaxon; 3 ppb malaoxon or 2 ppm of carbaryl in either ethylene glycol or water. While the method does not allow the absolute differentiation between compounds which might be present, some classification is possible, i.e., for compounds which must be activated before they possess anticholinesterase activity [3].

An analytical method developed for detection of 33 compounds in domestic air, its drinking water, and from skin contact during pesticides application. Soxhlet extraction was used. The extraction procedure of U.S. Environmental Protection Agency (EPA) method 608 was used for water samples [4].

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A sampling and analytical method for organophosphorous pesticides using a combined filter/XAD-2 sorbent sampler and gas chromatography (GC)-flame photometric detection (FPD) was developed. The method was evaluated for 19 organophosphorus Pesticides based on the joint Occupational Safety and Health Administration/National Institute for Occupational Safety and Health Standards Completion Program methods evaluation protocol. The evaluation addressed analytic recovery, sampler capacity, sample stability, and precision and accuracy [5].

A systematic toxicological analysis was discussed in three steps. The first is sample workup isolation and concentration e.g., hydrolysis, digestion, liquid–liquid extraction, solid-phase extraction (SPE) and immunoaffinity chromatography. The second step, differentiation and detection can be performed by, for example, TLC, GC or HPLC. The final step is identification by comparison of results with libraries of reference substance data [6].

At the Centers for Disease Control and Prevention (CDC), scientists have acquired extensive experience in the development and application of specific techniques for biological monitoring of a variety of toxicants, including many of the contemporary-use pesticides. They have used these methods to measure the internal dose of pesticides received by people in acute and chronic incidents resulting from both environmental and industrial exposure. Additionally, they have established normative values, or reference ranges, of several pesticides based on measurements of their metabolites in the urine of randomly selected adults in the US population. These data have been successfully used to distinguish overt exposures from 'background' exposure [7].

The scientist reported that biological monitoring is becoming an increasingly important element of field studies designed to assess the risk from occupational pesticide exposure for preventive purposes. Selection of suitable biomarkers of exposure to pesticides, development of detection methods, and validation of measurement and interpretation of results are of utmost concern among current issues [8].

An analytical method for precise identification and quantitation of 10 pesticides in human blood was developed. The pesticides studied, which have appeared frequently in actual cases, were endosulfan, lindane, parathion, ethyl-azinphos, diazinon, alachlor, tetradifon, fenthion and dicofol (o-p' and p-p' isomers). The current method replaces an earlier method which involved liquid-liquid extraction with a mixture of n-hexane-benzene (1 + 1). The extraction was performed by solid-phase extraction, with C18 cartridges and 2 internal standards, permethane and triphenylphosphate. Eluates were analyzed by gas chromatography (GC) with nitrogen-phosphorus and electrochemical detectors. Results were confirmed by GC-mass spectrometry in the electron impact mode. Blank volunteer studies with 1 mg oral doses of chlorpyrifos, diazinon and malathion. In non-occupationally exposed people, 95% of urinary alkyl phosphates do not exceed 72 micromol/mol creatinine. In occupationally exposed people, the corresponding 95th percentile of total urinary alkyl phosphates is 122 micromol/mol creatinine. In volunteer studies with 1 mg oral doses of chlorpyrifos, diazinon and propetamphos the mean peak values were 160, 750 and 404 micromol/mol creatinine, respectively, and were not associated with any reduction in blood cholinesterase activity. The levels of OP metabolites seen in urine from workers potentially exposed to OPs were generally low and unlikely to cause significant reduction in blood cholinesterase activity [11].

A multiresidue method for the determination of insecticides in honeybees was studied. The developed method was based on the matrix solid-phase dispersion technique. A total number of 12 insecticides (azinphos-methyl, buprofezin, chlorpyrifos, chlorpyrifos-methyl, diazinon, ethion, fenitrothion, fipronil, methidathion, phosalone, pirimicarb, propoxur) were used to distinguish overt exposures from 'background' exposure [7].

A simultaneous analytical method which was examined for carbofuran and its derivative pesticides in water was reported. Since carbofuran derivatives were hydrolyzed to carbofuran in water, the liquid-liquid extraction method was used to obtain an accurate concentration value. Moreover, since these compounds were easily decomposed at the GC/MS injection port, temperature programmable inlet on-column injection was used. By combining the two methods, a sensitive analytical method was established without hydrolysis and thermal decomposition. As a result of recovery experiments using distilled water, river water and tap water, acceptable recovery rates and favorable reproducibility were obtained [13].

Anticholinesterase pesticides are widely used, and as a result they are involved in numerous acute and even fatal poisonings. The aim of this study was the development, optimization, and validation of a simple, rapid, specific, and sensitive gas chromatography–mass spectrometry method for the determination of 11 anticholinesterase pesticides (aldicarb, azinphos methyl, carbocurian, chlorpyrifos, dialifos, diazinon, malathion, methamidophos, methidathion, methomyl, and tebufos) in blood. Only 500 µL of blood was used, and the recoveries after liquid–liquid extraction (toluene/chloroform, 4:1, v/v) were more than 65.6%. The calibration curves were linear (R(2) ≥ 0.996). Limit of detection and limit of quantifications were found to be between 1.00-10.0 and 3.00-30.0 µg/L, respectively. Accuracy expressed as the %E(r) was found to be between -11.0 and 7.8%. Precision expressed as the percent relative standard deviation was found to be <9.4% [14].

Conclusion
Analysis of insecticide is important not just for an investigation in which a foul play is suspected it is equally essential for determining accidental deaths and suicides. Many noteworthy analytical techniques have been reported by the toxicologist based on the several chromatographic and spectroscopic methods. The techniques involve
analysis of insecticide from urine and visceral materials for both qualitative as well as quantitative interest, which not only have high resolution values but also greater separation efficiency. Almost all the techniques are highly sensitive and require minimal sample requirement in addition of easy sample preparation. Gas Chromatography Mass Spectrometry and Gas Chromatography tandem Mass Spectrometry are the recent emerging trends in the simultaneous analysis. Besides all the techniques reported so far there is a need to develop other economical techniques with the better sensitivity and finest resolution in order to provide speedy determination of insecticides.

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