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RESEARCH ARTICLE

Isolation of Organophosphorus Pesticides from Biological Material by Accelerated Solvent Extractor (ASE-200) and its Determination by Gas Chromatographic Method

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ABSTRACT

Accelerated Solvent Extractor is an improved technique over the traditional extraction technique have been used for isolation of organophosophorus pesticides from biological materials. Accelerated Solvent Extractor ASE-200 from DIONEX Corporation, Sunnyvale, California, equipped with:33 ml. extraction cells, 60 ml Collection vials, A 2-litre glass reservoir for solvent and Cellulose filter disks were used for this purpose. Analysis of all the compounds by this method showed better resolution of peaks, appropriate identification with distinct peak pattern for each compound. Further, peaks at room temperature between 11-18 min are specific for organophosphosprus pesticides compounds were analysed. It can thus replace the customary extraction methods using less solvent and less time from a small amount of biological material. The exposure of the working staff to the health hazardous atmosphere and toxic solvents is greatly reduced. The use of elevated temperature and pressure give better extraction results.

Key words: Accelerated Solvent Extractor (ASE-200), Gas chromatography (GC) pesticides, Viscera, Organophosphorus compounds: Dichlorovos, Monocrotophos, Malathion, Chlorpyriphos, Dimetheoate.

INTRODUCTION

The importance of thin layer chromatography was recently reviewed (Miroslav, 2010; Sherma, 2007) Liquid chromatography was used to detect concentrations as low as 0.0083 μg/L of pesticides in groundwater (Mughari, 2007). Gas chromatography was used for pesticides in milk (Cerkvenik, 2000) The standard spectrophotometric Ellman's method may also be used to assay for pesticides (Arduini, 2005; Markmee, 2006; O'Farrell, 1977). Such a test was used for monitoring pesticides in surface waters (Drevenkar, 1981) and furthermore, it was employed to examine the cholinesterase activity in canine sera (Furlanello, 2007) . When the enzymatic activity was inhibited by metal ions, Ellman's assay for the cholinesterase activity was commonly used with another chromogen, i.e. o-nitrophenyl acetate (Frasco, 2005).

The cholinesterase activity may be measured by m-nitrophenol reaction with the formed acetate (Pohanka, 2007). Another way of detection of toxic compounds is that of biosensors presented recently (Pohanka, 2008). Biosensors for pesticides based on cholinesterase were thoroughly reviewed (Pohanka and Jun, 2007) and one of this type of biosensor was used for a pharmacological study of a cholinesterase reactivator and its impact Nevertheless, cheap and reliable multichannel methods for paraoxon and pesticides, in general, remain a challenge for practical use. In the present work, we introduce the multichannel spectrophotometry technique to assay for the pesticide paraoxon. Paraoxon is a pesticide in common use and the risk of contamination of water supplies remains in some countries. Spiked beverages and drinking water were chosen as commodities that will probably contain paraoxon when water supplies are polluted.

The toxic effects of organophosphorus insecticides have long been intensively studied (Ray, 2001). Forensic Toxicology is one of the important disciplines of forensic science that deals with the detection and determination of residual poisonous substances in a suspected poisoning case. These poisonous compounds are primarily isolated from the biological material such as viscera, stomach contents and blood preserved after postmortem in the fatal poisoning case by various methods (Moraes, 1994; Wang, 2009). The isolation and determination of the poison is a complex and challenging task as the amount of poison available for estimation is merely a representative of what remains of the original amount in the body after death. As after its entry into the body, the poison gets eliminated by vomiting, purging or by other channels of elimination. It undergoes metabolic changes or may be detoxified (Pohanka 2008; 2009). Also, it is next to impossible to analyse entire tissues of the body. Thus, the foremost important step before proceeding for the analytical findings is isolation of the poison from the biological material. Once the poison is isolated, the next significant step is its qualitative and quantitative determination.

The most conventional methods for isolation of organic poisons from biological tissue material are liquid-liquid extraction or solid-phase extraction (wrist-shaking extraction methods). These techniques have disadvantages associated with the handling and disposal of large volumes of flammable and hazardous organic solvents along-with handling of the visceral material, exposing the toxicologist to the health hazardous atmosphere (Valdes, 2008) .

Accelerated Solvent Extractor is an improved technique over these traditional extraction techniques. It accelerates these extraction processes by using organic solvents at elevated temperature and pressure to deliver extractions equivalent to traditional techniques, but in shorter amount of time, with reduced solvent use, and with automation of the extraction process. It can thus replace the customary extraction methods using less solvent and less time from a small amount of biological material. The exposure of the working staff to the health hazardous atmosphere & toxic solvents is greatly reduced. The use of elevated temperature & pressure give better extraction results.

The most commonly encountered organic poisons in Maharashtra & Central parts of India are insecticides, pesticides and drugs. GC provides a quick and efficient method for determination of various groups of insecticides.

MATERIALS AND METHODS

Extraction method by Accelerated Solvent Extractor, ASE-200 : The pre-requisites for extraction of organic poison by ASE-200 are :

Cell preparation*:* A cellulose disk is first inserted at the bottom of the extraction cell. 20gm viscera is cuts into small pieces and mixed with diatomaceous earth to get a well dried mass that can be easily transferred into the extraction cell. The whole mass is loaded into the extraction cell which is then tightly capped and loaded on the tray slots of ASE. A clean and properly capped collection vial is placed on the lower carousel at appropriate number for collection of the extract.

Extraction parameters: The following parameters are entered on the ASE 200 to initiate the run by following method :

Preheat	0	Temperature :	70^{O} C
Heat	5 mins	Cycles :	3
Static time	2 min	Rinse between samples	on
Flush %	60	Pressure	1200 Psi
Purge	20 secs		

Concentrating the extracts: The extracts obtained by above methods was passed through sodium sulfate to remove any water content and almost reduced to dryness at atmospheric temperature and pressure.

Gas Chromatographic method :
Equipment : Nucon gas chromatograph 5765,
Detector : NPD, processed with Winchrom software using Windows 98
Column : SE-30, S.S 2 mts, 1/8" OD, 2 mm ID, Chromosorb W H.P., mesh range 80-100
Carrier Gas : Nucon gas chromatograph 5765,

Method: Temperature programming Injection volume: 0.05, 0.1, 0.2, 0.3, 0.5µ

Oven temperature-1 : 150^{O}C Injection amount : 0.05, 0.1, 0.2, 0.3, 0.5, 1.0, 2.0 µg

Oven temperature-2 : 180^{O}C

Oven temperature-3 : 200^{O}C

Oven temperature-4 : 230^{O}C

Oven temperature-5 : 250^{O}C

Injector temperature : 200^{O}C

Detector temperature : 270^{O}C

RESULTS AND DISCUSSIONS

A majority of toxic OP's are esters of phosphoric, phosphorothioic, or phosphonic acids, or of their anhydrides, halides or amides. All are therefore, potentially hydrolysable, the most labile bonds being the anhydride or halide, the next alkoxy and then amide. Their liability depends on factors such as pH, temperature etc. Some OP's have carboxyester or carboxyamidic linkages that are reasonably labile.

The available commercial products are often formuolations having a definite percentage of active ingredient (ie: the OP compounds which is acting as poison) dissolved in solvents such as Kerosene-oil, Cyclohexanone etc. The OP compound might degrade during the storage period. Also, after ingestion, it undergoes different metabolic changes. Thus, after extraction, the isolated poison can be the compound itself or a mixture of the compound and its known or unknown metabolized or degradation products, that is to be analysed by GC. The goal of GC determination is to analyze a complex mixture of the OP compound along with its metabolized and degraded products. Hence a programmed method with different temperatures with definite time holds was attempted. Analysis of all the compounds by this method showed better separation of peaks, proper identification with definite peak pattern for each compound. Further, peaks at RT between 11-18 min are specific for OP compounds were analysed :

1. Malathion:

Malathion is also known as carbophos, maldison and mercaptothion. It is frequently encountered in suicidal poisoning cases. Technical malathion is a clear amber liquid which decomposes in strong acid or high humidity. Althrough stable in light, it decomposes at high temperatures. It has a very low melting point (2.85 ^{O}C). Its chemical name is diethyl (dimethoxy thiophosphorylthio) succinate i.e. $C_{10}H_{19}O_6PS_2$. It is soluble in most organic solvents and slightly soluble in water. It is metabolized by conversion to malaoxon, the toxic keto analogue, and by hydrolysis to malathion *a*-mono-and dicarboxylic acids which are the major metabolites. Other hydrolysis products include dimethylthiophosphoric acid (DMTP) and dimethyldithiophosphoric acid (DMDTP).

Malathion shows a typical pattern of three distinct peaks initially. Peaks between RT 17-18 are conspicuous. (Fig. 1). An amount of 0.05ug could be detected by this method and showed 99% recovery of malathion after its isolation by ASE-200.

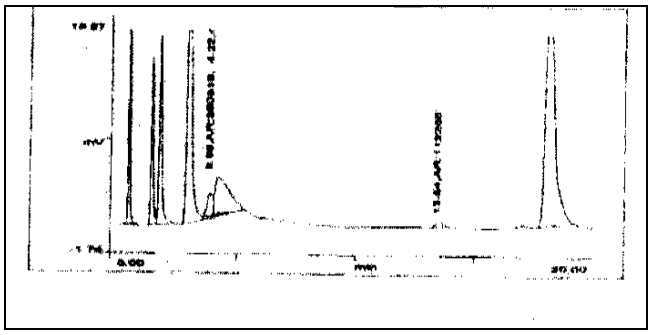

Fig. 1: Typical Pattern of Peaks of Malthion

2. Chlorpyriphos:

Chlorpyriphos is very toxic, general use pesticide. It is, O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorotioate, $C_2H_{11}Cl_{13}NO_3PS$. It is practically insoluble in water but soluble in organic solvents. Choloropyriphos is readily absorbed into the bloodstream from the gastrointestinal tract, through the lungs and through the skin and is eliminated primarily through the kidneys. The major metabolites observed are 3, 5, 6-trichloro-2-pyridinol (TCP), diethylphosphate, and diethylthio-phosphate. Chloropyriphos and its metabolites are eliminated rapidly from the bodyn via urine and faeces. It melting point is 41.5-44^{0}C. Research suggests that this insecticide is unstable in water and the rate at which it is hydrolyzed increases with temperature. The rate of hydrolysis is constant in acidic to neutral waters, but increases in alkaline waters.

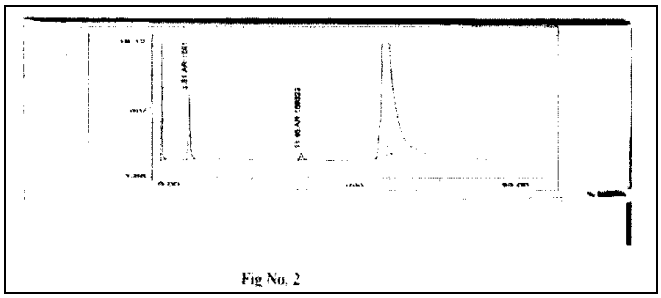

Fig. 2: Typical chrmatographic peaks of Chlorpyriphos.

Detection of Chlorpyriphos by the method shows a distinct peak at RT 2.5 or 2.6 and another peak at RT 18. (Fig.2). The detection limit is 0.05ug. After its isolation by Ase-200, the recovery was found to be 90%.

3. Monocrotophos:

Monocrotophos is also known as Nuvacron, is (E)-Phosphoric acid dimethyl [1-methyl-3-(methylamino)-3-oxo-1-propenyl] ester, $C_7H_{14}NO_5P$, with melting point 25^0 C to 30^0C for commercial product or 54^0C to 55^0C for pure product. It is miscible in water (1 kg/kg at 20^0C) and soluble e in acetone, ethanol, diesel oils, kerosene, dichloromethane, methanol, and toluene. It slowly decomposes in water and gets rapidly hydrolysed under alkaline conditions. It decomposes on some inert materials. Monocrotophos is Quickly metabolized by N-demethylation, Q- demethylation and cleavage of the vinyl phosphate bond. One metabolite identified is dimethylphosphate (DMP).

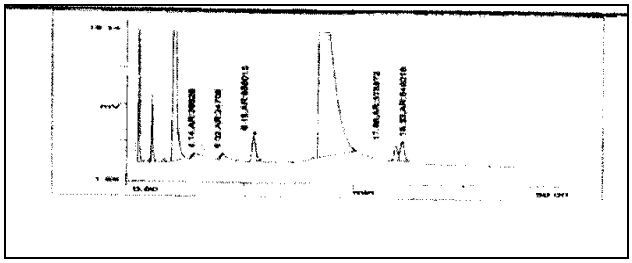

Fig.1: Typical chromatographic peaks of Monocrotphos

Monocrotophos gives a distinct peak between RT 2-3. Peaks at RT 17-18 are conspicuous (Fig.3). Detection limit is 0.5µg. The recovery percentage was found to be 90% for monocrotophos when isolated by ASE-200.

4. Dimethoate:

Also known as Rogor, it is O,O- dimethyl S-methyl-carbamonyl-methyl phosphorodithiolate $C_5H_{12}NO_3PS_2$. Technical dimethoate is a grey white crystalline solid at room temperature and its melting point is 43-45°C. It is readily soluble in most organic solvent s and solubility in water is 25gm/L Dimethioate is readily soluble in aqueous media at pH 2-7 but gets hydrolysed in alkaline solutions (50% hydrolysis occurs in12 days at pH 9). It decompose on heating. In mammals the carboxy derivatives and dimethoate acid $[(CH_3O)P(S)SCH_2COOH]$ was the major metabolite. Other metabolites found are O,O dimethyl phosphorodithioate $[(CH_3O)_2P(S)SH]$, O,O dimethyl phosphorothioate $[(CH_3O)_2P(S)SOH]$, dimethyl phosphate $[(CH_3O)_2P(O)OH]$ and desmethyl derivative.

At lower concentration of 0.05µg only two distinct peaks are obtained alongwith the peak at RT 12. In addition to these peaks another peaks are also obtained with increase in concentration with conspicuous peaks beyond 12 (Fig.4). Determination of Rogor by this method gave 72% recovery after its isolation by ASE-200

5. Dichlorovos (Nuvan)

Dichlorovos is 2, 2-dichlorovinyl dimethyl phosphate, $C4H_7Cl_2O_4P$. It is colourless - to-amber liquid with an aromatic odour having boiling point 234.1^0C. It is completely miscible with aromatic hydrocarbons, chlorinated hydrogen phosphate and dichloro-acetaldehyde.

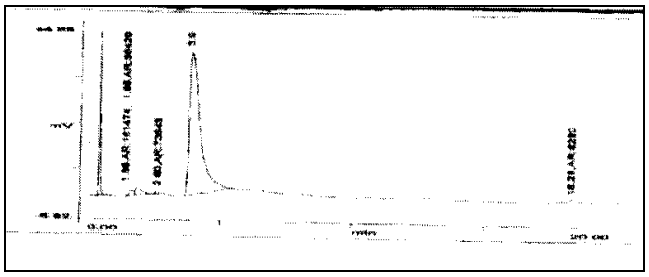

Fig. 4: Typical chromatographic peaks of Nuvan

An amount of 0.05ug could be detected by this method. A distinct peak between 3 & 4 is obtained. (Fig 4). The percent recovery of Nuvan by this method was found to be 85% after its isolation by ASE-200.

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