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Research Article

HPLC WITH SOLID PHASE EXTRACTION FOR IDENTIFICATION AND DIAGNOSIS OF ORGANOPHOSPHOROUS POISONING IN GOATS

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ABSTRACT: High performance liquid chromatographic determination of organophosphorous compound has been done by reverse phase chromatography in goats. The goats were dying showing the symptoms of organophosphorous poisoning. The viscera and stomach contents sample were received from Project Co-Ordinator, Animal Disease Research Institute, Phulnakhara, Cuttack, Orissa. The analysis of samples by HPLC with UV detector after cleaning up in Solid Phase Extraction (SPE revealed presence of malathion that was later quantified.

Key words: HPLC, SPE, Malathion, Goats.

INTRODUCTION

The toxicological impact of insecticidal compounds is increasing day by day as a consequence of their rapid and widespread application. It is used as ectoparasiticide in animal, production of agricultural crops and also public health programme. The consequence of indiscriminate use include apart from health hazards to non-target organisms, pollution of ecosystem and accidental poisoning. In field condition the pesticides poisoning is common due to accidental, malicious poisoning and improper use of pesticides by farmer, but the detection is very rare or undetected due to lack of analytical laboratory for identification and quantification of residue of pesticides and other toxin. Present study was undertaken to ascertain whether the cause of death of large number of goats was due to pesticide poisoning and if so, the identification and quantification of the pesticides by HPLC method.

MATERIALS AND METHODS

The viscera of six (6) animals (random sampling) like stomach contents and liver, lungs also blood smear were received from project co-coordinator, Animal Disease Research Institute, Cuttack, Orissa for diagnosis of causes of death of goats in the farm. It was suspected that, surrounding the farm field the insecticide

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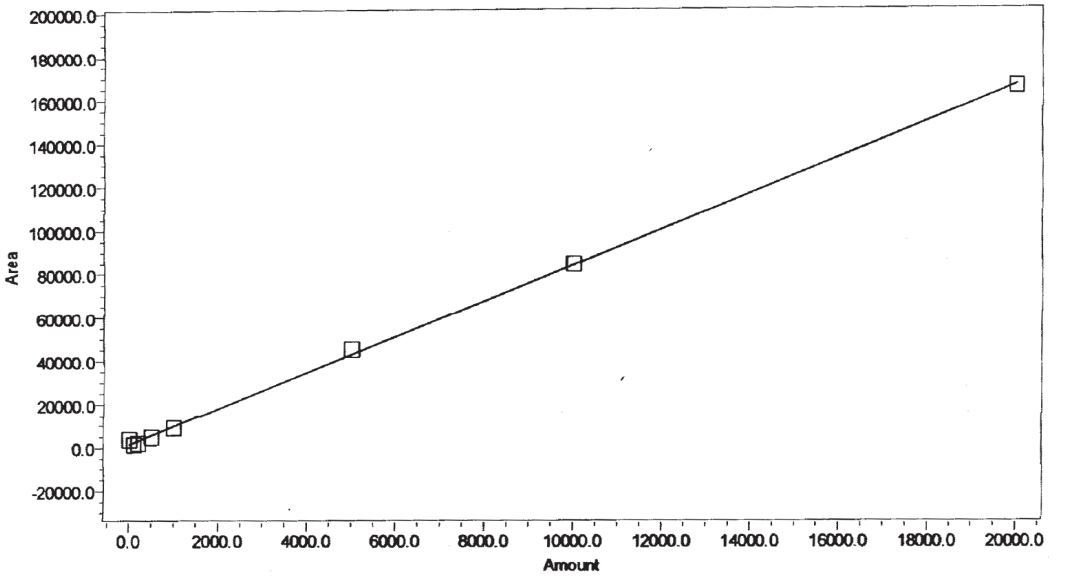
IAH & VB, KOLKATA-37

Project Name: PESTICIDE_ANALYSIS
Reported by User: System

Method:	MALA UV Ch1	Project Name:	PESTICIDE_ANALYSIS
Date Calibrated:	12/24/2007 4:07:32 PM	Channel:	2487Channel 1
		Channel Desc.:	



Name: Malathion Time: 7.461 Fit Type: Linear (1st Order) R: 0.999697 R²: 0.999395 Equation: Y = 8.22e+000 X + 1.69e+003

Peak: Malathion

	Sample Name	Peak Name	Level	Amount	Response	Calc. Amount	% Deviation	Manual Point	Ignore Point
1	Malathion 1ppb	Malathion		1.000	3.786e+003	255.490661	25449.066	No	No
2	Malathion 100ppb	Malathion		100.000	1.350e+003	-40.953421	-140.953	No	No
3	Malathion 200ppb	Malathion		200.000	2.107e+003	51.091742	-74.454	No	No
4	Malathion 0.5ppm	Malathion		500.000	4.886e+003	389.327724	-22.134	No	No
5	Malathion 1ppm	Malathion		1000.000	9.137e+003	906.709976	-9.329	No	No
6	Malathion 5ppm	Malathion		5000.000	4.511e+004	5285.585693	5.712	No	No
7	Malathion 10ppm	Malathion		10000.000	8.410e+004	10031.063230	0.311	No	No
8	Malathion 20ppm	Malathion		20000.000	1.654e+005	19922.684395	-0.387	No	No

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Fig. 1: LC Calibration report of Malathion.

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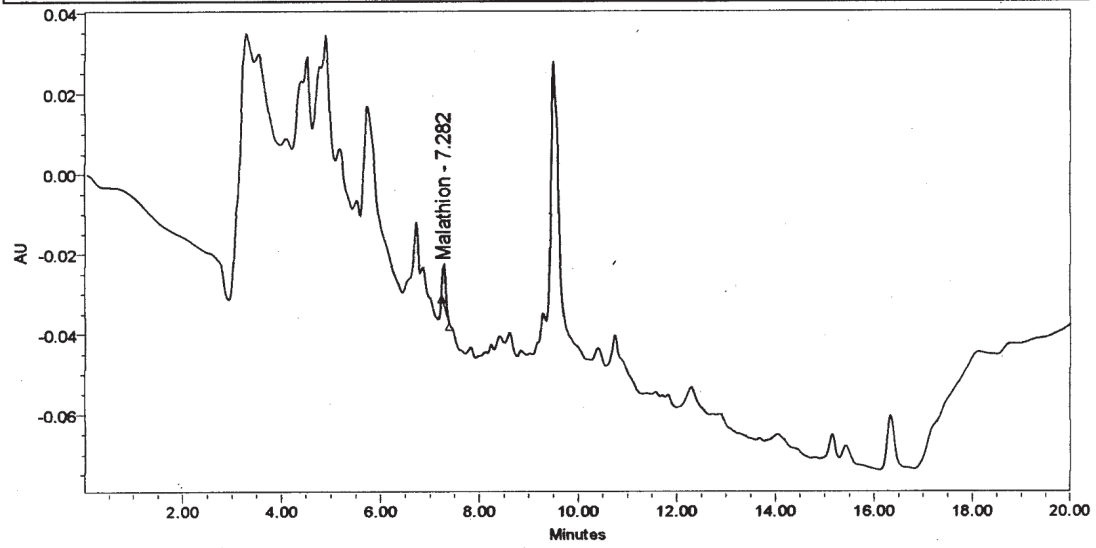
Project Name: PESTICIDE_ANALYSIS
Reported by User: System

SAMPLE INFORMATION

Sample Name:	G Sample3	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	8/26/2008 1:00:59 PM
Vial:	1	Acq. Method:	MALA
Injection #:	3	Date Processed:	8/26/2008 5:07:41 PM
Injection Volume:	20.00 ul	Channel Name:	2487Channel 1
Run Time:	20.00 Minutes	Sample Set Name:	



	Peak Name	RT (min)	Area (µV*sec)	% Area	Height (µV)	% Height	Amount	Units
1	Malathion	7.282	46049	100.00	10103	100.00	5277.803	ppb

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Fig. 2: Chromatogram of unknown sample.

may be sprayed and after the return from grazing the animals showed frothy salivation, with nervous symptoms, colic, constriction of pupil, tetany etc. and out of 150 goats, there was death of 90 goats. The blood smears were examined. The liver and lungs were taken for histopathological examination.

The liquid chromatography consisted of Millipore Waters (Milford, USA) model 1525 pumps, 2487UV detector. The column was a Waters reversed phase C18 (Nova pack) stainless steel column (150mm × 3.9mm I.D,

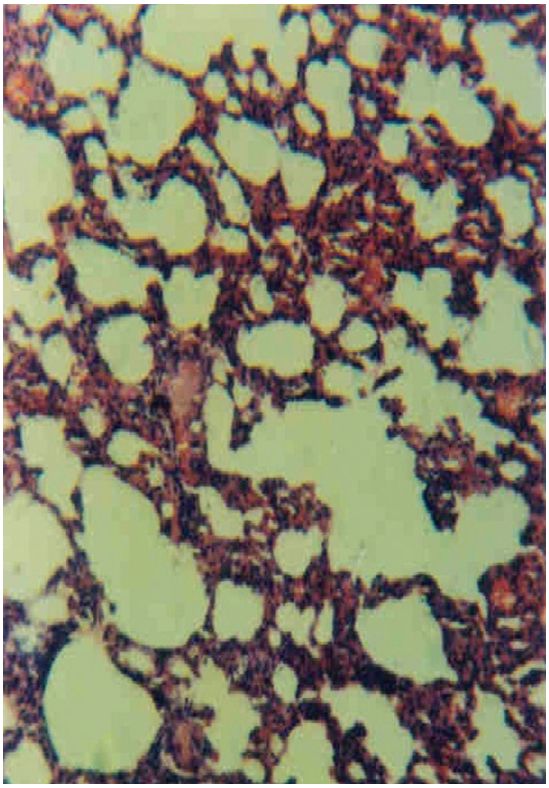

Fig. 3. Photomicrograph of lungs of goat showing congestion (H&E, 100 X).

Fig. 4. Photomicrograph of liver of goat showing mild congestion of the blood vessels (H&E, 100 X).

5µm) equipped with a waters guard pack column module. Solid Phase Extraction (SPE, Waters) was used for purification and clean up of the sample. The analytical method was modified and developed according to method of Abu-quare *et al*. (2001).

Reagents: HPLC grade acetonitrile, water and all other reagents / chemicals were procured from E. Merck (India). The standard compound of pesticides like malathion, etc were obtained from (Supelco-Sigma-Accustandard) and residue of organophosphorus compound in sample was compared to external standard.

Residue level determination: The tissue residue level of the unknown compound was estimated by the method of Manna *et al*. (2006), and also this method (HPLC) is now widely used according to Watanabe *et al*. (2014). Tissues (lungs and liver) were extracted for 4 min with acetonitrile and anhydrous sodium sulfate (0.4%) using a homogenizer. The extract was filtered through anhydrous sodium sulfate (0.5 g) and the tissues were re-extracted twice with acetonitrile . The extract was clarified by centrifugation and filtered through anhydrous sodium sulfate. The combined acetonitrile extracts were concentrated to 20 ml and partitioned with hexane. The hexane phases were discarded and the acetonitrile phase was evaporated to dryness using a rotary vacuum evaporator. The volumes were finally adjusted to 5 ml with acetonitrile for HPLC estimation

after purified and clean up by solid phase extraction (SPE). A stock solution of 1 mg per litre of malathion (analytical grade >99%) was prepared as an external standard. Acetonitrile and water were used as mobile phase in gradient manner and the retention time (RT) of malathion was 7.46 min. The data were recorded and calibration curve of malathion has been done (Fig. 1). The method has been repeated 4 times for validation of the assay With 10 ml Cap. Hamilton Syringe, 2 ml of standard and samples were injected into HPLC.

Histopathological examination: Small pieces of lungs, liver, were fixed in 10% neutral buffered formalin. Sections of 3-5m thicknesses were cut and stained with haematoxylin and eosin (H&E) for observation under light microscope.

RESULTS AND DISCUSSION

The antimortem symptoms were frothy salivation, nasal discharge, nervous symptoms, colic, constriction of pupil etc. The postmortem symptoms were bloat and inflammation in stomach and intestine, haemorrhages (Fig. 3, 4) in lungs and intestine. The histopathological changes in liver and lungs showed congestion, hemorrhages. The examination of blood smear showed no pathogenic organisms. The analysis confirmed the presence of malathion and the method of extraction –purification gave very good results without fortification of the sample (Fig.2) The detected residue level (average within six samples) of malathion was 5277ppb, and the death of the animals were due to organophosphorous compound poisoning (malathion The oral LD50 for malathion in rat has been reported to 5400mg/kg in male and 5700mg/kg in female according to Zeid *et al*. (1993).

The detection of pesticides and its metabolites in animal tissue more accurately and specifically using Mass Spectrometry (MS) may be taken into consideration in future.

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