

# INVESTIGATION OF SOME INSECTICIDES IN POSTMORTEM BIOLOGICAL MATERIALS USING TOXI TUBE AND SOLID PHASE EXTRACTION (SPE) METHODS



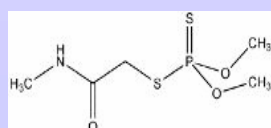
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## ABSTRACT

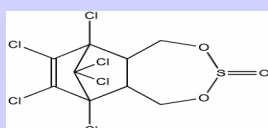
In this study, SPE and Toxi tube extraction methods were examined for the investigation of some insecticides in postmortem biological materials. These methods have been used frequently for pesticides analysis in postmortem materials in the world, that has'tn't been applied yet at Forensic Medicine Council Chemistry Department in Turkey. Firstly, LLE method was applied to postmortem materials (blood, urine, organ and stomach) that belong to three different cases and dimethoate, endosulfan and cypermethrin were found with GC/MS. Then SPE with Oasis and C<sub>18</sub> extraction cartridges and toxi tube (Toxi-A and Toxi-B) extraction methods were applied to the same postmortem materials and analyzed with GC/MS. As a result, compared to classical LLE, SPE and toxi tube extraction methods are faster, use less solvent, eliminate emulsions and give more sensitive results.

## INTRODUCTION

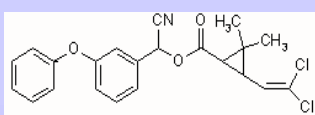
For many years, liquid-liquid extraction (LLE) has been a popular sample preparation procedure for pesticides analysis from postmortem materials. Routine methods used in pesticide analysis employ extraction protocols that are time and solvent consuming, cumbersome, and prone to experimental errors. Furthermore, these methods cause some extraction problems like phase emulsions, impure and wet extract, nonquantitative extractions. Solid phase extraction (SPE) and Toxi tube extraction methods eliminate these problems and provide clean extracts and high recoveries. Dimethoate is a widely used organophosphate insecticide used to kill insects on contact. Like other organophosphates, dimethoate is an anticholinesterase which disables cholinesterase, an enzyme essential for central nervous system function. Dimethoate is moderately toxic by ingestion, inhalation and dermal absorption. As with all organophosphates, dimethoate is readily absorbed through the skin. There was no cholinesterase inhibition in an adult human who ingested 18 mg (about 0.26 mg/kg/day) of dimethoate/day for 21 days. No toxic effects and no cholinesterase inhibition were observed in individuals who ingested 2.5 mg/day (about 0.04 mg/kg/day) for 4 weeks. In another study with humans given oral doses of 5, 15, 30, 45 or 60 mg/day for 57 days, cholinesterase inhibition was observed only in the 30 mg/day or higher dosage groups. Endosulfan is a neurotoxic organochlorine insecticide of the cyclodiene family of pesticides. It is an endocrine disruptor, and it is highly acutely toxic. Doses as low as 35 mg/kg have been documented to cause death in humans, and many cases of sub-lethal poisoning have resulted in permanent brain damage. Cypermethrin is a synthetic compound primarily used as an insecticide. It acts as a fast-acting neurotoxin in insects. It is easily degraded on soil and plants but can be effective for weeks when applied to indoor inert surfaces. The toxic oral dose in mammals is greater than 100-1000mg/kg, and the potentially lethal acute oral dose is 10-100g.



Dimethoate,



Endosulfan



Cypermethrin

## MATERIALS AND METHODS

### Sample Preparation

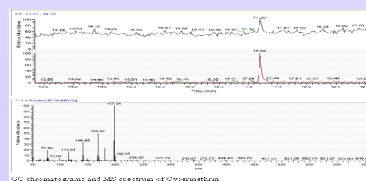
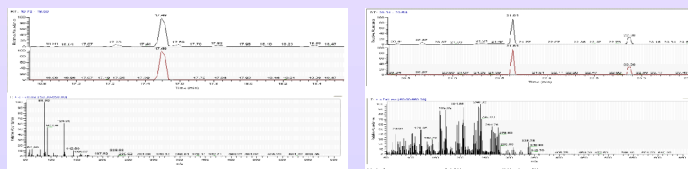
The samples (1 mL blood, urine and 5 g organ, stomach) were homogenized by diluting with 0.1 M KH<sub>2</sub>PO<sub>4</sub>, vortex and centrifuged at 4000 rpm for 10 min. The supernatants were transferred to clean tubes and used for SPE and Toxi extraction. The SPE cartridges (Oasis and C<sub>18</sub>) were conditioned with 2 mL methanol, followed by 2 mL phosphate buffer. The supernatants were loaded on to the columns at a flow rate of 1.5 mL/min, followed by 2 mL of phosphate buffer (0.04 M, pH 2) for C<sub>18</sub> cartridge, and by 2 mL H<sub>2</sub>O, then 2 mL 0.1 N HCl and then 2 mL 5% MeOH in H<sub>2</sub>O for Oasis cartridge. The cartridges were dried with vacuum suction for 10 min. The analytes were eluted out and collected using 3 mL of dichloromethane:ethyl acetate (1:1) for C<sub>18</sub> cartridge and 1.2 mL 5% NH<sub>4</sub>OH in 70:30 ACN/MeOH for Oasis cartridge. The elution solvents were completely dried under nitrogen stream. The analytes were reconstituted with dichloromethane, 1 µL of were analyzed by GC/MS. For Toxi tube extraction; the supernatants transferred to Toxi-A and Toxi-B extraction tubes, vortex and centrifuged at 4000 rpm for 10 min to avoid the proplems of emulsion formation. The organic layers were transferred to glass vials, evaporated till dryness at room temperature. The dry residues were reconstituted in methanol or dichloromethane, 1 µL of were analyzed by GC/MS.

### GC/MS Conditions

The GC/MS column used for the analysis was a TR-5MS column (30 m x 0.25 mm ID x 0.25 µm Film). The GC/MS parameters were as follows: initial temperature, 50 °C hold for 2 min; then increased at 10 °C/min to 290 °C; hold for 10 min at 290 °C. Injector port, transfer line and source temperatures were set at 250 °C, 290 °C and 250 °C, respectively.

## RESULTS AND DISCUSSION

LLE method was applied to postmortem materials (blood, urine, organ and stomach) that belong to three different cases and dimethoate, endosulfan and cypermethrin were found with GC/MS. Then SPE with Oasis and C<sub>18</sub> extraction cartridges and toxi tube (Toxi-A and Toxi-B) extraction methods were applied to the same postmortem materials and analysed with GC/MS.



Case	Sample	LLE	SPE (Oasis)	SPE (C <sub>18</sub> )	Toxi-A	Toxi-B
1	Blood	Dimethoate	Dimethoate		Dimethoate	Dimethoate
	Stomach	Dimethoate	Dimethoate	Dimethoate	Dimethoate	Dimethoate
		Omethoate	Omethoate	Omethoate	Omethoate	Omethoate
Organ	Dimethoate	Dimethoate	Dimethoate	Dimethoate	Dimethoate	Dimethoate
	-	Omethoate	Omethoate	Omethoate	Omethoate	Omethoate
2	Stomach	Endosulfan	Endosulfan	Endosulfan	Endosulfan	Endosulfan
	Organ	Endosulfan	Endosulfan	Endosulfan	Endosulfan	Endosulfan
3	Urine	-	-			
	Stomach	Cypermethrin	Cypermethrin	Cypermethrin	Cypermethrin	Cypermethrin
	Organ	-	-	-	-	-

As a result, compared to classical LLE, SPE and toxi tube extraction methods are faster, use less solvent, eliminate emulsions and give more sensitive results.