Original Paper

Development of Spray Reagent for TLC Detection of Pyrethroid Insecticides using Picric Acid

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ABSTRACT

There have been a variety of approaches developed since the 1970s to detect pyrethroid pesticides based on gas chromatography (GC), thin layer chromatography (TLC), and high performance liquid chromatography (HPLC) with ultraviolet detection. A new thin layer chromatographic method was developed for the detection of common pyrethroids in forensic toxicology case exhibits. Various solvent systems were tried as mobile phase with different spray reagents; picric acid was used as specific spray reagent for deltamethrin, fenvalerate, and cypermethrin. The method developed is rapid and sensitive and can be used for routine case analysis with better separation. A new modified spray reagent was developed for the detection of synthetic pyrethroids containing a hydrolysable nitrile group such as deltamethrin, fenvalerate and cypermethrin using picric acid, that produces reddish-orange spots on a yellow background.

Key Words: Pyrethroid, Thin Layer Chromatography, TLC

Introduction

Pyrethroid insecticides are widely used in agriculture to protect crops, in the household to control pests, and in public health system to control diseases caused by vectors or intermediate hosts.[1,2] Pyrethroids accounted for about 25% of the worldwide insecticide market in 1998.[3] Mosquito control professionals mix pyrethroids with water or oil and apply it as an ultra low-volume spray that kills flying adult mosquitoes. When used properly, pyrethroids have been found to pose very little risk to human health and the environment. Pyrethroids' popularity also stems from their insecticidal potency, slow development of pest resistance, and relatively low toxicity of most congeners in mammals. The quantities of pyrethroid insecticides used in agriculture are rapidly increasing, due in part to their limited toxicity to mammals, and also their good spectrum of activity against crop damaging pests.[4] However, adverse effects in humans may still occur following exposure to these compounds, with neurotoxicity being the primary side effect following acute exposure.[5] Most pyrethroids (including deltamethrin, fenvalerate and cypermethrin) act as neurotoxic agents. They act by delaying closure of sodium channels, resulting in a tail current that is characterized by a slow influx of sodium during the end of neuronal depolarization.[6,7]

Various methods are available in literature for qualitative and quantitative analysis of pyrethroids, such as chromatography (GC),[8,9] thin layer chromatography (TLC),[4,10] and high performance liquid chromatography (HPLC) with ultraviolet detection in formulations, and agricultural and forensic exhibits. Relatively few TLC methods are however available for clear separation, leading to ambiguous detection of the new generation synthetic pyrethroids in forensic exhibits. In addition to the available TLC methods, it was felt necessary to develop a rapid and sensitive method for the detection of these pyrethroids in forensic toxicology samples. The present work

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describes the selection of the best solvent system as a mobile phase specific, and detection of spots of these pyrethroids from complex visceral matrix. At present, there is concern that pyrethroids, like certain organophosphates, are received in forensic science laboratories that are developmental neurotoxicants in infants and children.[11]

Materials and Methods

Chemicals

Standards of Deltamethrin (A), Fenvalerate (C) and Cypermethrin (B) were procured from from Supelco Company. Cyclohexane, Methanol, Toluene, Picric Acid, and Sodium Hydroxide of AR grade, as well as TLC plates (aluminium backed sheets) with 0.25 mm thickness, GF 254 were purchased from Merck India.

Preparation of Stock

Standard stock solutions were prepared in methanol at a final concentration of 0.5 µg /mL. The stock solution was stored in a freezer, though deltamethrin, fenvalerate, and cypermethrin are generally believed to be stable for at least 6 months at room temperature.[2]

Experimental Procedure

Different solvent systems were tried as outlined in **Table 1**, for the separation of the pyrethroids, and it was found that the Cyclohexane: Toluene system was better than the other systems. The detection of the separated compounds was carried out by fluorescence under UV light, and the spots were marked under long and short wavelength ranges. The plates were sprayed with alkali, followed by picric acid. After exposure to 105^0C for 10 minutes, completion of reaction was indicated by the appearance of reddish-orange spots on a yellow background.

Results & Discussion

Different solvent systems were tried for the separation of some common pyrethroids, and the Cyclohexane: Toluene system was found to be the best. Detection of the separated compounds was carried out by fluorescence under UV light, and the spots were marked under long and short wavelength ranges.

A new spray reagent was developed for the detection of synthetic pyrethroids containing an ether group, for e.g.,

Table 1 Rf Values of Samples A, B & C and the Mobile Phases Used

Sample: Rf Values / Mobile Phase	Sample A		Sample B				Sample C			
	Spots		*Spots*				*Spots*			
	1st	2nd	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Hexane	No Elution		No Elution				No Elution			
Hexane: $HCCl_3$: : 5 : 5	Streak		Streak				Streak			
Hexane: $HCCl_3$: : 5 : 5	Complete Elution		Complete Elution				Complete Elution			
Hexane: Acetone : : 5 : 5	Complete Elution		Complete Elution				Complete Elution			
Hexane: Benzene : : 5 : 5	0.65	0.68	0.55	0.59	0.65	0.68	0.45	0.52	0.60	0.65
Cyclohexane: Toluene: : 5 : 5	0.750*	0.80	0.50	0.60	0.75*	0.80	0.40	0.55	0.70*	0.85

**Only these spots were identified by the spraying reagent; remaining were identified under UV.*
Sample A: Deltamethrin, Sample B: Cypermethrin, Sample C: Fenvalerate

deltamethrin, fenvalerate, and cypermethrin. These synthetic pyrethroids, on treatment with alkaline hydroxide solution and picric acid, yield a coloured derivative. The new spray reagent developed is recommended as an additional reagent that can be used for the detection of pyrethroids encountered routinely in forensic science laboratories, and agricultural laboratories.

Conclusion

A new spray reagent has been developed for the detection of some synthetic pyrethroids in forensic toxicology-related cases. The method developed will be useful for routine examination of samples, as well as residue analysis.

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