Original Paper

Analysis of Pyrethroids in Mosquito Mats, Coils and Spray Canisters

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ABSTRACT

Use of mosquito mats, coils and spray are popular methods of avoiding mosquito bites. There are many commercially available mosquito mats, coils and spray canisters which contain pyrethroids, that can cause nervous system toxicity on substantial exposure. Prolonged use of these mosquito repellents can affect human health. When cases of pyrethroid poisoning are reported to a forensic toxicologist, it is very important to be able to extract, isolate, separate and detect the pyrethroid compound. Thin Layer Chromatography (TLC) is widely used today for the detection of pyrethroids. More sophisticated techniques such as Gas Chromatography-Mass Spectrometry (GC-MS) or High Pressure Liquid Chromatography (HPLC) can be used for the confirmation of the compound. It is therefore important to create a laboratory database, which can be helpful in forensic examinations in future.

Key Words: Mosquito mat; Mosquito coil; Mosquito spray; Pyrethroid; Thin layer chromatography; TLC

Introduction

Pyrethrins were originally derived from pyrethrum, which is an active principle in East African Chrysanthemum, that is known to possess insecticidal activity. The flowers of the plant are harvested shortly after blooming and are either dried and powdered, or the oils within the flowers are extracted with solvents. Pyrethroids are synthetic forms of pyrethrins. The resulting pyrethrins containing dusts of extracts usually have an active ingredient content of about 30%. These active insecticidal components are collectively known as "pyrethrins". There are two main pyrethrin groups: pyrethrin-I and pyrethrin-II. Pyrethrins have four different active ingredients: cinerin I & II and jasmolin I & II. Pyrethrins and pyrethroids are used primarily to control human lice, mosquitoes, cockroaches, beetles and flies. They are also widely used as home and garden insecticides, pets and livestock insecticides, pesticidal treatment of transport vehicles, and for treatment of some ectoparasitic diseases.

How do pyrethroids work?

Nerve cell membranes have a specific electrical charge. Altering the amount of ions passing through ion channels causes the membranes to depolarize, which in turn causes a neurotransmitter to be released. Neurotransmitters help nerve cells communicate. Electrical messages sent between nerve cells allow them to generate a response in the form of muscular movement in an animal, or locomotory movement in an insect. Pyrethroids affect the nervous system of insects by causing multiple action potentials in the nerve cells by delaying the closing of ion channels.

Materials and Methods

Sample Preparation: In this study, the pyrethroids that were analysed included prallethrin, allethrin, cypermethrin, deltamethrin, and transfluthrin. Extraction of pyrethroids was done by direct extraction method. Samples were extracted after overnight incubation. Mats were broken and extracted in 1,2-dichloro ethane. The next day, the samples were filtered and concentrated. This material was used for the analysis. In the same way, coils and

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sprays were extracted in hexane. Cypermethrin was extracted in acetone, while deltamethrin was extracted in dioxane.

Instrumentation: Thin layer chromatography (TLC), Gas chromatography-mass spectrometry (GC-MS), Ultraviolet visible (UV) spectroscopy, and Infrared (IR) spectroscopy.

1. Thin Layer Chromatography: TLC is one of the most widely used techniques for the separation and identification of pyrethroids. It is a method in which a mobile phase moves by capillary action across a uniform thin layer of finely divided stationary phase bonded on to a plate. TLC is a method for identifying substances and testing the purity of compounds. It is a useful technique because it is relatively quick and requires small quantities of material.

   Stationary phase: Activated silica gel is used as stationary phase in thin layer chromatography.

   Solvent/mobile/developing system: Hexane : Acetone (95 : 5), Hexane : Acetone (80 : 20), Hexane : Acetone (70 : 30), Hexane : Acetone (50 : 50).

   Spray reagents: di-phenyl amine (DPA) and 2,4-dinitro phenylhydrazine

   Colour of spots: Green coloured spots are observed for pyrethroids.

2. Gas Chromatography-Mass Spectrometry: GC-MS is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC-MS is a combination of two powerful analytical tools: GC for the highly efficient gas-phase separation of components in complex mixtures, and mass spectrometry for the confirmation of identity of these components as well as for the identification of unknowns.

   Column: DB-1 (Dodecyl Benzene-1)

   Type of mass spectrometer detector: Quadruple ion detector
   Type of ionization: Electron ionization
   Carrier gas: Helium
   Injector temperature: 200 C
   Oven temperature: 150 C
   Injection volume: 0.2 microlitre
   Flow rate: 7 ml/minute
   Scan rate: 0.5

3. Ultraviolet-Visible Spectroscopy: It uses light in the visible and adjacent near-UV and near-infrared ranges. It involves the spectroscopy of photons in the UV-Visible region. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. UV-Visible spectrophotometer measures the intensity of light passing through a sample and compares it to the intensity of light before it passes through the sample.

4. Infrared Spectroscopy: IR spectroscopy is also called vibrational spectroscopy. IR spectroscopy deals with the infrared region of the electromagnetic spectrum. It can be used to identify the compounds or investigate sample compositions. It is a chemical analytical technique which measures the infrared intensity versus wavelength of light.

Results and Discussion

Pyrethroids are one of the more difficult classes of organic compounds to analyze in trace levels with a high degree of specificity and sensitivity. For the identification and detection, and for the purpose of collecting reliable data, all selected pyrethroids were analyzed using TLC, UV and IR spectroscopy and GC-MS.

All the pyrethroids cannot differentiate in one solvent system in TLC. Four different solvent systems were employed for the separation of the selected five pyrethroids. Prallethrin, cypermethrin and transfluthrin were best separated in hexane:acetone (95:5). Deltamethrin was best separated in hexane:acetone (50:50), while allethrin was best separated in hexane:acetne (70:30). In this way, all pyrethroids could be best separated and proper identification could be done.

Specific identification of a compound can rarely be made on the basis of UV spectral evidence alone. Often, the spectrum serves as confirmatory evidence of identity in support of other analytical data. Lambda max values differ for all the five pyrethroids. Proper identification can be done using UV spectrophotometer.

GC-MS was used as a confirmative technique. In GC, all the pyrethroids give different retention times. In MS, all pyrethroids show different principal peaks at m/z

ratio. But some pyrethroids show similar principal peaks at m/z ratio. So the peaks of their fragments are considered for proper identification. Thus by comparing retention times, principal peaks at m/z ratio, and peaks of their fragments with the library available with the instrument, all the pyrethroids could be identified accurately. There was 100% confirmation of every compound tested.

GC-MS RESULTS

COMPOUND	RETENTION TIME (Min)	Principal peaks
Prallethrin	12.800	69,81,95,109,121,136,149,161,175,203 ,231,257,273,285,297,316,328,352,368 ,384,396,410
Cypermethrin	11.8	51,65,77,91,109,115,127,152,163,181
Transfluthrin	10.867	65,83,91,109,129,149,163,181,191,204 ,232,244,320,335,358,371,401,414,430
Deltamethrin	13.8	65,77,93,103,115,137,152,181,208,253 253
Allethrin	9.90	55,67,79,91,107,123,136,153,167

Fig 1

IR spectroscopy is also a confirmative technique. In IR all the pyrethroids give different principal peaks. Thus by comparing these principal peaks, all the pyrethroids could be identified accurately.

IR RESULTS

COMPOUND	PRICIPAL PEAKS
Prallethrin	1.)C=CH : 964(980-6900 2.)C=0 : 1734(1600-1760) 3.)C-CH3 : 2926(3000-2800) 4.) ≡C-H : 3315(3010-3300)
Cypermethrin	1.)C-Cl : 784(800-700) 2.) C=CH : 3003(3100-3000) 3.)C=O : 1745(1780-1660) 4.)C6H6 : 849 (870-670) 5.)Aromatic hydrocarbon : 694(700-900)
Transfluthrin	1.)C-Cl : 699(800-700) 2.)C=C :1630(1640-1620) 3.)C=O : 1717(1760-1680) 4.)C-F : 1489(1000-1400)

Fig 2

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