

Gas Chromatography

Author:

Kira Yang

PerkinElmer

Shanghai, PRC

Determination of 10 Pyrethroid Pesticides in Soil by Soxhlet Extraction-GC/MS

Introduction

Pyrethroids are a type of synthetic insecticides which were developed in the late 1960s following research on natural pyrethrin. Since initial development, nearly 80 pyrethroids have been successfully synthesized, including permethrin, cypermethrin, deltamethrin and permethrin. Pyrethroids work by attacking arthropod insects' nervous system, with a knockdown and cidal effect that is 10~100 times higher than that of older generation insecticides, such as organochlorine (OC), organophosphorous (OP) and carbamate pesticides. The pyrethroid dosage required to cause arthropod toxicity, however, is only a tenth of that of other pesticides. Pyrethroids have been widely used in agriculture, public health and grain storage owing to the high efficiency and broad spectrum. At present, pyrethroid pesticides have become one of the three most widely used pesticides, second only to OP pesticides.

Early studies suggested that pyrethroid pesticides were environmentally friendly, with slow degradation and long validity, making them generally safe to human and livestock. However, later studies conducted following the expanded use of pyrethroid pesticides found that the residues left in the environment were more dangerous than initially reported. Recent studies indicate that pyrethroid pesticides can bioaccumulate in some species, and that prolonged exposure may result in chronic disease, even with relatively low-dose exposure.¹ Pyrethroid pesticides have also been found to be harmful to some beneficial insects, such as bees.

Owing to the potential environmental impact of pyrethroid pesticides, a number of countries have developed regulatory guidelines associated with safe levels of pyrethroid pesticide residues in the environment. In the United States, soil screening levels (SSLs) and ecological soil screening levels (Eco-SSLs) have been established by EPA for dozens of pesticides. In the EU, soil guideline values (SGVs) and soil screening values (SSVs) have been set for pesticides permitted in soil.

Gas chromatography coupled to mass spectrometry (GC/MS) is often considered the most traditional analytical method for the determination of pyrethroid pesticides. EPA Method 8270D is a highly sensitive and selective method for pyrethroid pesticide analysis, and also outlines a number of preparation techniques, such as Soxhlet extraction (EPA Methods 3540 and 3541). Soxhlet extraction is a common and robust method of sample preparation, and is employed in most commercial environmental laboratories. Chinese environmental standard HJ 1023-2019 also describes a method for the determination of ten pyrethroid pesticides in soil and sediment using Soxhlet extraction-GC/MS.

In this study, a Soxhlet extraction technique is used to extract target pesticides from soil samples. Following extraction, the extracts are analyzed by liquid injection into a PerkinElmer Clarus® SQ 8 GC/MS system. The results demonstrate that the method is accurate and reliable in the analysis of pyrethroid pesticides in soil samples.

Experimental

Sample Preparation and Extraction

The external (10 pyrethroid pesticides) and the internal standards (two deuterated polycyclic aromatic hydrocarbons) were purchased from ANPEL Laboratory Technologies Inc. (Shanghai). Chromatographic-grade acetone and n-hexane were used for sample extraction and standard dilutions to produce a range of concentrations required for the experiments. The soil sample analyzed in this study was provided by a private laboratory.

The extraction procedure used is as follows:

1. 10 g of soil sample was blended with 10 g of anhydrous sodium sulfate and placed in an extraction thimble.
2. 200 mL of extraction solvent acetone/n-hexane (1:1) (v/v) was placed into a flask containing two clean boiling chips. The flask was attached to the extractor, and the sample was extracted for eight hours at 4-6 cycles/hour.
3. The extract was allowed to cool after the extraction was completed.
4. The flask was attached to a rotary evaporator. The rotary evaporator was then placed in a warm water bath (30-35 °C) so that the flask was partially immersed in the water.
5. The extract was concentrated to 1-2 mL and allowed to cool.
6. The flask was rinsed with the extraction solvent several times. The extract and eluent were merged and concentrated by nitrogen blowdown technique. The internal standard was added, and the final volume was adjusted to 1 mL for analysis by GC/MS.

As a best practice, a purification process by a solid phase extraction (SPE) technique should be completed before adding the internal standard if the extract is cloudy.

The precision of the method was investigated by spiking six blank soil samples with the standards to achieve concentrations of 1.00, 5.00, 10.00 mg/kg in the soil samples. The instrument detection limits were determined based on a signal-to-noise ratio of three for the peaks in selected ion chromatograms of all target compounds by injecting diluted calibration standards. The recovery was investigated by spiking six blank soil samples with standards to get 1.00 mg/kg of soil samples.

Instrumentation

A PerkinElmer Clarus GC, connected to a PerkinElmer Clarus SQ 8 MS in electron ionization (EI) mode, was used to perform these experiments, with the conditions presented in Table 1. A capillary split/splitless deactivated glass liner with deactivated wool and a tapered end (Part No. N6502042) was used for pyrethroid pesticide determination in this study. The deactivated wool is fixed in the liner to avoid movement owing to solvent volume expansion by evaporation. The tapered end avoids sample vapor contact with metal parts which can prevent pesticides from adsorbing or degrading in the GC inlet. A PerkinElmer Elite 1701 column (30 m × 0.25 mm × 0.25 μm) was used to separate the target compounds.

Table 1. Analytical parameters.

GC Parameters	
Injector Type	Capillary injector with a capillary split/splitless deactivated glass liner with deactivated wool and tapered end
Analytical Column	Elite -1701 (30 m × 0.25 mm × 0.25 μm)
Inlet Temp	270 °C
Injection Volume	1 μL
Carrier Gas Flow Rate	1.4 mL/min
Split Ratio	10:1
Initial Oven Temp	40 °C
Oven Hold	1.0 min
Ramp	30 °C/min
2 nd Oven Temp	130 °C
Oven Hold	0 min
Ramp	5 °C/min
3 rd Oven Temp	250 °C
Oven Hold	10 min
Ramp	10 °C/min
3rd Oven Temp	280 °C
Oven Hold	8 min
MS Parameters	
Mass Range (amu)	45 to 450
GC Inlet Line Temp	280 °C
Ion Source Temp	260 °C
Function Type	SIFI
Ionization	EI

Calibration

The calibration curve was prepared by diluting 10 pyrethroid pesticide standards to the proper concentration with acetone/n-hexane (1:1) (v/v) and adding the internal standards, resulting in a series of concentration of calibration standards solution (Table 2).

Table 2. Calibration points employed in this study.

GC Parameters					
Calibration level	1	2	3	4	5
Calibration standard concentrations (µg/mL)	10.0	20.0	50.0	75.0	100.0
Internal standard concentrations (µg/mL)	20.0	20.0	20.0	20.0	20.0

Results and Discussion

The total ion chromatogram of level five calibration standards is shown in Figure 1. Many pyrethroid pesticides with a cyclopropane ring, such as cypermethrin, contain chiral centers in the molecules. Therefore, they may exist as several stereoisomers, including enantiomers and diastereoisomers. Diastereoisomers can be separated by conventional GC columns, owing to their different physico-chemical properties. The application forms of cypermethrin comprise of four

diastereoisomers, thus giving four peaks in the chromatogram. Fenvalerate has four optical isomers (SR+RS+SS+RR), but only two peaks in conventional GC columns. Each peak contains two enantiomers. The peak areas of one pesticide are usually summed for quantitation purposes.

All target compounds were quantified using selected ion chromatograms. Table three shows the quantitation and qualitative ions for all targets. The calibration mixtures included 10 pyrethroid pesticides. The calibration curves were plotted as the peak area ratios between the quantification ions for the analytes and the respective internal standard, versus the amount of analytes. For all target pesticides, calibration results showed good linearity with regression coefficients (r^2) higher than 0.995 (table four). The method precision (%RSD) was found to be 3.15-9.59% for target compounds at the three concentration levels. The instrument detection limits were calculated to be in the range of 0.001–0.287 mg/kg; the recoveries were in the range of 88.1 – 105.5 %. The results for precision, linearity, recovery and detection limit are excellent for all compounds, and meet or even exceed method criteria for EPA method 8270D and HJ 1023-2019.

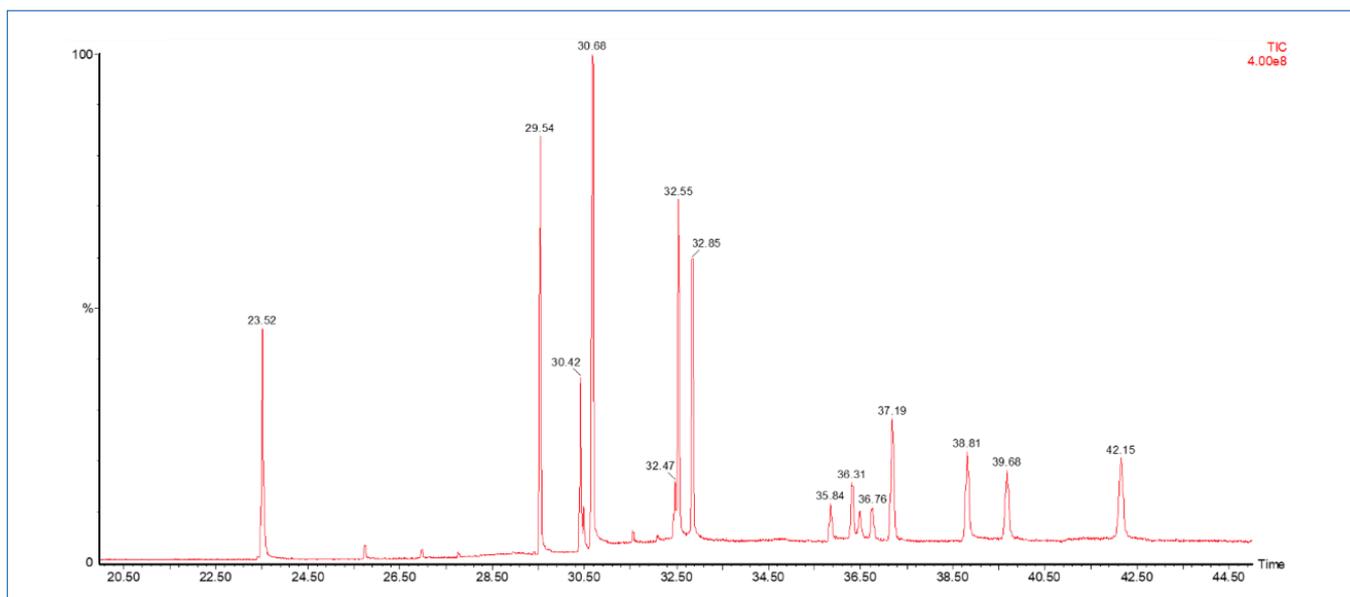


Figure 1. The total ion chromatogram of a pyrethroid pesticide standard.

Table 3. The qualitative and quantitative ions of ten pyrethroid pesticides and internal standards.

Compound Name	CAS NO	RT	Quantitation Ion	Qualitative Ion
s-Bioallethrin	28434-00-6	23.51	123	136, 79
cis-Bifenthrin	82657-04-3	29.54	181	165, 166
Chrysene-d12 (IS1)	1719-03-5	30.41	240	236, 229
Tetramethrin	7696-12-0	30.48, 30.66	164	123, 107
Fenpropathrin	39515-41-8	30.68	97	125, 55
Pyrethrins	8003-34-7	31.55	107	121, 167, 93
Permethrin	52645-53-1	32.47, 32.85	183	163
lambda-Cyhalothrin	91465-08-6	32.55	181	197, 208
Cypermethrin	52315-07-8	35.84, 36.32, 36.48, 36.75	165	163, 181, 209
Pyrene-d12 (IS 2)	203578-33-0	37.19	264	260, 265, 263
Fenvalerate	51630-58-1	38.82, 39.67	125	167, 152, 225
Deltamethrin	52918-63-5	42.15	253	209, 255

Table 4. Results for linearity, precision, recovery and instrument detection limit.

Compound Name	RSD%			Regression coefficient (r ²)	Instrument Detection Limit mg/kg	Recovery %
	1.00 mg/kg	5.00 mg/kg	10.0 mg/kg			
s-Bioallethrin	6.93	4.95	3.36	0.9994	0.005	91.2
cis-Bifenthrin	6.85	4.53	4.97	0.9981	0.001	94.3
Tetramethrin	6.29	3.75	5.81	0.9997	0.045	88.1
Fenpropathrin	6.62	5.04	3.15	0.9965	0.018	93.0
Pyrethrins	9.24	5.92	4.91	0.9952	0.287	89.1
Permethrin	6.16	5.91	5.33	0.9988	0.009	92.0
lambda-Cyhalothrin	5.72	3.23	5.46	0.9998	0.014	90.2
Cypermethrin	8.39	5.64	6.02	0.9951	0.120	105.5
Fenvalerate	9.59	4.04	5.76	0.9957	0.090	103.2
Deltamethrin	6.01	5.16	4.59	0.9965	0.099	88.4

Summary

In this paper, a method for the determination of 10 pyrethroid pesticides in soil samples was established using a Soxhlet extraction and GC/MS technique. The precision, recovery and linearity results demonstrate that this system delivers the performance and stability needed to analyze soil samples for pyrethroid pesticides in both high and low throughput laboratory environments. Further, the turnkey solution presented herein, including robust instrumentation, detector, software and fit-for-purpose column, reduces unnecessary complexity while ensuring compliance with regulatory programs.

Resources

1. Aznar-Alemany Ò., Eljarrat E. (2020) Bioavailability and Bioaccumulation of Pyrethroid Insecticides in Wildlife and Humans. In: Eljarrat E. (eds) Pyrethroid Insecticides. The Handbook of Environmental Chemistry, vol 92. Springer, Cham. https://doi.org/10.1007/698_2020_466