Sample Preparation Method for Analysis of Environmental Pesticides (Simazine, Thiobencarb and Thiuram (Thiram)) in Water Using ISOLUTE® 101 Solid Phase Extraction Columns

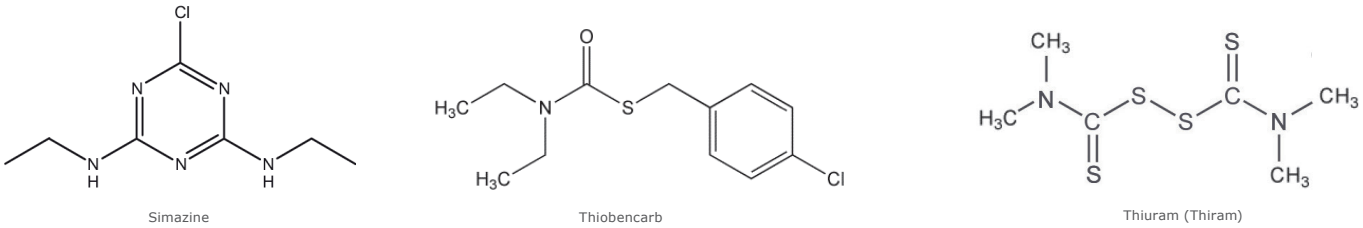

Figure 1. Analyte Structures.

Introduction

This application note describes a method for the extraction of pesticides from environmental water samples using ISOLUTE® 101 solid phase extraction columns.

Simazine, a triazine herbicide, Thiobencarb, a thiocarbamate herbicide, and Thiuram (alternatively named Thiram), a dithiocarbamate bactericide are widely used pesticides (Figure 1). As typical pesticides detected in the environment, they are designated as "Target Setting Items for Water Quality Management" in the Japanese Water Supply Law, "Environmental Standards for the Protection of Human Health" in the Environmental Standards for Water Pollution, and "Class III Specified Hazardous Substances" in the Soil Pollution Control Law, and compliance with the standard values set by each of them is required.

ISOLUTE® 101 is a solid phase extraction media consisting of an unmodified form of polystyrene divinylbenzene copolymer. Since its hydrophobic interactions are stronger than with ODS (C18) type media, it is possible to efficiently collect organic compounds with a wide range of hydrophobicities contained in water samples. In addition, as the column can easily be dried with nitrogen gas purge, the overall analysis time can be shortened which increases efficiency.

This method has been prepared in accordance with Japanese official law of the Ministry of the Environment, Notice No. 59, Annex 5 and Annex 6, 1971.

Analytes

Simazine, CAS: 122-34-9
Thiobencarb, CAS: 28249-77-6
Thiuram (Thiram), CAS: 137-26-8

Sample Preparation Procedure

Format

ISOLUTE® 101 200 mg/6 mL, part number 101-0020-C

Sample Pre-Treatment

To 200 mL of the sample water, add 4 mL of a solution of 10% EDTA·2Na. If thiuram is to be measured, adjust to pH 3.5 with hydrochloric acid.

Condition

Condition the column with Methanol (5 mL).

Equilibration

Equilibrate the column with 10% EDTA·2Na solution (4 mL) followed by purified water (15 mL).

Sample Loading

Load the sample at a flow rate of 100 mL/min using a vacuum pump.

Interference Elution

Wash the column with purified water (10 mL) at a flow rate of 100 mL/min using a vacuum pump, and dry the column using a flow of nitrogen for 30 minutes.

Elution

Elute simazine and thiobencarb with acetone (5 mL)

Elute thiuram with acetonitrile (5 mL)

Evaporation and Reconstitution

Simazine, Thiobencarb: Concentrate the resulting eluate to 1 mL under nitrogen gas in a 35 °C water bath. After evaporation, redissolve the residue in 5 mL of hexane, concentrate again to 1 mL under nitrogen gas in a 35 °C water bath. Add 20 µL of 10 mg/L phenanthrene-D_{10} and 20 µL of 5,000 mg/LPEG (1: 1 mixture of PEG 200 and PEG 300) as the internal standard, and perform the GC/MS analysis.

Thiuram: Concentrate the resulting eluate to 1 mL under nitrogen gas in a 35 °C water bath. Transfer the concentrated eluate to vials as it is, and perform HPLC/UV measurements.

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GC/MS Conditions (Simazine, Thiobencarb)

Instrument
Agilent 7890A/5975C GC-MSD system

Column
HP-5 MS (30 m x 0.25 mm, membrane pressure 0.25 µm, Agilent)

Carrier
Helium

Column Flow Rate
1.1 mL/min

Vaporizing Chamber Temperature
150 °C

Interface Temperature
280 °C

Ion Source Temperature
230 °C

Ionization Method
EI

Injection Volume
1 µL (Splitless)

Table 1. GC Column Heating Conditions.

Time (min)	Temperature
0 → 1	50 °C
0 → 11.5	(20 °C/min)
0 → 12.5	280 °C

Table 2. MS Conditions.

	For Assay (m/z)	For Detectability (m/z)
Simazine	201	173
Thiobencarb	100	72
Phenanthrene-D_{10}	188	160

HPLC/UV Conditions (Thiuram)

Equipment
Agilent 1100 HPLC system

Column
XDB-C18 (75 mm x 3.0 mm, 3.5 µm, Agilent)

Column Temperature
40 °C

Mobile Phase
A: Water

B: Acetonitrile

Detection Wavelength
272 nm

Injection Volume
10 µL

Table 3. HPLC Mobile Phase Conditions.

Time (min)	%A	%B
0 → 2	80	20
0 → 8	80 → 10	20 → 90
0 → 12	10	90

Results

The mass chromatogram of Simazine and Thiobencarb mixed standard and the UV chromatogram of Thiuram standard are shown in Figures 3 and 4. Good peaks are obtained for each. In addition, Figure 5 shows calibration curves using the standard solutions. Good linearity is obtained in the corresponding concentration range when the sample is concentrated 200 times. Reproducibility and recovery at n = 5 were also obtained at the lower limit of the generated calibration curve concentrations (Table 4). Good recoveries and reproducibility have been obtained for all samples. We have also confirmed the validity of this method through spiking and recovery tests on real samples.

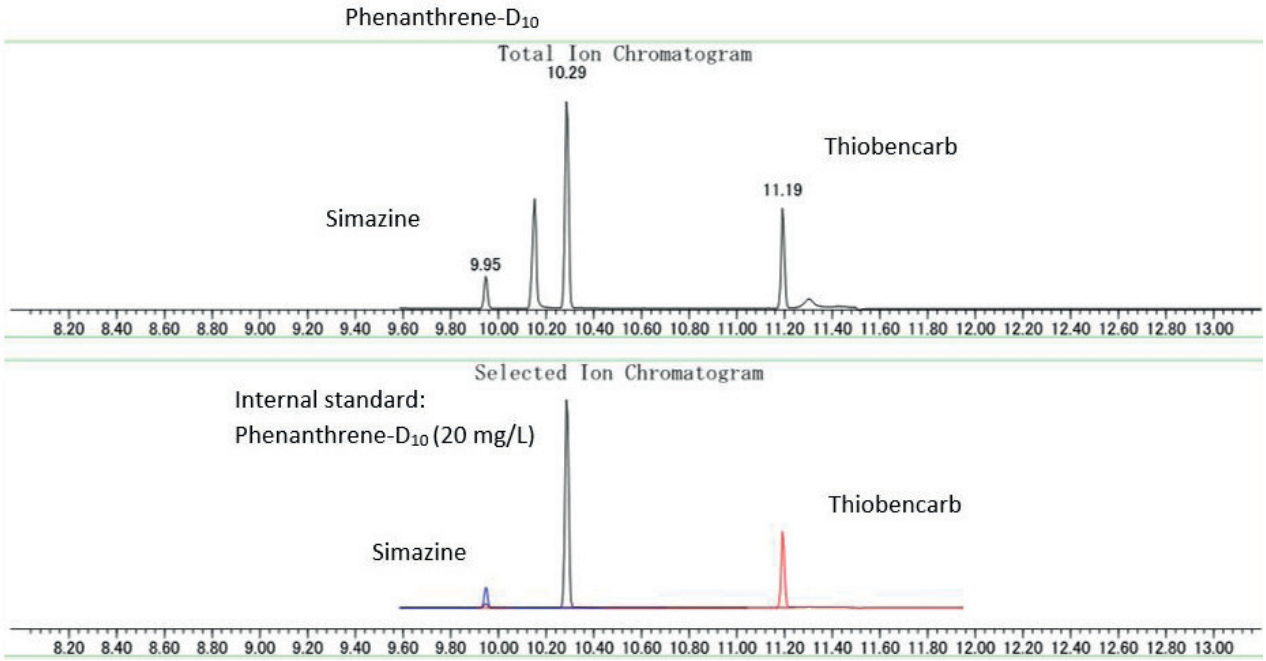

Figure 3. Mass chromatogram of Simazine and Thiobencarb mixed standard solution (0.2 mg/L).

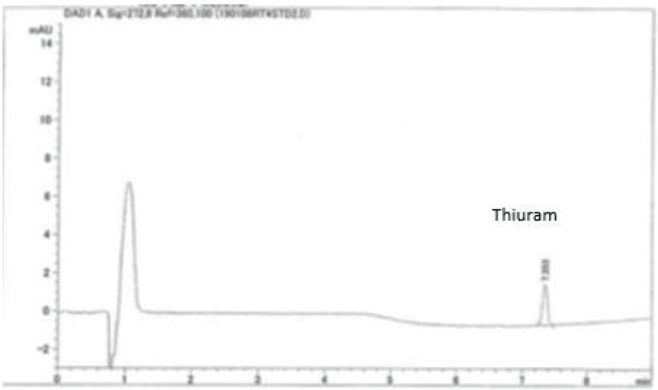

Figure 4. Chromatogram (λ = 272 nm) of Thiuram standard solution (0.25 mg/L).

Table 4. Reproducibility and recovery rate at the lower measurement limit of Simazine, Thiobencarb and Thiuram (n = 5).

	Mean (ug/L)	RSD (%)	Recovery Rate (%)
Simazine	0.3076	2.18	102.5
Thiobencarb	0.2954	2.64	98.5
Thiuram	0.5318	2.68	106.4

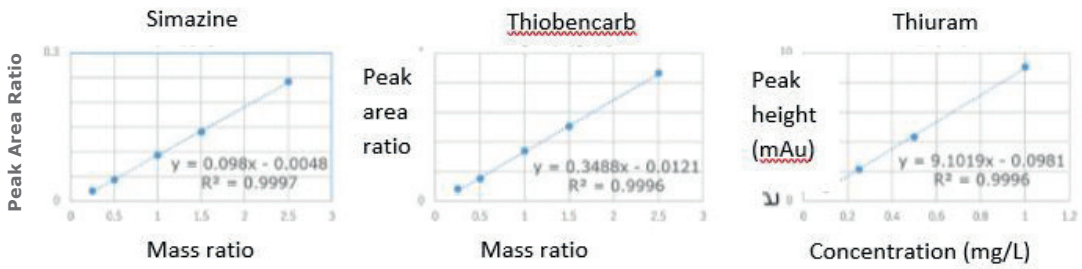

Figure 5. Calibration curves of Simazine, Thiobencarb (0.05-0.5 mg/L) and Thiuram (0.1-1.0 mg/L).

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Summary

The use of ISOLUTE® 101 allowed the sample to pass through at a superior flow rate (100 mL/min) than with the equivalent competitor product (13 mL/min). In addition, since column drying by centrifugation after column washing, which had been essential until now, has become unnecessary, it was possible to reduce the working time by about 50 minutes per batch while maintaining a recovery rate of approximately 100%.

Further efficiency can be achieved by using automated devices such as TurboVap® LV (Automated Evaporation System) and Biotage® Horizon SmartPrep (Automated Sample Preparation System).

Ordering Information

Part Number	Description	Quantity
101-0020-C	ISOLUTE® 101 200 mg/6 mL	30
415000	TurboVap® LV	1
41964	TurboVap® LV Multi Rack (48 Positions, 10–20 mm Tubes)	1
SPE2-2616	Biotage® Horizon SmartPrep II Extractor Base Module Configured with 6 mL Plunger Assembly	1

This application note was prepared in cooperation with Sougoukankyoubunseki Co., Ltd.

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