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Automated Solid-Phase Extraction (SPE) for Pesticides

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SCOPE AND APPLICABILITY

This technical system procedure (TSP) details the procedure for extracting pesticides from effluent and influent wastewater using automated solid-phase extraction (SPE). This method focuses on isolation and concentration of the pesticides listed in **Table 1**. These pesticides are analyzed using TSP-06.0017.00 - Pesticide Analysis by Gas Chromatography / Quadrupole Time of Flight Mass Spectrometry (GC/QToF) and TSP-06.0016.00 - Pesticide Analysis by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC/MS/MS).

Table 1. Target list for pesticide extraction

Analyte	CAS Registry No.	Analyte	CAS Registry
Bifenthrin	82657-04-3	Fipronil sulfone	120068-36-2
S-Bioallethrin	28434-00-6	Imidacloprid	138261-41-3
Chlorothalonil	1897-45-6	Novaluron	116714-46-6
Chlorpyrifos (Dursban)	2921-88-2	Permethrin	52645-53-1
Cyfluthrin	68359-37-5	Phenothrin	26002-80-2
Cyhalothrin	68085-85-8	Prallethrin	23031-36-9
Cypermethrin	52315-07-8	Propoxur	114-26-1
Cyphenothrin	39515-40-7	Pyrethrin 1	121-21-1
Deltamethrin	52918-63-5	Pyriproxyfen	95737-68-1
Esfenvalerate	66230-04-4	Tau-Fluvalinate	102851-06-9
Etofenprox	80844-07-1	Tetrachlorvinphos	22248-79-9
Fenpropathrin	39515-41-8	Tetramethrin	7696-12-0
Fipronil	120068-37-3	Chlorpyrifos-D10 (Surrogate)	285138-81-0
Fipronil amide	205650-69-7	Diuron-D6 (Surrogate)	1007536-67-5
Fipronil desulfinyl	205650-65-3	Fonofos (Surrogate)	944-22-9
Fipronil desulfinyl amide		Methomyl-D3 (Surrogate)	1398109-07-3
Fipronil sulfide	120067-83-6	Simazine-D5 (Surrogate)	220621-41-0

PRINCIPLE

Wastewater samples are collected from various wastewater treatment plants throughout California. Effluent and influent wastewater samples are collected in 250 mL and 150 mL amber glass bottles, respectively. Upon receipt, the samples are treated with sodium thiosulfate for dechlorination and acidified to pH 4. These samples are then filtered through glass fiber filters to separate the solid phase from the aqueous phase and are extracted separately via different techniques.

The filtered wastewater samples are extracted by passing through preconditioned solid phase extraction (SPE) cartridges containing 500 mg of a universal polymeric reversed-phase sorbent. After sample loading, the organic compounds are eluted and collected from the solid phase with a small volume of ethyl acetate. The extracts are adjusted to a final volume of 1 mL using ethyl acetate and then divided for analysis on the GC-QToF and LC-MS-MS.

Glass fiber filters containing the solid phase are extracted with ethyl acetate using Soxhlet extraction. The extracts are concentrated to a final volume of 1 mL using ethyl acetate and then divided for analysis on the GC-QToF and LC/MS/MS.

For GC-QToF analysis, analyte protectants and internal standards are added to 0.5 mL of the sample extracts prior to analysis. For LC-MS-MS analysis, 0.5 mL of the sample extracts are solvent exchanged to methanol and internal standards are added prior to analysis.

SAFETY

Precautions

Ethyl acetate, methanol, and acetone are hazardous and volatile, avoid direct inhalation of the solvents.
Be aware of the location of exit door, eye wash and shower station, spill kit, First Aid Kit, and closest phone to call in case of emergency.

Personal Protective Equipment (PPE)

Wear lab coat, glasses, and gloves as necessary. Use fume hood whenever possible.

INTERFERENCES

Solvents, reagents, glassware, and other sample processing equipment may contain artifacts and/or interferences that may affect sample analysis. All these materials are demonstrated to be free from interferences under the conditions of the analysis.

All reagents and apparatus must be free from contaminants, especially phthalates. Avoid using plastic materials such as soft polyvinyl chloride (PVC) and rubber because phthalates are commonly used as plasticizers and are easily extracted from plastic materials.

PRESERVATION AND HOLDING TIMES

Store samples at < 6°C in Teflon sealed glass containers. Add 80 mg/L sodium thiosulfate pentahydrate to each wastewater sample when samples are received at ECL. Acidify wastewater sample to about pH 4

prior to filtration. Wastewater samples are extracted within 14 days of the sampling date and the extracts are analyzed within 40 days.

EQUIPMENT AND SUPPLIES

SPE-03 8-Channel System, PromoChrom
Oasis HLB SPE cartridges, Waters
Membrane filtration apparatus, Kontes
Balance, capable of weighing to 0.01 g
Spatula, stainless steel
pH indicator strips, range pH 0-14
Glass microfiber filters (GF/F), diameter 90mm, Whatman or equivalent
Pasteur pipets, disposable glass
Pipet bulbs
Centrifuge tubes, graduated, 15 mL (ground-glass stopper is used to prevent evaporation of extracts)
Glass vials with PFTE-lined screw caps, various sizes
Volumetric flasks, various sizes
Organomation N-EVAP 111
SyncorePlus, Buchi
Digital Vortex mixer, VWR
Autosampler vials, 2 mL with caps of PTFE-lined septa
Centrifuge vials, Microsolv Technology Corporation
Heavy wall vials, 2mL, Microsolv Technology Corporation
Soxhlet extractors, 40 mm ID
Condensers with tubing to circulate cold water
Erlenmeyer flasks, 500 mL
Boiling chips, solvent-extracted, approximately 10/40 mesh, silicon carbide or equivalent
Hot plates
Glass Extraction Thimbles, PYREX™

REFERENCE MATERIALS AND REAGENTS

Reference Materials (RM) and Working RM

Refer to Table 2 for Working Reference Materials (WRM) for the surrogates and spikes.

Reagents

Ethyl acetate, HPLC quality or equivalent.
Methanol, HR-GC grade or equivalent.
Acetone, Optima® or equivalent.
Hydrochloric acid, Aristar® Plus or equivalent
Sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$), $\geq$ 99.0% purity or equivalent
Analyte Protectant solution in acetone: 10 mg/mL 3-*O*-ethyl-L-ascorbic acid, 10 mg/mL 6-*O*-stearoyl-L-ascorbic acid, 10 mg/mL 6-*O*-palmitoyl-L-ascorbic acid, 50 mg/mL polyethylene glycol 300.

METHOD PROCEDURE

Batch Quality Control (QC) Samples

Refer to QSP-02.0024.00, Quality Control, for required QC samples.
Batch QC samples are method blank, laboratory control sample, laboratory control sample duplicate, matrix spike, matrix spike duplicate and sample duplicate.
Surrogates are added to the method blank, samples, sample duplicate, laboratory control sample (LCS), laboratory control sample duplicate (LCSD), matrix spike (MS), and matrix spike duplicate (MSD). Refer to Table 2 for suggested amounts.
Note: There are two postspike samples: Postspike(LSC), which is used to calculate the matrix correction factor of the commercial synthetic wastewater, and Postspike(Matrix), which is used to calculate the matrix correction factor of influent wastewater or effluent wastewater.

Sample Pre-Preparation

Check out wastewater samples on the Internal Chain of Custody. Allow samples to equilibrate to room temperature.
Check balance prior to weighing if it has not been checked that day.
Measure the weight of each of the sample (i.e., wastewater and bottle) and record in FRM-07.0326.00, Sample Preparation Log for Solid-Phase Extraction.
Set-up membrane filtration manifolds with new glass fiber filters.
Mix samples thoroughly and centrifuge, if necessary, before filtration.
Filter the samples through the glass fiber filters. The aqueous phase portion is extracted with SPE, refer to the "SPE Extraction of Wastewater" section, below. The solid phase on the glass fiber filters is extracted with Soxhlet extraction, refer to the "Soxhlet Extraction of Glass Fiber Filter" section, below.
Rinse the filter apparatus.
Measure the weight of the empty bottles and record in FRM-07.0326.00.
Rinse syringes with methanol at least three times between each usage for reference material preparation and/or sample preparation.
Check syringes before use. Properly discard any damaged or inaccurate syringes.

SPE Extraction of Wastewater

For method blank, postspike(LCS), LCS and LCSD, the sample matrix is a commercially available synthetic wastewater. Record the amount on the sample preparation log sheet, FRM-07.0326.00.
Dilute and filter commercial synthetic wastewater twentyfold before use.
Add 20 µL of the 5 µg/mL surrogate solution into each sample and batch quality control sample. See Table 2 for amounts. The final concentration of the surrogates should be 100 µg/L in the analysis.
Add 20 µL of the 5 µg/mL analyte spike WRM to the LCS, LCSD, MS and MSD. See Table 2 for amounts. The final concentration of the spike should be 100 µg/L in the analysis.
Do not spike surrogate and target analyte on Postspike(LCS) and Postspike(Matrix) at this stage.
Record the reference materials used for surrogates, LCS/LCSD and MS/MSD on the sample preparation log sheet, FRM-07.0326.00.
Install new Oasis HLB cartridges on the SPE-03 8-channel system.
Fill solvent bottle #1 with DI water, bottle #2 with methanol and bottle #3 with ethyl acetate.
Put eight clean 15mL centrifuge tubes on the first row of the collection area.
Place samples on both side of the SPE03 system with sampling tubing in each sample.

On the SPE-03 8-channel system, scroll to the "method 2" and tap ►. This is a semi-automated SPE process. Refer to Table 3 for method 2 parameters.
After sample loading is complete, manually rinse sample container with 15 mL DI water. Tap "resume" to pass the rinsate through the SPE cartridge to the aqueous waste bottle.
Repeat rinsing the sample bottle with DI water two more times as in 8.3.12.
Elute the SPE cartridge with ethyl acetate as the program proceeds.
After collection of ethyl acetate sample extracts is complete, remove the 15 mL centrifuge tubes and place new centrifuge tubes on the first row of the collection area.
Manually rinse the sample containers with 5 mL ethyl acetate and press "resume". The ethyl acetate rinsate will pass through the SPE cartridge and collect in the 15mL centrifuge tubes.
Repeat sample bottle rinse with 5 mL ethyl acetate two more times as in 8.3.16.
Using a disposable glass pipet, transfer the ethyl acetate extract from 8.3.14 to a 250 mL evaporation tube. Leave any residual aqueous layer in the bottom of the 15mL centrifuge tube.
To recover any trace level of pesticides left in the aqueous layer, add 2 mL of ethyl acetate and vortex the aqueous bottom layer in the 15mL centrifuge tube. Remove the top solvent layer and add it to the extract in the 250 mL evaporation tube.
Add the ethyl acetate rinsate from 8.3.16. and 8.3.17. to the extract in the 250 mL evaporation tube.
Evaporate the extract to about 1 mL using the 12 position SyncorePlus evaporator. Rinse the internal wall of the tube with ethyl acetate.
Note: At the beginning of the program, set pressure to about 750 mbar to degas solvent for about 2 minutes. Then, gradually manually lower the pressure to a desired value. Refer to **Table 5** for SyncorePlus parameters.
Transfer the extract to a 2 mL autosampler vial and further evaporate the extract to less than 1 mL using the N_2 Evaporate Blower (N-EVAP 111).
Adjust the final volume to 1 mL with ethyl acetate.
Note: For Influent wastewater samples, extracts may contain precipitate and need to be centrifuged to pellet the precipitate. Transfer the top layer (supernatant) to a new autosampler vial.
Spike 20 µL of the 5 µg/mL surrogate and 20 µL of the 5 µg/mL target analyte to the Postspike(LCS) and Postspike(Matrix) and bring volume to 1 mL.
For GC/QToF analysis, take 0.5 mL of the extract and add 10 µL analyte protectant and 2.5µL of the 2 µg/mL GC-QTOF internal standard solution.
For LC/QQQ analysis, solvent exchange 0.5 mL of the extract into methanol. Add LC-QQQ internal standard solution.
Store extracts in the refrigerator at < 6°C until analysis.
Proceed with TSP-06.0017.00: Pesticide Analysis by Gas Chromatography / Quadrupole Time of Flight Mass Spectrometry (GC/QToF) and TSP-06.0016.00: Pesticide Analysis by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC/QQQ) for analysis.

Soxhlet Extraction of Glass Fiber Filter

After filtering each sample, allow the glass fiber filters to dry in the dark in the fume hood.
Put 230 mL ethyl acetate into a 500 mL flask with about two boiling chips for each sample.
Prepare a glass Soxhlet thimble with 10 g anhydrous sodium sulfate for each sample.
Cut the filter into pieces that are about 1 centimeter square and add them to the prepared thimble.
Add 20 µL of the 5 µg/mL surrogate to each sample and batch quality control sample. See Table 2 for amounts. The final concentration of the surrogates should be 100 µg/L in the analysis.
Add 20 µL of the 5 µg/mL analyte spike WRM to the LCS, LCSD, MS and MSD. See Table 2 for amounts. The final concentration of the spike should be 100 µg/L in the analysis.
Put the thimble in a Soxhlet extraction unit and attach the 500 mL flask with at the bottom.

Turn on the chiller and the heaters. Set the temperature of the hot plate so it is greater than the solvent boiling point.
Extraction time starts when the Soxhlet unit completes one cycle of extraction. An extraction cycle is defined as when the Soxhlet chamber fills with solvent and then empties automatically by a siphon side arm, with the solvent running back down into the distillation flask.
Extract the sample for 16 – 24 hours at 4-6 cycles per hour (about 10-15 minutes per cycle). The extraction may be stopped and continued the next day. If stopped, make sure the extractor units are cooled before turning off the chiller.
After the extraction is complete, turn off the heater and allow the units to cool before removal. Turn off the chiller and remove the unit (i.e., Soxhlet still connected to the Erlenmeyer flask) from the condenser.
Drain any remaining solvent in the Soxhlet unit into the Erlenmeyer flask by tilting the whole Soxhlet/flask unit at a slight angle.
Use long tweezers to carefully remove the extraction thimble from the Soxhlet unit. Let any remaining solvent drain into the flask. If necessary, rinse the Soxhlet unit with extraction solvent and drain into the flask as well.
Put a clean stopper on the sample extract contained in the Erlenmeyer flask. Store in refrigerator at < 6°C until the concentration step.
Concentrate the extracts to about 1 mL using the Syncore Plus evaporator.
Transfer the extract to a 2 mL autosampler vial and further evaporate the extract to less than 1 mL using the N_2 Evaporate Blower (N-EVAP 111).
Adjust the final volume to 1 mL with ethyl acetate.
Proceed with TSP-06.0017.00: Pesticide Analysis by Gas Chromatography / Quadrupole Time of Flight Mass Spectrometry (GC/QToF) and TSP-06.0016.00: Pesticide Analysis by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC/QQQ) for analysis.

Record Keeping

Keep record of extraction on FRM-07.0326.00, SPE Extraction Sample Preparation Log Sheet.
Keep a record of reference material preparation on FRM-07.0329.00, Pesticide Reference Material Preparation Log and working reference material on FRM-07.0330.00, Pesticide Working Reference Materials (WRM) Preparation Log.
Keep a record of ICAL preparation on FRM-07.0327.00 for GC-QTOF and FRM-07.0328.00 for LC-QQQ.

WASTE DISPOSAL

After extraction, allow the sample containers and HLB cartridge to dry in the fume hood. The dried waste then goes into the waste bag. Solvent waste goes to the appropriate waste bottle.
Extracts are stored in the sample refrigerator until cleared for disposal in the solvent waste bottle.

MAINTENANCE

Ensure that all heating/cooling systems and instruments are turned off after use.
On the SPE-03 8-channel system, scroll to the “method 4” and tap ► to clean up the system.
Rinse dirty glassware with the extraction solvent to remove contaminants and then proceed to washing, drying, and storing until next usage.
Stock up on chemicals, reagents and consumables when running low.
Check if waste bottles and bags are full and with proper labeling. Full bottles and bags can be transferred for disposal. Replace with new empty waste bottles and bags with proper labeling.

REFERENCES

EPA Method 3500C, Organic Extraction and Sample Preparation.
http://www.epa.gov /osw/hazard/testmethods/sw846/pdfs/3500c.pdf.

EPA Method 8000D, Determinative Chromatographic Separations.
https://www.epa.gov /hw-sw846/sw-846-test-method-8000d-determinative-chromatographic-separations.

EPA Method 3535A, Solid-Phase Extraction (SPE).
https://www.epa.gov/hw-sw846/sw-846-test-method-3535a-solid-phase-extraction-spe.

EPA Method 3540C, Soxhlet Extraction.
https://www.epa.gov/hw-sw846/sw-846-test-method-3540c-soxhlet-extraction.

Table 2. Suggested Amounts of Working Reference Materials (WRM) for each analytical method:

Compound	Sample Amount (mL)		Primary Reference Materials/Working Reference Materials	Spike Working Reference Materials (WRM)	Spike Amount (WRM) (µL)	Final Conc. /Final Volume
Pesticides	**100 mL influent or 200 mL effluent**	**Surrogates:**	07.0xxx.00_rev0_SC_Pest Reference Material Log Sheet: Chlorpyrifos-D10 (6), Diuron-D6 (7), Fonofos (8), Methomyl-D3 (9), Simazine-D5 (10)	**5 mg/L Surrogate Working Solution (A)**	20 µL	100 µg/L /1 mL
		Spikes:	07.0xxx.00_rev0_SC_Pest Reference Material Log Sheet: Custom Pesticides Standard (1), Fipronil amide (3), Fipronil desulfinyl amide (4), Pyrethrin 1 (5)	**5 mg/L 1° Pesticide Working Solution (C)**	20 µL	100 µg/L /1 mL

Table 3. Parameters for Method 2 **on the SPE03 Unit:**

Action	**Inlet1**	**Inlet2(ratio)**	**Flow** (mL/minute)	**Volume** (mL)
Elute W2	Solvent 3	-	5	10
Elute W2	Solvent 2	-	5	10
Elute W1	Solvent 1	-	5	10
Add Samp W1	Sample	-	5	210
Wait	-	Manual resume	-	-
Add Samp W1	Sample	-	5	15
Wait	-	Manual resume	-	-
Add Samp W1	Sample	-	5	15
Wait	-	Manual resume	-	-
Air-Purge W1	Air	-	10	100
Collect 1	Solvent 3	-	3	13
Air-Purge 1	Air	-	5	10
Air-Purge W1	Air	-	5	3
Wait	-	Manual resume	-	-
Collect 1	Sample	-	5	7
Wait	-	Manual resume	-	-
Collect 1	Sample	-	5	5
Wait	-	Manual resume	-	-
Collect 1	Sample	-	5	4
Air-Purge 1	Air	-	10	10

Table 4. Parameters for Method 4 (cleanup) **on the SPE03 Unit:**

Action	**Inlet1**	**Inlet2(ratio)**	**Flow** (mL/minute)	**Volume** (mL)
Elute W2	Solvent 3	-	5	10
Elute W2	Solvent 2	-	5	10
Add Samp W2	Sample	-	5	10
Air-Purge W1	Air	-	10	10

Table 5. Parameters on the SyncorePlus Evaporator Unit:

Components	Parameters
Chiller	-2 ºC
Heater Cover	40 ºC
Heater Base	50 ºC
Rotation	180-220 rpm

RE-APPROVAL

Signatures	**Date**
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