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Pesticide Analysis by Gas Chromatography / Quadrupole Time-of-Flight Mass Spectrometry (GC/QToF)

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SCOPE AND APPLICABILITY

Monitoring pesticides in wastewater, surface water, and drinking water is an important process of water quality control. Pesticides may be harmful to humans, animals, and the environment because of their toxicity. These pesticides may be used in crops, buildings, ornamental plants, and lawns. The routes of exposure include oral, inhalation, and dermal. To minimize the risk of exposure to these chemicals, Congress passed the Food Quality Protection Act of 1996 to allow EPA to regulate the use of pesticides.

This technical system procedure (TSP) describes steps to determine the concentration of a group of mainly pyrethroids pesticides from extracts prepared from aqueous liquid matrix. Target pesticides in wastewater samples are extracted using TSP-04.3535.00, Automated Solid-Phase Extraction for Pesticides, with target pesticides listed in Table 1. The extracts are then divided into two aliquots and analyzed by gas chromatography quadrupole time-of-flight mass spectrometry (GC-QToF-MS) in negative chemical ionization (NCI) mode using methane as collision gas as well as liquid chromatography triple quadrupole mass spectrometry (LC-QQQ). This procedure focuses on data acquisition, data analysis and reporting for GC-QToF-MS.

PRINCIPLE

Sample extracts containing pesticides of interest are injected into a gas chromatograph for separation and quantitated with a mass spectrometer-time of flight detector in negative chemical ionization (NCI) mode. Compounds are separated in a narrow bore fused silica capillary GC column in a temperature-controlled oven. Each target analyte eluted from the capillary column is transferred into ion source where it is fragmented to produce ions.

The quadrupole is operating as a mass filter for the selection range of specific ions based on their mass-to-charge ratio (m/z). The collision cell is in total transmission ion (TTI) mode without subsequent fragmentation of ions. After leaving the collision cell, ions are accelerated into the ion modulator region of the time-of-flight analyzer where they are pulsed by an electric field and accelerated orthogonally to their original direction. All ions having acquired the same kinetic energy now enter the flight tube which is a field free drift region where mass separation occurs. Ions exhibiting a lighter mass will have a shorter time of flight, whereas heavier ions will take longer to traverse the flight path towards the detector.

Analytes eluting from the GC column are identified by comparing their mass spectra and retention time to reference spectra and retention times. Reference spectra and retention times for analytes are obtained

from the calibration standards under the same conditions used for samples. Concentration of the target analytes and surrogates are being quantitated with internal standard calibration method.

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(s), eye wash and shower station(s), spill kit, first aid kit and closest phone to call in case of emergency.

Personal Protective Equipment (PPE)

Wear lab coat, glasses, and gloves at all times. Fume hood is used whenever possible.

INTERFERENCES

Ensure glassware and consumables are free of contamination. Plastics may cause phthalate contamination.

PRESERVATION AND HOLDING TIMES

Storage and Holding Times

Aqueous liquid samples are stored at $<6^{\circ}\text{C}$ in Teflon-sealed glass containers.
Aqueous liquid samples have 14 days to be extracted and extracts should be analyzed within 40 days.

EQUIPMENT AND SUPPLIES

Hardware

Agilent GC-QToF System: Agilent 7890B Gas Chromatograph (GC), Agilent 7200 Quadrupole Time-of-Flight Mass Spectrometer (QToF), Agilent 7693 Autosampler, Agilent MassHunter Software.
Agilent J&W HP-5ms Ultra Inert fused silica capillary column, 15m x 0.25mm ID x 0.25 μm film thickness

Instrumentation Conditions

Pesticide identification parameter, Table 1.
Gas Chromatography Parameters for Pesticides (NCI), Table 2.

Consumables

Autosampler vials – 2 mL with caps with PTFE-lined septa.
Latex or nitrile gloves. Avoid vinyl gloves.
Gas tight syringes: 10 μL , 25 μL , 100 μL , 250 μL and 1 mL.

REFERENCE MATERIALS AND REAGENTS

Reference Materials (RM)

Refer to FRM-07.0329.00, Pesticide Reference Materials (RMs) Preparation Log, for list of RMs.
Refer to FRM-07.0330.00, Pesticide Working Reference Materials (WRMs) Preparation Log, for preparation and list of WRMs.
Refer to FRM-07.0327.00, Pesticide Calibration and Verification Solution Preparation for GC-QToF, for preparation and list of calibration and verification WRMs.

Reagents

Ethyl Acetate, HPLC grade, or equivalent

METHOD PROCEDURE

Instrument Requirements

Refer to QSP-02.0048.00, Instrument Checks, for requirements.

Batch Quality Control (QC) Requirements

Refer to QSP-02.0024.00, Quality Control, for requirements

Sample Preparation

Samples are extracted using TSP-04.3535.00, Automated Solid-Phase Extraction for Pesticides.
Allow sample extracts to warm to room temperature, if not already.
Prior to analysis, add 2.5 μL of 2 $\mu\text{g}/\text{mL}$ Internal Standard Working Solution to each 0.5 mL extract.
Place the samples in the autosampler.

MassHunter Data Acquisition and Analysis

Refer to TSP-06.0007.00, MassHunter Data Acquisition.

Data Reporting

Complete the applicable report: templates are located in the "U" drive/ECL Pasadena/Report Templates and Archive/Report Templates.
Refer to TSP-06.0007.00, MassHunter Data Acquisition Section 4.5. for a list of reports from MassHunter that are required.
Make copies of all supporting records. Refer to Report Package Contents (check-off) on FRM-07.0106.00, Initial Level Review Check List, when compiling the report package.

MAINTENANCE AND TROUBLE SHOOTING

Instrument Maintenance Schedule

Refer to FRM-07.0137.00 maintenance logbooks for Agilent 7890B/7200 GC/MS QTOF.

GC Consumables Maintenance

Common problems include carryover, loss of sensitivity, column bleed, retention time drift and loss of resolution.

To minimize carryover or loss of sensitivity: As necessary, replace septum, replace inlet liner, replace gold seal disc, clean inlet, bake out inlet, back out column, replace guard column, and/or replace new column.

To correct for retention time drift: As necessary, check for leaks, replace septum, replace the liner, replace lower injection port seal, check flow rate, and/or replace guard column. If problem remains, replace the column.

To correct for loss of resolution: Check for leaks, replace septum, liner, and inlet seal, replace guard column from the injector end. If issue remains, replace the column.

Mass Spectrometer Maintenance

A common problem is failed autotune. The ion source of mass spectrometer may need to be cleaned.

Waste Disposal

Solvent waste is poured into the appropriate waste bottle.

Extracts should be stored in the sample refrigerator for a minimum of 40 days before being disposed of in the solvent waste bottle.

Glass reference material vials are dried in the fume hood and disposed in a waste bag.

REFERENCES

United States Environmental Protection Agency, “*Method 3500C - Organic Extraction and Sample Preparation*”, SW-846, 2007.

United States Environmental Protection Agency, “*Method 8000C - Determinative Chromatographic Separations*”, SW-846, 2003.

Table 1. Identification parameter for Pesticide Targets, Surrogates, and Internal Standards

Compound Name	RT	Quantifier ion	Qualifier ion 1	Qualifier ion 2	Type
Novaluron	6.206	182.9894	84.9913	162.9830	Target
Chlorothalonil	16.268	263.8827	265.8797	267.8769	Target
Fipronil desulfinyl	18.326	351.9980	353.9950	318.0351	Target
Chlorpyrifos	19.530	312.9524	169.0098	314.9496	Target
Fipronil sulfide	21.092	385.9671	383.9702	314.9739	Target
S-Bioallethrin	21.200	167.1094	134.0754	168.1119	Target
Fipronil	21.340	365.9380	330.9692	383.9710	Target
Prallethrin	21.739	167.1089	132.0588	168.1118	Target
Tetrachlorvinphos	22.122	221.9428	223.9400	126.0058	Target
Fipronil desulfinyl amide	23.644	370.0083	361.9703	372.0050	Target
Fipronil sulfone	23.657	415.9602	346.9645	382.9413	Target
Pyrethrins I I	24.992	167.1080	168.1112		Target
Pyrethrins I II	25.367	167.1087	168.1120		Target
Fipronil amide	26.792	409.9387	411.9356	401.9790	Target
Tetramethrin I	27.590	331.1794	165.0796	332.1822	Target
Tetramethrin II	27.851	331.1799	165.0799	332.1822	Target
Bifenthrin	27.862	386.1529	241.0261	205.0495	Target
Fenpropathrin	28.063	141.0969	142.0973	143.0989	Target
Phenothrin	28.787	167.1095	168.1128	294.1279	Target
Cyhalothrin	29.454	205.0495	241.0267	243.0231	Target
Gamma-Cyhalothrin	29.826	205.0495	241.0267	243.0231	Target
Lambda-Cyhalothrin-Total	29.550	205.0495			Target
Cyphenothrin I	30.676	167.1090	168.1118	169.1150	Target
Cyphenothrin II	30.758	167.1089	168.1115	169.1156	Target
Permethrin I	31.147	207.0005	208.9973	171.0228	Target
Permethrin II	31.400	207.0002	208.9974	171.0243	Target
Cyfluthrin(I)	32.337	206.9992	208.9964	171.0221	Target
Cyfluthrin(II)	32.522	206.9995	208.9965	171.0227	Target
beta-Cyfluthrin I	32.659	206.9994	208.9965	171.0222	Target
beta-Cyfluthrin II	32.746	206.9991	208.9962	171.0220	Target
Cypermethrin(I)	32.911	206.9992	208.9964	171.0223	Target
Cypermethrin(II)	33.115	206.9995	208.9964	171.0225	Target
Alpha-Cypermethrin(III)	33.253	206.9994	208.9964	171.0225	Target
Cypermethrin(IV)	33.333	206.9994	208.9964	171.0222	Target
Esfenvalerate	35.056	211.0545	167.0633	213.0518	Target
Tau-Fluvalinate I	35.083	294.0523	296.0494	295.0551	Target
Tau-Fluvalinate II	35.217	294.0523	296.0494	295.0551	Target
Deltamethrin I	35.673	80.9176	78.9188	294.8969	Target
Deltamethrin II	36.066	80.9176	296.8949	294.8969	Target
Fonofos	15.490	168.9929	109.0125	170.9883	Surrogate
Chlorpyrifos-d10	19.382	322.0062	324.0060	179.0717	Surrogate
4,4'-Dibromooctafluorobiphenyl	13.559	455.8243	453.8253	375.9144	ISTD
Fipronil desulfinyl 13C4	18.326	358.0021	359.9992	324.0418	ISTD
Fipronil sulfide 13C4	21.092	389.9750	391.9715	320.9789	ISTD

Compound Name	RT	Quantifier ion	Qualifier ion 1	Qualifier ion 2	Type
Fipronil 13C4	21.337	371.9427	389.9739	336.9740	ISTD
Fipronil sulfone 13C4	23.657	421.9637	388.9451	352.9678	ISTD

Table 2. Pesticides Analysis Data Acquisition Method by GC/QToF

GC Summary	
GC Run Time	40.5 min
GC Post Run Time	5 min
Post Run Temp	310°C
Oven Temp (initial)	60°C
Hold Time	1 min
Oven Program	
Rate #1	40°C/min
Value #1	120°C
Hold Time #1	0 min
Rate #2	5°C
Value #2	310°C
Hold Time #2	0.0 min
Collision Cell/Backflush	
He Quench Gas	4 mL/min
N2 Collision Gas	Off
Front SS Inlet He	
Mode	Pulsed Splitless
Heater	280°C
Pressure	10.362 psi
Total Flow	54.089 mL/min
Septum Purge Flow	3 mL/min
Septum Purge Flow Mode	Switched
Gas Saver	15 mL/min after 3 min
Injection Pulse Pressure	25 psi until 0.6 min
Purge Flow to Split Vent	50 mL/min at 0.7 min
Thermal Aux 2 (RIS MSD Transfer Line)	
Temperature	300°C
MS Parameters	
Source temp	150°C
Solvent Delay	3.5 min
Column 1	
	HP-5ms Ultra Inert (15 m x 250 µm x 0.25 µm)
Flow Mode	Constant Flow
Initial Flow	1.089 mL/min
Post Run	-1.374 mL/min
Pressure	10.362 psi
Flow	1.089 mL/min
Avg Velocity	27.789 cm/sec
Holdup Time	0.89964 min

GC Summary	
Column 2	HP-5ms Ultra Inert (15 m x 250 μ m x 0.25 μ m)
Flow Mode	Constant Flow
Initial Flow	1.3 mL/min
Post Run	1.7881 mL/min
Pressure	3.7889 psi
Flow	1.3 mL/min
Avg Velocity	59.052 cm/sec
Holdup Time	0.42335 min

RE-APPROVAL

Signatures

Date
