Status: Final **Approved by:**

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Pesticide Analysis by Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS-MS)

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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, 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) and by liquid chromatography triple quadrupole mass spectrometry (LC-MS-MS). This procedure focuses on data acquisition, data analysis and reporting for LC-MS-MS.

PRINCIPLE

This method quantifies pesticides using liquid chromatography triple quadrupole mass spectrometry (LC-MS-MS). The compounds of interest are separated using high performance liquid chromatography (HPLC) on a reverse phase C-18 column. After separation, the pesticides are ionized by electrospray ionization and directed into the mass spectrometer. The triple quadrupole mass spectrometer is operated in dynamic multiple reaction monitoring (dMRM) mode. In dMRM, each compound has a retention time specific time window for acquisition. During acquisition, a precursor ion mass is selected in the first quadrupole, fragmented in the collision cell, compound specific product ions are selected in the last quadrupole, and finally sent to the detector.

SAFETY

Precautions

Ethyl acetate, methanol, and ammonium fluoride 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 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°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 HPLC LC-MS-MS System: Agilent 1290 Infinity II HPLC, Agilent 6470 Triple Quadrupole Mass Spectrometer, Agilent MassHunter Software.
Zorbax RRHD Eclipse Plus C18 column: 2.1 × 100 mm, 1.8 µm

Instrumentation Conditions

LC-MS-MS dMRM Transitions for Pesticide Targets, Surrogates, and Internal Standards, Table 1
Acquisition Parameters for LC-QQQ, Table 2

Consumables

Autosampler vials – 2 mL with caps with PTFE-lined septa, 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)

Custom pesticide standard from Accustandard. See Table 3.
Refer to FRM-07.0329.00, Pesticide Reference Materials (RMs) Preparation Log, for list of RMs.
Refer to FRM-07.0330.00.00, Pesticide Working Reference Materials (WRMs) Preparation Log, for preparation and list of WRMs.
Refer to FRM-07.0328.00, Pesticide Calibration and Verification Solution Preparation for LC-QQQ, for preparation and list of calibration and verification WRMs

Reagents

Methanol, HPLC grade, or equivalent
Water, HPLC grade, or equivalent

Isopropanol, HPLC grade, or equivalent
Ammonium fluoride, mass spectrometry 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 necessary. Prior to analysis, add 10 µL of 5 µg/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 TROUBLESHOOTING

Mass Spectrometer Maintenance

Prior to sample analysis, rinse mass spectrometer source with 50% isopropanol in water, followed by methanol. After analysis, clean the nebulizer. Clean the mass spectrometer source as needed per manufacturer's instructions.

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. LC-MS-MS dMRM Transitions for Pesticide Targets, Surrogates, and Internal Standards

Target Analyte	Retention Time (min)	Retention Window (min)	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Acceleration (V)	Polarity
Bifenthrin	9	0.6	442	181	100	16	2	Positive
Bifenthrin	9	0.6	442	166	100	35	2	Positive
Bifenthrin-D6 (ISTD)	9	0.6	446.2	181.1	68	16	2	Positive
Bifenthrin-D6 (ISTD)	9	0.6	446.2	166.1	68	50	2	Positive
Bioallethrin	5.26	0.4	303.2	135	80	10	3	Positive
Bioallethrin	5.26	0.4	303.2	107	80	20	3	Positive
Chlorpyrifos	5.45	0.4	349.9	197.92	100	20	5	Positive
Chlorpyrifos	5.45	0.4	349.9	96.95	100	41	5	Positive
Chlorpyrifos-D10 (Surr)	5.45	0.4	360	198.9	102	20	2	Positive
Chlorpyrifos-D10 (Surr)	5.45	0.4	360	99	102	44	2	Positive
Cyfluthrin	6.4	0.8	451.3	434.1	58	4	2	Positive
Cyfluthrin	6.4	0.8	451.3	191	58	13	2	Positive
Cyfluthrin-13C6 (ISTD)	6.68	0.8	439.1	422.1	68	4	2	Positive
Cyfluthrin-13C6 (ISTD)	6.68	0.8	439.1	197	68	16	2	Positive
Cyhalothrin	6.68	0.8	467.13	450	100	5	3	Positive
Cyhalothrin	6.68	0.8	467.13	225	100	10	3	Positive
Cyhalothrin-D6 (ISTD)	6.51	0.8	473.2	456	100	5	2	Positive
Cyhalothrin-D6 (ISTD)	6.51	0.8	473.2	231	58	16	2	Positive
Cypermethrin	6.67	0.8	433.3	416	90	7	2	Positive
Cypermethrin	6.67	0.8	433.3	191	90	16	2	Positive
Cypermethrin-D6 (ISTD)	6.62	0.8	439.1	422.1	53	8	2	Positive
Cypermethrin-D6 (ISTD)	6.62	0.8	439.1	197	53	16	2	Positive
Deltamethrin	7	0.8	523	280.9	70	15	3	Positive
Deltamethrin	7	0.8	523	181	70	50	3	Positive
Deltamethrin-D6 (ISTD)	6.8	0.6	527	510	53	8	2	Positive
Deltamethrin-D6 (ISTD)	6.8	0.6	527	284.9	53	16	2	Positive
Diuron-D6 (Surr)	3.02	0.4	239.1	78.1	81	32	3	Positive
Diuron-D6 (Surr)	3.02	0.4	239.1	52.1	81	20	6	Positive
Esfenvalerate	7.12	0.5	437.16	167	100	10	3	Positive
Esfenvalerate	7.12	0.5	437.16	125	100	25	3	Positive
Etofenprox	8.5	0.6	394.2	177.3	90	8	2	Positive
Etofenprox	8.5	0.6	394.2	107.1	90	40	2	Positive
Etofenprox-D5 (ISTD)	8.45	0.6	399.3	182.1	53	12	2	Positive
Etofenprox-D5 (ISTD)	8.45	0.6	399.3	108	53	50	2	Positive
Fenpropathrin	6.2	0.4	350.2	125.1	115	10	3	Positive
Fenpropathrin	6.2	0.4	350.2	97.1	115	32	3	Positive
Fenpropathrin-D6 (ISTD)	6.2	0.4	373.2	356.2	53	4	2	Positive
Fenpropathrin-D6 (ISTD)	6.2	0.4	373.2	131.1	53	16	2	Positive
Fipronil	3.6	0.4	434.9	330	106	16	2	Negative

Target Analyte	Retention Time (min)	Retention Window (min)	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Acceleration (V)	Polarity
Fipronil	3.6	0.4	434.9	250	106	32	4	Negative
Fipronil-[13C]4[15N]2 (ISTD)	3.6	0.4	440.9	336	115	16	2	Negative
Fipronil-[13C]4[15N]2 (ISTD)	3.6	0.4	440.9	252	115	32	4	Negative
Fipronil-amide	3	0.5	454.96	385.9	121	20	2	Negative
Fipronil-amide	3	0.5	454.96	290	121	40	2	Negative
Fipronil-desulfinyl	3.48	0.4	387	351	86	12	2	Negative
Fipronil-desulfinyl	3.48	0.4	387	282	86	36	2	Negative
Fipronil-desulfinyl amide	2.85	0.4	405	369	82	8	2	Negative
Fipronil-desulfinyl amide	2.85	0.4	405	329	82	20	2	Negative
Fipronil-desulfinyl-[13C]4[15N]2 (ISTD)	3.48	0.4	393	357	101	12	2	Negative
Fipronil-desulfinyl-[13C]4[15N]2 (ISTD)	3.48	0.4	393	288	101	36	2	Negative
Fipronil-sulfide	3.66	0.4	418.9	383	101	12	2	Negative
Fipronil-sulfide	3.66	0.4	418.9	262	101	32	2	Negative
Fipronil-sulfide-[13C]4[15N]2 (ISTD)	3.66	0.4	424.9	389	115	12	2	Negative
Fipronil-sulfide-[13C]4[15N]2 (ISTD)	3.66	0.4	424.9	265	115	32	2	Negative
Fipronil-sulfone	3.78	0.4	450.9	415	115	16	2	Negative
Fipronil-sulfone	3.78	0.4	450.9	282	115	28	4	Negative
Fipronil-sulfone-[13C]4[15N]2 (ISTD)	3.78	0.4	456.9	421	130	16	2	Negative
Fipronil-sulfone-[13C]4[15N]2 (ISTD)	3.78	0.4	456.9	288	130	32	4	Negative
Fonofos (Surr)	3.99	0.4	247.04	137.02	80	5	3	Positive
Fonofos (Surr)	3.99	0.4	247.04	108.99	80	15	3	Positive
Imidacloprid	1.78	0.8	256	208.9	80	12	4	Positive
Imidacloprid	1.78	0.8	256	175	80	12	4	Positive
Imidacloprid-D4 (ISTD)	1.75	0.8	260.1	213	67	16	2	Positive
Imidacloprid-D4 (ISTD)	1.75	0.8	260.1	179.1	67	24	2	Positive
Linuron-D6 (ISTD)	3.2	0.4	255.1	185	91	20	3	Positive
Linuron-D6 (ISTD)	3.2	0.4	255.1	159.9	91	20	3	Positive
Methomyl-D3 (Surr)	1.2	0.6	166.1	105.9	48	8	3	Positive
Methomyl-D3 (Surr)	1.2	0.6	166.1	88	48	8	3	Positive
Novaluron	4.52	0.4	493	158.1	90	16	3	Positive
Novaluron	4.52	0.4	493	141.1	90	56	3	Positive
Permethrin	7.71	0.4	391.09	355	100	5	3	Positive
Permethrin	7.71	0.4	391.09	183	100	5	3	Positive
Permethrin-D6 (ISTD)	7.66	0.4	414.1	361.1	48	4	2	Positive

Target Analyte	Retention Time (min)	Retention Window (min)	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Acceleration (V)	Polarity
Permethrin-D6 (ISTD)	7.66	0.4	414.1	183	48	20	2	Positive
Prallethrin	4.4	0.4	301.18	169	80	5	3	Positive
Prallethrin	4.4	0.4	301.18	105	80	20	3	Positive
Propoxur	2.8	0.4	210.11	168.1	48	4	4	Positive
Propoxur	2.8	0.4	210.11	153.1	48	4	3	Positive
Propoxur-D3 (ISTD)	2.8	0.4	213.1	171.1	106	4	4	Positive
Propoxur-D3 (ISTD)	2.8	0.4	213.1	111	106	16	3	Positive
Pyrethrin 1	6.21	0.4	329.2	161	67	8	3	Positive
Pyrethrin 1	6.21	0.4	329.2	133.1	67	20	3	Positive
Pyriproxyfen	5.3	0.4	322.2	185	110	20	2	Positive
Pyriproxyfen	5.3	0.4	322.2	96	110	12	2	Positive
Simazine-D5 (Surr)	2.8	0.4	207.1	137	105	20	3	Positive
Simazine-D5 (Surr)	2.8	0.4	207.1	129	105	20	4	Positive
Tau-Fluvalinate	7.8	0.4	503.13	208.08	80	15	3	Positive
Tau-Fluvalinate	7.8	0.4	503.13	181.06	80	25	3	Positive
Tetrachlorvinphos	3.75	0.4	364.9	203.9	120	40	3	Positive
Tetrachlorvinphos	3.75	0.4	364.9	127	120	16	3	Positive
Tetrachlorvinphos-13C6 (ISTD)	3.75	0.4	370.9	209.9	121	50	2	Positive
Tetrachlorvinphos-13C6 (ISTD)	3.75	0.4	370.9	127	121	16	2	Positive
Tetramethrin	4.9	0.8	332.19	164.07	100	15	3	Positive
Tetramethrin	4.9	0.8	332.19	135	100	15	3	Positive

Table 2. Acquisition Parameters for LC-MS-MS

LC Parameters	
Injection volume	2 µL
Needle wash	Flush port, 6 s
Column temperature	55 °C
Maximum pressure	800 bar
Flow rate	0.4 mL/min
Eluent A	Water, 1 mM ammonium fluoride
Eluent B	Methanol
Gradient	
Time	% B
0.00	30 %
1.00	30 %
2.00	75 %
10.00	90 %
10.20	100 %
Stop time	12.00 min
Post Time	3.00 min
Source Parameters	
Ion Source	AJS ESI
Gas Temp (°C)	200
Gas Flow (L/min)	13
Nebulizer (psi)	55
Sheath Gas Temp (°C)	200
Sheath Gas Flow (L/min)	10
Capillary (V)	5000 (+) / 3500 (-)
Nozzle (V)	0

Table 3. Custom Pesticide Reference Materials

Compound	CAS ID	Concentration in mix (ug/mL)
Bifenthrin	82657-04-3	100
S-Bioallethrin	28434-00-6	50
Chlorothalonil	1897-45-6	50
Chlorpyrifos	2921-88-2	50
Cyfluthrin	68359-37-5	50
Cyhalothrin	68085-85-8	100
Cypermethrin	52315-07-8	50
Cyphenothrin	39515-40-7	250
Deltamethrin	52918-63-5	250
Esfenvalerate (Asana)	66230-04-4	100
Etofenprox	80844-07-1	50
Fenpropathrin (Danitol)	39515-41-8	250
Fipronil	120068-37-3	50
Fipronil-desulfinyl	205650-65-3	50
Fipronil-sulfide	120067-83-6	50
Fipronil-sulfone	120068-36-2	50
Imidacloprid	138261-41-3	50
Novaluron	116714-46-6	50
Permethrin	52645-53-1	2500
Phenothrin	26002-80-2	2500
Prallethrin	23031-36-9	50
Propoxur (Baygon)	114-26-1	50
Pyriproxyfen	95737-68-1	50
Tau-fluvalinate	102851-06-9	50
Tetrachlorvinphos	22248-79-9	50
Tetramethrin	7696-12-0	50

RE-APPROVAL

Signatures	Date
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