Determination of Selected Pesticides Collected on XAD-4 Resin by Ultra Performance Liquid Chromatography Mass Spectrometry and Gas Chromatography Mass Spectrometry

1. Scope

This section method (SM) is for the analysis of the selected pesticides trapped in XAD-4 resin and extracted with ethyl acetate. These pesticides are acephate, bensulide, chlorpyrifos OA, DEF, diazinon, diazinon OA, dimethoate, dimethoate OA, diuron, malathion OA, methidathion, metolachlor, molinate, norflurazon, oryzalin, oxydemeton-methyl, phosmet, propanil, simazine, thiobencarb, DDVP, EPTC, trifluralin, chlorothalonil, dacthal, malathion, chlorpyrifos, dicofol, Endosulfan I, oxyfluorfen, endosulfan sulfate, propargite, iprodione, permethrin, and cypermethrin. It is to be followed by all authorized section personnel. The reporting limits vary from 0.2 µg to 0.5 µg depending on the instrument sensitivity for individual compounds.

2. Principle:

Residues of the selected pesticides are extracted from XAD-4 resin cartridge using ethyl acetates. The first twenty compounds are determined by the injection of sample extract into an HPLC equipped with a HSS T3 column and a mass spectrometer (LC-MS). The remaining compounds are determined by the injection of sample extract into a GC equipped with a mass selective detector (GC-MSD). The confirmation of compound identity on LC-MS is achieved simultaneously with collision-induced dissociation to produce a product ion for each of the analytes. The confirmation of compound identity with GC-MSD is achieved by the ratio of selected ions.

3. Safety:

3.1 All general laboratory safety rules for sample preparation and analysis shall be followed.
3.2 All solvents should be handled with care in a ventilated area.

4. Interferences:

The response and peak shape of some compounds in matrix extracts versus in plain solvent shows great differences. To be consistent, standards were prepared in matrix extract for GC-MS and LC-MS analysis.

5. Apparatus and Equipment:

5.1 Rotary evaporator (Büchi/Brinkman or equivalent)
5.2 Nitrogen evaporator (Meyer N-EVAP Organomation Model # 112 or equivalent)
5.3 Vortex-vibrating mixer
5.4 Separatory funnel, 250 mL
5.5 Conical tube with glass stopper, 15-mL graduated
5.6 Boiling flask, 500-mL
5.7 Funnel, 15 cm diameter
5.8 Disposable Pasteur pipettes, and other laboratory ware as needed
5.9 Liquid chromatograph: Waters model Acquity UPLC and auto-sampler with column heater and remote control through MassLynx system.
5.10 Gas chromatograph: equipped with a mass spectrometer

6. Reagents and Supplies: (All reagents shall meet the minimum requirement in HPLC, residue and pesticide analysis)

6.1 Acetic acid: glacial, analytical grade
6.2 Ethyl acetate, (Fisher, reagent grade or equivalent)
6.3 Nitrogen, refrigerated liquid or nitrogen generator with capacity of delivering 20 liters per minute
6.4 Standards: The individual 1.0 mg/mL stock standards of each compound were obtained from the CDFA/CAC Standard Repository.

Acephate	CAS Number 06560-19-1
Bensulide	CAS Number 741-58-2
Chlorothalonil	CAS Number 1897-45-6
Chlorpyrifos	CAS Number 2921-88-2
Cypermethrin	CAS Number 52315-07-8
Dacthal	CAS Number 1861-32-1
DEF	CAS Number 78-48-8
Diazinon	CAS Number 333-41-5
Dicofol	CAS Number 115-32-2
Dimethoate	CAS Number 60-51-5
Diuron	CAS Number 330-54-1
Endosulfan I	CAS Number 959-98-8
Endosulfan sulfate	CAS Number 1031-07-08
EPTC	CAS Number 759-94-4
Iprodione	CAS Number 36734-19-7

Malathion	CAS Number 121-75-5
Methidathion	CAS Number 950-37-8
Metolachlor	CAS Number 51218-45-2
Molinate	CAS Number 2212-67-1
DDVP	CAS Number 62-73-7
Norflurazon	CAS Number 27314-13-2
Oryzalin	CAS Number 19044-88-3
Oxydemeton-methyl	CAS Number 301-12-2
Oxyfluorfen	CAS Number 42874-03-3
Permethrin	CAS Number 52645-53-1
Phosmet	CAS Number 732-11-6
Propanil	CAS Number 709-98-8
Propargite	CAS Number2312-35-8
Simazine	CAS Number 122-34-9
Thiobencarb	CAS Number 28249-77-6
Trifluralin	CAS Number 1582-09-8
Chlorpyrifos OA	CAS Number 0000-00-00
Diazinon OA	CAS Number 962-58-3
Dimethoate OA	CAS Number 1113-02-6
Malathion OA	CAS Number 0000-00-00

6.5 Water, MS grade, Burdick & Jackson or equivalent

6.6 Analytical column for UPLC-MS: Waters Acquity UPLC HSS T3 1.8 µm, 2.1x100 mm (part# 186003539). No guard column was used.

6.7 Analytical column for GC-MSD: HP-5MS 30m x 0.25mm x 0.25µm

7. Standards Preparation:

7.1 Dilute the 1.0 mg/mL standards, obtained from the CDFA/CAC Standards Repository, with ethyl acetate. The concentration of each individual standard is 10 ng/µL.

7.2 The CDFA/CAC Standards Repository prepares two combination standards. One contains all compounds without the oxygen analogies (A). The other standard contains the 4 oxygen analogies (B). Each component of the combinations is 100 µg/mL prepared in ethyl acetate.

7.3 A combined stock solution is prepared by mixing equal amount of (A) and (B). This combined stock solution is for preparing working standards and QC spiking.

7.4 Working standards are prepared in sample matrix solution. The dilution ratios are:

Concentration of Working Standards in sample matrix (µg/mL)	Amount of 50 µg/mL stock solution (µL)	Amount of matrix extract (µL)	Final volume (mL)
0.05	2	1998	2.0
0.25	10	1990	2.0
0.5	20	1980	2.0
1.0	40	1960	2.0
2.5	100	1900	2.0

8. Sample Preservation and Storage:

Store all samples waiting for extraction in a designated freezer. Extracts shall be stores in a designated refrigerator (4 ± 3 °C).

9. Test Sample Preparation:

9.1 Sample Preparation

9.1.1 Remove samples from freezer and allow them to reach ambient temperature.

9.1.2 Clamp the sample tube on a laboratory rack in a hood. Remove the cap from both ends of the tube, connect a stopcock to the exit end of the sample tube with a modified disposable pipet tip. Place a 250 mL boiling flask with a funnel just under it.

9.1.3 Pour 100 mL ethyl acetate into the sample cartridge while the stopcock is closed. Open the stopcock and adjust the flow to a rate about 2-4 mL/min.

9.1.4 Allow the entire 100 mL of solvent to run through the cartridge. After the tube has finished draining, apply air pressure to remove any remaining solvent.

9.1.5 Evaporate the solvent to about 10 mL on a rotary vacuum evaporator at 40-45°C and 24 inches vacuum.

9.1.6 Quantitatively transfer the solution to a 15 mL conical centrifuge tube and evaporate on a N-evap at 40°C to about 1 mL.

9.1.7 Adjust final volume to 2.0 mL with ethyl acetate. Vortex for 20 seconds and transfer to three auto-sampler vials, two with insert for analysis and one without insert for storage for in case of re-analysis.

10 Instrument Calibration:

10.1 The calibration standard curve consists of five levels. The lowest level must be at or below the corresponding reporting limits. The working standard levels are 0.05, 0.1, 0.5, 1.0, and 2.5 ng/µL, as prepared in 7.4.

10.2 The calibration curves for the LC-MS and GC-MS are generally obtained using linear regression. Quadratic fit may be used if the response of certain compounds exhibited quadratic behavior.

11 Analysis:

11.1 Injection Scheme

Both LC-MS and GC-MS may need to be conditioned with a matrix standard or a sample extract 1 to 5 runs before running the following sequence: A set of calibration standards in matrix, a matrix blank, a matrix spike, a set of up to 12 test samples, then a set of standards in matrix, etc.

11.2 UPLC Instrumentation

11.2.1 Waters model Acquity UPLC and auto-sampler with column heater and remote control through Mass Lynx system.

11.2.2 Column: Waters Acquity UPLC HSS T3 1.8 µm, 2.1 x 100 mm column

11.2.3 Column Temperature: 50°C

11.2.4 Sample Tray Temperature: 10°C

11.2.5 Mobile Phase: Gradient
Solvent A1: 0.04% acetic acid in water
Solvent B1: 0.04% acetic acid in methanol
Flow rate: 0.6 mL/min
Gradient:

Time (min)	Flow rate (mL/min)	A1	B1	Curve
0	0.6	90	10	
0.5	0.6	90	10	11
7.0	0.6	10	90	6
8.8	0.6	10	90	6
9.5	0.6	90	10	11
10.50	0.6	90	10	11

11.2.6 Injection Volume: 1 µL

11.2.8 Liquid Chromatography Mass spectrometer (LC-MS) and Operating Parameters

Model:	Waters Acquity UPLC system and Xevo TQ MS
Ion Source Type:	Electrospray Ionization (ESI)
Source Polarity:	Positive
Cone voltage	20 V
Desolvation Temp:	500°C
Cap	1.5kv
Desolvation Gas	650 liters/hour
Cone Gas:	0 liters/hour
Mode of Operation:	MS/MS
Collision gas flow	0.15 mL/min

The run parameters of each compound's retention time, parent ion, daughter ion, dwell time, cone voltage and collision voltage are listed below:

	Retention time (min)	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone(v)	Collision (v)
Acephate	1.41	183.9800	142.8932	0.061	10	8
		205.9161	164.9203	0.061	20	10
Bensulide	6.45	398.16	158.01	0.013	14	34
		398.16	314.02	0.013	14	16

	Retention time (min)	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone(v)	Collision (v)
Chlorpyrifos OA	6.32	334.0958	197.9541	0.013	24	28
		334.0958	277.955	0.013	24	16
DEF	7.78	315.1919	57.0125	0.128	24	24
		315.1919	168.9082	0.128	24	14
Diazinon	6.59	305.1958	153.0104	0.024	28	20
		305.1958	169.0278	0.024	28	20
Diazinon OA	5.57	289.1958	83.9585	0.021	30	36
		289.1958	153.0707	0.021	30	22
Dimethoate	3.27	230.0042	124.948	0.128	14	20
		230.0042	198.9361	0.128	14	10
Dimethoate OA	1.66	214.0319	124.9585	0.061	18	22
		214.0319	182.9991	0.061	18	10
Diuron	5.24	233.0319	46.029	0.021	26	14
		233.0319	71.9684	0.021	26	14
Malathion OA	4.71	315.1958	98.9423	0.06	22	22
		315.1958	127.0488	0.06	22	12
Methidathion	5.41	302.9299	144.8944	0.021	10	8
		325.0576	84.907	0.021	24	24
Metolachlor	6.32	284.2235	176.1394	0.013	20	26
		284.2235	252.1164	0.013	20	14
Molinate	5.99	188.0958	55.028	0.013	20	22
		188.0958	126.0393	0.013	20	12
Norflurazon	5.34	304.1596	160.0417	0.021	42	30
		304.1596	284.1132	0.021	42	22
Oryzalin	6.14	347.2235	288.0372	0.013	26	16
		347.2235	305.2145	0.013	26	12
Oxydemeton-methyl	1.98	246.983	168.9396	0.061	18	14
		268.98	190.9867	0.061	16	12
Phosmet	5.54	318.0958	132.043	0.021	12	14
		318.0958	159.9881	0.021	12	10
Propanil	5.6	218.0319	126.9635	0.016	26	24
		218.0319	162.0047	0.016	26	16
Simazine	4.57	202.0958	124.0307	0.06	34	16
		202.0958	131.9775	0.06	34	18
Thiobencarb	6.71	258.0958	100.0593	0.024	20	12
		258.0958	125.0141	0.024	20	18

Note: The real retention times are expected within 15 seconds of that stated above when the column is new. The column conditions, temperature, mobile phase, etc. may slightly shift retention times.

11.3 GC-MSD Instrumentation:

11.3.1 Agilent GC-MSD model HP6890 with auto sampler. Operating software is Enviroquant ChemStation version G1701B.01.00
11.3.2 Column: HP-5MS 30m x 0.25mm x 0.25 µm
11.3.3 Pre-column: not used
11.3.4 Temperature program
Injector Temperature: 250°C
Oven Temperature:

Oven Ramp	Program (°C/min)	Temperature (°C)	Hold (min)
initial		70	2
Ramp 1	15	190	5
Ramp 2	15	250	5
Ramp 3	15	270	8

11.3.5 Injection volume: 2 µL
11.3.6 Column flow rate: 1.0 ml/min
11.3.7 Retention times and ions selected for SIM acquisition:

Compound name	Retention time	Selected ions	Starting time
DDVP	7.25	109,145,185	5.0
EPTC	8.35	128,189,86	8.10
Trifluralin	11.02	264, 306,335	10.8
Chlorothalonil	12.76	266, 229, 109	12.50
Dacthal	14.04	301, 303, 332	13.50
Malathion	15.83	173, 125, 93	15.75
Chlorpyrifos	15.98	314, 197, 258	15.75
Dicofol p, p’	16.46	139, 250, 111	16.28
Endosulfan 1	18.02	195, 339, 241, 261	17.80
Oxyfluorfen	18.68	252, 300, 361	18.50
Endosulfan Sulfate	20.04	272, 387, 229, 422	19.90
Propargite	20.5-20.6	135, 173, 350	20.45
Iprodione	20.57	314, 316, 187	20.45
Permethrin	24.8,25.09	183, 163, 127	24.80
Cypermethrin	26.41	163, 181,209	26.00

12. Quality Control:

12.1 Each set of samples shall have a matrix blank and minimum of one matrix spike sample. Each set contains up to 12 samples.

12.2 The matrix blank shall be free of target compounds.

12.3 The recoveries of the matrix spike should be within the control limits.

12.4 The retention time shall be within ± 10 seconds of that of the standard.

12.5 To maintain the instrument performance, trim the front 10 inches off the GC column every 50-80 injections. The response of the 0.05 ng/µL combination standards is a good measure of the instrument performance.

12.6 The sample extract shall be diluted if results fall outside the linear range of the standard curve.

12.7 Method Detection Limits (MDL)

The method detection limit refers to the lowest concentration of analyte that a method can detect reliably. To determine the MDL, 7 replicate XAD-4 sample tubes are spiked at 0.1 µg per sample. The standard deviation from the spiked sample recoveries are used to calculate the MDL for each analyte using the follow equation:

$$MDL = tS$$

Where t is the Student t test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicate used to determine the MDL, t=3.143.

12.8 Reporting limit (RL):

The reporting limit (RL) refers to the level at which reliable quantitative results may be obtained. The MDL is used as a guide to determine the RL. In general, the RL is chosen in a range 1-10 times the MDL. The response reproducibility of each compound is also considered to determine the RL. MDL data and the RL are tabulated in Appendix 1.

12.9 Method Validation Recovery Data and Control Limits:

12.9.1 The method validation consisted of five sample sets. Each set included 4 levels of fortification (0.5, 1.0, 2.0 and 5.0 µg) and a method blank. All spikes, method blank and samples were processed through the entire analytical method.

12.9.2 Control charts were generated using the data from the method validation for each analyte. The upper and lower warning and control limits are set at ± 2 and 3 standard deviations of the percent recovery, respectively, shown in Appendix 2.

13. Calculations:

13.1 The quantification is based on the sum of area counts of the product ion and the precursor of the compound analyzed. The calculation is based on external standard (ESTD).

13.2 Quantitation is based on an external standard (ESTD) calculation using either the peak area or height. The LCMS and GCMS software uses a linear or Quadratic curve fit. Alternatively, at the chemist's discretion, sample results may be calculated using the response factor for the standard.

$$y = mx+b$$

Where: y = peak response
m = slope
b = intercept
x = concentration of compound

When the unit and the dilution factor are entered correctly in the analysis sequence, the software will then correctly generate the results.

13.2 Results can be manually calculated by a single point standard. The unit is µg/sample. This calculation is to verify the results derived from the

software The general equation is as follows:

$$\mu g = \frac{(\text{sample peak area})\ (\text{std. conc. } \mu g/mL)\ (\text{std. vol. Injected}(\ \mu L))\ (\text{sample final vol., (mL)})}{(\text{std. peak area})\ (\text{sample vol. Injected}(\ \mu L))}$$

14. Reporting Procedure:

14.1 Perform Quantification with Targetlynx in the Mass lynx for LCMS result.

14.1.1 Use the Targetlynx to create a processing method when the analytic method is created. This processing method will be associated with the analytic method.

14.1.2 Open a sample list file with raw data to be quantified. Delete sample files you do not want to be included in the report (for example: solvent injection between samples). Identify calibration files and their correct levels. Check if all sample dilution factors are correct. Highlight all raw files to be processed. Go to Targetlynx; click run and verify the correct processing method is chosen. Click OK. The result file will appear on the screen.

14.1.3 Review the calibration curves and all sample results.

14.1.4 If any compound was integrated poorly, the processing method must be modified. Changing the integration parameter usually can correct the problem.

14.1.5 Choose your reporting format and print it.

14.2 Perform Quantification with EnviroQuant: for GCMS results

14.2.1 Load a standard data file
Integrate the data file
Edit compounds based on retention time and identity
Review the window range of each compound and adjust it as needed.
Reintegrate the data file based on the new method
Update levels
View the calibration curves
Save as a new method

14.2.2 Load a sample data file
Do quantification with this new method with new calibration curves
Review each compound and do integration correction
Save this reviewed file
Print this reviewed data file

14.3 Acceptance Criteria:

14.3.1 Peak retention time between standards, QC spikes and unknowns shall be within 10 seconds. If there is a known reason for retention time shifting, an explanation note shall be included.

14.3.2 Peak response shall be within the calibration range

14.3.3 Recoveries of spike QC shall be within the established control range, otherwise a rerun of the entire set shall be performed. If problems remain, an explanation note shall be included.

14.3.4 Manual single point calculation result is acceptable with explanation

14.4 Reporting:

Sample results are reported out according to the client's analytical laboratory specification sheet.

15 Discussion

15.1 The GC column maintenance: Trim 10 inches from the front end of the column after every 50-80 injections. To compensate the retention time change due to a shorter column we add 0.1 – 0.15 minutes to the initial temperature holding time. Then check the retention time for each compound to make sure it falls within the integration window.

15.2 The new LCMSMS is extremely sensitive. An injection of 1µL satisfies our sensitivity needs. The small injection volume provides us the advantage of using ethyl acetate as the final solvent.

15.3 By using ethyl acetate as final solvent instead of methanol, the response of phosmet is greatly improved. We are able to obtain a linear calibration curve consistently.

15.4 A storage stability study was done with this project. The storage stability study consisted of a 10 µg spike level and 3 replicates over a 28 day period. Twelve resin cartridge tubes were spiked and then stored in the freezer until analyzed on 0, 7, 14 and 28 days. This storage study showed no significant degradation for these compounds within the 28 days. Another storage study was preformed with this project when five new compounds were added. This storage study was similar to the previous one, but had more frequent intervals. Spiked samples were analyzed on 0, 3, 6, 10, 14, 21 and 28 days. The oxygen analogs were not spiked so that breakdown of the parent compound could be monitored. No significant breakdown occurred. Results for the storage study are shown in Appendix 3.

15.5 A trapping efficiency study was also done with this project. It consisted of 3 replicates of 10, 5, and 2 µg levels. The concentration of the spiking solution was 0.1 µg/µL for each component. A 100µL syringe was used for spiking. Each spiking was dispersed into 10 spots on the surface of resin bed. The tubes were spiked while air was pumped through them and allowed to run for 24hrs. All spikes were processed through the entire analytical method. The oxygen analogs were not spiked so that breakdown of the parent compound could be monitored. No significant breakdown occurred and all compounds showed sufficient trapping recovery. Results are shown in Appendix 4
One sample of the 5 µg had only about 60% recovery for all compounds. It was determined that the low recovery was not due to trapping related issue.

15.6 The resin used in the sample cartridges for this project was washed following the instructions in Appendix 5. After the resin was washed and dried a cartridge was packed and processed through the entire method to check for any interferences.

16. References:

Lee, Paul; *Determination of Selected Pesticides Collected on XAD-4 Resin by High Performance Liquid Chromatography Ion trap Mass Spectrometry and Gas Chromatography Mass Spectrometry,* 2005 Environmental Analysis Method, Center for Analytical Chemistry, CDFA