Appendix K. Chemical Analytical Method for Candidate Pesticides, California Department of Food and Agriculture

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Revised:

Determination of Selected Organophosphate Pesticides in Air on XAD-4 Resin

Scope: This method is for the determination of dimethoate, diazinon, malathion, chlorpyrifos, diazinon oxygen analog, and malathion oxygen analog in 30 mL XAD-4 resin. The reporting limit is 0.2 μg for all compounds.

Principle: The organophosphates are extracted from XAD-4 resin with ethyl acetate. One half of the extract is evaporated to dryness. The resulting residues are redissolved in 2 mL ethyl acetate. A GC equipped with Flame Photometric Detectors in the phosphorus mode is used to analyze these organophosphates.

Reagents, Equipment and Instrument:

Reagents:

1. Organophosphate standards Stock solutions (1 mg/mL) : obtained from Standards Repository CDFA Center for Analytical Chemistry, 3292 Meadowview Rd. Sacramento, CA. 95832
   Chlorpyrifos, CAS number 2921-58-5
   Dimethoate, CAS number 60-51-5
   Diazinon, CAS number 333-41-5
   Diazinon OA, CAS number 962-58-3
   Malathion, CAS number 121-75-5
   Malathion OA, CAS number 1634-78-2
2. Ethyl Acetate, pesticide residue grade
3. Purified XAD-4 resin, acid washed, Soxhlet extracted with methanol and ethyl acetate, and vacuum dried by UCD IR-4 Laboratory.

Standards:

1. Stock solutions are stored in freezer, working standards are stored in refrigerator
2. Working standards are diluted from stock solution with same solvent by volume ratio.

Equipment:

1 Brown bottle, 500 mL, wide month, diameter 8 cm.
2. Flat bottom boiling flask, 125 mL
3. Rotary evaporator, Buchi/Brinkmann, RE111
4. Nitrogen evaporator Organomation Model #112
5. Rotary platform shaker with appropriate clamps
6. Resin Cartridge, Teflon, 4.1 cm i.d. with teflon screen at ends

Instrument:

Hewlett Packard 5890 GC with Flame Photometric Detector, controlled by Chem Station

Analysis:

Sample Preservation and storage:

Each sample cartridge is protected with a zip lock bag. Store samples in freezer. Bring them to ambient temperature just prior to extraction. Store extracts in freezer.

Sample Extraction:

1. Open cartridge end cap. Remove Teflon screen from resin cartridge with a forcept and pour resin into a 500 mL wide mouth brown jar. Put the Teflon screen in the jar.
2. Carefully rinse the cartridge and forcept with 100 mL of ethyl acetate and add the solvent to the jar. Cap the jar with a Teflon lined lid
3. Swirl for one hour on a rotary platform shaker at 200 rpm
4. Quantitatively transfer a 50 mL aliquot to a 125 mL flat bottom boiling flask
5. Evaporate the solvent to near dryness using a rotary evaporator at 45 °C and 25 inches vacuum. Blow gently to just dryness on a nitrogen evaporator at room temperature.
6. Pipet 2.0 mL of ethyl acetate to the flask, cap and swirl.
7. Transfer the solution from the flask to two auto sampler vials, one with an insert for analysis, the other one for storing in freezer for possible future analysis.

Equipment Conditions:

Hewlett Packard 5890 GC with FPD
Column: HP-1 (100% methyl polysiloxane) 30 m x 0.53 mm x 0.88 μm
Carrier gas: helium, column flow rate 10 mL/min
Injector temperature: 220 °C
Detector temperature: 250 °C
Column oven temperature:

Initial temperature:	120°C hold for 2 min.
Ramp rate:	20°C / min.
Final temperature ramp:	250°C hold for 3 min

Injection volume: 3 μL

Retention times:

Chemicals	Retention Time
Dimethoate	12.2±0.1
DiazinonOA	13.1±0.1
Diazinon	13.6±0.1
MalathionOA	14.7±0.1
Malathion	15.5±0.1
Chlorpyrifos	15.7±0.1

Calculations:

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

Instrument Calibration:

1. Establish the retention time of each analyte by injecting appropriate concentration individual standard
2. Determine the retention time of the analytes by injecting a combination of standard solutions at low concentration into the GC. If the retention time differs from the previous recorded value by 0.2 minute or response differs from the previous recorded value by 15 %, an instrument diagnosis shall be performed.
3. Calibrate the instrument by injecting standards to construct a calibration curve from instrument response. A new curve must be re-established when a new septum, insert, or column is installed.

Data acceptance Criteria:

1. Recoveries of spiked QA samples must be within control limit.
2. During a bracketed series analysis, the average standard response of each analyte before and after samples shall vary no more than 10 % of its mean.
3. During a sequence analysis, the analyte retention time should vary no more than 2 percent from their average.

Method Performance:

Quality Control:

1. A 3 - 5 point calibration curve shall be obtained at the beginning and the end of each set of samples.
2. Each sample shall be injected two times to insure reliability of the analysis. Standards and samples are injected twice sequentially. If the signal of a sample is greater than that of the highest standard, dilute the sample. Reinject the diluted sample together with standards twice more, sequentially. A sample set consist of not more than 10 samples, a blank and a spike.
3. This method has the quality control charts listed in the appendix .

Method Detection Limit:

Method Detection Limit (MDL) refers to the lowest concentration of analytes that a method can detect reliably in either a sample or blank.. To determine the MDL, 7 samples each containing 30mL of XAD-4 resin were spiked separately with 0.4 μg of dimethoate diazinon OA, diazinon, malathion OA, malathion, chlorpyrifos. These spiked samples along with a blank were analyzed using the described method. The standard deviation derived from the 7 spiked samples was used to calculate the MDL using the following equation:

$$\mathbf{MDL} = t\,S$$

where:
t is the Student 't' value for the 99% confidence level with n-1 degrees of freedom (n-1, 1 - α = 0.99). n represents the number of replicates
S denotes the standard deviation obtained from replicate analyses.

Results for the standard deviation and the MDL are in appendix 1.

Reporting Limit:

Report Limit (RL) refers to the level above which quantitative results may be obtained. In this method the RL is set 0.2 ug for dimethoate, diazinon OA, diazinon, malathion OA, malathion and chlorpyrifos.

Method Validation:

Method validation data are in appendix 2.

Discussion:

An insert without glass wool shall be used in the gas chromatograph inlet A 150% recoveries of malathion OA has been experienced when we useinsert with glass wool. After replacing a new insert without glasswool, the problem of extremely high recovery did not re-occur.

We sometimes experience temperature variation in our laboratory as much as 20 degree. It affect the density of solvent significantly. Therefore, we weigh the solvent, which has a quantation importance, when solvent is added or aliquot is taken. .

Reference:

1. A fax copy of UCD IR-4 Lab proposed analytical procedure forward to the CAC from DPR on May 2, 2000.
2. Communication with Mat Hengel (530 752-2402) of UCD IR-4 Lab on 6-15-2000.

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Appendix 1
MDL Determination

compound	spk #	spk level	results ug	% recovery		
Dimethoate	1	0.40	0.398	99.5		
	2	0.40	0355	88.7		
	3	0.40	0.371	92.8		
	4	0.40	0.424	106.1		
	5	0.40	0.398	99.4	average	0.38
	6	0.40	0.319	79.8	stdev	0.035
	7	0.40	0.401	100.4	MDL	0.11
Diazinon OA	1	0.40	0.379	94.9		
	2	0.40	0.336	84.2		
	3	0.40	0.368	92.0		
	4	0.40	0.414	103.7		
	5	0.40	0.389	97.3	average	0.37
	6	0.40	0.305	76.3	stdev	0.036
	7	0.40	0.383	95.9	MDL	0.11
Diazinon	1	0.20	0.188	93.9		
	2	0.20	0.175	87.8		
	3	0.20	0.177	88.5		
	4	0.20	0.201	100.6		
	5	0.20	0.196	98.3	average	0.18
	6	0.20	0.101	50.5	stdev	0.039
	7	0.20	0.213	106.6	MDL	0.12
Malathion OA	1	0.80	0.826	103.3		
	2	0.80	0.767	95.9		
	3	0.80	0.801	100.2		
	4	0.80	0.893	111.7		
	5	0.80	0.864	108.0	average	0.82
	6	0.80	0.753	94.2	stdev	0.050
	7	0.80	0.831	103.9	MDL	0.16
Malathion	1	0.40	0.379	94.9		
	2	0.40	0.353	88.3		
	3	0.40	0.372	93.1		
	4	0.40	0.414	103.7		
	5	0.40	0.395	98.9	average	0.37
	6	0.40	0.298	74.6	stdev	0.039
	7	0.40	0.399	100.0	MDL	0.12
Chlorpyrifos	1	0.20	0.198	94.4		
	2	0.20	0.187	87.2		
	3	0.20	0.207	91.2		
	4	0.20	0.191	102.4		
	5	0.20	0.206	95.2	average	0.18
	6	0.20	0.119	59.5	stdev	0.032
	7	0.20	0.201	100.9	MDL	0.10

Appendix 2

Recovery Data for Method Validation

Spiked (µg)	Set	Dimethoate (ug)	%	Diazinon OA	%	Diazinon	%
0.40	1	0.393	98.4	0.425	106	0.449	112
	2	0.377	94.5	0.477	119	0.427	107
	3	0.408	102.2	0.438	110	0.432	108
4.0	1	3.69	92.5	2.95	73.9	3.45	86.4
	2	4.19	104.9	3.92	98.0	4.06	101.5
	3	3.86	96.5	3.71	93.8	3.75	93.8
40	1	42.2	105.6	37.6	94.1	39.9	99.9
	2	42.0	105.1	40.5	101.5	39.2	98.2
	3	37.1	92.9	36.9	92.3	37.9	94.9

Spiked (µg)	Set	Malathion	%	Chlorpyrifos	%	Spiked (µg)	Malathion OA	%
0.4	1	0.393	98.2	0.371	92.8	0.8	0.869	108
	2	0.352	88.2	0.348	87.1		0.823	103
	3	0.392	98.2	0.396	99.0		0.935	117
4.0	1	3.73	93.3	3.85	96.2	8.0	7.89	98.7
	2	4.05	101	3.96	99.0		8.75	109
	3	3.83	95.8	3.92	97.9		7.99	100
40	1	43.7	109	42.3	105	80	83.5	104
	2	40.7	102	39.4	98.5		85.7	107
	3	38.5	96.3	39.7	99.2		80.5	101