California Department of Food and Agriculture
Inspection Services
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Method #: 51.1
Original Date: 05/03/02
Revised:

Determination of Methyl Parathion and its Oxygen Analog (OA) in Air on XAD-4 Resin

Scope: This method is for the determination of methyl parathion and its oxygen analog (OA) in low volume air sampler tube containing XAD-4 resin. The reporting limit is 0.1 µg for methyl parathion and 0.2 µg for methyl parathion OA.

Principle: The methyl parathion and its oxygen analog are eluted from XAD-4 low-volume tubes with acetone. The eluants are evaporated to dryness. The resulting residues are redissolved in ethyl acetate and brought to a final volume of 1 .O mL A GC equipped with dual Flame Photometric Detectors in the phosphorus mode are used to analyze these compounds.

Reagents:

1. Organophosphate Standards Stock solutions (1 mg/mL): obtained from Standards Repository (Center for Analytical Chemistry, California Department of food and Agriculture)
2. Acetone, pesticide residue grade
3. Ethyl Acetate, pesticide residue grade

Safety:

Most of the reagents used and analyzed for this method have not been completely characterized. All general laboratory safety rules must be followed.

Equipment: 4

1. Separator-y funnel, 125 mL
2. Flat bottom boiling flask, 250 mL
3. Graduated conical centrifuge tube, 15 mL
4. Rotary evaporator, Buchi/Brinkmann, RE1 11
5. Nitrogen evaporator Organomation Model #112
6. Vortex mixer

I n s t r u m e n t :

Hewlett Packard 5890 GC with Dual Flame Photometric Detectors

Interference:

The background air samples had small peaks on the chromatograms that fell close to the retention time of the compounds of interest but didn't interfere with the quanitation at method validation,

Standard Preparation:

1. The 1 mg/mL standards are diluted to 100 and 10 µg/mL with ethyl acetate for spiking purposes.
2. Working standards may be made in ethyl acetate or in blank matrix solution. Dilute the mg/mL standard into a series of desired standard concentrations that will be used for instrument calibration and sample calculation.
3. Store all standards in the designated refrigerator while not in used.
4. The shelf life of standards is six months.

Procedure:

1. Clamp the low vol sample tube in a vertical position. The outlet of the tube should be about 5 mm into the mouth of the 250 mL collection flask. A 125 mL separatory funnel is set up above the sample tube. The tip of the separatory funnel should be – 5mm into the top of the sample tube.
2. Measure 100 mL acetone and transfer it into the 125 mL separatory funnel.
3. Turn the stopcock to allow the solvent to flow into the tube. Make sure to maintain a solvent plug above the resin so that channeling doesn't occur.
4. Collect the eluate into a 250 mL round bottom flask. Use air pressure to blow the last drop of solvent from the resin into the flask.
2. Evaporate the extract just to dryness on a rotary evaporator at about 35 °C water bath and 23 inches vacuum.
3. Dissolve the residue in about 3 - 5 mL ethyl acetate and quantitatively transfer it to a 15 mL conical tube. Rinse the flask twice with a total of 4 - 6 mL ethyl acetate and combine to the same conical tube.
4. Concentrate the solution to a final volume of 1 mL under a gentle stream of nitrogen in a 45 °C water bath. Vortex to mix well. Transfer extract into autosampler vials for analysis.

Instrument Conditions:

Hewlett Packard 5890 GC with FPD
Column: HP-l (100% methyl polysiloxane) 10 m x 0.53 mm 2.65 µm
Column: HP - 17 (Crosslinked 50% Ph Me Silicone) 10 m x 0.53 mm x 2.0 µm
Carrier gas: helium, column flow rate 20 mL/min
Injector temperature: 220 °C
Detector temperature 280 °C
Column oven temperature:

Initial temperature:	140 °C hold for 1 min.
Ramp rate:	20 °C / min.
Final temperature ramp:	260 °C hold for 4 min

Injection volume: 3 µL

Retention times:

HP-l

Chemicals	Retention Time
M Parathion	~4.3min
M Parathion OA	~4.8min

HP-17

Chemicals	Retention Time
M Parathion	~5.3min
M Parathion OA	~5.6 min

Analysis:

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 two more times, sequentially. A sample set is usually comprised of 10 samples, a blank and a spike.

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 15 mL of XAD-4 resin were spiked with 0.25µg of methyl parathion, and OA. 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:

$$MDL = t\,S$$

where:

t is the Student 't' value for the 99% confidence level with n-l degrees of freedom (n-l, 1 - a = 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 I.

Reporting Limit:

Report Limit (RL) refers to the level above which quantitative results may be obtained. In this method the RL is set 0.1 µg for methyl parathion and 0.2 µg for OA.

Method Validation:

Method validation was performed by spiking 5 different levels of methyl parathion and OA standards into the blank XAD-4 resin tubes in five time replicates. Results of the method validation are summarized in Appendix II.

Calculations:

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

Discussion:

In this project, a storage stability study was done. The storage stability study was carried out at a level of 1 µg/tube, with three replicate samples per time point over 32 days period. The spiked samples were stored in the walk-in freezer and then analyzed on days 3, 7, 12, 18, 24, and 32. We found that the recoveries of methyl parathion and OA were stable for up to 32 days of storage time. The results for the storage stability study are shown in appendix III. From the chromatograms, we noticed that methyl parathion and its OA in sample matrix solution gave a stronger response than in pure solvent. We prepared the working standards in blank matrix solution and also diluted high level samples with blank matrix solution. (e.g. to make 1 µg/mL standard, 100 µL of 10 µg/mL standard was diluted to a final volume of 1 mL with blank matrix solution.) We also observed that the analysis responses changed significantly after a long period of chromatography. In order to maintain the quantitation stability, an insert in the gas chromatograph inlet needed to be replaced more often and frequent single point standard calibration was used.

Reference:

1. Lee, Paul, *Determination of Acephate and Methamidophos in Air on* XAD-2,2-14-95, Environmental Monitoring Methods, Center for Analytical Chemistry, California Department of Food and Agriculture.
2. White, Jane, *Determination of Selected Organophosphate Pesticides in Air on XAD-2 Resin,* 1999, Environmental Monitoring Methods, Center for Analytical Chemistry, California Department of Food and Agriculture.

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Appendix I

MDL Determination

compound	spk #	Spiked ug	Results ug	% Recovery		
Methyl Parathion	1	0.25	0.188	75.1		
	2	0.25	0.218	87.3		
	3	0.25	0.153	61.4		
	4	0.25	0.244	97.6		
	5	0.25	0.230	92.2	average	0.208
	6	0.25	0.195	78.2	stdev	0.02902
	7	0.25	0.229	91.7	MDL	0.091
M. Parathion OA	1	0.25	0.183	78.4		
	2	0.25	0.213	85.2		
	3	0.25	0.138	85.6		
	4	0.25	0.237	90		
	5	0.25	0.218	84.8	average	0.199
	6	0.25	0.190	95.6	stdev	0.03012
	7	0.25	0.217	80	MDL	0.095

Appendix II

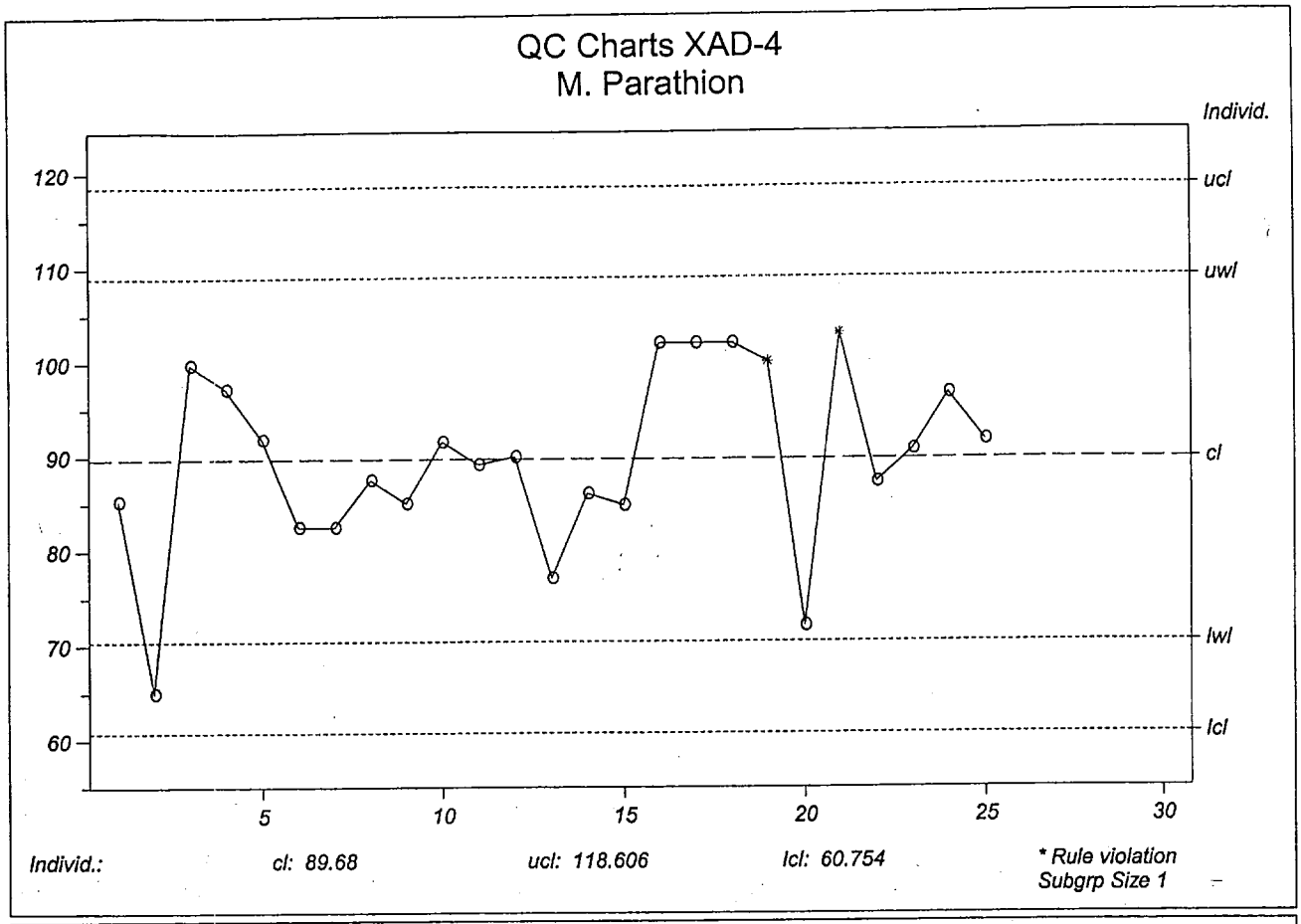
Recovery Data for Method Validation

Spike level ug	Methyl Parathion ug	Recovery %	Methyl Parathion OA ug	Recovery %
0.5	0.427	85.3	0.417	83.4
	0.325	65.0	0.343	68.6
	0.499	99.8	0.514	103
	0.486	97.2	0.470	94.0
	0.459	91.8	0.448	89.6
2.0	1.65	82.5	1.31	65.5
	1.65	82.5	1.40	70.0
	1.75	87.5	1.48	74.0
	1.70	85.0	1.37	68.5
	1.81	90.5	1.77	88.5
10	8.91	89.1	8.40	84.0
	8.99	89.9	8.56	85.6
	7.71	77.1	7.26	72.6
	8.60	86.0	9.04	90.4
	8.48	84.8	8.66	86.6
20	20.3	102	21.7	109
	20.4	102	24.7	124
	20.4	102	20.5	103
	20.0	100	19.9	100
	14.4	72.0	15.0	75.0
50	51.3	103	53.5	107
	43.6	87.2	42.9	85.8
	45.3	90.6	45.9	91.8
	48.3	96.6	48.4	96.8
	45.8	91.6	47.8	95.6

Appendix III

Recoveries for Storage Stability Study:

Day	Sample	Methyl Parathion %R	M. Parathion OA %R
0	Blank	ND	ND
	Spike 1	85.3	87.6
	Spike 2	97.8	102
	Spike 3	94.3	97.8
3	Blank	ND	ND
	Spike 1	97.2	96.1
	Spike 2	92.6	90.2
	Spike 3	90.4	84.6
7	Blank	ND	ND
	Spike 1	90.7	89.7
	Spike 2	71.6	70.7
	Spike 3	71.5	71.4
12	Blank	ND	ND
	Spike 1	95.0	101
	Spike 2	107	111
	Spike 3	93.7	94.8
18	Blank	ND	ND
	Spike 1	101	93.9
	Spike 2	92.2	85.6
	Spike 3	101	92.0
24	Blank	ND	ND
	Spike 1	105	116
	Spike 2	89.1	87.6
	Spike 3	94.6	94.5
32	Blank	ND	ND
	Spike 1	97.0	90.4
	Spike 2	110	111
	Spike 3	100	101


QC Charts XAD-4
M. Parathion
Individ.
120
110
100
90
80
70
60
ucl
uwl
cl
lwl
lcl
5
10
15
20
25
30
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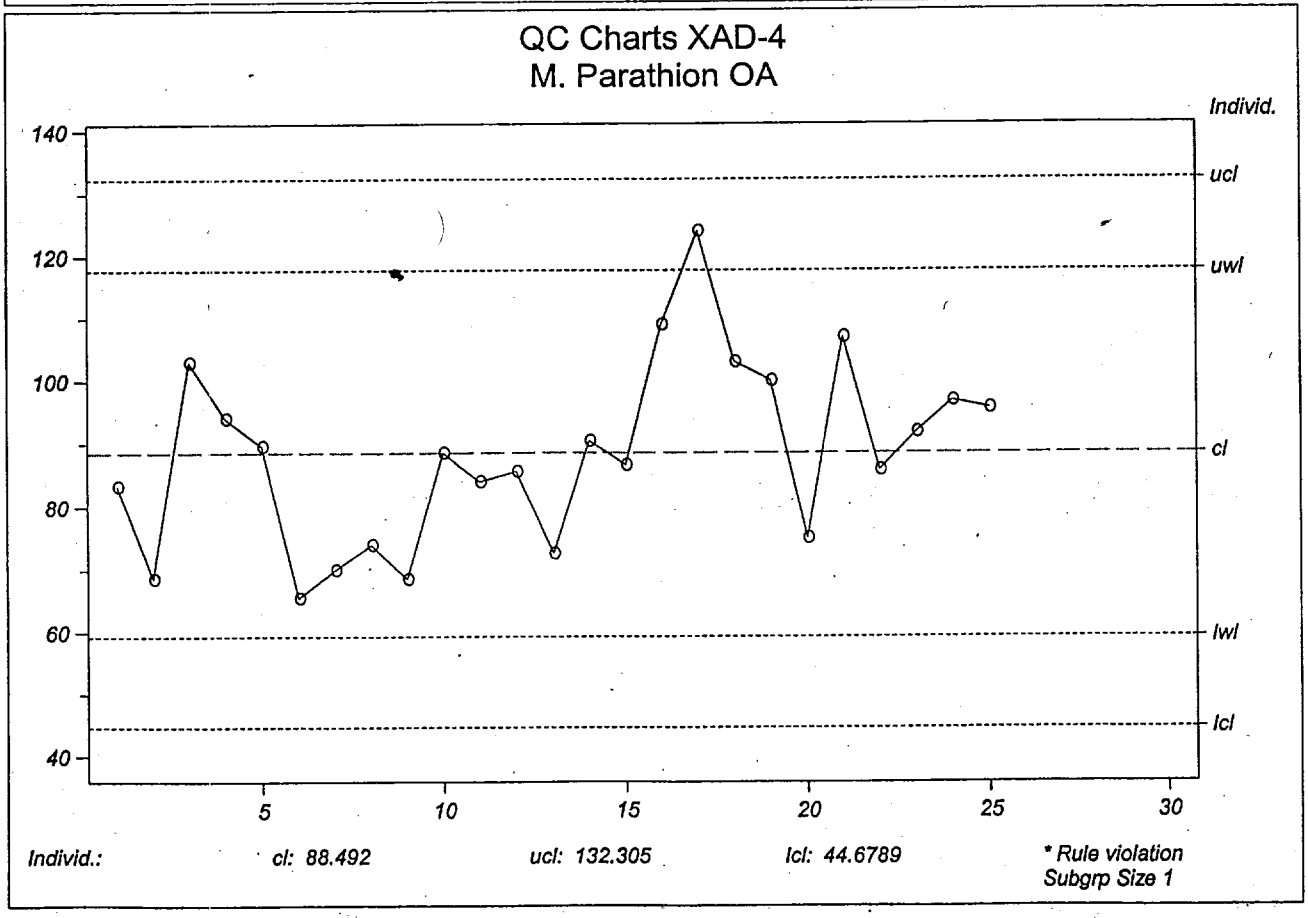
ucl: 118.606
lcl: 60.754
* Rule violation
Subgrp Size 1


QC Charts XAD-4
M. Parathion OA
Individ.
140
120
100
80
60
40
ucl
uwl
cl
lwl
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5
10
15
20
25
30
Individ.:
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* Rule violation
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