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Method #: EM 37.6
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Determination of Residues of Alachlor and Metolachlor and Selected Metabolites in Well Water by Liquid Chromatography-Mass Spectrometry

Scope: This method is for the determination of the residues of Alachlor and Metolachlor and selected metabolites in well water. These metabolites are ((2-ethyl-6-methylphenyl) (2-methoxy-1-methylethyl)amino) oxo-acetic acid, ((2-ethyl-6-methylphenyl) (2-methoxy-1-methylethyl)amino) 2-oxo-ethanesulfonic acid, ((2,6 diethylphenyl) (methoxymethy)amino) oxo-acetic acid, and ((2,6-diethyl phenyl) (methoxymethyl)amino) 2-oxo-ethanesulfonic acid. These six compounds are analyzed by liquid chromatography with a C-8 reverse phase column with ion trap mass spectrometry in MS/MS mode The reporting limit is 0.05 µg/L for all compounds. The lowest validated spiking level is 0.1 µg/L for all compounds in well water.

Principle: A 150 mL aliquot of well water is passed through a C-18 solid phase extraction columns (1 g). The analytes and the adsorbed water are eluted with methanol. The methanol is evaporated at 45 °C with a gentle stream of nitrogen to just 0.4 mL. A 0.1 mL acetonitrile is added and the final extract volume is adjusted to 0.5 mL with water. The extract is analyzed by LC/MS/MS using a C-8 column and acidified mobile phase. All metabolites are analyzed using ESI negative ion mode. The residues of Alachlor and Metolachlor are analyzed using APCI positive ion mode.

Reagents:

Use residue grade solvents for sample extraction and ultra pure grade solvents (Burdick & Jackson or equivalent) and reagents for HPLC elution and Mass Spectrometry detection.

1. Alachlor, CAS # 015972-60-8, 1.0 mg/mL in methanol, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture
2. Metolachlor, CAS #051218-45-2 , 1.0 mg/mL in methanol, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture
3. Metolachlor OXA, CAS #152019-73-3, 1.0 mg/mL in water, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture, its chemical name is ((2-ethyl-6-methylphenyl) (2-methoxy-1-methylethyl)amino) oxo-acetic acid.
4. Metolachlor ESA, CAS # not known, 1.0 mg/mL in water, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and

Agriculture, its chemical name is ((2-ethyl-6-methylphenyl) (2-methoxy-1-methylethyl)amino) 2-oxo-ethanesulfonic acid

5. Alachlor ESA, CAS # not known, 1.0 mg/mL in water, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture, its chemical name is ((2,6-diethyl phenyl) (methoxymethyl)amino) 2-oxo-ethanesulfonic acid
6. Alachlor OXA, CAS # not known, 1.0 mg/mL in water, provided by the Standards Repository, Center for Analytical Chemistry, California Department of Food and Agriculture its chemical name is (2,6 diethylphenyl) (methoxymethy)amino) oxo-acetic acid
7. Methanol, ultra pure grade from Burdick & Jackson, Cat #230-4 or equivalent
8. Acetonitrile, ultra pure grade from Burdick & Jackson, Cat #o18-04 or equivalent
9. Water, ultra pure grade, Burdick & Jackson, Cat #365-4 or equivalent
10. Acetic acid, HPLC grade Fisher Cat #A35-500 or equivalent
11. Acrodisc® 0.2 μm, Gelman Laboratory, Cat # 09730191.
12. C-18 Solid phase extraction cartridge (1g), Waters Sep-Pak Vac 6 cc, Part #36905 or equivalent

Safety:

No known carcinogens are used in this method. All general laboratory safety procedures must be followed (e.g. wear safety glasses, gloves, use ventilation hood, etc...)

Equipment:

1. Vacuum manifold, Supelco 24 port model, Cat # 913-0445
2. Larger Volume Sampler, Supelco, Cat #57275
3. Vacuum pump or in-house vacuum, at least 25 inches vacuum
4. Balance, analytical
5. Graduated cylinders
6. Nylon Acrodisc, 0.2 um, Gelman, Part #4436
7. Graduated conical test tube, 15 mL, calibrated for 0.5 mL
8. Nitrogen evaporator, Organomation, Model 112
9. Vortex mixer, Fisher Scientific, Model Vortex-Genie 2
10. Autosampler vial, Waters total recovery vial, 12X32mm and cap with preslit PTFE/Silicon septa, Part #186000385

Instrument: (see detail in operating parameters)

1. HPLC with autosampler and column oven
2. Mass spectrometer
3. Computer

Interference:

The MS/MS detection of all these analytes is specific. Multiple factors are used to eliminate possible interferences. The factors are retention time, specific parent mass $(M-H)^-$, $(M+H)^+$ and specific daughter ions:

	Parent mass m/z	Parent ion	Daughter ions m/z	Retention time (min)
Alachlor OXA	264	(M-H)-	192, 160	13.11
Alachlor ESA	314	(M-H)-	121	13.86
Metolachlor OXA	278	(M-H)-	206	14.88
Metolachlor ESA	328	(M-H)-	121, 192	13.80
Alachlor	270	$(M+H)^+$	238	11.50
Metolachlor	284	$(M+H)^+$	252	12.50

Standard Preparation:

The individual stock standards of 1.0 mg/mL are obtained from the Standards Repository, CAC, CDFA. Alachlor and Metolachlor are prepared in methanol. The four metabolites are prepared in water. They are sealed in ampules and are stored in a refrigerator (less than 5 °C). The working standards of the four metabolites are prepared by mixing equal amount of stock solutions, then diluted with water to the following concentration by volume and ratio: 30.0, 1.0, 0.5, 0.2, 0.1, 0.05 ng/μL The working standards of the two parent compounds are prepared in the same manner as the metabolites, but in methanol.

Sample Preservation and storage:

Check and record sample temperature upon arrival. Store all samples in a locked designated area in the walk-in refrigerator (less than 5 °C). Return samples to the refrigerator immediately after subsample is taken.

Sample Extraction:

1. Measure and record a 150±0.1 mL subsample into a 500 mL beaker.
2. Do sample spike at this step, if required (such as for MDL, validation, and continuing QC).
3. Set up a Supelco 24 channels manifold extraction device.
4. Connect a C-18 SPE columns (1 gram) to each channel. Turn off the unused channels of the manifold. Pre-condition the SPE columns by passing 10 mL of methanol followed by 20 mL of D.I. water. Do not allow the columns to go dry.
5. Apply the sample at the rate of 5-10 mL per minute by adjusting the vacuum. The typical operating pressure is about 10-15 inch Hg. Maintain at least 1 cm water level in the column until all sample has passed through the cartridge.
6. As soon as the sample has passed through the column, rinse the beaker with 10 mL of D.I. water and continue the extraction until all the rinsate has passed through the columns. Make sure all the columns are properly labeled before disconnecting them.
7. Remove the sampling tube. Apply a 25 inches vacuum for 5 minutes to allow excess water to be removed.
8. Elute the columns with 10 mL methanol and collecte into a 15 mL graduated conical centrifuge tube. Filter the solution through a 0.2 micron Acrodisc and rinse the tube with 2 mL methanol. Pass the rinsate through the same Acrodisc filter and combine the filtrates

9. Evaporate the eluant on a water bath at 45 °C with a gentle stream of nitrogen. Continue the evaporation to just 0.4 mL. Further evaporation will result in a significant low recovery of Alachlor.
10. Add 0.1 mL of acetonitrile and vortex for 20 seconds. Add water to a final volume of 0.50 mL and vortex for 15 seconds.
11. Transfer the entire content to a Waters total recovery autosampler vial.

Equipment Conditions:

1. HPLC System and Operating Parameters

Instrument: Waters Model 2690 HPLC, gradient pump, autosampler, column heater with remote control through the Finnigan Xcalibur system
Detector: Finnigan LCQ Deca Mass spectrometer
Column: Zorbax SB-C8 4.6 x 150mm 3.5 Micron (part number: Agilent 863953-906)
Precolumn: Phenomenex C-18 4 mm L x 2.0 mm ID cartridge (part number: AJO-4286)
Column Temperature: 40 °C
Solvent: Isocratic: 65% solvent A and 35 % solvent B,
Solvent A: 0.1% acetic acid in methanol (Burdick & Jackson or equivalent)
Solvent B: 0.1% acetic acid in ultra pure water (Burdick & Jackson or equivalent)

Flow rate: 0.6 mL/ min
Injection volume: 25 µL
Retention time:

Alachlor OXA:	13.11 min
Alachlor ESA:	13.86 min
Metolachlor OXA:	13.80 min
Metolachlor ESA:	14.88 min
Alachlor	11.50 min
Metolachlor	12.50 min

The retention time may change from day to day, but the elution order should be the same.

Note: An alternative C-8 column will probably work. The retention times may be different.

2. Mass Spectrometry System and Operating Parameters:

Instrumentation:

Finnigan LCQ Deca, ion trap mass spectrometer with ESI ion in negative ion mode for the analysis of metabolites and with APCI ion source in positive ion mode for the analysis of Alachlor and Metolachlor.
Instrument control and data handling: Gateway computer model E-4200 with 10 MB hard disk.
Software: Xcalibur Version 1 SR1.

MS run time (min): 8
Divert valve: in use during run

Divert Time (min)	Valve State
0	To waste
10	To source
18	To waste

Contact Closure: not used during run

MS Detector Settings 1: ESI ion source for the analysis of the metabolites
Acquisition start Delay (min): 10.00
Segment 1 Information
Duration (min): 8.0
Number of Scan Events: 4
Tune Method: ESI negative high flow tune10-10-00acidwavefor2.LCQT (Noted in detail in tune method section)
Scan Event Details:
1. Negative
Scan Mode:MS/MS
Amp. 20% Q 0.250 Time (μsec) 30.000 IsoW 2.0
Scan Type: Full (278.0)- > (75.0-350.0)
2. Negative
Scan Mode:MS/MS
Amp. 35% Q 0.250 Time (μsec) 30.000 IsoW 2.0
Scan Type: Full (328.0)- > (90.0-350.0)
3. Negative
Scan Mode:MS/MS
Amp. 31% Q 0.250 Time (μsec) 30.000 IsoW 2.0
Scan Type: Full (264.0)- > (70.0-350.0)
4. Negative
Scan Mode:MS/MS
Amp. 35% Q 0.250 Time (μsec) 30.000 IsoW 2.0
Scan Type: Full (314.0)- > (85.0-350.0)

Tune method: ESI negative high flow tune10-10-00acidwavefor2.LCQT

ESI Source Settings:

Sheath Gas Flow Rate (arb)	20
Aux Gas Flow Rate (arb)	0
I Spray Voltage (kV)	6.0
Spray Current (μA)	0.01
Capillary Temp (°C)	225
Capillary Voltage (V)	-7.0
Tube Lens Offset (V)	-60

Ion Optics Settings:

Octapole 1 Offset (V)	7.75
Lens Voltage (V)	34.00
Octapole 2 Offset (V)	12.50
Octapole RF Amplitude (V p-p)	370.00
Entrance Lens (V)	36.00

Automatic Gain Control: on

Full Mass Target	5 x e7
SIM	2 x e7
MSn Target	2 x e7
Zoom Target	2 x e7
Inject Waveform	Type 1
Total microscans	3
Maximum inject time(μsecond)	50.00

MS Detector Settings 2: APCI ion source for the analysis of Alachlor and Metolachlor.

Acquisition start Delay (min): 8.00min

Segment 1 Information

Duration (min): 7.0

Number of Scan Events: 2

Tune Method: APCI high flow 238 tune.LCQT (Noted in detail in tune method section)

Scan Event Details:

1. Positive

Scan Mode:MS/MS

Amp. 25% Q 0.250 Time (μsec) 30.000 IsoW 3.0

Scan Type: Full (270.0)- > (75.0-290.0)

2. Positive

Scan Mode:MS/MS

Amp. 35% Q 0.250 Time (μsec) 30.000 IsoW 3.0

Scan Type: Full (284.0)- > (150.0-290.0)

Tune method: APCI high flow 238 tune.LCQT

APCI Source Settings:

Vaporizer Temp((°C)	500.00
Sheath Gas Flow Rate (arb)	89
Aux Gas Flow Rate (arb)	3
Discharge Current (μA)	5
Capillary Temp (°C)	150.00
Capillary Voltage (V)	44.00
Tube Lens Offset (V)	55.00

Ion Optics Settings:

Octapole 1 Offset (V)	-4.75
Lens Voltage (V)	-16.00
Octapole 2 Offset (V)	-8.00
Octapole RF Amplitude (V p-p)	400.00
Entrance Lens (V)	-52

Automatic Gain Control: on

Full Mass Target	5 x e7
SIM	2 x e7
MSn Target	2 x e7
Zoom Target	2 x e7
Inject Waveform	off
Total microscans	1
Maximum inject time(μsecond)	300

Instrument Calibration:

A 5 level standard curve is run before and after each sample set. The concentration of working standards are 0.05, 0.1, 0.2, 0.5 and 1.0 ng/μL

Analysis:

Build a sequence table and inject the first standard at least twice to condition the instrument. Input the correct dilution factors. The typical sequence order is standards, blank, spikes, 10 samples and standards, then repeat the order for the second injection .

Calculations:

Calculate the concentration of chemical(s) of a sample as follows:

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

The LCQuan software in Xcalibur is used for calculations.
In general, std vol. injected = sample vol. injected.
final volume =0.50 mL
sample vol.= 150 mL

Method Performance:

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, each of the 7 samples containing 150 mL of background well water obtained from Auburn, California for Study GW-1 (matrix blank) were spiked separately with 0.1 µg/L (15 ng) of Alachlor OXA, Alachlor ESA, Metolachlor OXA and Metolachlor ESA, Alachlor and Metolachlor. These spiked samples along with a blank were analyzed using the described method. The standard deviation derived from the analytical results of 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.

Reporting Limit:

Report Limit (RL) refers to the level above which quantitative results may be obtained usually 1-5 times the MDL. In this case, the reporting limit is 0.05 µg/L for all six compounds.

Spiking solution and spiking volume:

MDL, method validation and QC spikes are made by spiking 150.0 mL of background well water obtained for Study GW-1, which is from Auburn well.
The concentration of mixed standard for spiking is 1.0 ng/µL and 30.0 ng/µL for all six compounds. The volumes spiked are as in the following table.

	Sample Size (mL)	Volume Added (µL)		Analyte Spiked (ng)	Equivalent to (µg/L)
Spiking Solution		1.0 ng/µL	30 ng/µL		
MDL	150	15		15	0.1
Validation level 1	150	15		15	0.1
Validation level 2	150	30		30	0.2
Validation level 3	150	75		75	0.5
Validation level 4	150		5	150	1.0
Validation level 5	150		10	300	2.0
Set QC	150	45		90	0.3

MDL Data:
Appendix 1

Method Validation Data:
Appendix 2

Acceptance Criteria:

1. The standard curves at the beginning and end of each sample set should not have a percent change greater than 20%. The % change in response is calculated as follows:
   % Change in response = absolute value of [slope of (STD curve before - STD curve after)/ STD curve before] x 10
2. The sample results are calculated based on the average of two adjacent calibration curves using Xcalibur software. When the difference between the calculated results of the two injections is less than 15%, either result can be reported. Additional injection is required if the differences is greater than 15%.

Discussion:

In the beginning, we developed the parameters of analysis for these metabolites with a used C-18 column. As we changed to an identical new column to run the analysis, the method did not work. The reason is unknown. A renewed effort to use a C-8 column and an isocratic mobile phase, as described in this method, provide us with reproducible and reliable results. We are now satisfied with this method.

It was a difficult task to develop a method to analyze these highly water soluble acidic compounds. In order to get an acceptable chromatogram, addition of acid into the mobile phase is necessary, but too much acid reduces the negative ion ionization. We found that 0.1 % acetic acid in mobile phase gives good chromatograms and the required sensitivity.

The evaporation step, in the sample preparation section (step 9), to reduce the volume to 0.4 mL is critical. We experienced a significant low recovery of Alachlor and slightly low recovery of Metolachlor, if the evaporation continues.

We choose to use isocratic elution in the HPLC operation, which provides us a wide, but symmetrical bell shape peak and stable response. It also provides us more data points across each peak and reproducible results.

In order to achieve sensitivity and stable response, we have to analyze Alachlor and Metolachlor with APCI ion source and their metabolites with ESI ion source.

This method provides acceptable results, as measured by the average recovery at all spiking level for all six analytes. No residues or interferences are found in background water

Reference:

1. Method of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group-Update and Additions to the Determination of Chloroacetanilide Herbicide Degradation Compounds in Water Using High-Performance Liquid Chromatography/Mass Spectrometry. By E.A. Lee, J.L. Kish, L.R. Zimmerman, and E.M. Thurman
   U.S. Department of the Interior, U.S. Geological Survey.
   Open-File report 01-10
2. Determination of Metolachlor (CGA-24705)and CGA-77102, and their Degradates CGA-50212, CGA-354743, CGA-380168, CGA-37735, CGA-67125, and CGA-41638 in Water by High Performance Liquid Chromatography with Mass Spectrometric Detection Including Validation Data.
   Method Number: AG-682

Written By: Paul Lee

Paul Lee

Title: Agricultural Chemist III

Approved By: Catherine Cooper

Catherine Cooper

Title: Agricultural Chemist III
Supervisor

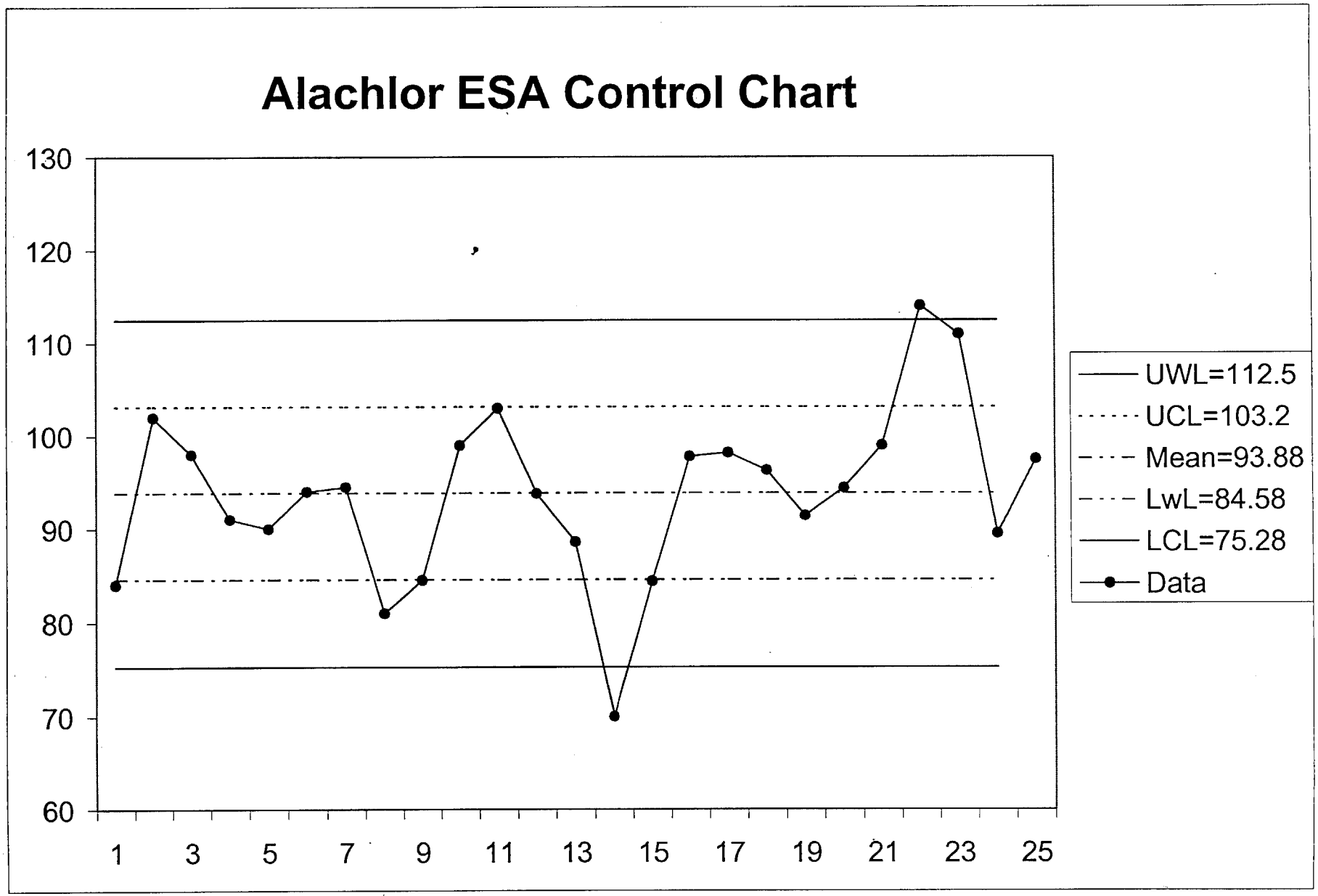
Appendix 1:
MDL Results (µg/L) for well water

	Spike level (µg/L)	Alachlor OXA found (µg/L)	Alachlor ESA found (µg/L)	Metolachlor OXA found (µg/L)	Metolachlor ESA found (µg/L)	Alachlor Found (µg/L)	Metolachlor Found (µg/L)
Blank	0	ND	ND	ND	ND	ND	ND
1	0.1	0.117	0.111	0.091	0.107	0.073	0.093
2	0.1	0.104	0.091	0.076	0.088	0.071	0.090
3	0.1	0.101	0.084	0.070	0.085	0.066	0.087
4	0.1	0.103	0.086	0.070	0.084	0.076	0.096
5	0.1	0.115	0.109	0.098	0.109	0.075	0.094
6	0.1	0.114	0.101	0.085	0.091	0.077	0.096
7	0.1	0.107	0.092	0.082	0.088	0.076	0.097
Average		**0.109**	**0.096**	**0.082**	**0.093**	**0.073**	**0.093**
STDEV		**0.006**	**0.011**	**0.011**	**0.010**	**0.004**	**0.004**
MDL = 3.143 x STDEV		**0.020**	**0.034**	**0.033**	**0.033**	**0.012**	**0.011**
RL		**0.05**	**0.05**	**0.05**	**0.05**	**0.05**	**0.05**

Appendix: 2
Method Validation Results and Recovery on background well water

	Alachlor OXA		Alachlor ESA		Metolachlor OXA		Metolachlor ESA	
Spike Level (µg/L)	Result (µg/L)	Recovery (%)	Result (µg/L)	Recovery (%)	Result (µg/L)	Recovery (%)	Result (µg/L)	Recovery (%)
0.1	0.092	92.0	0.084	84.0	0.078	78.0	0.090	90.0
	0.117	117	0.102	102	0.089	89	0.104	104
	0.107	107	0.098	98.0	0.087	87.0	0.104	104
	0.099	99.0	0.091	91.0	0.089	89.0	0.090	90.0
	0.080	80.0	0.090	90.0	0.074	74.0	0.087	87.0
0.2	0.192	96.0	0.188	94.0	0.170	85.0	0.188	94.0
	0.216	108	0.189	94.5	0.176	88.0	0.211	105
	0.194	97.0	0.162	81.0	0.133	66.5	0.189	94.5
	0.154	77.0	0.169	84.5	0.181	90.5	0.184	92.0
	0.159	79.5	0.198	99.0	0.192	96.0	0.190	95.0
0.5	0.562	112	0.514	103	0.52	104	0.52	104
	0.403	80.6	0.469	93.8	0.495	99.0	0.515	103
	0.373	74.6	0.443	88.6	0.415	83.0	0.431	86.2
	0.316	63.2	0.350	70.0	0.334	66.8	0.391	78.2
	0.390	78.0	0.422	84.4	0.444	88.8	0.427	85.4
1.0	1.09	109	0.978	97.8	1.04	104	0.996	99.6
	0.732	73.2	0.982	98.2	0.966	96.6	0.940	94.0
	0.753	75.3	0.963	96.3	0.948	94.8	0.942	94.2
	0.747	74.7	0.914	91.4	0.893	89.3	1.01	101
	0.898	89.8	0.944	94.4	0.914	91.4	0.916	91.6
2.0	2.01	100	1.98	99.0	1.96	98.0	1.85	92.5
	2.23	112	2.28	114	2.28	114	2.26	113
	2.08	104	2.22	111	2.21	111	2.31	115
	1.95	97.5	1.79	89.5	1.75	87.5	1.79	89.5
	2.05	102.6	1.95	97.5	2.01	100	1.98	99.0

	Alachlor		Metolachlor	
Spike Level (ppb)	Result (ppb)	Recovery (%)	Result (ppb)	Recovery (%)
0.1	0.108	108	0.122	122
	0.077	77.0	0.092	92.0
	0.103	103	0.112	112
	0.079	79.0	0.089	89.0
	0.068	68.0	0.086	86.0
0.2	0.148	74.0	0.167	83.5
	0.141	70.5	0.164	82.0
	0.194	97.0	0.196	98.0
	0.147	73.5	0.164	82.0
	0.146	73.0	0.178	89.0
0.5	0.496	99.2	0.520	104
	0.346	69.2	0.393	78.6
	0.484	96.8	0.484	96.8
	0.373	74.6	0.379	75.8
	0.394	78.8	0.444	88.8
1.0	0.750	75.0	0.854	85.4
	0.865	86.5	0.915	91.5
	0.912	91.2	0.897	89.7
	0.826	82.6	0.851	85.1
	0.845	84.5	0.913	91.3
2.0	1.55	77.5	1.60	80.0
	2.04	102	2.09	105
	1.80	90.0	1.99	99.5
	1.56	78.0	1.69	84.5
	1.87	93.5	1.91	95.6


Alachlor ESA Control Chart
130
120
110
100
90
80
70
60
1
3
5
7
9
11
13
15
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19
21
23
25
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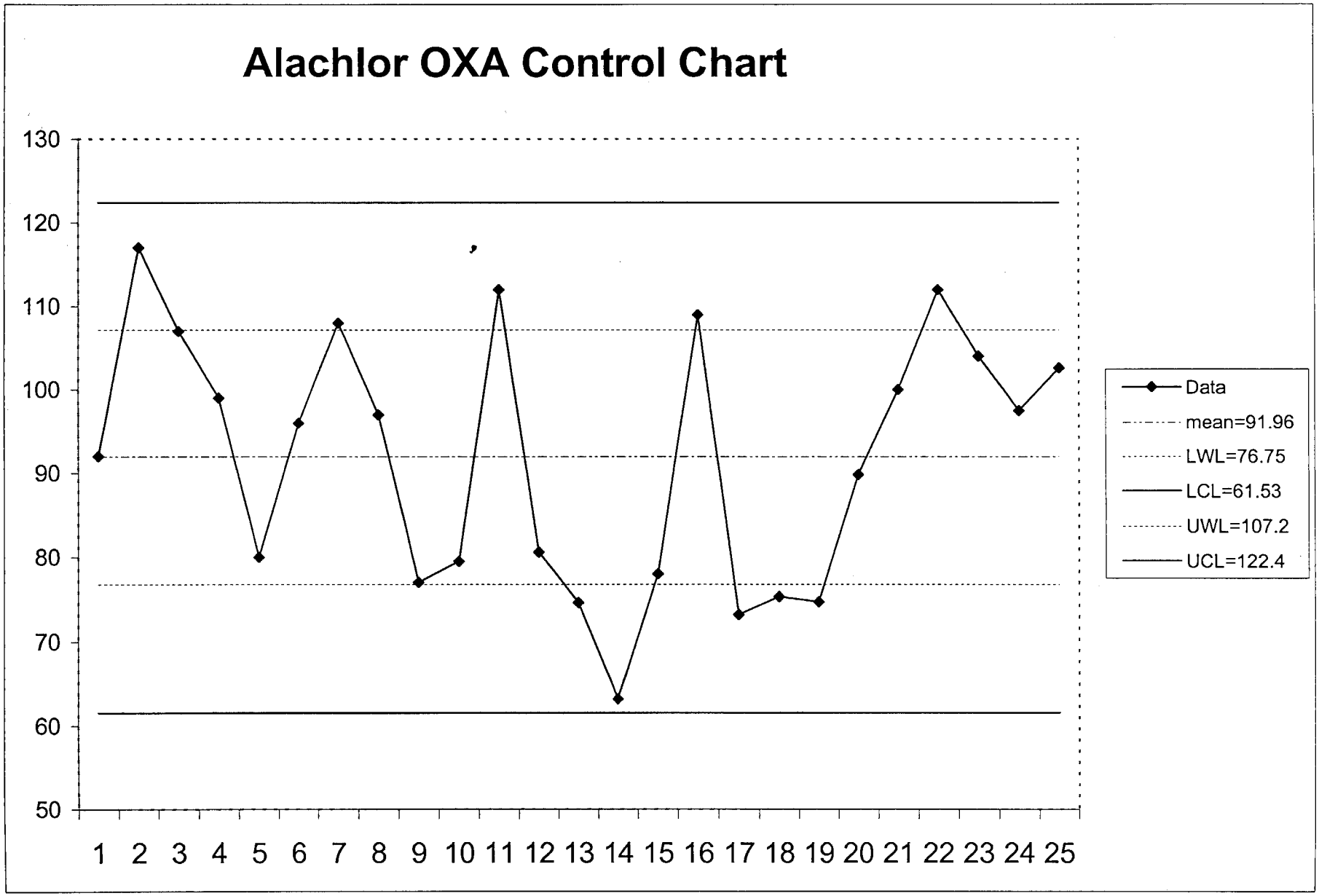
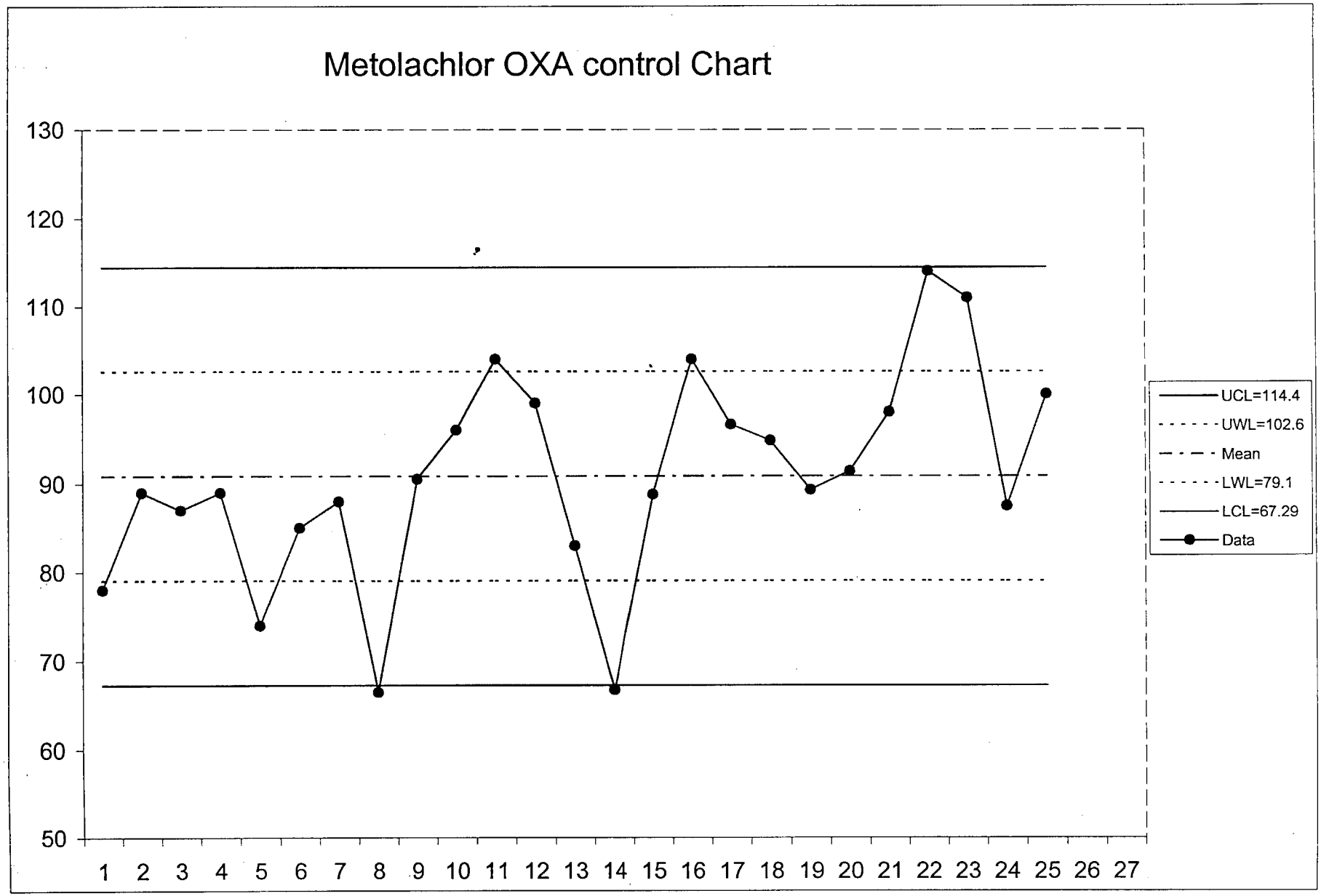
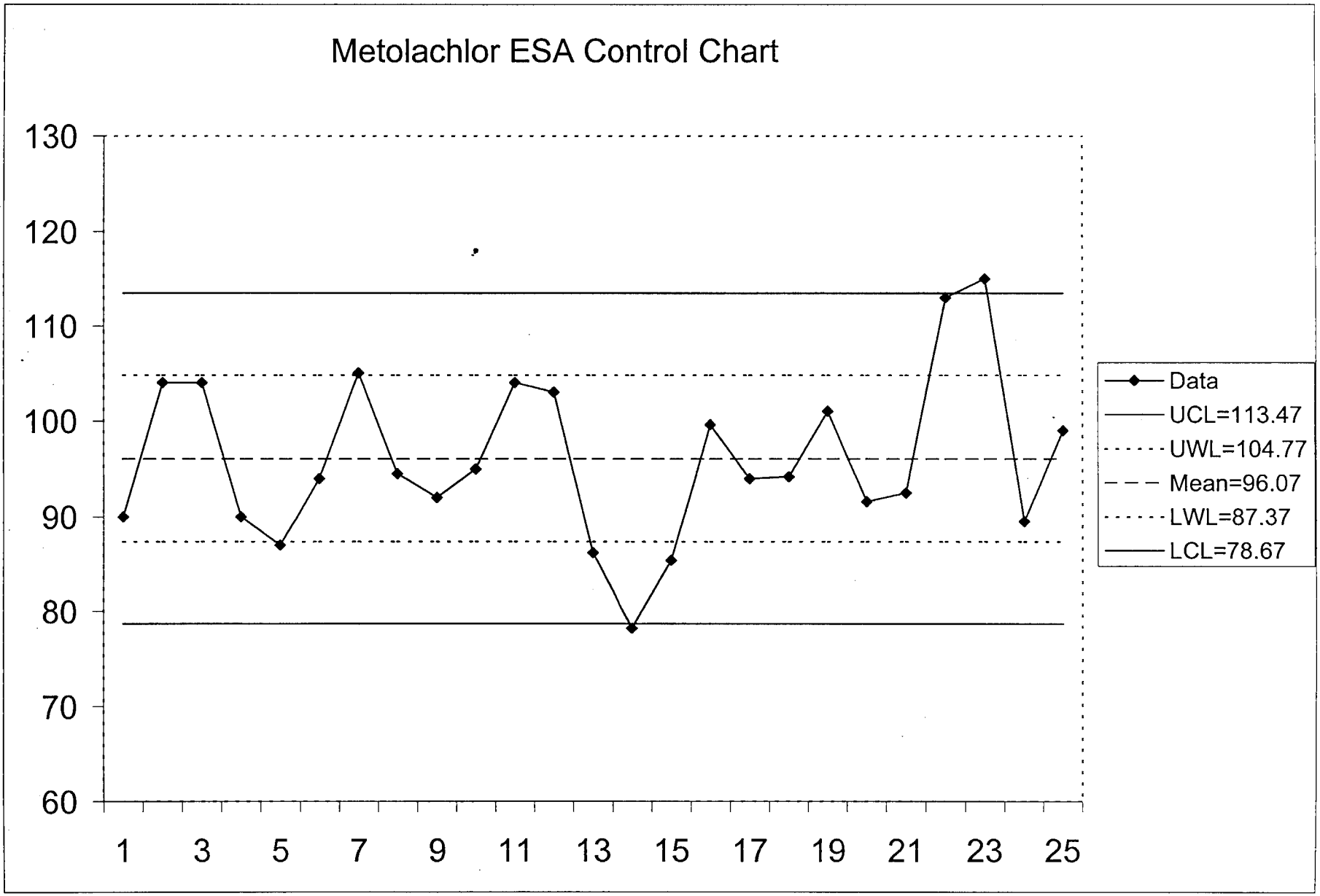
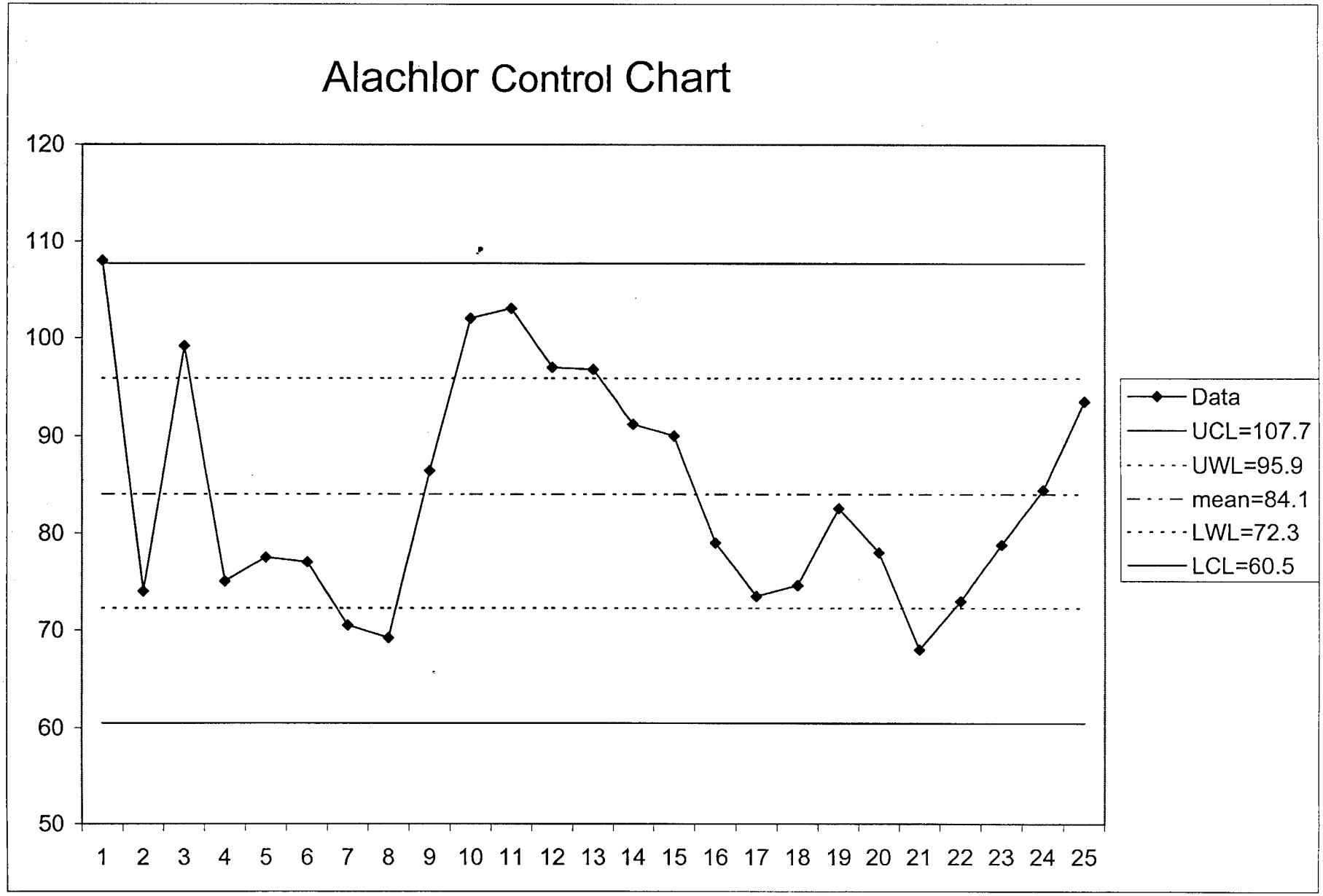
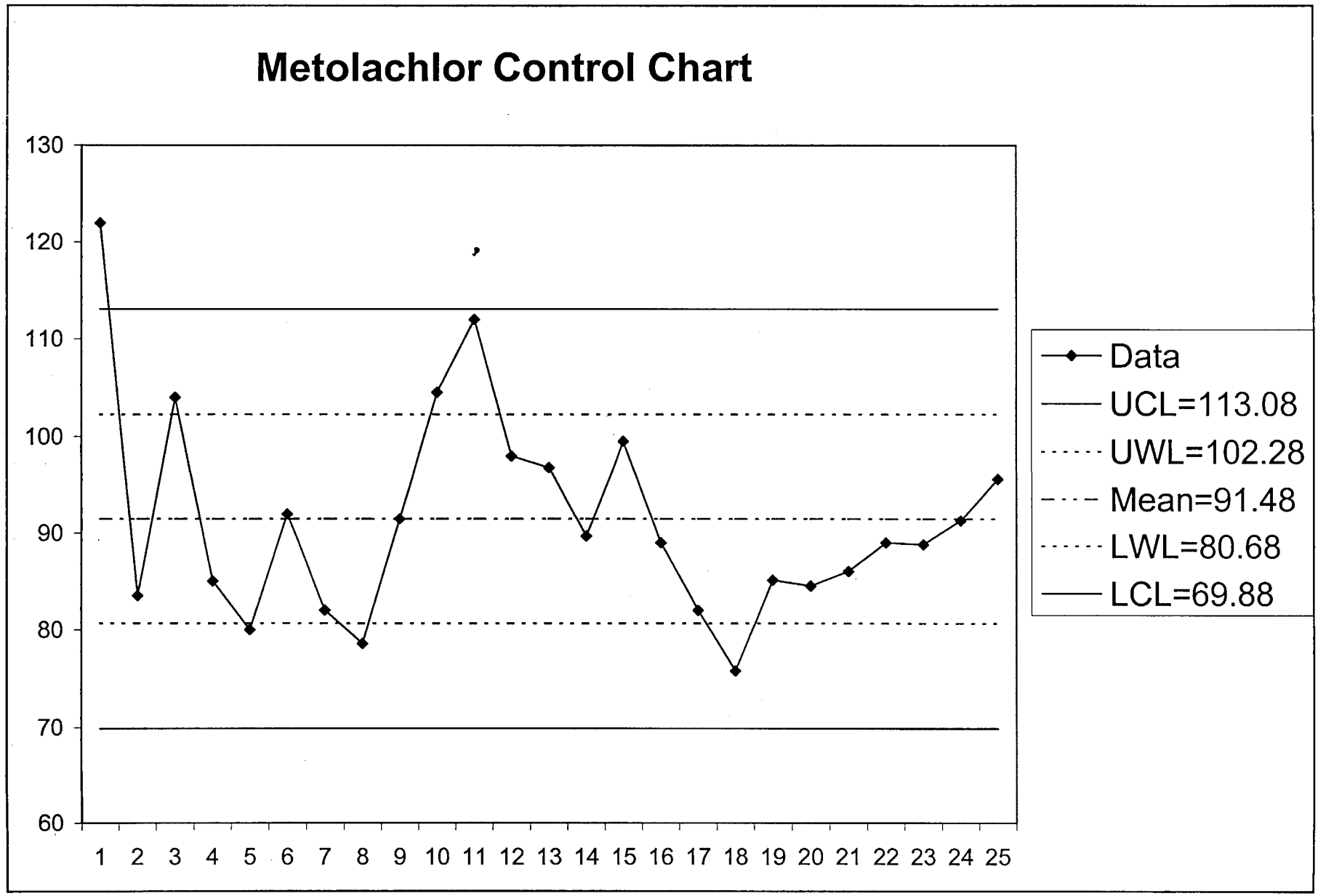
UCL=103.2
Mean=93.88
LwL=84.58
LCL=75.28
Data


Alachlor OXA Control Chart
130
120
110
100
90
80
70
60
50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Data
mean=91.96
LWL=76.75
LCL=61.53
UWL=107.2
UCL=122.4


Metolachlor OXA control Chart
130
120
110
100
90
80
70
60
50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
UCL=114.4
UWL=102.6
Mean
LWL=79.1
LCL=67.29
Data


Metolachlor ESA Control Chart
130
120
110
100
90
80
70
60
1
3
5
7
9
11
13
15
17
19
21
23
25
Data
UCL=113.47
UWL=104.77
Mean=96.07
LWL=87.37
LCL=78.67


Alachlor Control Chart
120
110
100
90
80
70
60
50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Data
UCL=107.7
UWL=95.9
mean=84.1
LWL=72.3
LCL=60.5


Metolachlor Control Chart
130
120
110
100
90
80
70
60
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Data
UCL=113.08
UWL=102.28
Mean=91.48
LWL=80.68
LCL=69.88