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December 17, 2001

MEMORANDUM

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SUBJECT: Acetamiprid (NI-25) and IC-0 Lab Method Evaluation
Report No. ECM0176S1-S2

FROM: Aubry E. Dupuy, Jr., Chief *Aubry E. Dupuy Jr.*
BEAD/Environmental Chemistry Lab

TO: Dana Spatz
EFED/Environmental Risk Branch 4 (7507-C)

As requested ECL has completed an Environmental Chemistry Lab evaluation for acetamiprid (NI-25) and its metabolite IC-0 in soil, MRID #449885-16, using a method submitted by Aventis Cropscience (formerly Rhône-Poulenc Ag. Co.), entitled "NI-25: Method of Analysis for NI-25 and Its Metabolite IC-0 in Soil Using LC/MS/MS".

This method was evaluated with soil fortified at three levels, 3.33, 10.0 and 100 ppb, and with quadruplicate analyses at each level.

The attached method lab evaluation report includes three parts:

Part I: Summary and conclusions

In this section any problems encountered with the method and how they were handled are discussed. ECL's opinion of how well the method performed is also presented.

Part II: Analytical Results

In this section the individual results of each sample at each spiking level are listed. The average percent recovery and relative standard deviation (RSD) for each spiking level is also presented here.

Part III: Experimental Details

In this section any modifications that were made, instrument parameters, representative sample calculations and standard curve are listed and/or discussed.

If you have any questions concerning this report, please contact Henry Shoemaker at (228) 688-1222 or Aubry Dupuy at (228) 688-3212.

Attachments

cc: Christian Byrne, QA Officer
BEAD/Environmental Chemistry Lab

Henry Shoemaker, Chemist
BEAD/Environmental Chemistry Lab

ENVIRONMENTAL CHEMISTRY METHOD EVALUATION REPORT
NUMBER ECM 0176S1-S2

Acetamiprid(NI-25)and IC-0 in Soil

Final Report

ENVIRONMENTAL CHEMISTRY LABORATORY
BIOLOGICAL AND ECONOMIC ANALYSIS DIVISION

November 28, 2001

Prepared by: Henry Shoemaker
Henry Shoemaker, ECL Chemist

Date: 11/28/01

Reviewed by: Christian Byrne
Christian Byrne, ECL QA Officer

Date: 12/05/01

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PART I

SUMMARY AND CONCLUSIONS

The Environmental Chemistry Laboratory (ECL) has completed an Environmental Chemistry Method Laboratory Evaluation of Acetamiprid (NI-25) and its metabolite IC-0 in soil. This method, MRID# 449885-16, submitted by Rhône-Poulenc Ag Company, is titled "Method of Analysis for NI-25 and its Metabolite, IC-0 in Soil Using LC/MS/MS".

In order to evaluate this method ECL fortified a soil matrix with NI-25 and IC-0 at 3.33, 10 and 100 ppb. All samples were done in replicates of four at each level. ECL estimated the method limit of detection (LOD) to be 3.33 ppb for each compound. The registrant's method limit of quantitation (LOQ) of 10 ppb in soil for both compounds was confirmed by our data. ECL found the precision to be well within our target limits of $\leq 20\%$ relative standard deviation (RSD) at or above the (LOQ). For example, the (RSD's) are 4.61% and 2.39% for NI-25 and IC-0, respectively, at the (LOQ) of 10 ppb and 2.62% and 1.46% at the 100 ppb level. The mean recoveries for NI-25 of 89.0% at the 10 ppb level and 90.0% at the 100 ppb level are well within the target range of 70% to 120%. The mean recoveries for IC-0 were a bit low at 65.7% at the (LOQ) and 74.5% at the 100 ppb level.

This method involves extraction of soil samples by accelerated solvent extraction technology (ASE). This technology utilizes high pressure and temperature to extract soils with a minimum amount of solvent yet yielding a clean extract that needs little or no clean-up. The analysis is done by liquid chromatography using a Sciex API300 MS/MS detector.

ECL believes that this method can be used for monitoring for NI-25 and IC-0 at the suggested levels.

PART II
ANALYTICAL RESULTS FOR ACETAMIPRID (NI-25)

EPA RECOVERIES IN SOIL

Sample	Added (ppb)	Found (ppb)	Recovery (%)	Statistics
AC 20	0	0	method blank	
AC 21	0	0	matrix blank	
AC 22	0	0	matrix blank	
AC 23	0	0	matrix blank	
AC 24	3.33	2.78		
AC 25	3.33	2.55		
AC 26	3.33	2.76		
AC 27	3.33	3.07		
AC 28	10.0	8.68	86.8	mean(Rec) = 89.0% SD = 4.099 RSD = 4.61%
AC 29	10.0	9.16	91.6	
AC 30	10.0	9.32	93.2	
AC 31	10.0	8.44	84.4	
AC 32	100	89.8	89.8	mean(Rec) = 90.0% SD = 2.357 RSD = 2.62%
AC 33	100	89.2	89.2	
AC 34	100	87.6	87.6	
AC 35	100	93.2	93.2	

ANALYTICAL RESULTS FOR METABOLITE (IC-0)

EPA RECOVERIES IN SOIL

Sample	Added (ppb)	Found (ppb)	Recovery (%)	Statistics
AC 20	0	0	method blank	
AC 21	0	0	matrix blank	
AC 22	0	0	matrix blank	
AC 23	0	0	matrix blank	
AC 24	3.33	1.21		
AC 25	3.33	1.56		
AC 26	3.33	1.25		
AC 27	3.33	1.71		
AC 28	10.0	6.42	64.2	mean(Rec) = 65.7% SD = 1.570 RSD = 2.39%
AC 29	10.0	6.68	66.8	
AC 30	10.0	6.72	67.2	
AC 31	10.0	6.44	64.4	
AC 32	100	75.6	75.6	mean(Rec) = 74.5% SD = 1.088 RSD = 1.46%
AC 33	100	74.8	74.8	
AC 34	100	73.0	73.0	
AC 35	100	74.4	74.4	

PART III

EXPERIMENTAL SUMMARY

(a) Method Procedure

Soil samples of 30.0 grams containing NI-25 and IC-0 were mixed with 8 mL of hydromatrix and extracted by accelerated solvent extractor with approximately 50 mL of 50% acetonitrile/water. The extract was then built to exactly 60 mLs with 50% acetonitrile/water. A 5 mL portion was diluted to 10 mL with 50% acetonitrile/water and then filtered with a nylon acrodisc syringe filter. The analysis was done by liquid chromatography using a Sciex API 300 MS/MS detector.

(b) Source of Analytical Reference Standard

Analytical standards of NI-25 and IC-0 were obtained from Aventis Cropscience (formerly Rhône-Poulenc), Research Triangle Park, North Carolina.

Acetamiprid (NI-25); Lot no. TS-00110, 99.8% purity, expiration date; Dec. 2002

Metabolite (IC-0); Lot no. MF-4267, 99.7% purity, expiration date; May 2007

(c) Source of Sample Matrix

The soil used was Iowa State University soil, Batch 5, (see Appendix 2) characterized by A&L Analytical Laboratory, Memphis, TN.

(d) Instrumentation for Quantitation

1. Perkin Elmer Sciex API 300 LC/MS/MS system.
2. The column used was a YMC ODS-AQ 3.0 X 150mm, 5µm particle size, 120A pore size.

(e) Modification of Method

The registrant used a Perkin Elmer Sciex API III LC/MS/MS system for analysis. We used the API 300 system which is a less sensitive instrument.

(f) Analyses Time

The time for preparing and analyzing a set of sixteen samples, including standards and controls is two days for two persons.

(g) Calculations

1. Standard Curve

The instrument software contains preprogrammed data processing capabilities which calculates a standard curve for each analytical set using the responses of the calibration standards; which had acetamiprid (NI-25) and IC-0 concentrations of 1.0 ng/mL, 2.5 ng/mL, 7.5 ng/mL, and 25 ng/mL. The calibration standard sets were analyzed three times during the sample set with all points being included in the calibration curve. The calibration curve is constructed using linear regression with the concentration (ng/mL) on the X-axis and the response on the Y-axis.

The regression equation is: $Y = mX + b$

Where: Y = peak area
X = analyte concentration (ng/mL)

2. Calculation of Analyte in Samples

The instrument software calculated the concentration (ng/mL) of NI-25 and IC-0 in each sample extract from the analyte area counts by using the calibration equation generated by the calibration standards in the analytical set. Each sample was injected and analyzed twice with the average concentration used to calculate the ppb.

To find the sample concentration (S) in ppb of the analyte use the formula:

$$S = \{ C \} \{ V / W \} \{ \text{dilution factor} \}$$

C = average conc. of analyte in the
sample extract (ng/mL)

V = final extract vol. (60 mLs)

W = sample weight (30.0 grams)

Dilution factor = 2

3. Example Calculation

Sample no. AC 28 for acetamiprid (10 ppb)

Concentrations of acetamiprid from two runs are
2.24 ng/mL and 2.10 ng/mL.

Average conc. = 2.17 ng/mL

$$S = \{ 2.17 \text{ ng/mL} \} \{ 60 \text{ mL} / 30.0 \text{ g} \} \{ 2 \}$$

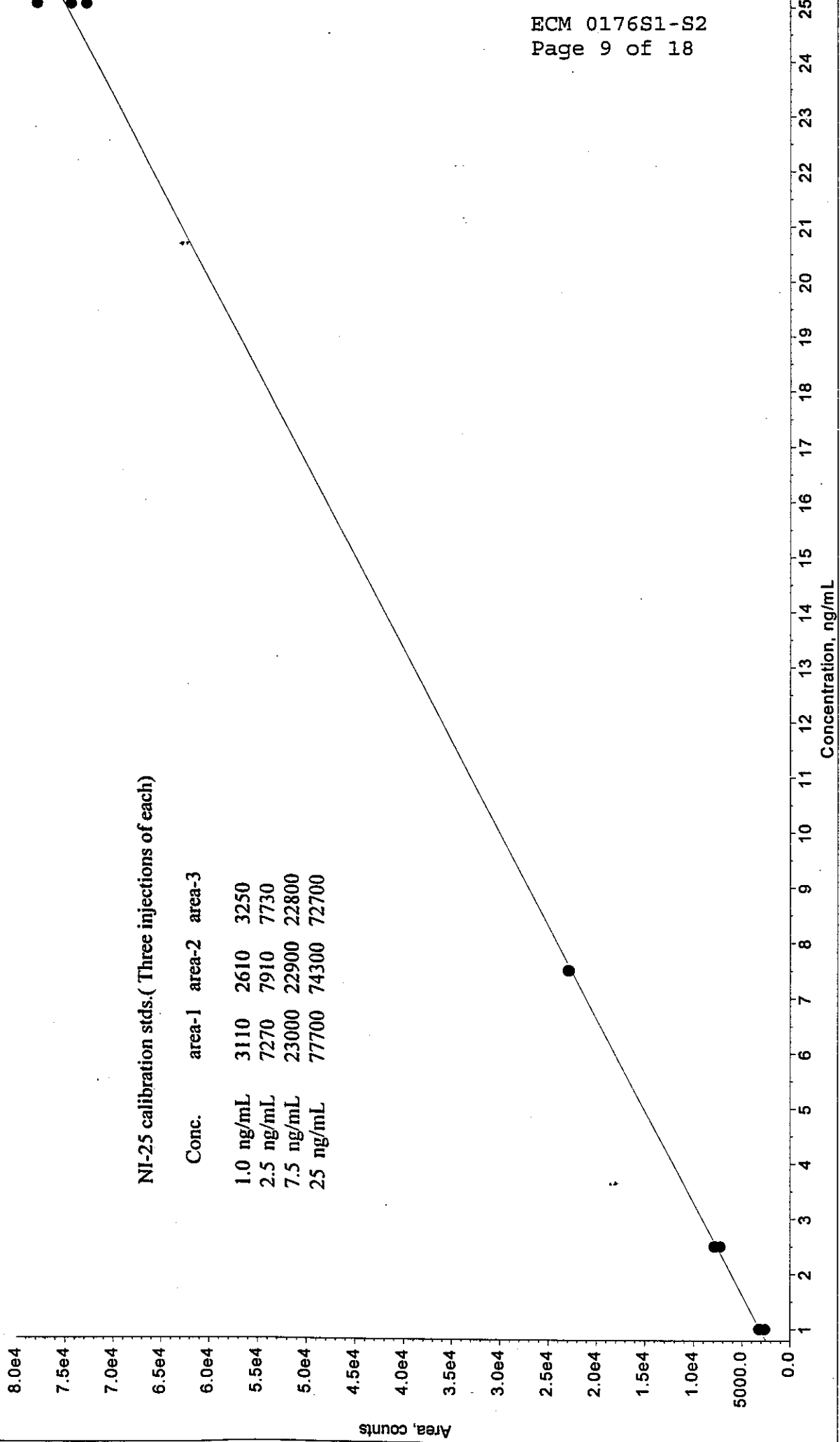
$$S = 8.68 \text{ ng/g}$$

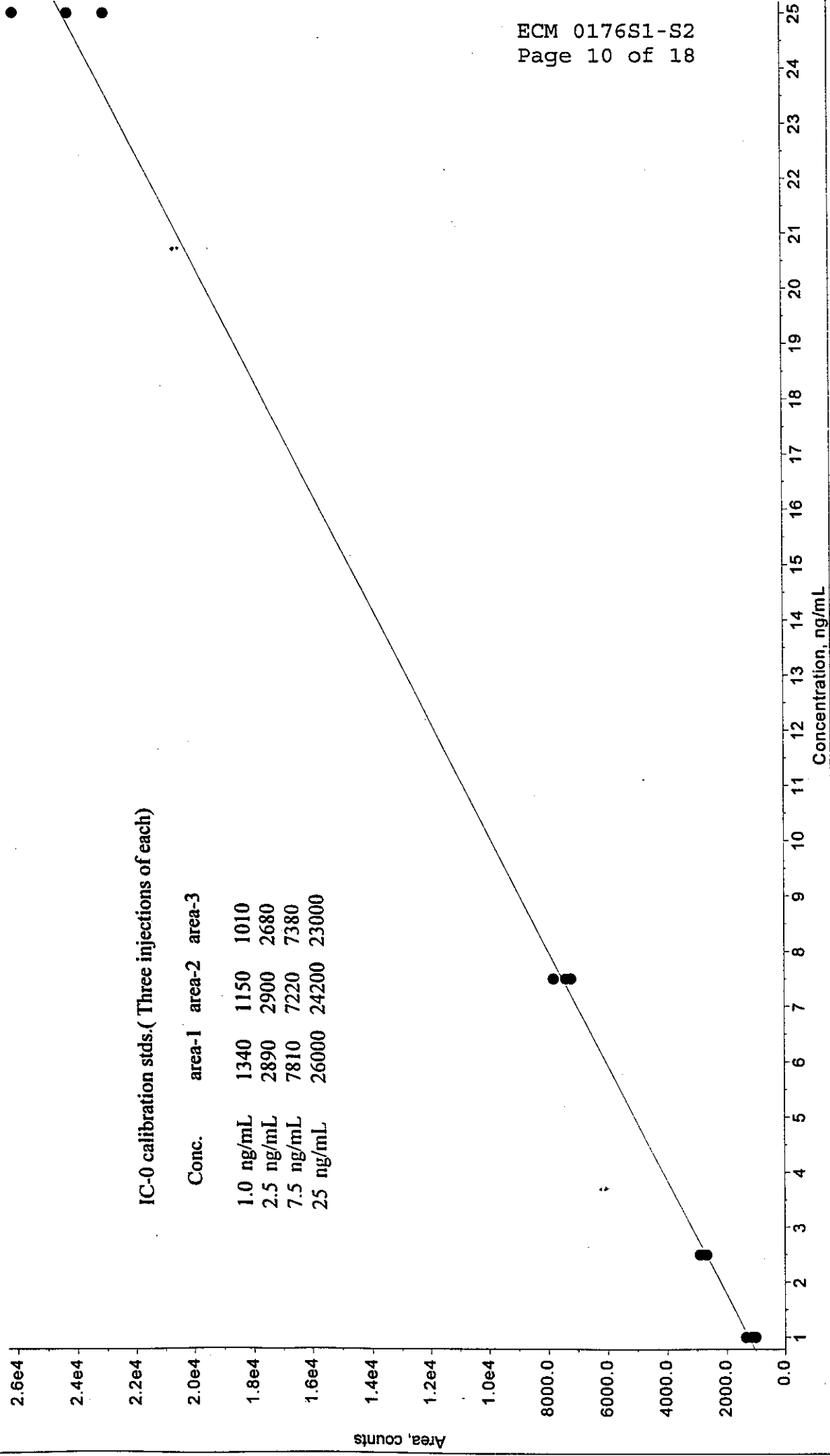
$$S = 8.68 \text{ ppb}$$

(h) Graphs and Data

The following pages contain a print-out of the calibration curve and data generated by the NI-25 and IC-0 standards and representative samples.

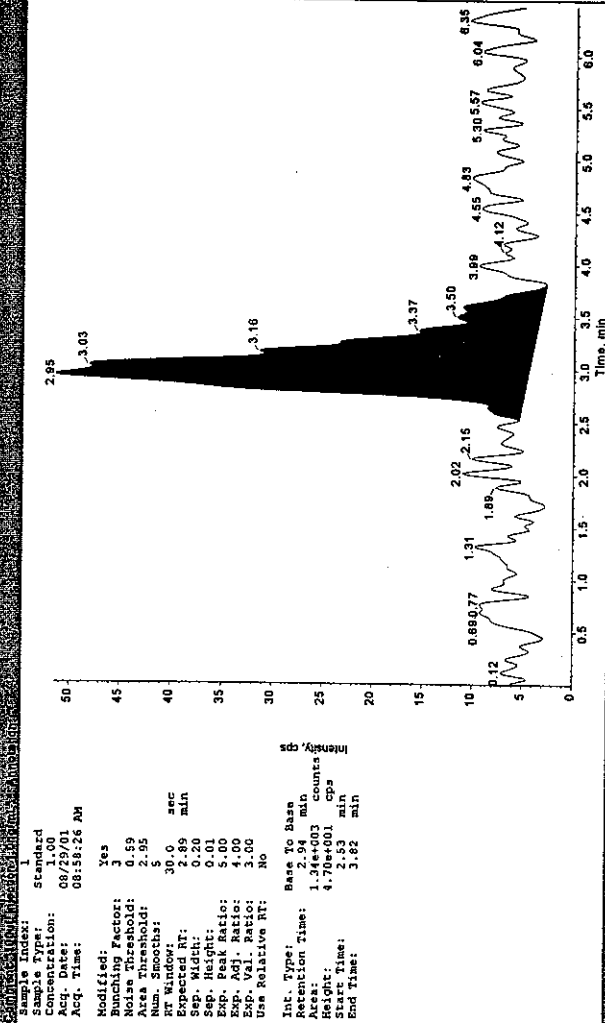
1. Pages 9 thru 12, calibration curves and raw data for calibration standards.
2. Pages 13 thru 16, chromatograms and raw data for selected samples.





IC-0 calibration stds. (Three injections of each)

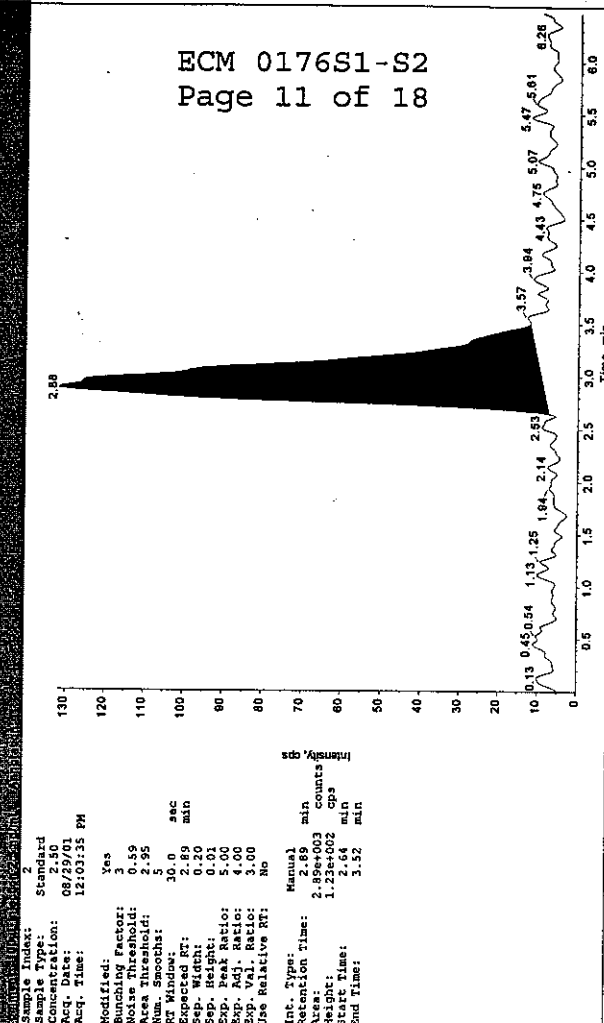
Conc.	area-1	area-2	area-3
1.0 ng/mL	1340	1150	1010
2.5 ng/mL	2890	2900	2680
7.5 ng/mL	7810	7220	7380
25 ng/mL	26000	24200	23000



Sample Index: 1
 Standard
 Concentration: 1.00
 Acq. Date: 08/29/01
 Acq. Time: 08:58:26 AM

Modified: No
 Bunching Factor: 4
 Noise Threshold: 0.30
 Area Threshold: 1.50
 Num. Smooths: 5
 RT Window: 30.0 sec
 Expected RT: 3.15 min
 Sep. Width: 0.20
 Sep. Height: 0.01
 Exp. Peak Ratio: 5.00
 Exp. Adj. Ratio: 4.00
 Exp. Val. Ratio: 3.00
 Use Relative RT: No

Int. Type: Base To Base
 Retention Time: 2.94 min
 Area: 1.34e+003 counts
 Height: 4.70e+001 cps
 Start Time: 2.83 min
 End Time: 3.82 min



Sample Index: 2
 Standard
 Concentration: 2.50
 Acq. Date: 08/29/01
 Acq. Time: 12:03:35 PM

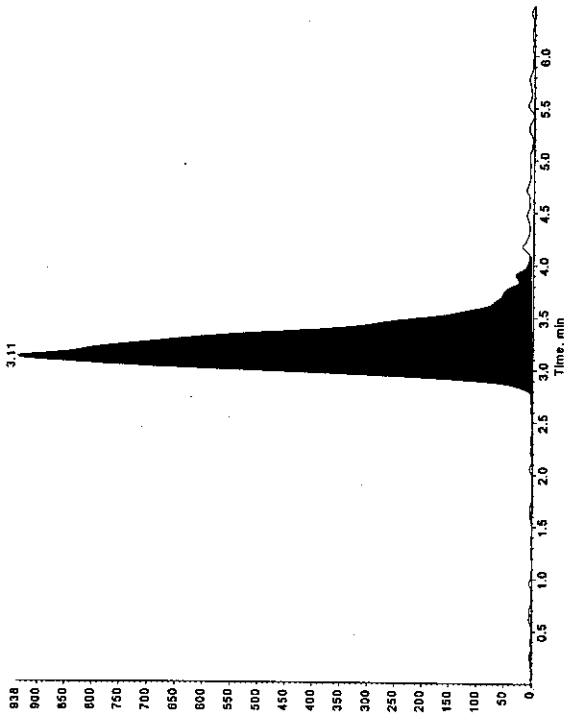
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 Area Threshold: 1.50
 Num. Smooths: 5
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 Sep. Width: 0.20
 Sep. Height: 0.01
 Exp. Peak Ratio: 5.00
 Exp. Adj. Ratio: 4.00
 Exp. Val. Ratio: 3.00
 Use Relative RT: No

Int. Type: Manual
 Retention Time: 2.89e+003 min
 Area: 1.23e+002 counts
 Height: 2.64 cps
 Start Time: 2.64 min
 End Time: 3.52 min

Sample Index: 3
Standard
Sample Type: Standard
Concentration: 7.50
Acq. Date: 08/29/01
Acq. Time: 12:13:00 PM

Modified: No
Bunching Factor: 4
Noise Threshold: 0.30
Area Threshold: 1.50
Num. Smooths: 5
RT Window: 30.0 sec
Expected RT: 3.15 min
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Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 3.00
Use Relative RT: No

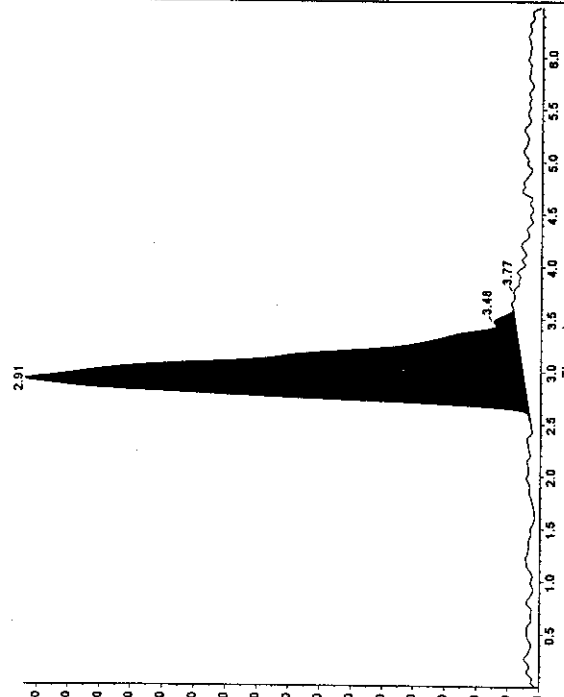
Int. Type: Valley
Retention Time: 3.11 min
Area: 7.01e+004 counts
Height: 9.39e+002 cps
Start Time: 2.44 min
End Time: 4.09 min



Sample Index: 3
Standard
Sample Type: Standard
Concentration: 7.50
Acq. Date: 08/29/01
Acq. Time: 12:13:00 PM

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Bunching Factor: 4
Noise Threshold: 0.59
Area Threshold: 2.95
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Exp. Val. Ratio: 3.00
Use Relative RT: No

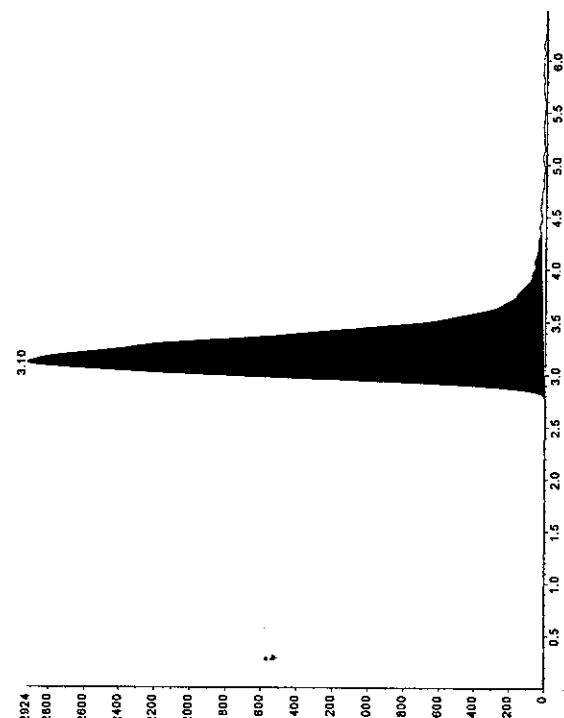
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Retention Time: 2.91 min
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Height: 3.19e+002 cps
Start Time: 2.44 min
End Time: 3.60 min



Sample Index: 4
Standard
Sample Type: Standard
Concentration: 25.0
Acq. Date: 08/29/01
Acq. Time: 12:22:26 PM

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Bunching Factor: 3
Noise Threshold: 0.59
Area Threshold: 2.95
Num. Smooths: 5
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Use Relative RT: No

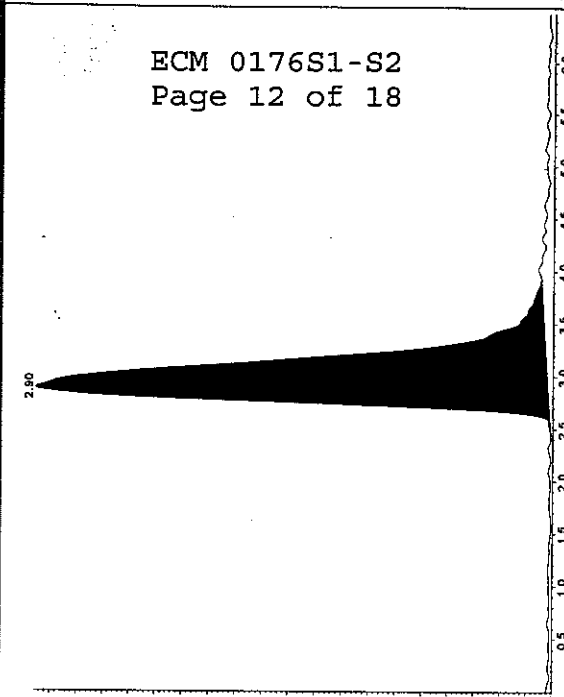
Int. Type: Base To Base
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Start Time: 2.44 min
End Time: 3.34 min

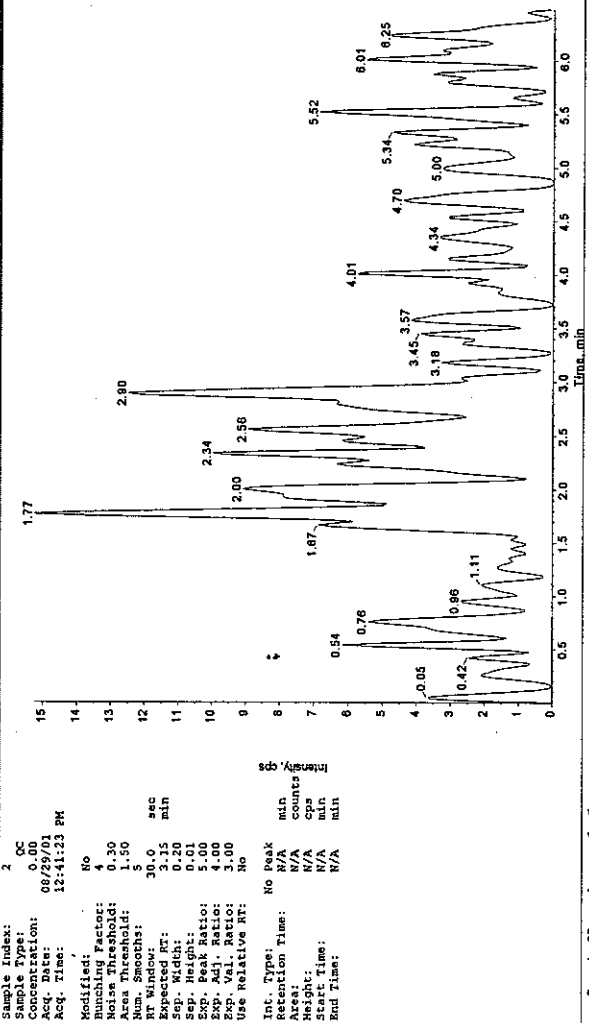
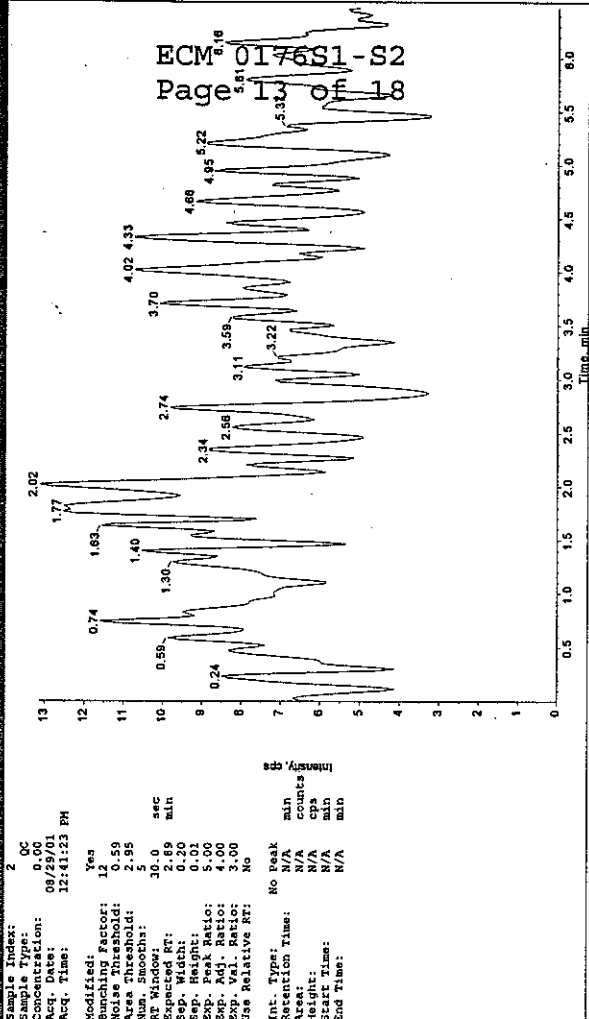
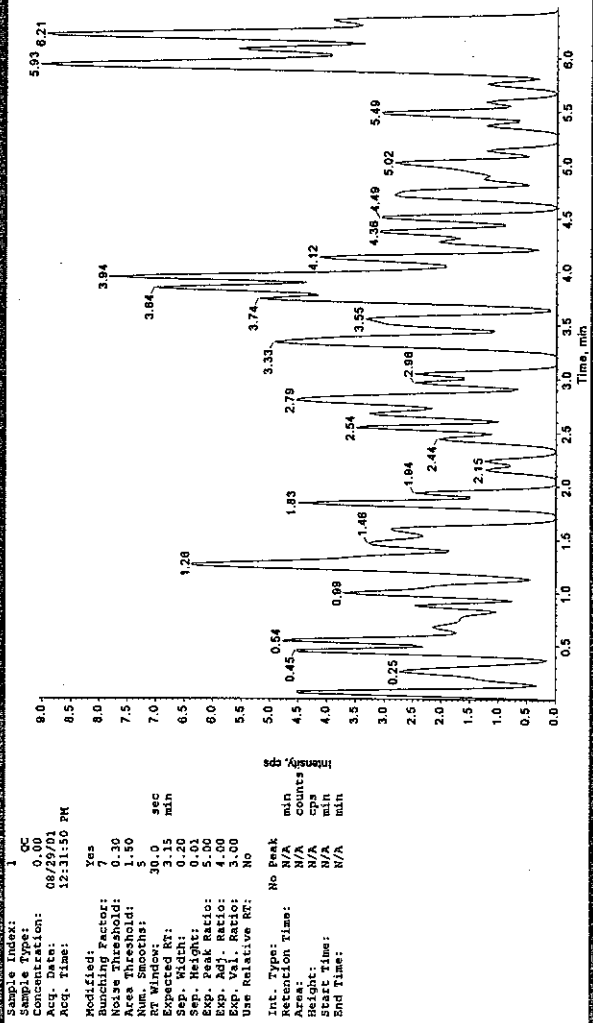
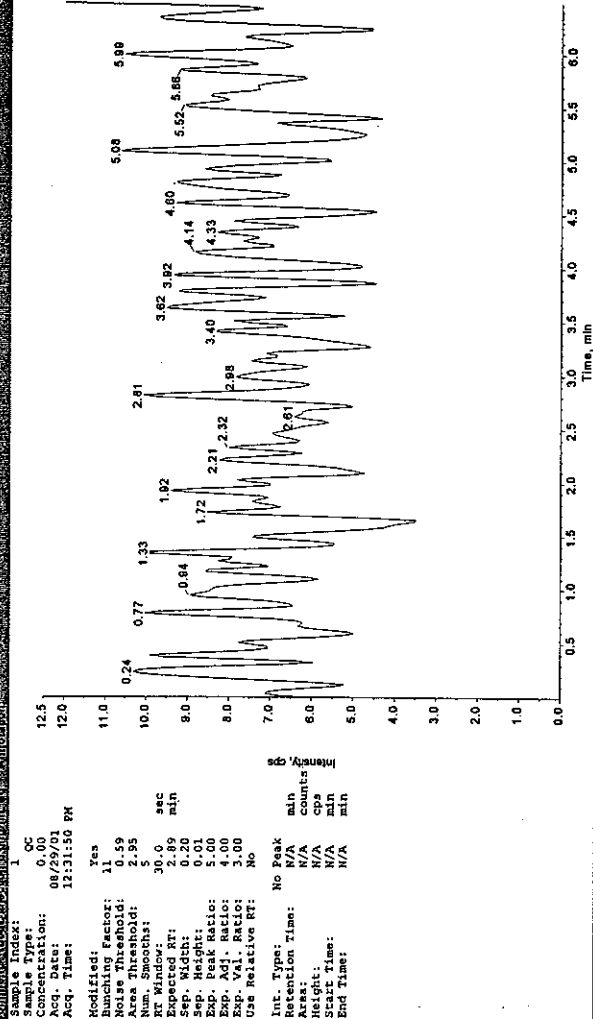


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Standard
Sample Type: Standard
Concentration: 25.0
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Acq. Time: 12:22:26 PM

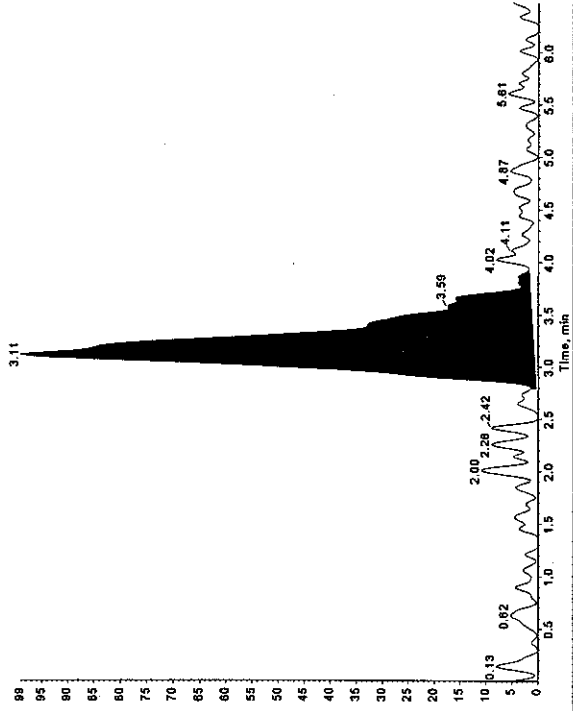
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Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 3.00
Use Relative RT: No

Int. Type: Base To Base
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Area: 2.66e+004 counts
Height: 5.70e+002 cps
Start Time: 2.44 min
End Time: 3.34 min

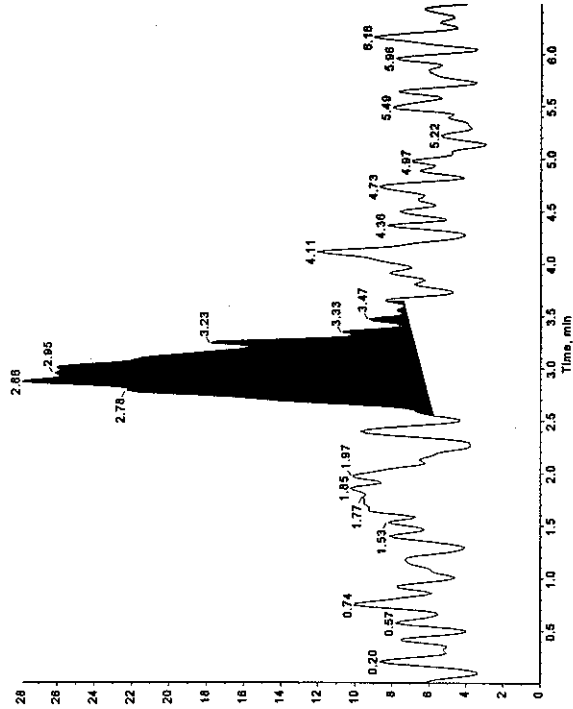




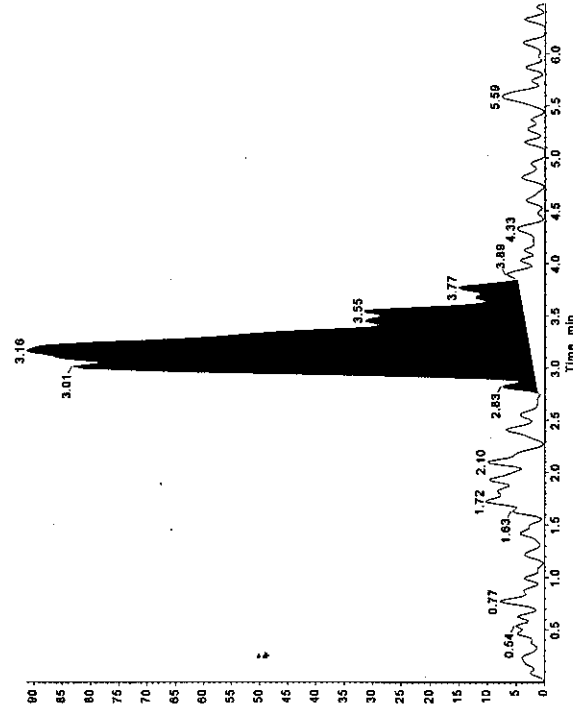
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Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 3.00
Use Relative RT: No
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Height: 9.69e+001 cps
Start Time: 2.79 min
End Time: 3.34 min



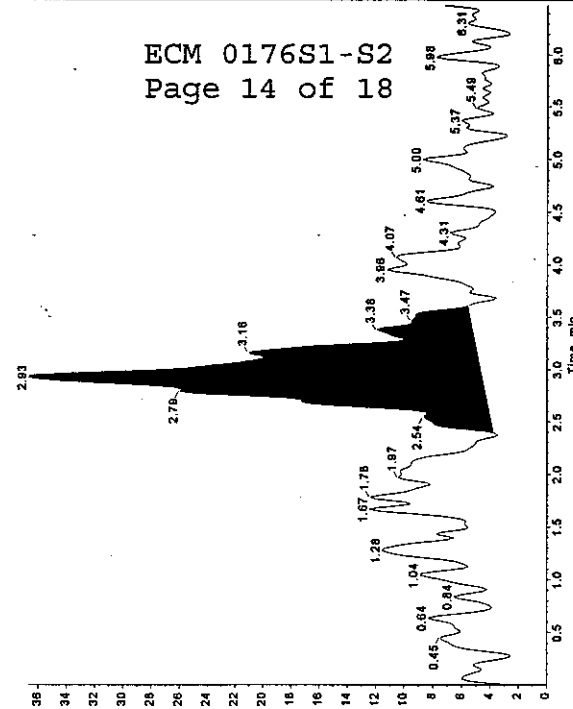
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Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 3.00
Use Relative RT: No
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Start Time: 2.54 min
End Time: 3.64 min

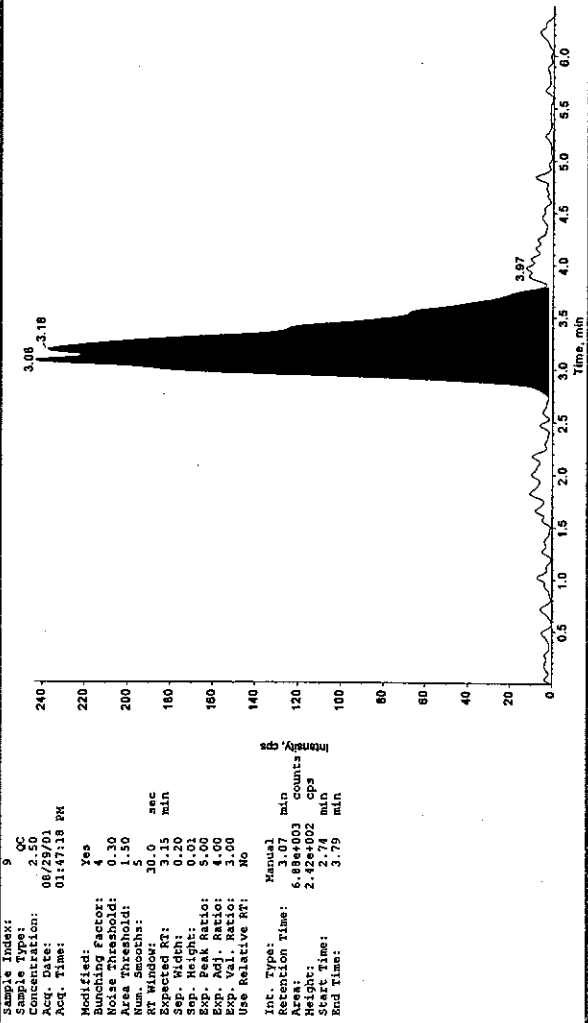
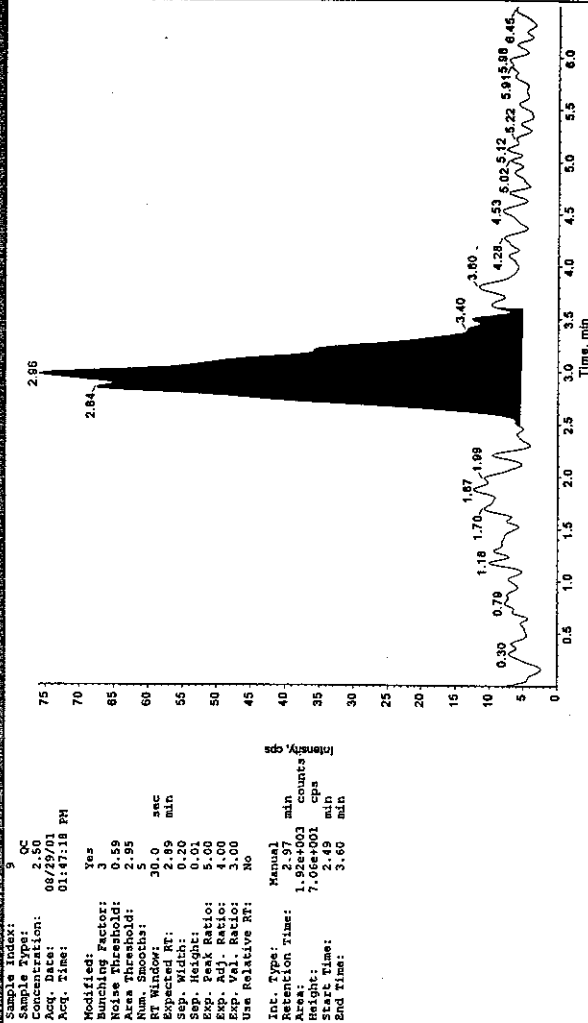


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Concentration: 08/29/01
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Acq. Time: 01:37:52 PM
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Area Threshold: 1.50
Num. Smoother: 5
RT Window: 30.0 sec
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Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 3.00
Use Relative RT: No
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Height: 8.87e+001 cps
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End Time: 3.54 min

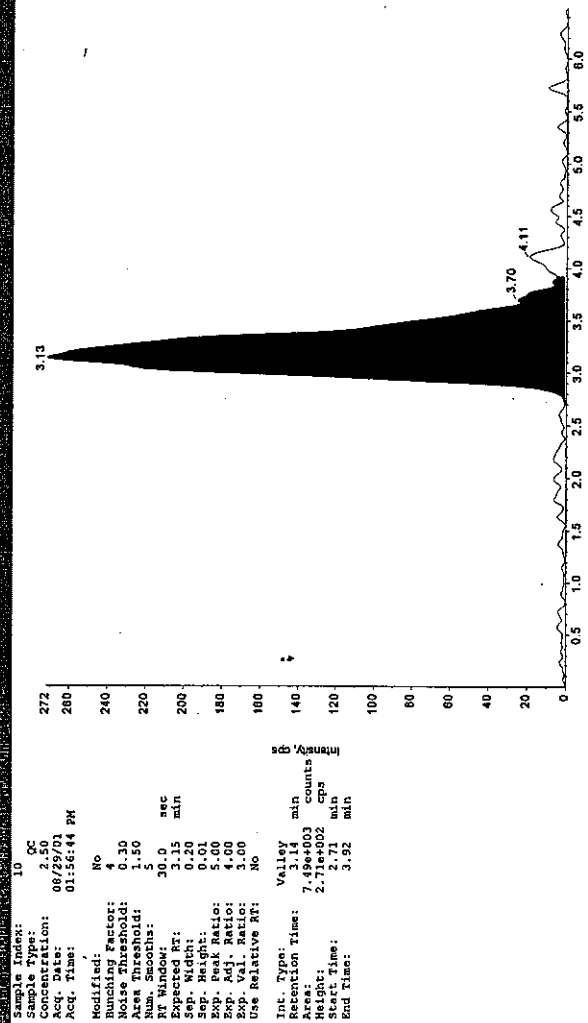
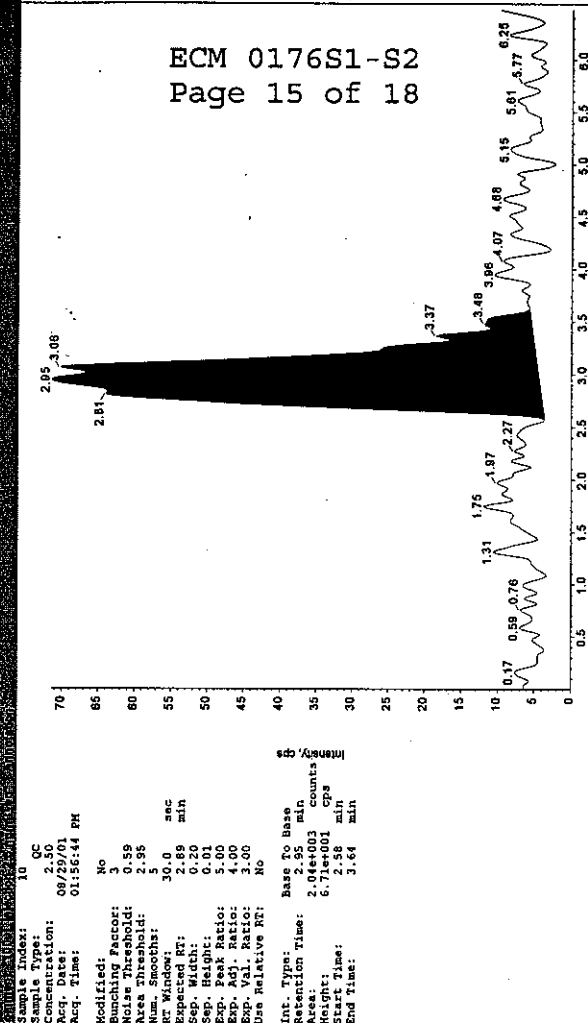


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Concentration: 08/29/01
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Noise Threshold: 0.59
Area Threshold: 2.95
Num. Smoother: 5
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Sep. Width: 0.20
Sep. Height: 0.01
Exp. Peak Ratio: 5.00
Exp. Adj. Ratio: 4.00
Exp. Val. Ratio: 3.00
Use Relative RT: No
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Height: 3.22e+001 cps
Start Time: 2.39 min
End Time: 3.64 min

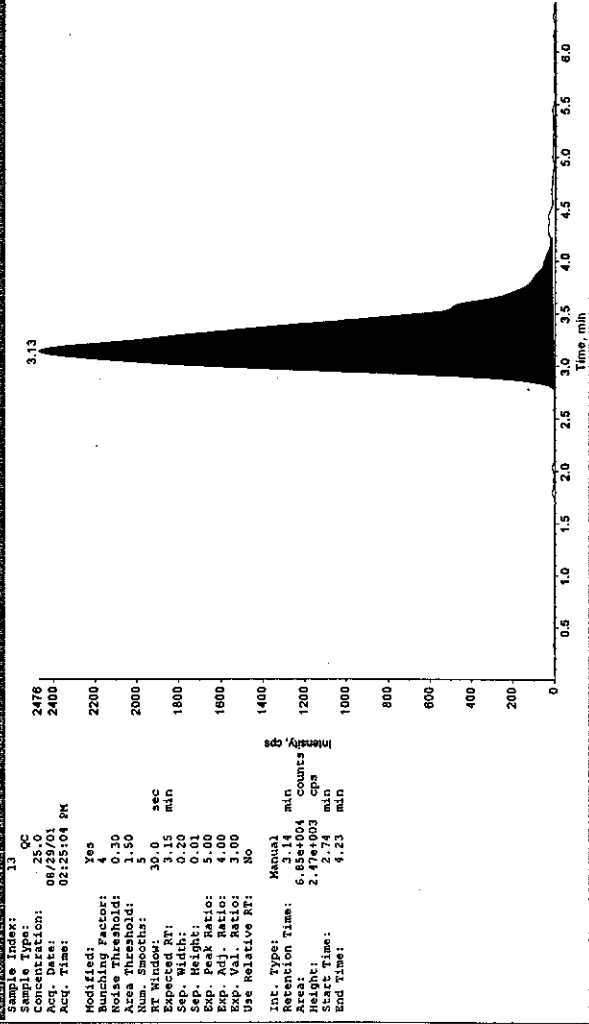
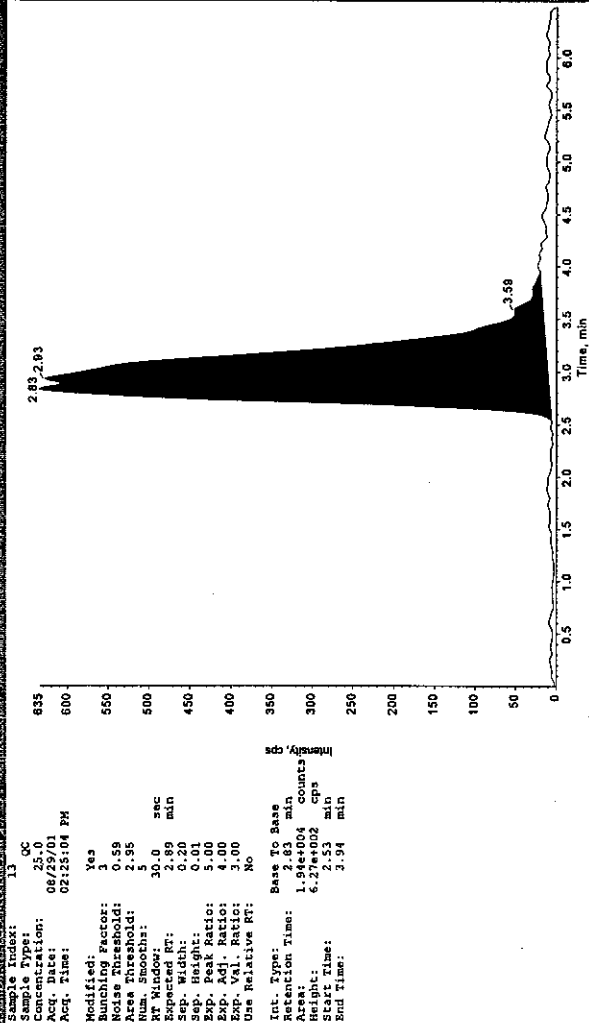




Sample Index: 9
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 Concentration: 2.50
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 Modified: Yes
 Bunching Factor: 4
 Noise Threshold: 0.30
 Area Threshold: 1.50
 Num. Smooths: 5
 RT Window: 30.0 sec
 Exp. Peak Ratio: 5.00
 Sep. Height: 0.01
 Exp. Adj. Ratio: 4.00
 Exp. Val. Ratio: 3.00
 Use Relative RT: No
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 Height: 7.06e+001 cps
 Start Time: 2.49 min
 End Time: 3.60 min



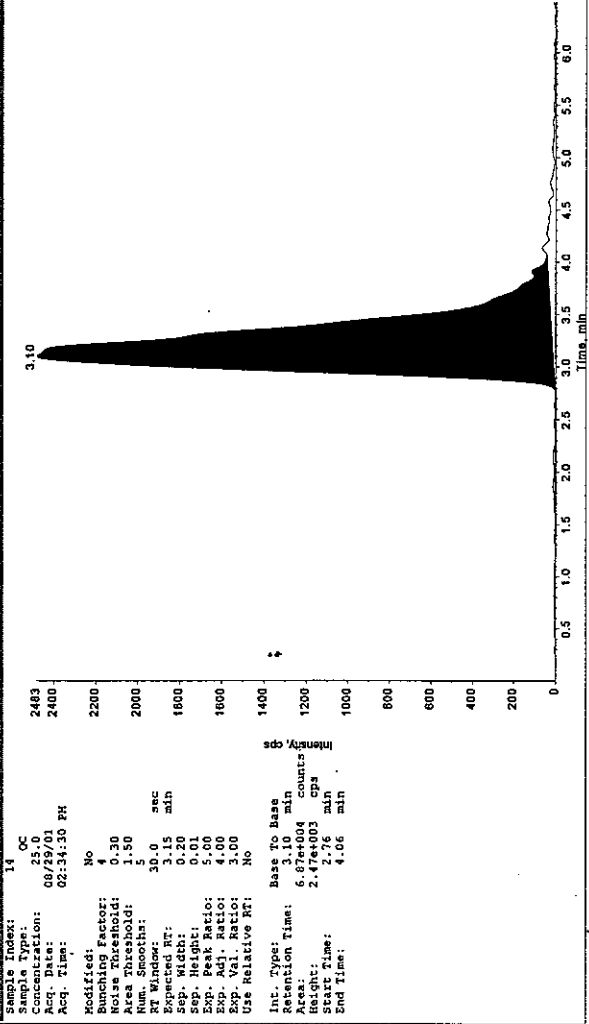
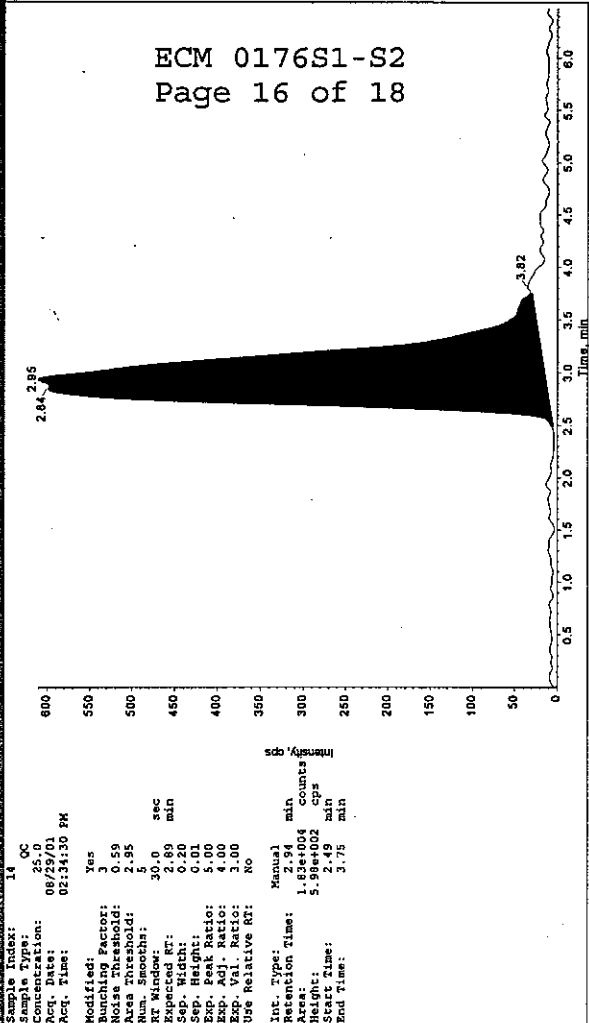
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 Area Threshold: 2.95
 Num. Smooths: 30.0 sec
 RT Window: 30.0 sec
 Exp. Peak Ratio: 5.00
 Sep. Height: 0.01
 Exp. Adj. Ratio: 4.00
 Exp. Val. Ratio: 3.00
 Use Relative RT: No
 Int. Type: Base to Base
 Retention Time: 2.95 min
 Area: 6.71e+001 counts
 Height: 6.71e+001 cps
 Start Time: 2.58 min
 End Time: 3.64 min



Sample Index: 13
 Sample Type: QC
 Concentration: 25.0
 Acq. Date: 08/29/01
 Acq. Time: 08:25:04 PM

Modified: Yes
 Bunching Factor: 3
 Noise Threshold: 0.59
 Area Threshold: 1.35
 Num. Smoother: 5
 RT Window: 30.0 sec
 Expected RT: 2.89 min
 Sep. Width: 0.20 min
 Sep. Height: 0.01
 Exp. Peak Ratio: 4.00
 Exp. Adj. Ratio: 4.00
 Exp. Val. Ratio: 3.00
 Use Relative RT: No

Int. Type: Manual
 Base To Base: 2.89 min
 Retention Time: 1.94e+004 counts
 Area: 6.27e+002 cps
 Height: 2.53 min
 Start Time: 3.94 min
 End Time:



Sample Index: 14
 Sample Type: QC
 Concentration: 25.0
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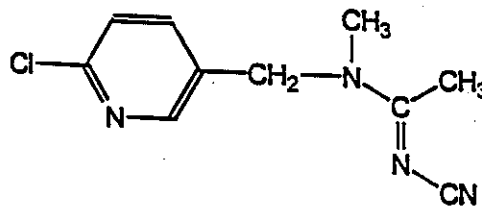
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 Area Threshold: 2.95
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 Sep. Height: 0.01
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 Exp. Adj. Ratio: 4.00
 Exp. Val. Ratio: 3.00
 Use Relative RT: No

Int. Type: Manual
 Base To Base: 2.94 min
 Retention Time: 1.82e+004 counts
 Area: 5.99e+002 cps
 Height: 3.75 min
 Start Time:
 End Time:

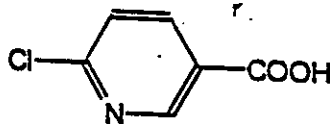
APPENDIX I

The structures of the compounds are as follows:

Name or Code: NI-25 (Acetamiprid)
Chemical Name: (E)-N¹-(6-chloro-3-pyridyl)methyl-N²-cyano-N¹-methylacetamide
CAS No.: 135410-20-7
Molecular Weight : 222.68



Name or Code: IC-0
Chemical Name: 6-chloronicotinic acid
CAS No.: 5326-23-8
Molecular Weight : 157.56



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085 P01 OCT 08 '98 17:12

IOWA
BATCH #5

A&L Analytical Laboratories, Inc.

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SOIL ANALYSIS

CLIENT U.S. EPA/ECS BLDG. 1105 STENNIS SPACE CNTR, MS 39529	GROWER	REPORT # 8-261-0520 DATE 09/22/98 ACCOUNT 15028 PAGE 1 A&L AGRONOMIST Richard Large
LAB NUMBER 22544	SAMPLE ID IOWA BATCH 5	DATE RECEIVED: 09/18/98
		SIGNATURE <i>Richard Large</i>

TEST	RESULTS	SOIL TEST RATINGS					CATION EXCHANGE CAPACITY
		Very Low	Low	Medium	Optimum	Very High	
Soil pH	5.0						28.3 meq/100g
Buffer pH	6.52						
Phosphorus (P)	14 ppm						CATION SATURATION %K 1.0 %Ca 4.4 %Mg 19.5 %H 15.0
Potassium (K)	123 ppm						
Calcium (Ca)	4614 ppm						SOIL TEST METHOD MEHLICH EXTRACTION
Magnesium (Mg)	718 ppm						
Sulphur (S)	9 ppm						
Boron (B)							
Copper (Cu)							
Iron (Fe)							
Manganese (Mn)							
Zinc (Zn)	2.1 ppm						
Sodium (Na)							
Soluble Salts							
Organic Matter	2.2 % ENR 88						
NO ₃ -N							

> ADD'L RESULTS TO FOLLOW

SOIL FERTILITY GUIDELINES

CROP:

YIELD GOAL:

LIME	N	P ₂ O ₅	K ₂ O	Mg	S	B	Cu	Mn	Zn

REPORT OF ANALYSIS

REPORT DATE: 09/22/98
DATE RECEIVED: 09/18/98

LAB NO	SAMPLE IDENTIFICATION	PERCENT SAND	PERCENT SILT	PERCENT CLAY	TEXTURAL CLASSIFICATION
22544	IOWA BATCH 5	47	32	21	LOAM