



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Analytical Chemistry Section
Building 306, BARC-East
Beltsville, Maryland 20705

2/29/92

MEMORANDUM

SUBJECT: PP#9F3787. Abamectin in/on Pears. (MRID#'s 412064-01, 411885-01 to -16, DEB #5700). Petition Method Validation Report.

FROM: Mark W. Law, Chemist *MWL*
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Analytical Chemistry Section

THRU: Harvey K. Hundley, Head *Harvey K. Hundley*
Analytical Chemistry Section

THRU: Donald A. Marlow, Chief *DM*
Analytical Chemistry Branch

TO: Phillip V. Errico, Head
Chemistry Branch I - Tolerance Support
Health Effects Division (H7509C)

INTRODUCTION

The Analytical Chemistry Branch was requested to run a method validation trial of Merck & Co. Method No. 8000 ("HPLC-Fluorescence Determination for Avermectin B₁ and its Delta 8,9 Isomer in Pears", dated November 18, 1987) using control pears and pears fortified with abamectin and its delta 8,9 isomer at the 0.035 and 0.070 ppm levels. A second Merck & Co. document, "Suggestions for the Analyst Performing Merck Residue Method No. 8000", dated November 18, 1987, accompanied the method.

METHOD SUMMARY

Pear samples (10 g) are hydrolyzed with pectinase, homogenized with 50:50 acetonitrile/water, and filtered to extract the residues. After the filtrate is diluted with water and passed through a C8 solid phase extraction (SPE) column, the residues are eluted from the column with CH₃CN, diluted with water, and extracted into hexane. The residues are further cleaned up on an aminopropyl SPE column, eluted with 1+1 acetone-CH₂Cl₂, and diluted to 10 ml with CH₂Cl₂.



The sample solution is then split, and half is reacted with a mixture of N,N-dimethylformamide, trifluoroacetic anhydride, and 1-methylimidazole, followed by reaction with methanolic ammonium hydroxide to form fluorescent derivatives of the residues. The derivatives are dissolved in chloroform and cleaned up on a silica gel SPE column, evaporated to dryness, dissolved in 5 ml of methanol, and analyzed by reverse phase HPLC using fluorescence detection.

COMMENTS

1. The term "delta 8,9 isomer of abamectin" is anomalous and a source of confusion in the discussions on the characterization of residues and their derivatives in the Merck documents. Abamectin (avermectin B₁) is a mixture of the homologs avermectin B_{1a} and avermectin B_{1b} (here abbreviated as B_{1a} and B_{1b}, respectively), both of which exist in the 8,9-E configuration and may undergo trans to cis olefin isomerization at carbons 8 and 9 to produce the corresponding 8,9-Z-avermectin B_{1a} (abbreviated 8,9-Z-B_{1a}) and 8,9-Z-avermectin B_{1b} (abbreviated 8,9-Z-B_{1b}), respectively. This standard nomenclature convention for the isomers is in keeping with the usage in current Merck and Co. publications and should be employed throughout the method.

2. We were unable to obtain purified analytical standards of abamectin, B_{1a}, B_{1b}, or 8,9-Z-B_{1a} from the EPA Pesticides and Industrial Chemicals Repository (PICR) or from the petitioner. Instead, both PICR and Merck & Co. provided us with samples of dilute standard solutions of abamectin (0.956% B_{1a} and 0.071% B_{1b} in glycerol formal) and 8,9-Z-B_{1a} (0.38% in glycerol formal). The samples received from PICR were not used in this trial because of lack of sufficient amounts of reference material for use as described in the method. Additionally, the samples had not been shipped under conditions consistent with the method's recommendations for maintaining their integrity and they were not properly labeled with the concentrations of the analytes. After these problems were discussed with PICR and the missing assay information was obtained, standards were requested directly from the petitioner as in previous abamectin method trials when similar problems were encountered with the standards from PICR. The standards sent by Merck & Co. were very elaborately packaged to assure that they would be cooled by dry ice throughout shipment. It may be impractical for PICR to use similar means to distribute abamectin standards to regulatory laboratories routinely, but if refrigerated shipment is necessary to assure their stability, the petitioner should instruct PICR on shipping requirements as well as on the amounts of the dilute standard solutions required in the method.

According to page 13 of "Suggestions for the Analyst...", that was provided by the registrant, solid Bla, Blb, or 8,9-Z-Bla are not available in easily handled forms, therefore, the analytical standards are provided as dilute solutions in glycerol formal. Merck personnel have claimed that high purity abamectin is hygroscopic and less stable than the dilute abamectin standard and that pure 8,9-Z-Bla is unavailable or very scarce. The dilute standard solutions were used in previous method trials for abamectin and the methods were approved as such. However, Merck's failure to provide purified analytical standards of abamectin and 8,9-Z-Bla is inconsistent with requirements provided in Sec. 171-13 of EPA's Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry and could potentially compromise analytical results obtained for abamectin residues in regulatory situations. EPA should be provided with adequate amounts of suitable reference standard materials, instructions for handling them, and the analytical methods required to assay the pure materials and the dilute standards in glycerol formal before any permanent tolerances are established for abamectin.

3. In the method, the Bla calibration curve is used to quantitate both Bla and Blb. This is not analytically correct and should not be accepted unless the two analytes are demonstrated to produce equivalent HPLC responses in the method. The petitioner claims (on p. 13 of "Suggestions for the Analyst...") that it is reasonable to use the Bla standard curve to quantitate Blb residues since the structure of the fluorescent derivatives is the same. This may not be true since the structures differ enough to give significantly different HPLC retention times. The method does not include relative response data for Bla and Blb, but it can be reasonably inferred from the recovery data provided in the method that the use of the Bla standard curve to quantitate Blb residues introduces a systematic method bias of at least 10% towards high results for Blb.

The petitioner also uses the Bla standard curve to quantitate 8,9-Z-Bla and attempts to justify this with two statements in the suggestions document: a statement on page 3 indicates the derivatives of the two compounds have the same retention time, and a statement on page 4 indicates the two compounds are converted to a common derivative.

The method also uses a very narrow range of concentrations in the curve used for calculations. The result is that samples must contain 5 to 10 mg/ml analyte in the final solution in order to match the curve. This will result in the need for repetitive analyses to obtain valid results. The curve should be run over a larger range of concentrations to be useful for analytical quantitation.

We did not use calibration curves to calculate our reported data. Instead, we bracketed sample injections between injections of standard solutions and calculated directly.

4. Merck's Method No. 8000 and "Suggestions for the Analyst.." should be consolidated into a single document containing all the information needed to apply the method in a regulatory laboratory. The revised method should eliminate discrepancies between the existing documents and provide any essential information from "protocol AB-P1" and "memo from Morgan to Tway (3/16/87)", which appear to be internal Merck documents and are cited in "Suggestions...."

5. We did not attempt to determine the limit of detection for the method, but from our linearity studies using low ppb level standards and from the appearance of our control sample chromatograms, the limit of detection appears to be less than 1 ppb.

6. The method required a minimum of two full days to prepare a set of known fortified samples for overnight analysis on a HPLC with an autosampler. Any unknown samples not fitting the narrow calibration curve would have to be repeated. The procedure marginally meets the guidelines for analysis within 24 hours provided a repeat analysis is not required.

7. If the method is rewritten in accord with the above comments, it would meet 40 CFR 158 and EPA's requirements as published in the Pesticide Assessment Guidelines, Subdivision O, for Residue Chemistry, Part 171-4 (b) as an enforcement method. //

8. No special safety hazards were noticed. Normal laboratory safety procedures were adhered to.

Attachments:

Table of Recovery Data
Representative Chromatograms

Method: "HPLC-Fluorescence Determination for Avermectin B₁ and its Delta 8,9-Isomer in Pears." Method No. 8000, Merck & Co., Inc., dated November 18, 1987, 24 pages.

Determination of abamectin* and 8,9-z-Bla** in fortified and unfortified pear homogenate samples.

Sample Number	Name of Chemical	Concentration of Chemical (PPB)		Percent Recovery
		Added	Found	
1.	Abamectin	None	N.D.	
	Bla	None	N.D.	
	Blb	None	N.D.	
	8,9, <u>z</u> -Bla	None	N.D.	
2.	Abamectin	None	N.D.	
	Bla	None	N.D.	
	Blb	None	N.D.	
	8,9, <u>z</u> -Bla	None	N.D.	
3.	Abamectin	35.0	32.5	92.0
	Bla	32.6	30.7	94.2
	Blb	2.4	1.79	74.6
4.	Abamectin	35.0	35.8	102.3
	Bla	32.6	33.4	102.5
	Blb	2.4	2.37	98.8
5.	Abamectin	70.0	70.4	100.6
	Bla	65.2	65.8	100.9
	Blb	4.8	4.61	96.0
6.	Abamectin	70.0	71.9	102.7
	Bla	65.2	67.6	103.7
	Blb	4.8	4.25	88.5
7.	8,9- <u>z</u> -Bla	35.0	32.9	94.0
8.	8,9- <u>z</u> -Bla	35.0	32.2	92.0
9.	8,9- <u>z</u> -Bla	70.0	65.9	94.1
10.	8,9- <u>z</u> -Bla	70.0	65.8	94.0

N.D. = Less than 2.0 ppb for abamectin and 1.0 ppb for the individual compounds.

* Abamectin is a mixture of two avermectin B₁ homologs, avermectin B_{1a} and B_{1b}, which are identified as Bla and Blb, respectively, in this table.

** 8,9-z-Bla is the abbreviation for the 8,9-z isomer of avermectin B_{1a}.

Method: "HPLC-Fluorescence Determination for Avermectin B₁
and its Delta 8,9-Isomer in Pears." Method No. 8000,
Merck & Co., Inc., dated November 18, 1987, 24 pages.

Modifications to method (major or minor):

None

Special precautions to be taken:

None

Source of analytical reference standards:

Merck & Company
(Standards provided by PICR were not useable)

If derived standard used, give source:

None

Instrumentation for quantitation:

Waters HPLC System with Maxima Operating System
Schoeffel/McPherson FL-750 Bx Fluorescence Detector

Instrumentation for confirmation:

None

If instrument parameters differ from method given, list
parameters used:

Flow rate reduced to 1.0 ml/min. to give retention time
of 12 minutes for peak of interest. All other parameters
are as per method.

Commercial source for any special chemicals or apparatus:

None

Comments:

See report

Chromatograms:

Sample chromatograms attached.

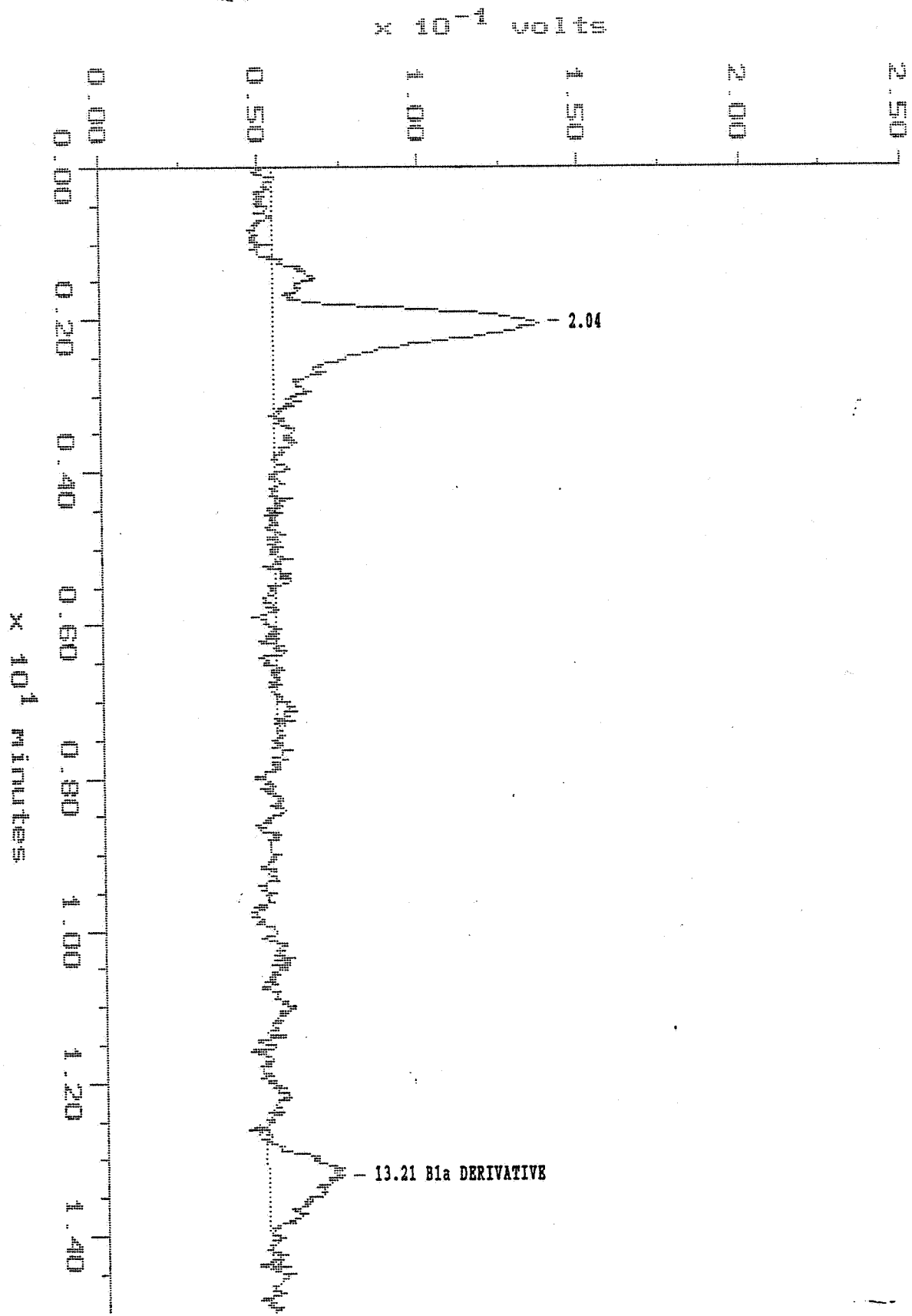
Representative
chromatograms
and standard
curves from
method trial
for abamectin
in pears.

new
diff
BJP

Sample: 892 522 2ng/ml
Acquired: 22-MAY-91 21
Inj Vol: 50.00
Comments: Abamectin PMV (B90-38)

Channel: FL-750 BX
Method: C:\MAX\DATA1\B90-3824
Injection vol: 50ul

Filename: ABA202
Operator: BJP
BART PUMA AND MARK LAW



Lowest level standard (2 ng/ml)
for 8,9-E-B1a standard curve

MAXIMA 820 PEAK INTEGRATION REPORT

Printed: 21-FEB-1992 9:03:09

PLE: 89Z 522 2ng/ml

#21 in Method: ABAECTIN

Acquired: 22-MAY-1991 21:43

Rate: 2.0 points/sec

Duration: 15.000 minutes

Operator: BJP

Type: STND

Instrument: Waters Maxima

Filename: ABA202

Index: 15

Injection Volume: 50.0

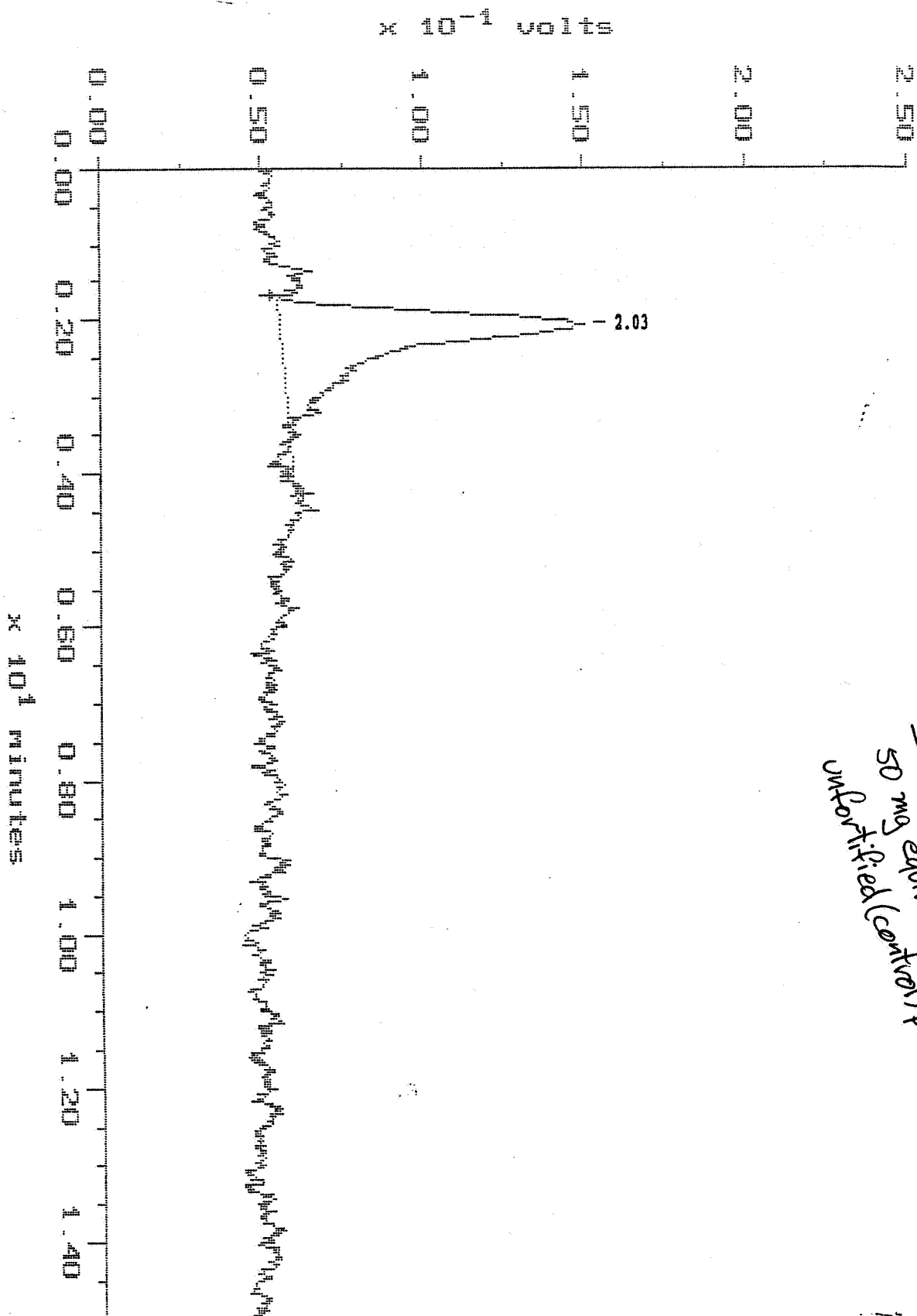
DETECTOR: FL-750 BX

ID#	Peak Start (minutes)	Peak End (minutes)	Retention (minutes)	Type	Peak Area (microvolt-sec)	Peak Height (microvolt)	Area Percent	Hght Percent	Component Name
	0.000	7.250	2.042	DB	2852520.9	82873.830	76.13	77.84	
1	12.600	14.425	13.208	BB	894366.04	23593.518	23.87	22.16	B1a DERIVATIVE
					3746886.9	106467.35			

Sample: Control-1, 50mg Channel: FL-750 BX
Acquired: 22-MAY-91 2: Method: C:\MAX\DATA1\B90-3824
Inj Vol: 50.00
Comments: Abamectin PMV (B90-38) Injection vol: 50ul BART PUMA AND MARK LAW

Filename: ABA208
Operator: BJP

3



Sample No. 1
50 mg equivalent injection of
unfortified (control) pear homogenate.

MAXIMA 820 PEAK INTEGRATION REPORT

Printed: 21-FEB-1992 9:06:58

NPLB: Control-1, 50mg

#26 in Method: ABAMECTIN

Acquired: 22-MAY-1991 23:04

Rate: 2.0 points/sec

Duration: 15.000 minutes

Operator: BJP

Type: UNKN

Instrument: Waters Maxima

Filename: ABA208

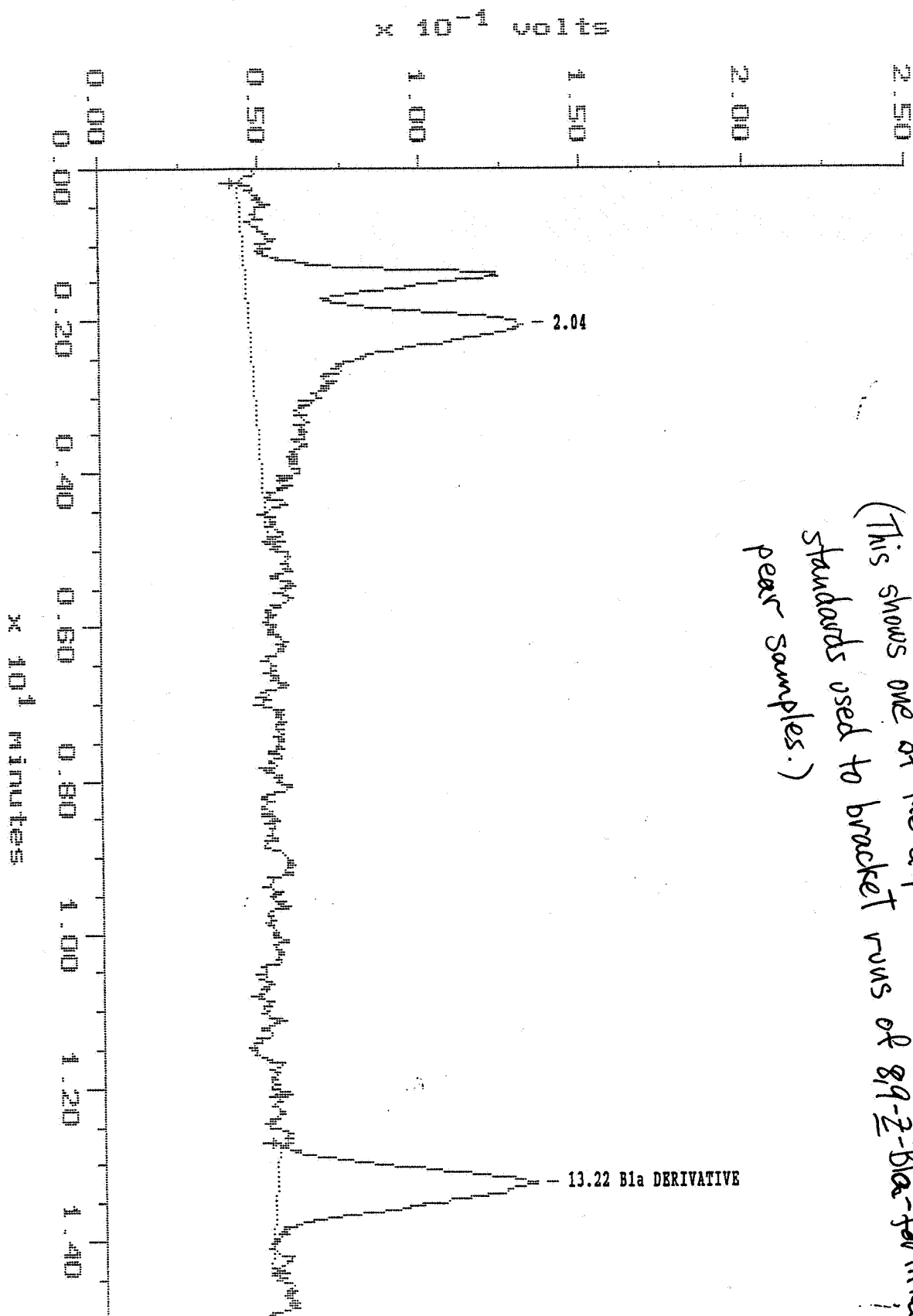
Index: 7

Injection Volume: 50.0

DETECTOR: FL-750 BX

ID#	Peak Start (minutes)	Peak End (minutes)	Retention (minutes)	Type	Peak Area (microvolt-sec)	Peak Height (microvolt)	Area Percent	Hght Percent	Component Name
1	1.683	4.242	2.033	BB	3102237.0	94437.907	100.00	100.00	
AL					3102237.0	94437.907			

Sample: 89Z 522a 7ng/ml Channel: FL-750 BX Filename: ABA210
Acquired: 22-MAY-91 23:3 Method: C:\MAX\DATA1\B90-38Z4 Operator: BJP
Inj Vol: 50.00
Comments: Abamectin PMV (B90-38) Injection vol: 50ul BART PUMA AND MARK LAW



8,9-Z-B1a standard (7 ng/mL)
(This shows one of the duplicate 7 ng/mL 8,9-Z-B1a
standards used to bracket runs of 8,9-Z-B1a-fortified
pear samples.)

MAXIMA 820 PEAK INTEGRATION REPORT

Printed: 21-FEB-1992 9:08:29

SAMPLE: 89Z 522a 7ng/ml

#28 in Method: ABAMECTIN

Acquired: 22-MAY-1991 23:36

Rate: 2.0 points/sec

Duration: 15.000 minutes

Operator: BJP

Type: UNKN

Instrument: Waters Maxima

Filename: ABA210

Index: 9

Injection Volume: 50.0

DETECTOR: FL-750 BX

#	ID#	Peak Start (minutes)	Peak End (minutes)	Retention (minutes)	Type	Peak Area (microvolt-sec)	Peak Height (microvolt)	Area Percent	Hght Percent	Component Name
1		0.175	4.867	2.042	BB	6128292.3	85080.752	69.62	51.16	
2	1	12.717	14.408	13.217	BB	2674326.7	81210.860	30.38	48.84	B1a DERIVATIVE
TAL						8802619.0	166291.61			

Sample: 89Z Lo-1, 10m

Channel: FL-750 BX

Filename: ABA211

Acquired: 22-MAY-91 2

Method: C:\MAX\DATA1\B90-38E4

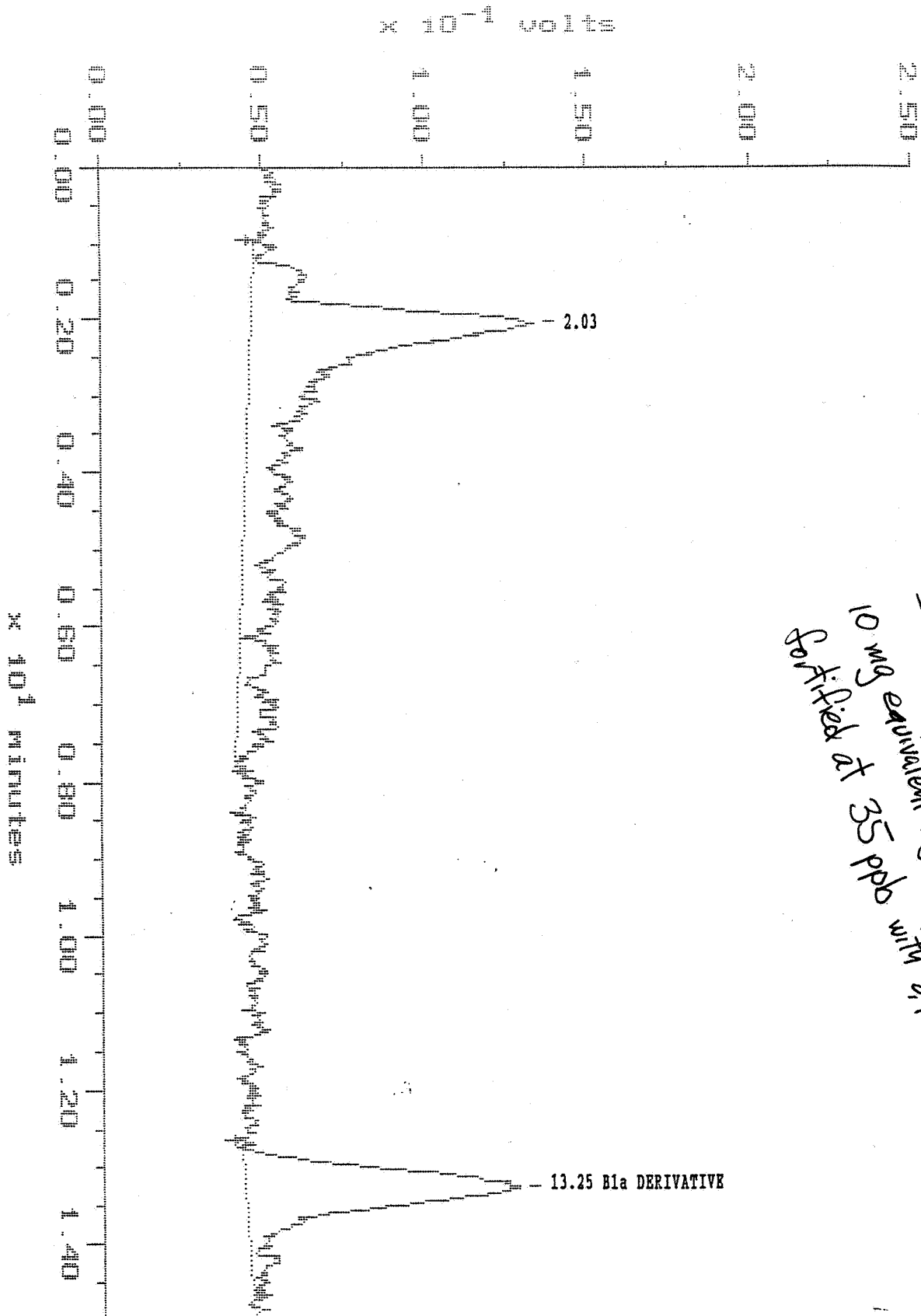
Operator: BJP

Inj Vol: 50.00

Comments: Abamectin PMV (B90-38)

Injection vol: 50ul

BART PUMA AND MARK LAW



Sample No. 7
10 mg equivalent injection of pear sample
fortified at 35 ppb with 8,9-E-Ba.

8

MAXIMA 820 PEAK INTEGRATION REPORT

Printed: 21-FEB-1992 9:09:15

NPLE: 89Z Lo-1, 10mg

#29 in Method: ABAMECTIN

Acquired: 22-MAY-1991 23:53

Rate: 2.0 points/sec

Duration: 15.000 minutes

Operator: BJP

Type: UNKN

Instrument: Waters Maxima

Filename: ABA211

Index: 10

Injection Volume: 50.0

DETECTOR: FL-750 BX

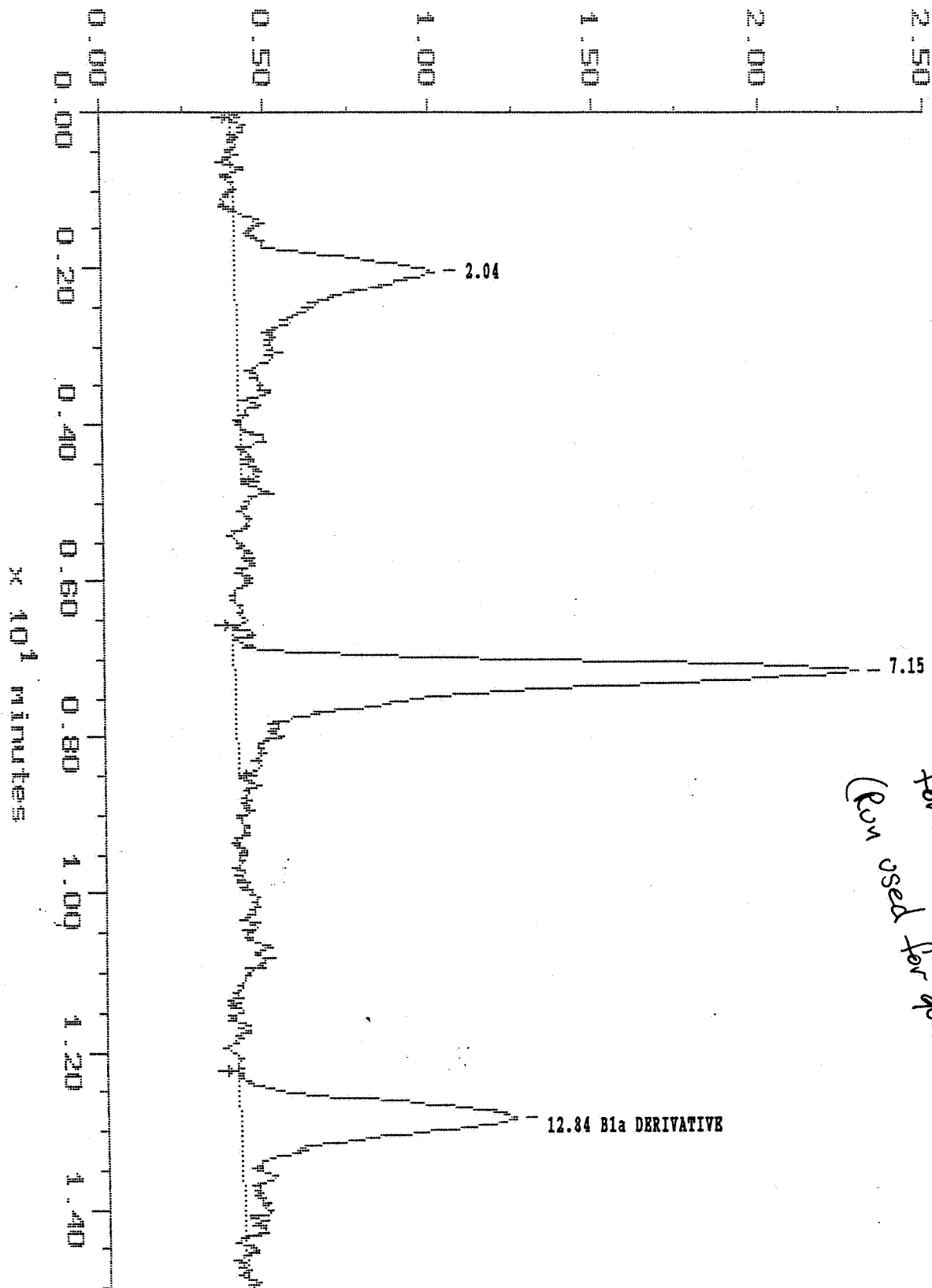
ID#	Peak Start (minutes)	Peak End (minutes)	Retention (minutes)	Type	Peak Area (microvolt-sec)	Peak Height (microvolt)	Area Percent	Hght Percent	Component Name
	0.950	7.842	2.033	BB	6489803.7	87033.937	68.81	50.94	
1	12.642	14.658	13.250	BB	2942170.0	83819.041	31.19	49.06	B1a DERIVATIVE
					9431973.7	170852.98			

15

Sample: ABA Lo-2, 10mg Channel: FL-750 BX
Acquired: 11-JUN-91 20:54 Method: C:\MAX\DATA1\B90-38X1
Inj Vol: 50.00
Comments: Abamectin PMV (B90-38) Injection vol: 50ul BART PUMA AND MARK LAW

Filename: ABA259
Operator: BJP

$\times 10^{-1}$ volts



Sample No. 4
10 mg equivalent injection of pear sample
fortified at 35 ppb with abamectin.
(Run used for quantitation of B1a.)

MAXIMA 820 PEAK INTEGRATION REPORT

Printed: 21-FEB-1992 10:49:58

SAMPLE: ABA Lo-2, 10mg

#21 in Method: ABANECTIN

Acquired: 11-JUN-1991 20:54

Rate: 2.0 points/sec

Duration: 16.000 minutes

Operator: BJP

Type: UNKN

Instrument: Waters Maxima

Filename: ABA259

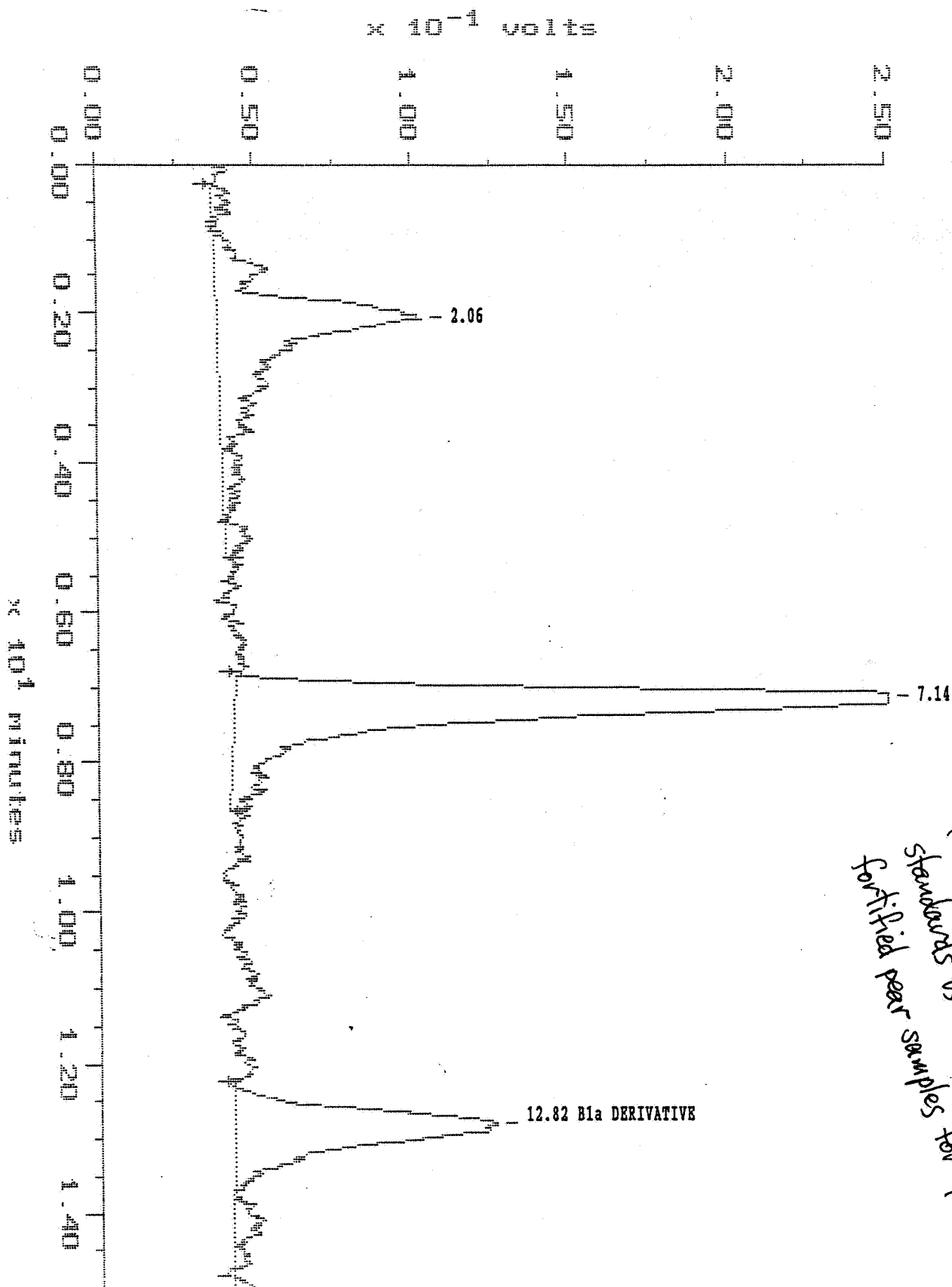
Index: 8

Injection Volume: 50.0

DETECTOR: FL-750 BX

PK#	ID#	Peak Start (minutes)	Peak End (minutes)	Retention (minutes)	Type	Peak Area (microvolt-sec)	Peak Height (microvolt)	Area Percent	Hght Percent	Component Name
1		0.067	4.717	2.042	BB	2732704.2	60139.960	24.96	18.02	
2		6.575	8.500	7.150	BB	5310451.8	189484.41	48.51	56.79	
3	1	12.225	15.117	12.842	BB	2904703.0	84061.244	26.53	25.19	B1a DERIVATIVE
TOTAL						10947859	333685.61			

Sample: ABA 611b 7ng/ml Channel: FL-750 BX Filename: ABA260
Acquired: 11-JUN-91 21:11 thod: C:\MAX\DATA1\B90-38X1 verator: BJP
Inj Vol: 50.00
Comments: Abamectin PMV (B90-38) Injection vol: 50ul BART PUMA AND MARK LAW



Abamectin standard (7 ng/ml)
(this shows one of the duplicate 7 ng/ml abamectin standards used to bracket runs of B1a.)
fortified pear samples for quantitation of B1a.

MAXIMA 820 PEAK INTEGRATION REPORT

Printed: 21-FEB-1992 10:50:46

SAMPLE: ABA 611b 7ng/ml

#22 in Method: ABAHECTIN

Acquired: 11-JUN-1991 21:11

Rate: 2.0 points/sec

Duration: 16.000 minutes

Operator: BJP

Type: UNKN

Instrument: Waters Maxima

Filename: ABA260

Index: 9

Injection Volume: 50.0

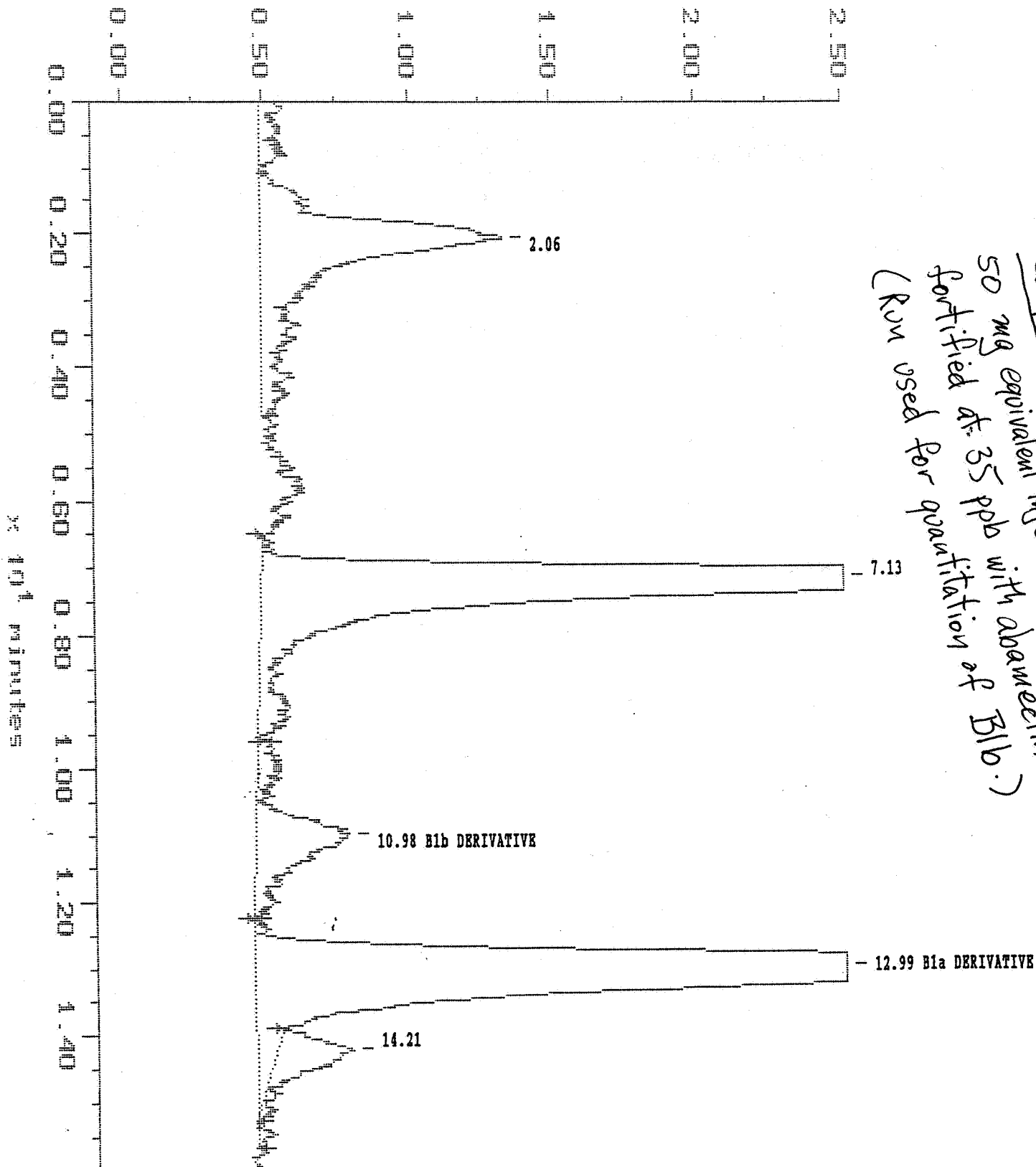
DETECTOR: FL-750 BX

PK#	ID#	Peak Start (minutes)	Peak End (minutes)	Retention (minutes)	Type	Peak Area (microvolt-sec)	Peak Height (microvolt)	Area Percent	Hght Percent	Component Name
1		0.242	5.258	2.058	BB	3517965.0	64433.373	27.27	16.91	
2		6.775	8.650	7.142	BB	6423169.6	233708.94	49.80	61.32	
3	1	12.250	15.175	12.817	BB	2957514.1	82978.168	22.93	21.77	B1a DERIVATIVE
TOTAL						12898649	381120.48			

Sample: ABA Lo-2, 50mg Channel: FL-750 BX
Acquired: 17-JUN-91 19:44 thod: C:\MAX\DATA1\B90-38X2
Inj Vol: 50.00
Comments: Abamectin PMV (B90-38) Injection vol: 50ul BART PUMA AND MARK LAW

Filename: ABA232
Operator: BJP

$\times 10^{-1}$ volts



MAXIMA 820 PEAK INTEGRATION REPORT

Printed: 21-FEB-1992 13:10:41

SAMPLE: ABA Lo-2, 50mg

#13 in Method: ABAMECTIN

Acquired: 17-JUN-1991 19:44

Rate: 2.0 points/sec

Duration: 16.000 minutes

Operator: BJP

Type: UNKN

Instrument: Waters Maxima

Filename: ABA282

Index: 13

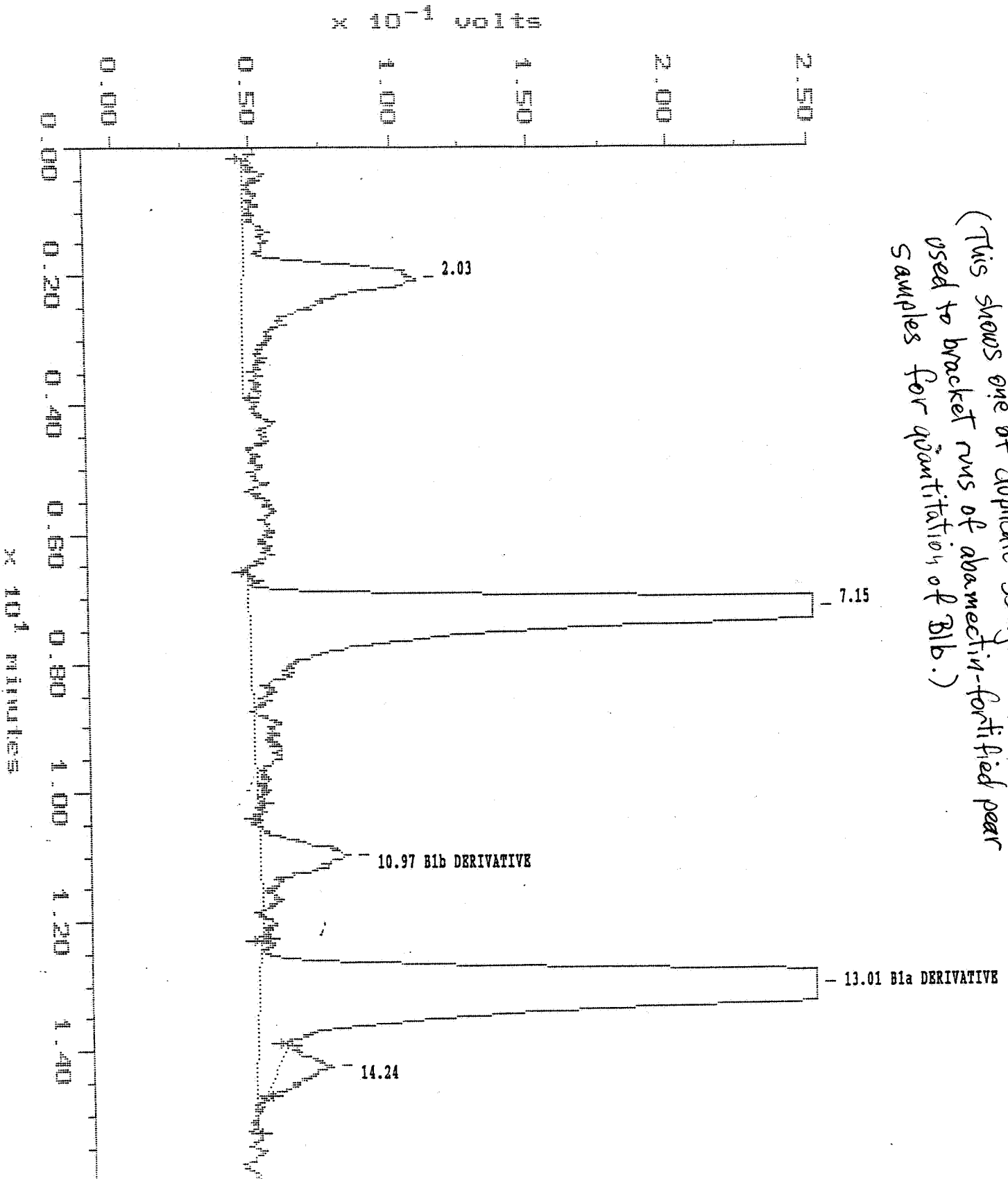
Injection Volume: 50.0

DETECTOR: FL-750 BX

PK#	ID#	Peak Start (minutes)	Peak End (minutes)	Retention (minutes)	Type	Peak Area (microvolt-sec)	Peak Height (microvolt)	Area Percent	Hght Percent	Component Name
1		0.000	4.733	2.058	DB	4500415.9	83866.247	14.99	9.61	
2		6.500	9.583	7.133	BP	10553401	365941.06	35.16	41.91	
3	1	9.583	12.242	10.975	PP	1660699.2	31785.521	5.53	3.64	B1b DERIVATIVE
4	2	12.242	15.667	12.992	PB	12472326	365247.17	41.55	41.84	B1a DERIVATIVE
5		13.875	15.283	14.208	SS	832428.86	26220.473	2.77	3.00	
TOTAL						30019271	873060.47			

Sample: ABA 617b35ng/ml nel: FL-750 BX Tenname: ABA283
 Acquired: 17-JUN-91 20:01 Method: C:\NAX\DATA1\B90-38X2 Operator: BJP
 Inj Vol: 50.00
 Comments: Abamectin PMV (B90-38) Injection vol: 50ul BART PUMA AND MARK LAW

5



Abamectin standard (55 ng/ml)
 (This shows one of duplicate 35 ng/ml abamectin standards
 used to bracket runs of abamectin-fortified pear
 samples for quantitation of B1b.)

MAXIMA 820 PEAK INTEGRATION REPORT

Printed: 21-FEB-1992 13:11:31

SAMPLE: ABA 617b35ng/ml

#14 in Method: ABAMECTIN

Acquired: 17-JUN-1991 20:01

Rate: 2.0 points/sec

Duration: 16.000 minutes

Operator: BJP

Type: UNKN

Instrument: Waters Maxima

Filename: ABA283

Index: 14

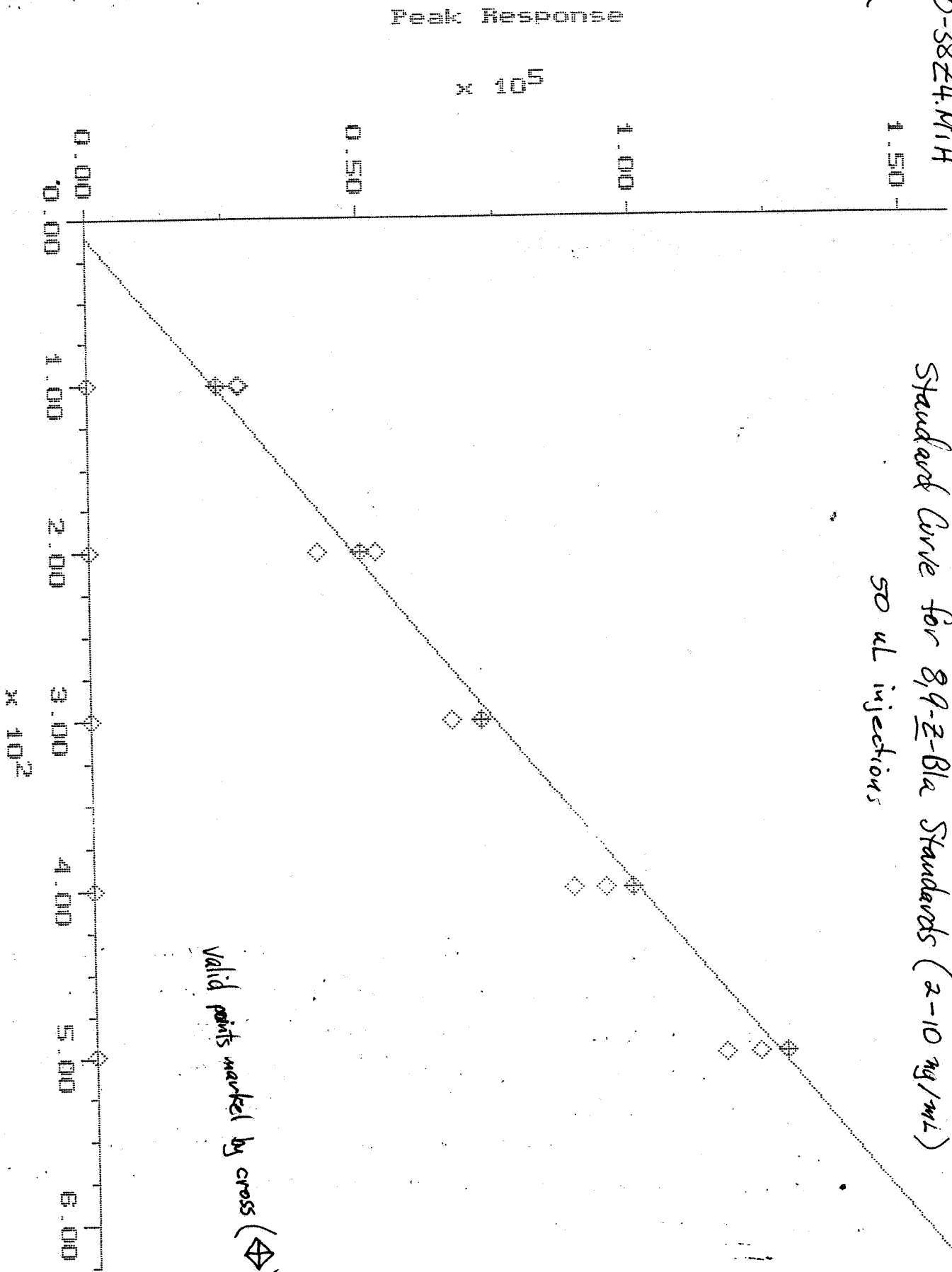
Injection Volume: 50.0

DETECTOR: FL-750 BX

PK#	ID#	Peak Start (minutes)	Peak End (minutes)	Retention (minutes)	Type	Peak Area (microvolt-sec)	Peak Height (microvolt)	Area Percent	Hght Percent	Component Name
1		0.167	3.883	2.033	BB	3010911.0	61952.329	10.72	7.10	
2		6.600	10.183	7.150	BB	10532774	371783.44	37.52	42.60	
3	1	10.417	12.258	10.967	BB	960696.01	31753.335	3.42	3.64	B1b DERIVATIVE
4	2	12.308	15.267	13.008	BB	13122836	387869.19	46.74	44.44	B1a DERIVATIVE
5		13.892	14.708	14.242	SS	447775.02	19427.242	1.59	2.23	
TOTAL						28074992	872785.54			

B90-38Z4.MTH

Standard Curve for 8,9-E-Bl_a Standards (2-10 ng/mL)
50 μ L injections



~~BL_a~~ DERIVATIVE CONC * INJ VOL ($\approx$ P3)

89Z

Bla DERIV. FIVE Calibrat on Report

Printed: 21-FEB-1992 9:43:18

Method file: B90-38Z4.MTH

18

Quant Basis: Height

Rejection Tolerance: None

Internal Standard: None

Curve Type: Linear

Weighting: None

Forced Through Origin: No

Y-axis Label: Response

Corr. Coef. (r): 0.9992332

Coef. of Determination (r²): 0.9984671

Equation: Conc*(Inj Vol) = 1.037072E+01 + 3.892722E-03 * R

These values are for the
8,9-Z-Bla derivative standard
curve indicated by valid points below.

Sample	File Name	Valid	Concentration	Response	Calc'd Concentration	% Deviation	Response Factor
31a 522 2ng/ml	ABA181	N	2.000000E+00	0.000000E+00	2.074144E-01	8.64E+02	Invalid
31a 522 4ng/ml	ABA182	N	4.000000E+00	0.000000E+00	2.074144E-01	1.83E+03	Invalid
31a 522 6ng/ml	ABA183	N	6.000000E+00	0.000000E+00	2.074144E-01	2.79E+03	Invalid
31a 522 8ng/ml	ABA184	N	8.000000E+00	0.000000E+00	2.074144E-01	3.76E+03	Invalid
31a 522 10ng/ml	ABA185	N	1.000000E+01	0.000000E+00	2.074144E-01	4.72E+03	Invalid
31a 522 2ng/ml	ABA197	N	2.000000E+00	2.7794125E+04	2.371310E+00	-1.57E+01	3.597883E-03
31a 522 4ng/ml	ABA198	N	4.000000E+00	5.2598660E+04	4.302454E+00	-7.03E+00	3.802378E-03
31a 522 6ng/ml	ABA199	N	6.000000E+00	7.1886430E+04	5.804092E+00	3.38E+00	4.173249E-03
31a 522 8ng/ml	ABA200	N	8.000000E+00	8.8523633E+04	7.099372E+00	1.27E+01	4.518567E-03
31a 522 10ng/ml	ABA201	N	1.000000E+01	1.1610050E+05	9.246353E+00	8.15E+00	4.306614E-03
31Z 522 2ng/ml	ABA202	Y	2.000000E+00	2.3593518E+04	2.044274E+00	-2.17E+00	4.238452E-03
31Z 522 4ng/ml	ABA203	Y	4.000000E+00	4.9809164E+04	4.085279E+00	-2.09E+00	4.015325E-03
31Z 522 6ng/ml	ABA204	Y	6.000000E+00	7.2024023E+04	5.814804E+00	3.18E+00	4.165277E-03
31Z 522 8ng/ml	ABA205	Y	8.000000E+00	9.9288414E+04	7.937458E+00	7.88E-01	4.028667E-03
31Z 522 10ng/ml	ABA206	Y	1.000000E+01	1.2729873E+05	1.011819E+01	-1.17E+00	3.927769E-03
31Z 522 2ng/ml	ABA218	N	2.000000E+00	2.7344855E+04	2.336333E+00	-1.44E+01	3.656995E-03
31Z 522 4ng/ml	ABA219	N	4.000000E+00	4.2077426E+04	3.483329E+00	1.48E+01	4.753142E-03
31Z 522 6ng/ml	ABA220	N	6.000000E+00	6.6609180E+04	5.393235E+00	1.13E+01	4.503884E-03
31Z 522 8ng/ml	ABA221	N	8.000000E+00	9.4375680E+04	7.554980E+00	5.89E+00	4.238380E-03
31Z 522 10ng/ml	ABA222	N	1.000000E+01	1.2219502E+05	9.720839E+00	2.87E+00	4.091820E-03

This calibration report was obtained from the same sample queue used to run the control pear samples and the 8,9-Z-Bla-fortified pear samples. A complete set of 5 Bla standards (2-10 ng/ml) and a complete set of 5 8,9-Z-Bla standards were derivatized with the pear samples and with duplicate 7 ng/mL 8,9-Z-Bla standards that were used to bracket the fortified samples in the sample queue. The Bla standard set was run before and after the group of pear samples and bracketing 8,9-Z-Bla standards; then the 8,9-Z-Bla standard set was run before and after the group of pear samples and bracketing standards was re-run.

25

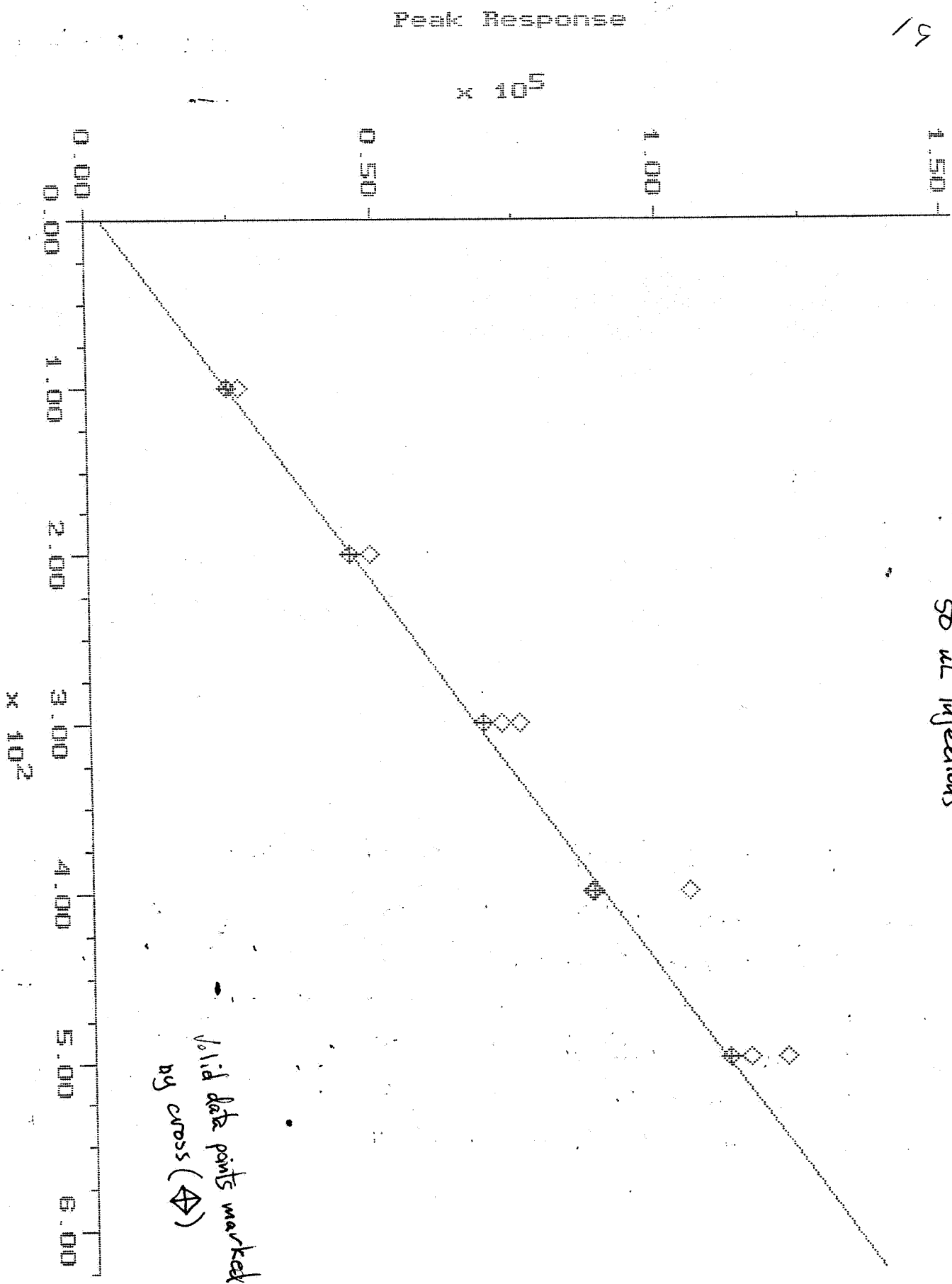
B9D-38X1.M1H

Standard Curve for avermectin Bla standards (2-10 ng/mL)

50 μ L injections

26

Bla DERIVATIVE conc * Inj Vol (= PG)



Bla DERIVATIVE Calibration Report

Printed: 21-FEB-1992 10:26:30

Method file: BAO-38X1.MTH

20

Quant Basis: Height
Curve Type: Linear
Y-axis Label: Response
Corr. Coef. (r): 0.9995644

Rejection Tolerance: None
Weighting: None

Internal Standard: None
Forced Through Origin: No

Coef. of Determination (r²): 0.9991290

Equation: Conc*(Inj Vol) = -1.295856E+01 + 4.615789E-03 * R

These values are for the Bla derivative standard curve indicated by the valid points shown below.

Sample	File Name	Valid	Concentration	Response	Calc'd Concentration	% Deviation	Response Factor
Bla 611 2ng/ml	ABA239	Y	2.000000E+00	2.4484279E+04	2.001114E+00	-5.57E-02	4.084253E-03
Bla 611 4ng/ml	ABA240	Y	4.000000E+00	4.5688215E+04	3.958572E+00	1.05E+00	4.377496E-03
Bla 611 6ng/ml	ABA241	Y	6.000000E+00	6.9136898E+04	6.123256E+00	-2.01E+00	4.339217E-03
Bla 611 8ng/ml	ABA242	Y	8.000000E+00	8.8094211E+04	7.873315E+00	1.61E+00	4.540593E-03
Bla 611 10ng/ml	ABA243	Y	1.000000E+01	1.1160512E+05	1.004374E+01	-4.36E-01	4.480081E-03
Bla 611 2ng/ml	ABA252	N	2.000000E+00	2.6641848E+04	2.200292E+00	-9.10E+00	3.753493E-03
Bla 611 4ng/ml	ABA253	N	4.000000E+00	4.9237047E+04	4.286185E+00	-6.68E+00	4.061982E-03
Bla 611 6ng/ml	ABA254	N	6.000000E+00	7.5542008E+04	6.714549E+00	-1.06E+01	3.971300E-03
Bla 611 8ng/ml	ABA255	N	8.000000E+00	1.0480035E+05	9.415556E+00	-1.50E+01	3.816781E-03
Bla 611 10ng/ml	ABA256	N	1.000000E+01	1.2155880E+05	1.096262E+01	-8.78E+00	4.113236E-03
Bla 611 2ng/ml	ABA265	N	2.000000E+00	2.4433318E+04	1.996410E+00	1.80E-01	4.092772E-03
Bla 611 4ng/ml	ABA266	N	4.000000E+00	4.5594070E+04	3.949881E+00	1.27E+00	4.386535E-03
Bla 611 6ng/ml	ABA267	N	6.000000E+00	7.2195148E+04	6.405581E+00	-6.33E+00	4.155404E-03
Bla 611 8ng/ml	ABA268	N	8.000000E+00	8.7540070E+04	7.822159E+00	2.27E+00	4.569336E-03
Bla 611 10ng/ml	ABA269	N	1.000000E+01	1.1520733E+05	1.037628E+01	-3.63E+00	4.340002E-03

This calibration report is for the first of three runs of a complete set of Bla standards (2-10 ng/mL) in the sample queue used for quantitation of Bla in abamectin-fortified pear samples. The initial runs of the pear samples (and bracketing 7 ng/mL abamectin standards that were used for quantitation) were made between the first and second runs of the complete Bla standard set. The pear samples and bracketing standards were run again between the second and third runs of the Bla standard set.

B90-38X2.MTH

2

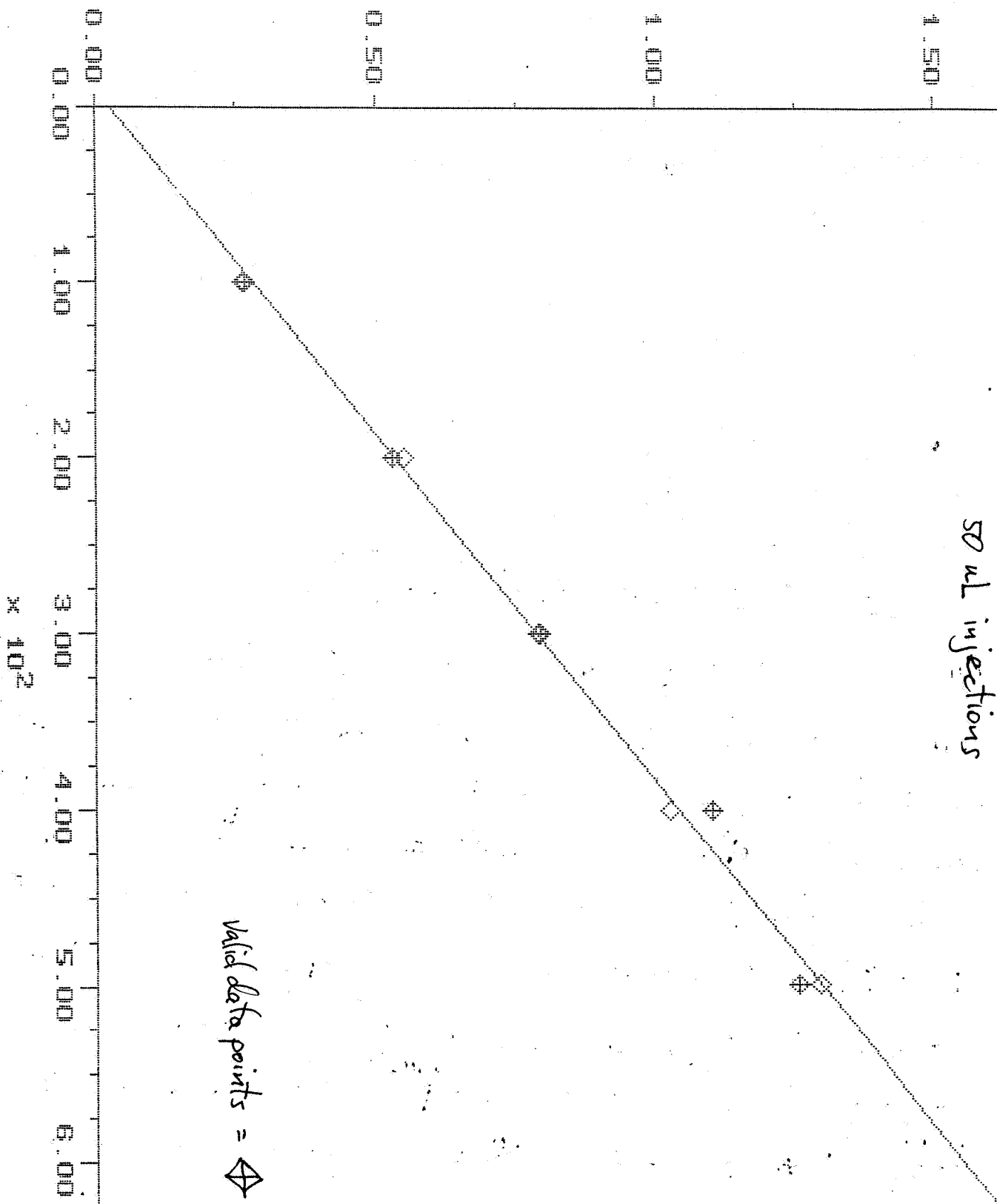
Standard Curve for avermectin B1b standards (2-10 ug/mL)

50 uL injections

SPK

Peak Response

$\times 10^5$



Valid data points = $\diamond$

B1b DERIVATIVE Conc * Inj Vol (=Pg)

B1b DERIVATIVE Calibration Report

Printed: 21-FEB-1992 13:01:04

Method file: B10-38X2.MTH

22

Quant Basis: Height
Curve Type: Linear
Y-axis Label: Response
Corr. Coef. (r): 0.9957583

Rejection Tolerance: None
Weighting: None

Internal Standard: None
Forced Through Origin: No

Coef. of Determination (r²): 0.9915346

Equation: Conc*(Inj Vol) = -9.554186E+00 + 3.930850E-03 * R

These values are for the B1b derivati-
std. curve indicated by the valid
points shown below

Sample	File Name	Valid	Concentration	Response	Calc'd Concentration	% Deviation	Response Factor
B1b 617 2ng/ml	ABA275	Y	2.000000E+00	2.6689324E+04	1.907151E+00	4.87E+00	3.746816E-03
B1b 617 4ng/ml	ABA276	Y	4.000000E+00	5.2665180E+04	3.949295E+00	1.28E+00	3.797576E-03
B1b 617 6ng/ml	ABA277	Y	6.000000E+00	7.9172109E+04	6.033191E+00	-5.50E-01	3.789213E-03
B1b 617 8ng/ml	ABA278	Y	8.000000E+00	1.1000437E+05	8.457131E+00	-5.41E+00	3.636219E-03
B1b 617 10ng/ml	ABA279	Y	1.000000E+01	1.2521867E+05	9.653234E+00	3.59E+00	3.993015E-03
B1b 617 2ng/ml	ABA302	N	2.000000E+00	2.6004287E+04	1.853296E+00	7.92E+00	3.845520E-03
B1b 617 4ng/ml	ABA303	N	4.000000E+00	5.4980461E+04	4.131316E+00	-3.18E+00	3.637656E-03
B1b 617 6ng/ml	ABA304	N	6.000000E+00	7.8965312E+04	6.016933E+00	-2.81E-01	3.799136E-03
B1b 617 8ng/ml	ABA305	N	8.000000E+00	1.0265700E+05	7.879503E+00	1.53E+00	3.896471E-03
B1b 617 10ng/ml	ABA306	N	1.000000E+01	1.2937020E+05	9.979614E+00	2.04E-01	3.864878E-03

of two

This calibration report is for the first ^{of two} runs of a complete set of 5 B1b standards (2-10 ng/mL) in the sample queue used for quantitation of B1b in abamectin-fortified samples. Each B1b standard contained Bla at a concentration of 13.46 times that of B1b so that the set of B1b standards contained Bla at levels from ca 26.9 to 134.6 ng/mL. Besides these B1b standards containing high levels of Bla, the sample queue for this method file (B90-38X2.MTH) was arranged to include three runs of a complete set of 5 Bla standards at 2-10 ng/mL. A standard curve for one run of the 2-10 ng/mL Bla standard set and a standard curve for ^{Bla in} one run of the B1b standard set containing ^{Bla at} 26.9-134.6 ng/mL are attached (both are labeled with the method file name B90-38X2.MTH).

Runs of ~~fortified~~ 50 mg equivalent samples of pears fortified with abamectin at 35 ppb and 70 ppb were bracketed with injections of duplicate 35 ng/mL and 70 ng/mL 2g abamectin standards, respectively, immediately ^{the first} after runs of the B1b standard set and immediately before the repeat runs of this set

B90-38X2.MTH

2

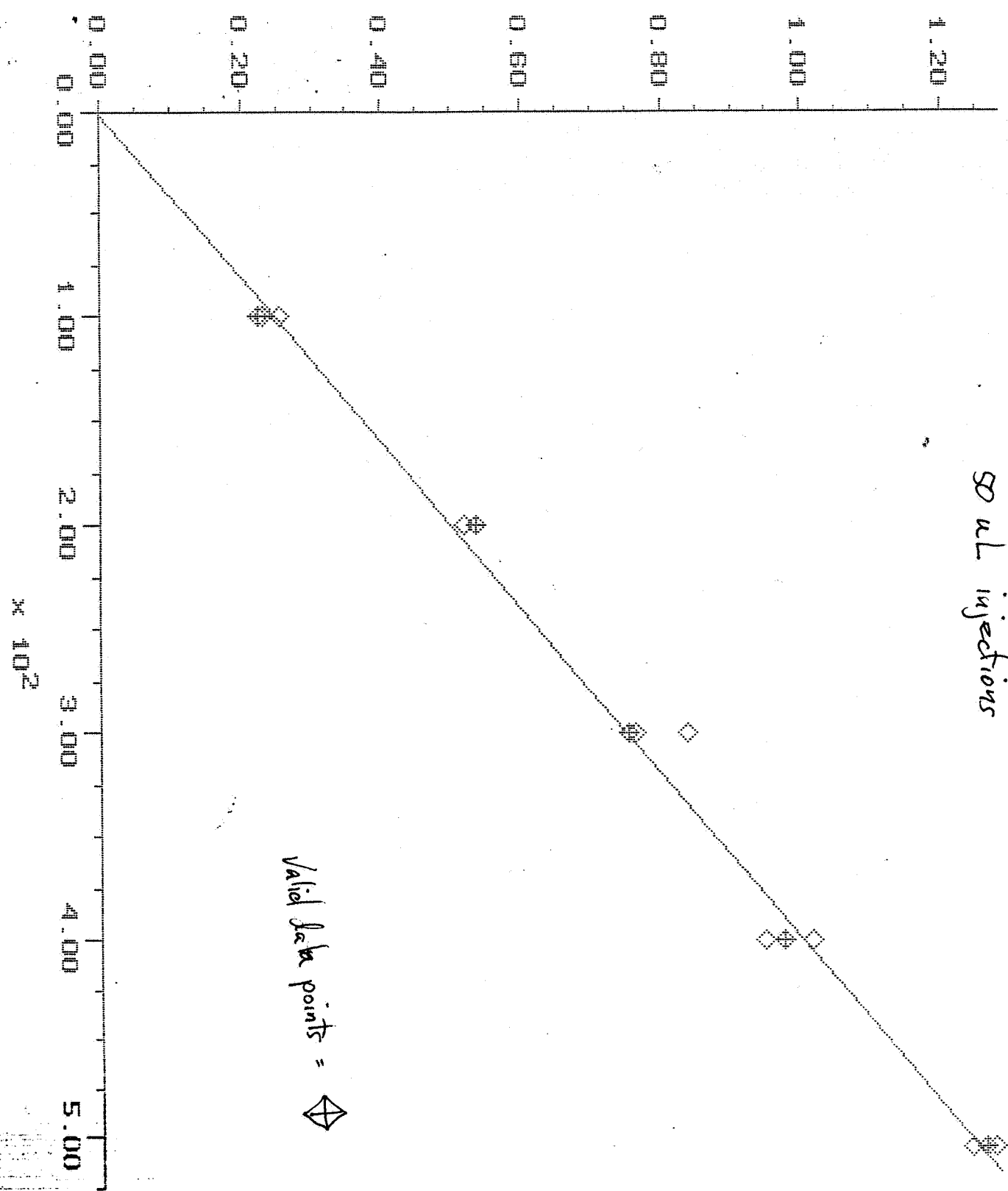
Standard Curve for avermectin Bla standards (2-10 ng/mL)

50 μ L injections

30

Peak Response

$\times 10^5$



R1a Derivative Conc * 10²

(= pg)

B1a DERIVATIVE Calibration Report

Printed: 21-FEB-1992 12:42:07 Method file: B90-38X2.MTH 24

Quant Basis: Height
Curve Type: Linear
Y-axis Label: Response
Corr. Coef. (r): 0.9979509

Rejection Tolerance: None
Weighting: None
Coef. of Determination (r²): 0.9959060

Internal Standard: None
Forced Through Origin: No

Equation: Conc*(Inj Vol) = 1.163702E+00 + 3.976333E-03 * R

These values are for the B1c std. curve given by the data points marked as valid (Y) in the 3rd column below.

Sample	File Name	Valid	Concentration	Response	Calc'd Concentration	% Deviation	Response Factor
B1a 617 2ng/ml	ABA270	Y	2.000000E+00	2.2598955E+04	1.820494E+00	9.86E+00	4.424983E-03
B1a 617 4ng/ml	ABA271	Y	4.000000E+00	5.3642039E+04	4.289247E+00	-6.74E+00	3.728419E-03
B1a 617 6ng/ml	ABA272	Y	6.000000E+00	7.5515992E+04	6.028809E+00	-4.78E-01	3.972668E-03
B1a 617 8ng/ml	ABA273	Y	8.000000E+00	9.7695453E+04	7.792668E+00	2.66E+00	4.094356E-03
B1a 617 10ng/ml	ABA274	Y	1.000000E+01	1.2631623E+05	1.006878E+01	-6.83E-01	3.958319E-03
B1b 617 2ng/ml	ABA275	N	2.692000E+01	3.0332112E+05	2.414539E+01	1.15E+01	4.437541E-03
B1b 617 4ng/ml	ABA276	N	5.384000E+01	6.7561956E+05	5.375305E+01	1.62E-01	3.984491E-03
B1b 617 6ng/ml	ABA277	N	8.076000E+01	1.0328721E+06	8.216415E+01	-1.71E+00	3.909487E-03
B1b 617 8ng/ml	ABA278	N	1.076800E+02	1.3673144E+06	1.087612E+02	-9.94E-01	3.937646E-03
B1b 617 10ng/ml	ABA279	N	1.346000E+02	1.6440430E+06	1.307685E+02	2.93E+00	4.093567E-03
B1a 617 2ng/ml	ABA288	N	2.000000E+00	2.5460066E+04	2.048028E+00	-2.35E+00	3.927720E-03
B1a 617 4ng/ml	ABA289	N	4.000000E+00	5.1941520E+04	4.154010E+00	-3.71E+00	3.850484E-03
B1a 617 6ng/ml	ABA291	N	6.000000E+00	8.3730797E+04	6.682105E+00	-1.02E+01	3.582911E-03
B1a 617 8ng/ml	ABA292	N	8.000000E+00	1.0172520E+05	8.113141E+00	-1.39E+00	3.932162E-03
B1a 617 10ng/ml	ABA293	N	1.000000E+01	1.2789659E+05	1.019446E+01	-1.91E+00	3.909408E-03
B1b 617 2ng/ml	ABA302	N	2.692000E+01	3.0647781E+05	2.439643E+01	1.03E+01	4.391835E-03
B1b 617 4ng/ml	ABA303	N	5.384000E+01	6.7486287E+05	5.369287E+01	2.74E-01	3.988959E-03
B1b 617 6ng/ml	ABA304	N	8.076000E+01	1.0326619E+06	8.214743E+01	-1.69E+00	3.910283E-03
B1b 617 8ng/ml	ABA305	N	1.076800E+02	1.3545125E+06	1.077431E+02	-5.86E-02	3.974862E-03
B1b 617 10ng/ml	ABA306	N	1.346000E+02	1.6291387E+06	1.295833E+02	3.87E+00	4.131017E-03
B1a 617 2ng/ml	ABA307	N	2.000000E+00	2.3028107E+04	1.854623E+00	7.84E+00	4.342519E-03
B1a 617 4ng/ml	ABA308	N	4.000000E+00	5.2111840E+04	4.167555E+00	-4.02E+00	3.837899E-03
B1a 617 6ng/ml	ABA309	N	6.000000E+00	7.6571445E+04	6.112746E+00	-1.84E+00	3.917910E-03
B1a 617 8ng/ml	ABA310	N	8.000000E+00	9.4964422E+04	7.575478E+00	5.60E+00	4.212104E-03
B1a 617 10ng/ml	ABA311	N	1.000000E+01	1.2437538E+05	9.914434E+00	8.63E-01	4.020088E-03

B90-38X2.MTH

Standard curve for avermectin Bla standards (26.9-134.6 mg/mL)

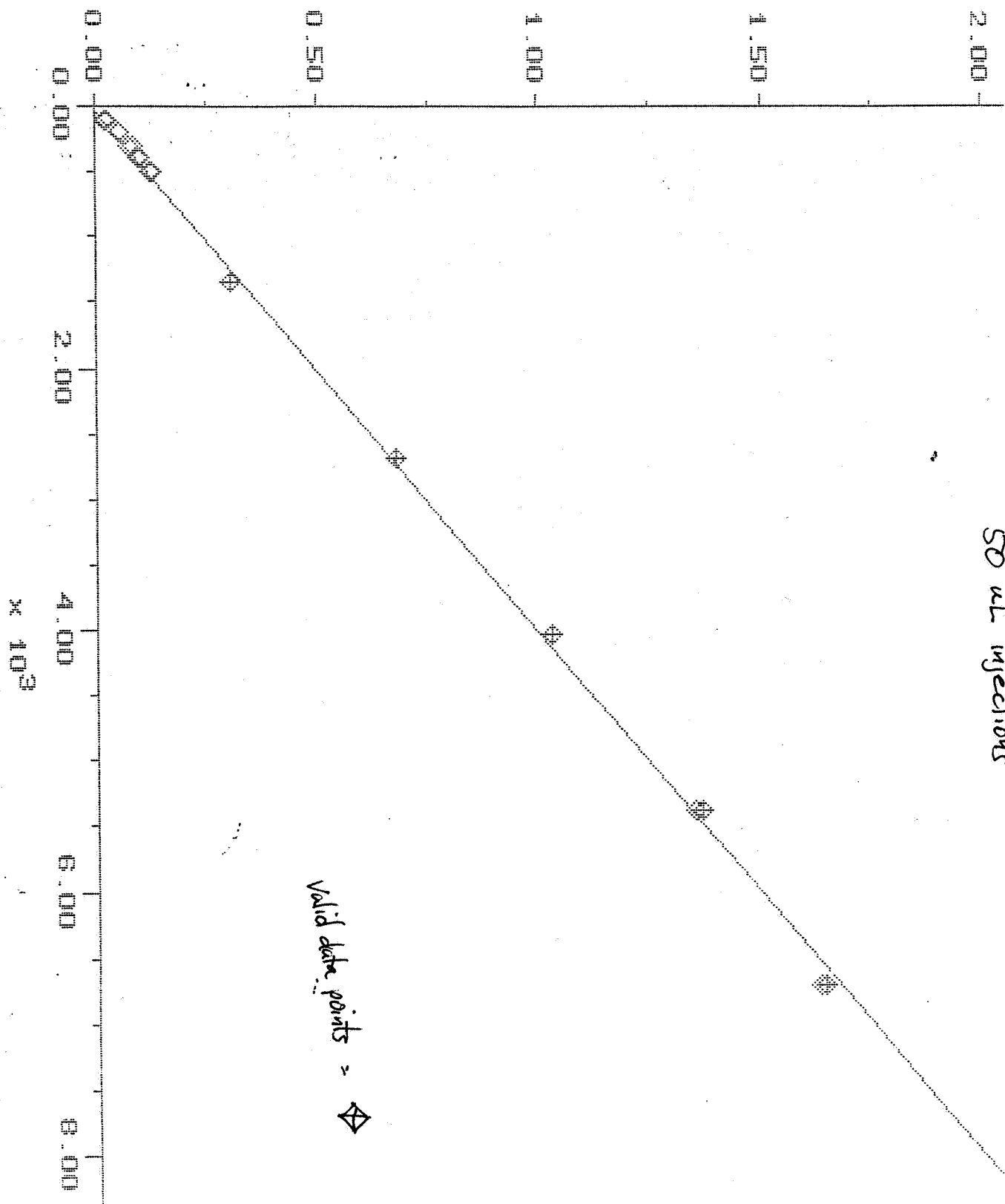
50 ul injections

5

32

Peak Response

$\times 10^6$



B1a DERIVATIVE Conc * Inj Vol (= P3)

Bla DERI ATIVE Calibre ion Report

Printed: 21-FEB-1992 12:57:34

Method file: B90-38X2.MTH

26

Quant Basis: Height
Curve Type: Linear
Y-axis Label: Response
Corr. Coef. (r): 0.9984820

Rejection Tolerance: None
Weighting: None

Internal Standard: None
Forced Through Origin: No

Coef. of Determination (r²): 0.9969664

Equation: Conc*(Inj Vol) = 2.915950E+01 + 3.990349E-03 * R

These values are for the Bla standard curve given by the data points show as valid (Y) in the third column below. (This Bla standard curve is for the high concentrations of Bla in the Blb standard

Sample	File Name	Valid	Concentration	Response	Calc'd Concentration	% Deviation	Response Factor
Bla 617 2ng/ml	ABA270	N	2.000000E+00	2.2598955E+04	2.386745E+00	-1.62E+01	4.424983E-03
Bla 617 4ng/ml	ABA271	N	4.000000E+00	5.3642039E+04	4.864200E+00	-1.78E+01	3.728419E-03
Bla 617 6ng/ml	ABA272	N	6.000000E+00	7.5515992E+04	6.609894E+00	-9.23E+00	3.972668E-03
Bla 617 8ng/ml	ABA273	N	8.000000E+00	9.7695453E+04	8.379970E+00	-4.53E+00	4.094356E-03
Bla 617 10ng/ml	ABA274	N	1.000000E+01	1.2631623E+05	1.066411E+01	-6.23E+00	3.958319E-03
Blb 617 2ng/ml	ABA275	Y	2.692000E+01	3.0332112E+05	2.479034E+01	8.59E+00	4.437541E-03
Blb 617 4ng/ml	ABA276	Y	5.384000E+01	6.7561956E+05	5.450235E+01	-1.22E+00	3.984491E-03
Blb 617 6ng/ml	ABA277	Y	8.076000E+01	1.0328721E+06	8.301360E+01	-2.71E+00	3.909487E-03
Blb 617 8ng/ml	ABA278	Y	1.076800E+02	1.3673144E+06	1.097044E+02	-1.85E+00	3.937646E-03
Blb 617 10ng/ml	ABA279	Y	1.346000E+02	1.6440430E+06	1.317893E+02	2.13E+00	4.093567E-03
Bla 617 2ng/ml	ABA288	N	2.000000E+00	2.5460066E+04	2.615081E+00	-2.35E+01	3.927720E-03
Bla 617 4ng/ml	ABA289	N	4.000000E+00	5.1941520E+04	4.728486E+00	-1.54E+01	3.850484E-03
Bla 617 6ng/ml	ABA291	N	6.000000E+00	8.3730797E+04	7.265493E+00	-1.74E+01	3.582911E-03
Bla 617 8ng/ml	ABA292	N	8.000000E+00	1.0172520E+05	8.701572E+00	-8.06E+00	3.932162E-03
Bla 617 10ng/ml	ABA293	N	1.000000E+01	1.2789659E+05	1.079023E+01	-7.32E+00	3.909408E-03
Blb 617 2ng/ml	ABA302	N	2.692000E+01	3.0647781E+05	2.504226E+01	7.50E+00	4.391835E-03
Blb 617 4ng/ml	ABA303	N	5.384000E+01	6.7486287E+05	5.444196E+01	-1.11E+00	3.988959E-03
Blb 617 6ng/ml	ABA304	N	8.076000E+01	1.0326619E+06	8.299682E+01	-2.70E+00	3.910283E-03
Blb 617 8ng/ml	ABA305	N	1.076800E+02	1.3545125E+06	1.086828E+02	-9.23E-01	3.974862E-03
Blb 617 10ng/ml	ABA306	N	1.346000E+02	1.6291387E+06	1.305998E+02	3.06E+00	4.131017E-03
Bla 617 2ng/ml	ABA307	N	2.000000E+00	2.3028107E+04	2.420994E+00	-1.74E+01	4.342519E-03
Bla 617 4ng/ml	ABA308	N	4.000000E+00	5.2111840E+04	4.742079E+00	-1.56E+01	3.837899E-03
Bla 617 6ng/ml	ABA309	N	6.000000E+00	7.6571445E+04	6.694126E+00	-1.04E+01	3.917910E-03
Bla 617 8ng/ml	ABA310	N	8.000000E+00	9.4964422E+04	8.162014E+00	-1.98E+00	4.212104E-03
Bla 617 10ng/ml	ABA311	N	1.000000E+01	1.2437538E+05	1.050921E+01	-4.85E+00	4.020088E-03

33