US EPA ARCHIVE DOCUMENT

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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
Bart J. Puma, Chemist
Analytical Chemistry Section

THRU: Harvey K. Hundley, Head
Analytical Chemistry Section

THRU: Donald A. Marlow, Chief
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 B_1 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 CH_3CN, diluted with water, and extracted into hexane. The residues are further cleaned up on an aminopropyl SPE column, eluted with 1+1 acetone-CH_2Cl_2, and diluted to 10 ml with CH_2Cl_2.

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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 B_1) is a mixture of the homologs avermectin B_{1a} and avermectin B_{1b} (here abbreviated as B1a and B1b, 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-B1a) and 8,9-Z-avermectin B_{1b} (abbreviated 8,9-Z-B1b), 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, B1a, B1b, or 8,9-Z-B1a 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% B1a and 0.071% B1b in glycerol formal) and 8,9-Z-B1a (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 B_1 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) Added	Found	Percent Recovery
1.	Abamectin	None	N.D.	
	Bla	None	N.D.	
	Blb	None	N.D.	
	8,9,Z-Bla	None	N.D.	
2.	Abamectin	None	N.D.	
	Bla	None	N.D.	
	Blb	None	N.D.	
	8,9,Z-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-Z-Bla	35.0	32.9	94.0
8.	8,9-Z-Bla	35.0	32.2	92.0
9.	8,9-Z-Bla	70.0	65.9	94.1
10.	8,9-Z-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 B_1 homologs, avermectin B_{1a} and B_{1b}, which are identified as Bla and Blb, respectively, in this table.

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

Method: "HPLC-Fluorescence Determination for Avermectin B_1 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:

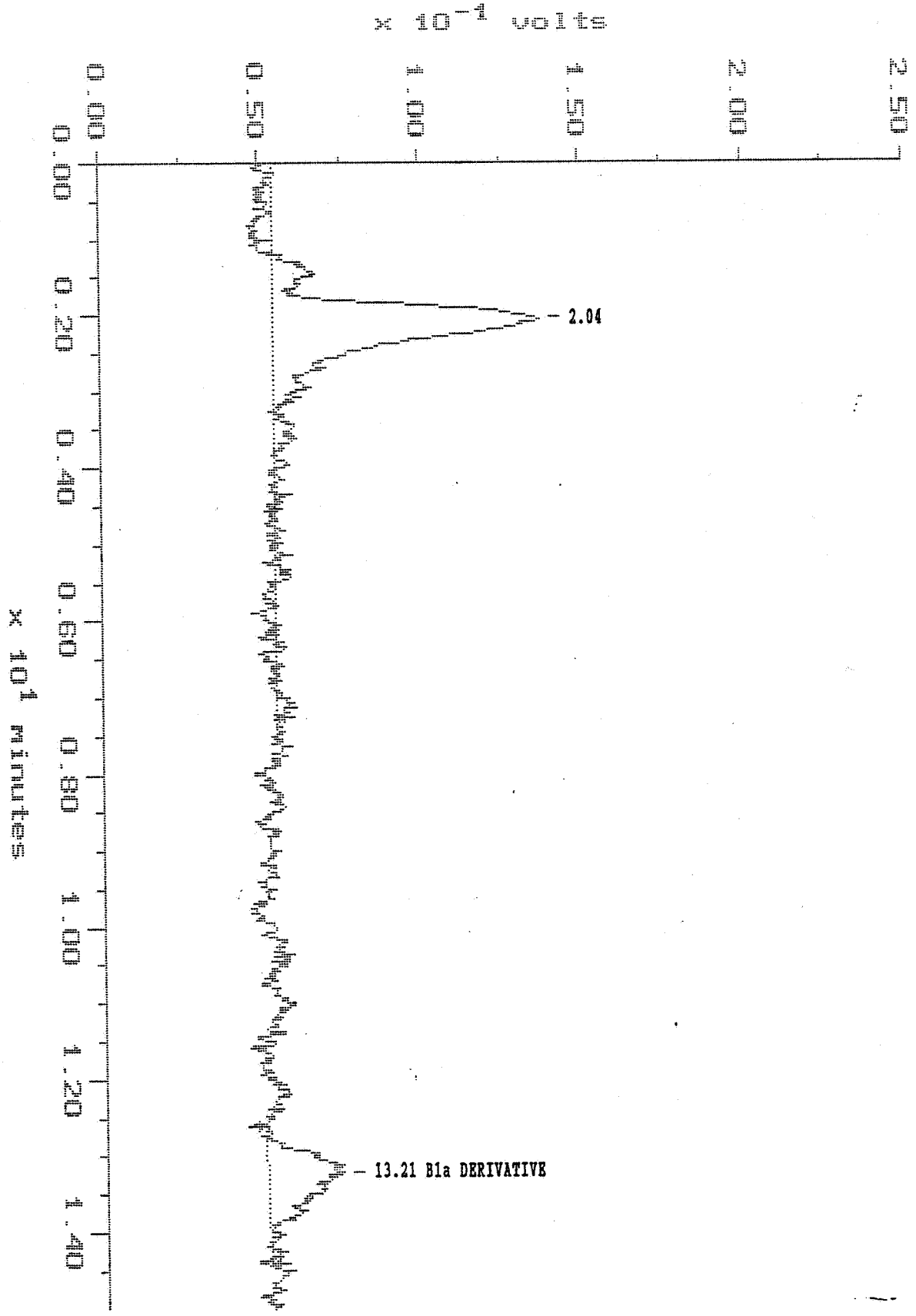
See report

Chromatograms:

Sample chromatograms attached.

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

Sample: 89Z 522 2ng/ml Channel: FL-750 BX Filename: ABA202
Acquired: 22-MAY-91 21 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



Lowest level standard (2 ng/mL) for 8,9-Z-B1a standard curve

8

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MAXIMA 820 PEAK INTEGRATION REPORT

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

PLE: 89Z 522 2ng/ml
#21 in Method: ABAMECTIN
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

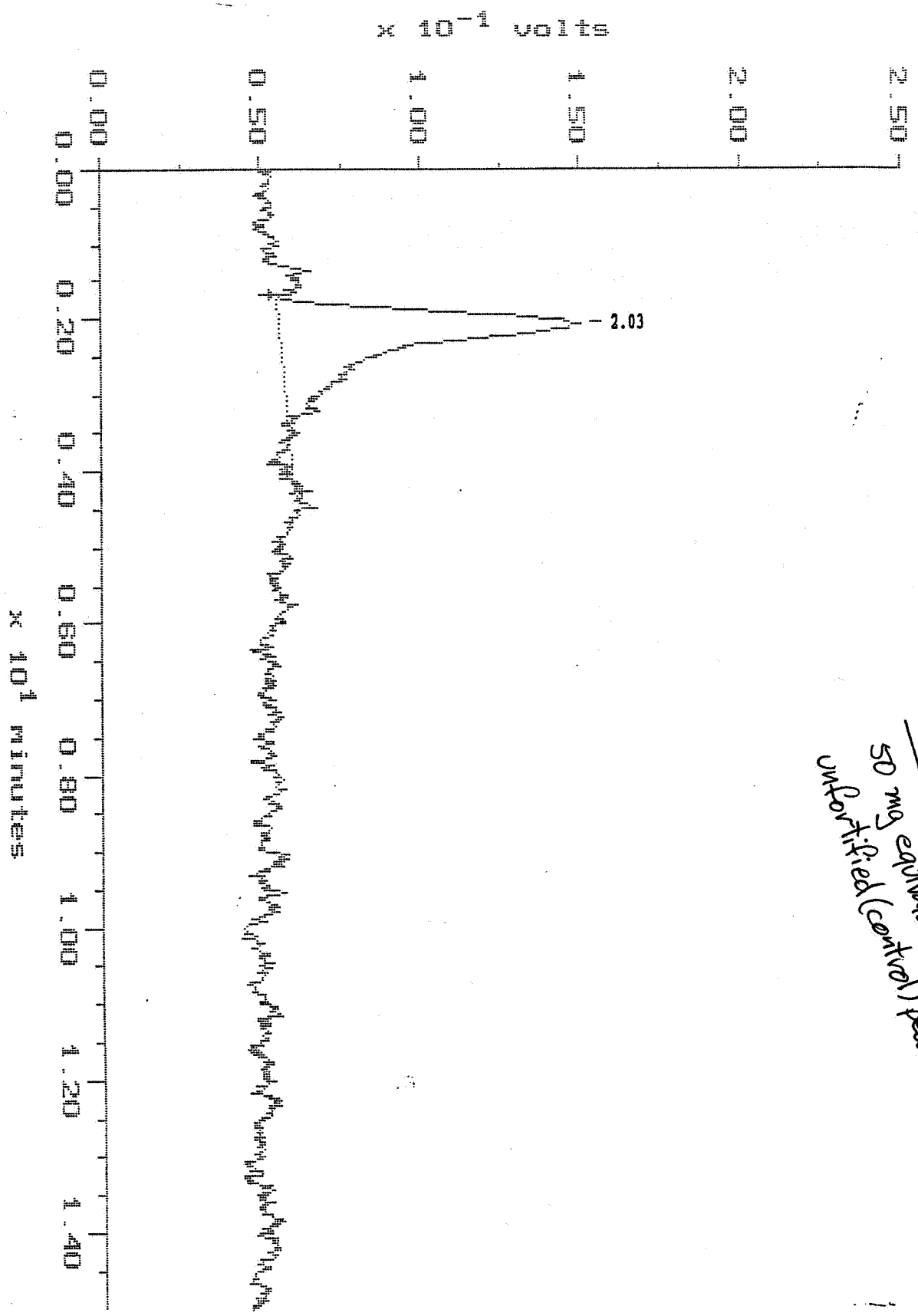
ETECTOR: FL-750 BX

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
L					3746886.9	106467.35			

9

Sample: Control-1, 50mg Channel: FL-750 BX Filename: ABA208
Acquired: 22-MAY-91 2: 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

3



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

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MAXIMA 820 PEAK INTEGRATION REPORT

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

MPLE: 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

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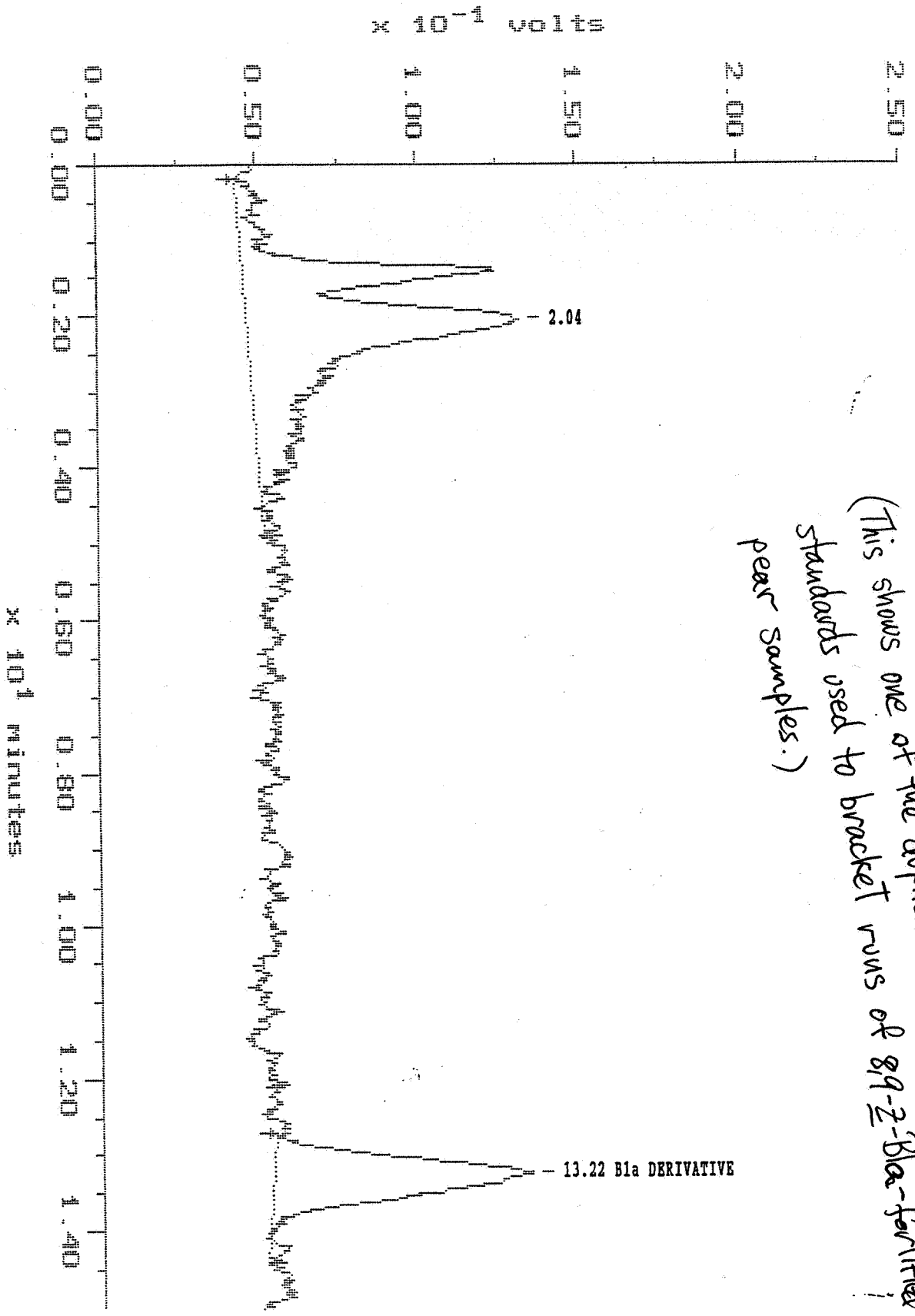

8,9-Z-B1a standard (7 ng/mL)
(This shows one of the duplicate 7 ng/mL 8,9-Z-B1a standards used to bracket runs of 8,9-Z-B1a-fortified pear samples.)

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MAXIMA 820 PEAK INTEGRATION REPORT

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

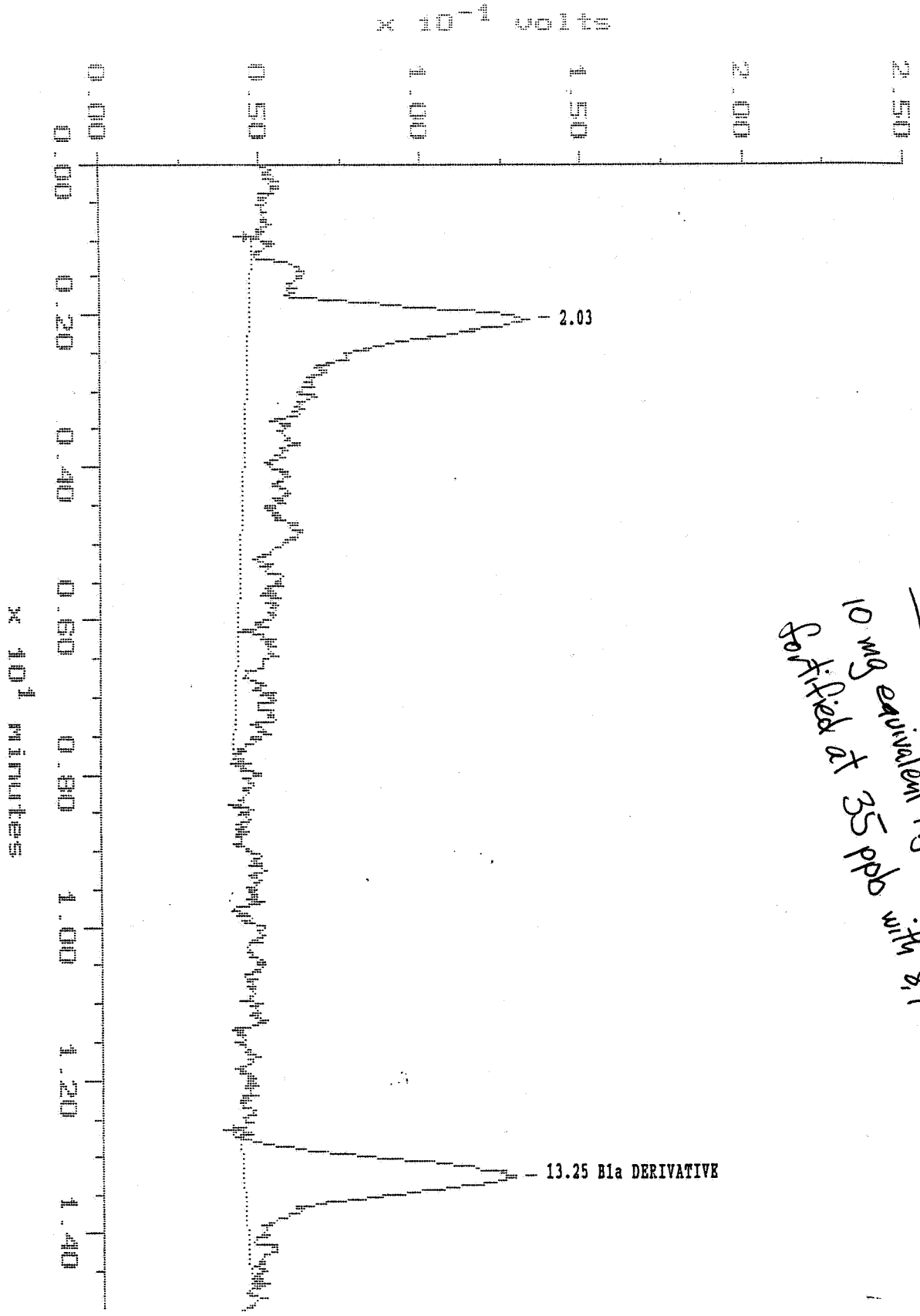
AMPLE: 89Z 522a 7ng/ml
#28 in Method: ABAMECTIN
Acquired: 22-MAY-1991 23:36
Rate: 2.0 points/sec
Duration: 15.000 minutes
Operator: BJP

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
:AL						8802619.0	166291.61			

Sample: 89Z Lo-1, 10mg 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 89-Z-Bla.

8

MAXIMA 820 PEAK INTEGRATION REPORT

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

MPLE: 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

ETECTOR: 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
L					9431973.7	170852.98			

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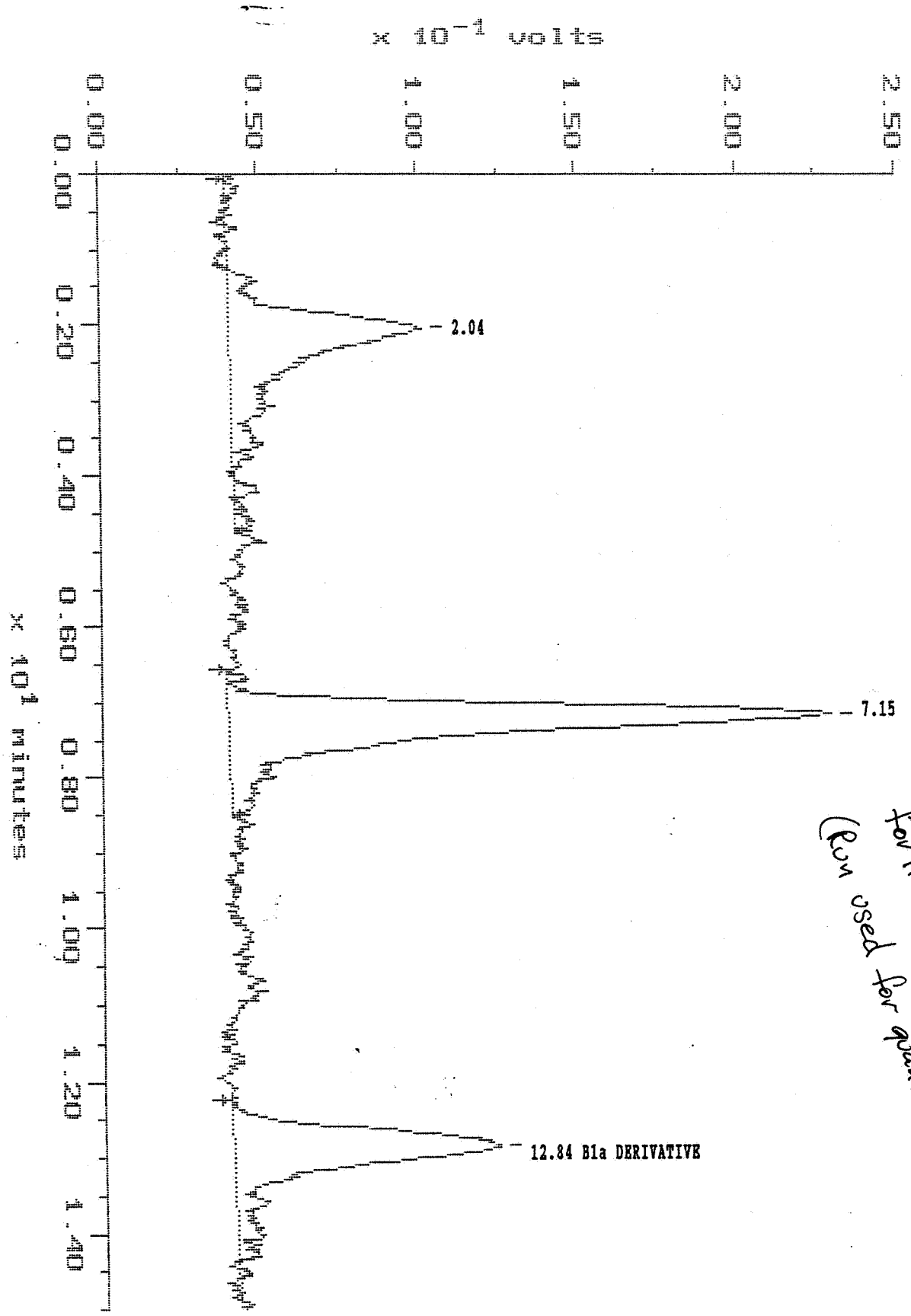
Sample: ABA Lo-2, 10mg Channel: FL-750 BX Filename: ABA259
Acquired: 11-JUN-91 20:54 Method: C:\MAX\DATA1\B90-38X1 Operator: BJP
Inj Vol: 50.00
Comments: Abamectin PMV (B90-38) Injection vol: 50ul BART PUMA AND MARK LAW



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

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MAXIMA 820 PEAK INTEGRATION REPORT

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

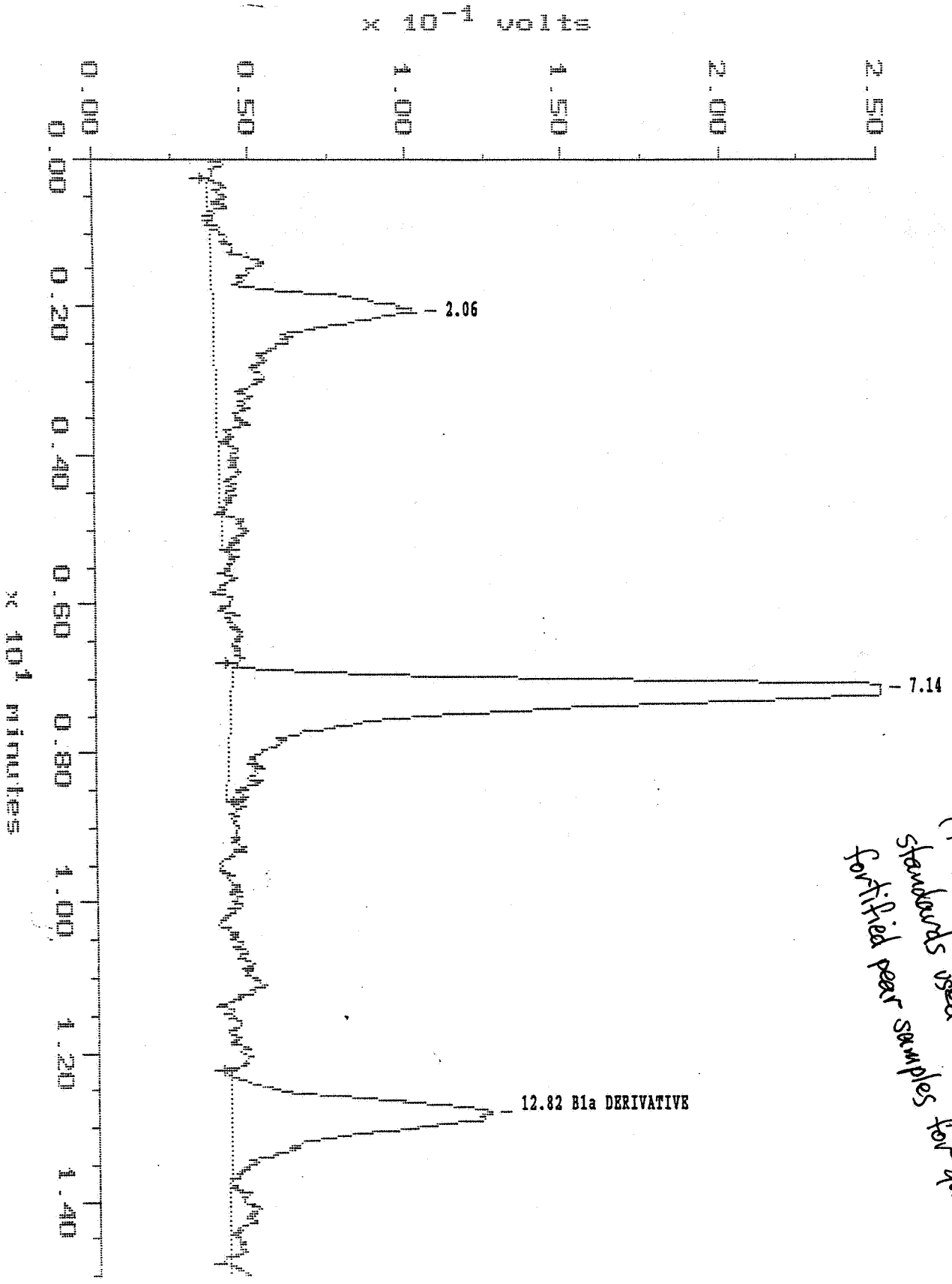
SAMPLE: ABA Lo-2, 10mg
#21 in Method: ABAMECTIN
Acquired: 11-JUN-1991 20:54
Rate: 2.0 points/sec
Duration: 16.000 minutes
Operator: BJP

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 erator: 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 abamectin-fortified pear samples for quantitation of B1a.)

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MAXIMA 820 PEAK INTEGRATION REPORT

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

SAMPLE: ABA 611b 7ng/ml
#22 in Method: ABAMECTIN
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 Filename: ABA282
Acquired: 17-JUN-91 19:44 thod: C:\MAX\DATA1\B90-38X2 erator: BJP
Inj Vol: 50.00
Comments: Abamectin PMV (B90-38) Injection vol: 50ul BART PUMA AND MARK LAW

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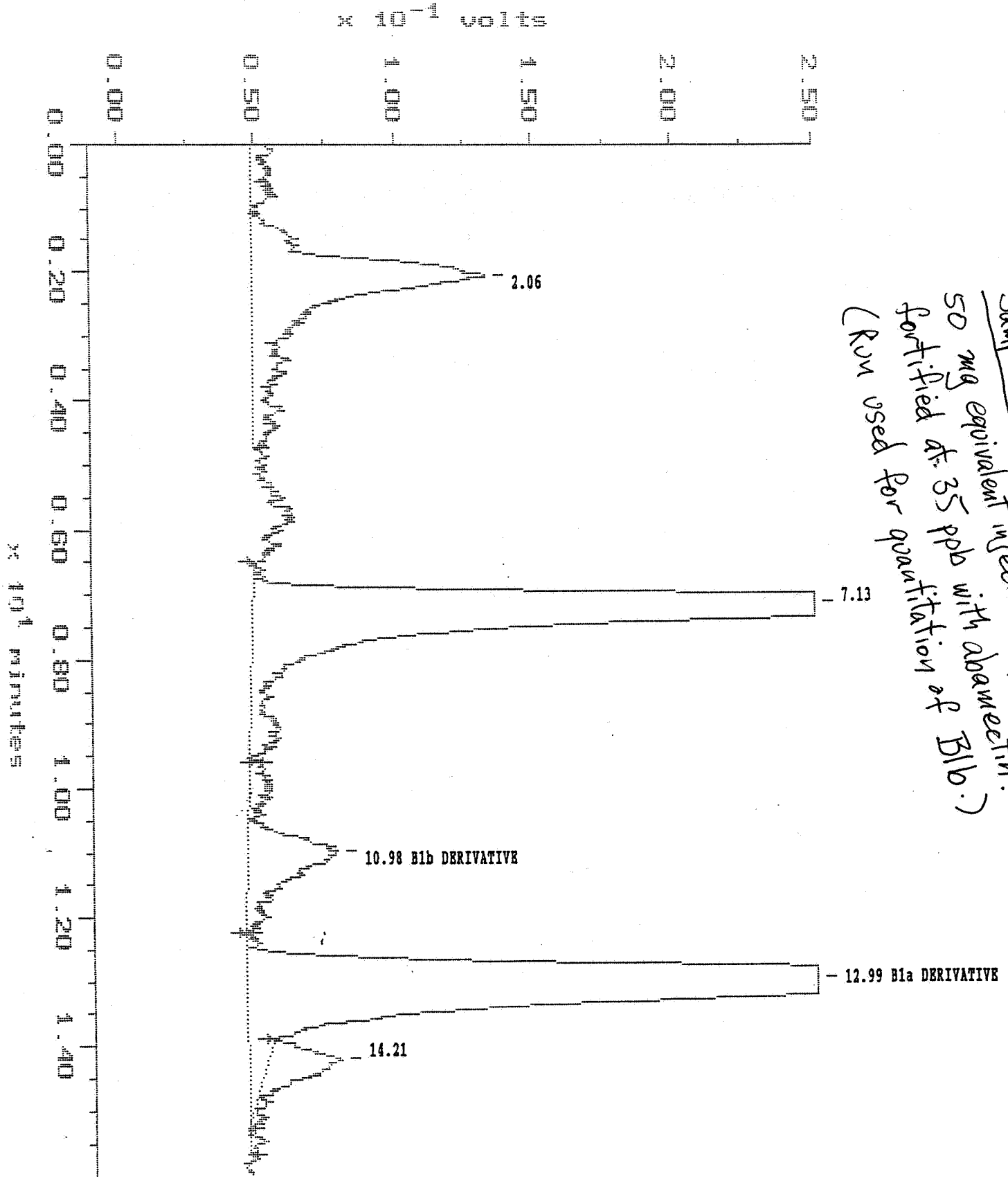

Sample No. 4
50 mg equivalent injection of pear sample fortified at 35 ppb with abamectin.
(Run used for quantitation of B1b.)

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MAXIMA 820 PEAK INTEGRATION REPORT

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

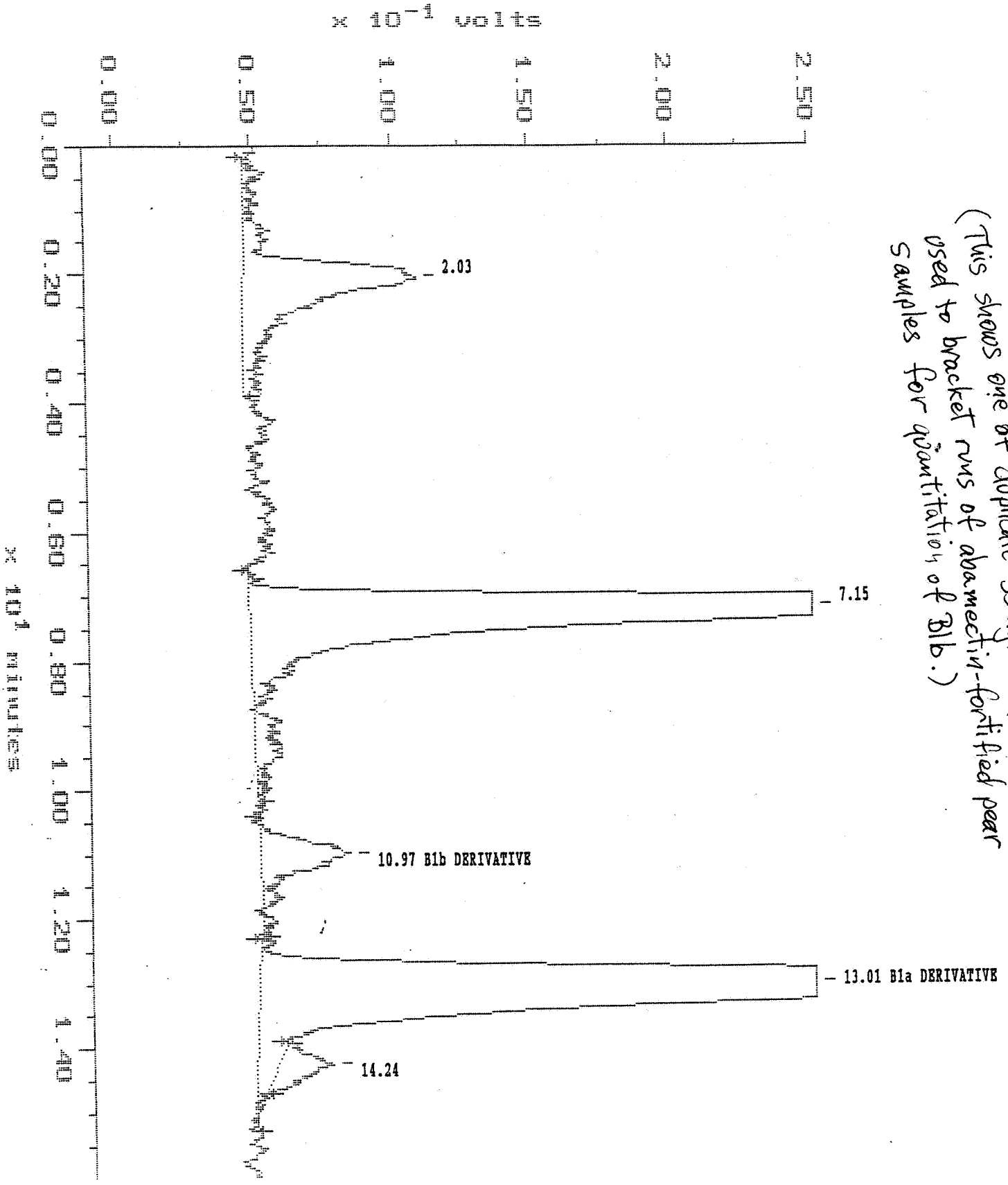
SAMPLE: ABA Lo-2, 50mg
#13 in Method: ABAMECTIN
Acquired: 17-JUN-1991 19:44
Rate: 2.0 points/sec
Duration: 16.000 minutes
Operator: BJP

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 lename: ABA283
Acquired: 17-JUN-91 20:01 ethod: C:\MAX\DATA1\B90-38X2 perator: BJP
Inj Vol: 50.00
Comments: Abamectin PMV (B90-38) Injection vol: 50ul BART PUMA AND MARK LAW



Abamectin standard (35 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.)

16

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			

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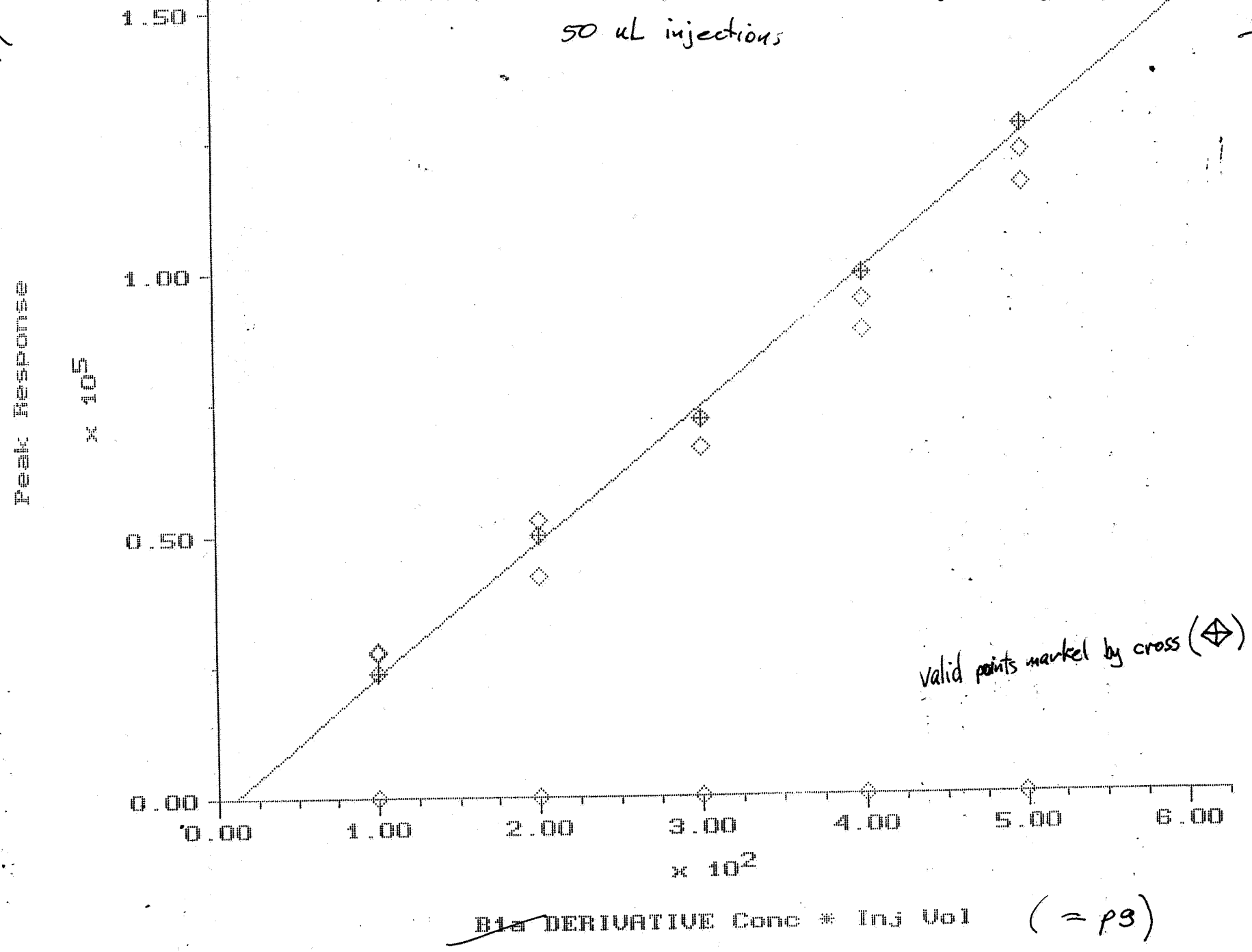
B90-38Z4.MTH

Standard Curve for 8,9-Z-Bla Standards (2-10 ng/mL)
50 uL injections



8,9-Z

~~Bla~~ DERIVATIVE Calibration Report

Method file: B90-38Z4.MTH

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

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Quant Basis: Height
Curve Type: Linear
Y-axis Label: Response
Corr. Coef. (r): 0.9992332

Rejection Tolerance: None
Weighting: None
Coef. of Determination (r^2): 0.9984671

Internal Standard: None
Forced Through Origin: No

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

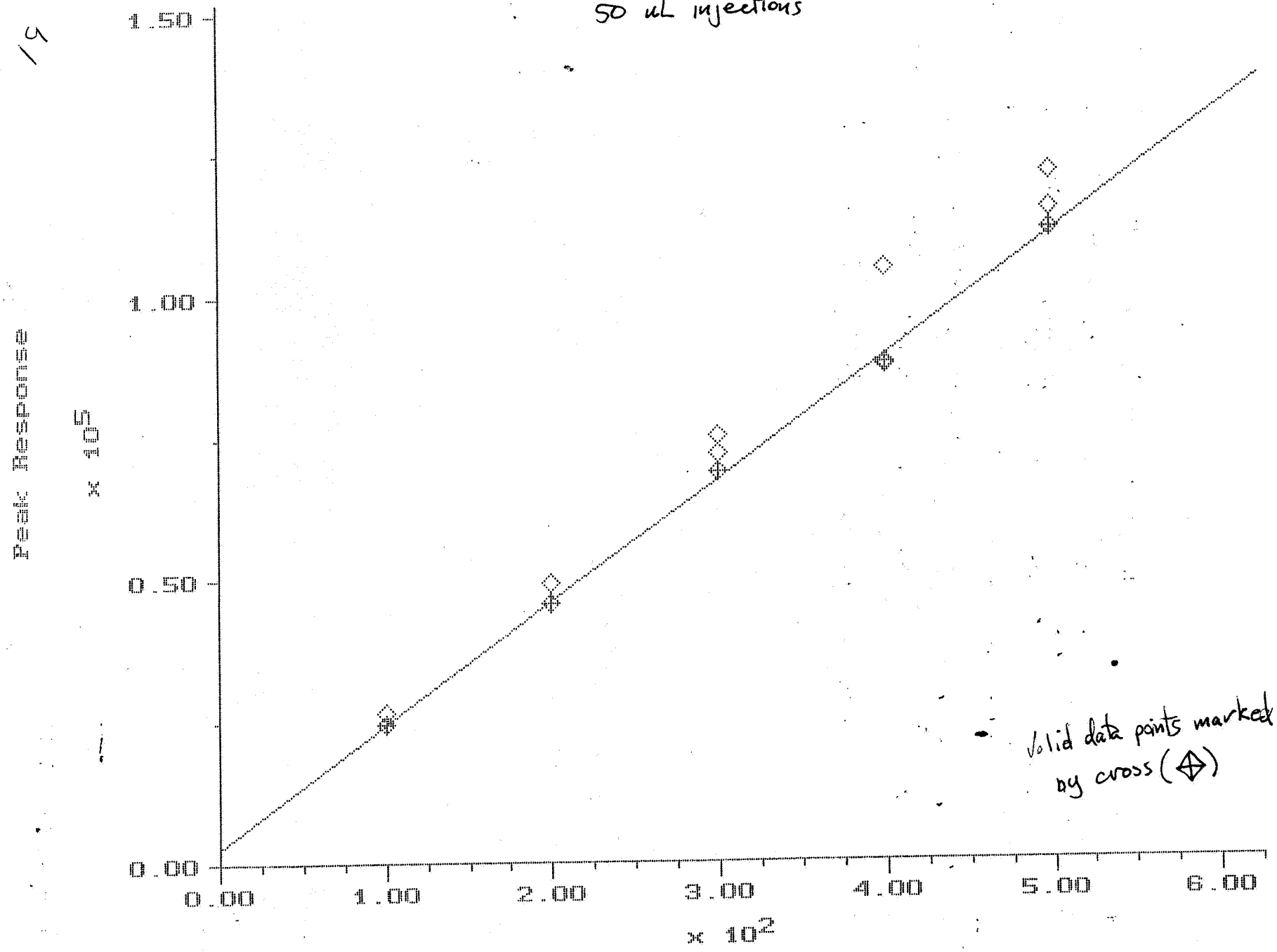
These values are for the 8,9-Z-Bla derivative standard curve indicated by valid points below.

Sample	File Name	Valid	Concentration	Response	Calc'd Concentration	% Deviation	Response Factor
Bla 522 2ng/ml	ABA181	N	2.000000E+00	0.0000000E+00	2.074144E-01	8.64E+02	Invalid
Bla 522 4ng/ml	ABA182	N	4.000000E+00	0.0000000E+00	2.074144E-01	1.83E+03	Invalid
Bla 522 6ng/ml	ABA183	N	6.000000E+00	0.0000000E+00	2.074144E-01	2.79E+03	Invalid
Bla 522 8ng/ml	ABA184	N	8.000000E+00	0.0000000E+00	2.074144E-01	3.76E+03	Invalid
Bla 522 10ng/ml	ABA185	N	1.000000E+01	0.0000000E+00	2.074144E-01	4.72E+03	Invalid
Bla 522 2ng/ml	ABA197	N	2.000000E+00	2.7794125E+04	2.371310E+00	-1.57E+01	3.597883E-03
Bla 522 4ng/ml	ABA198	N	4.000000E+00	5.2598660E+04	4.302454E+00	-7.03E+00	3.802378E-03
Bla 522 6ng/ml	ABA199	N	6.000000E+00	7.1886430E+04	5.804092E+00	3.38E+00	4.173249E-03
Bla 522 8ng/ml	ABA200	N	8.000000E+00	8.8523633E+04	7.099372E+00	1.27E+01	4.518567E-03
Bla 522 10ng/ml	ABA201	N	1.000000E+01	1.1610050E+05	9.246353E+00	8.15E+00	4.306614E-03
89Z 522 2ng/ml	ABA202	Y	2.000000E+00	2.3593518E+04	2.044274E+00	-2.17E+00	4.238452E-03
89Z 522 4ng/ml	ABA203	Y	4.000000E+00	4.9809164E+04	4.085279E+00	-2.09E+00	4.015325E-03
89Z 522 6ng/ml	ABA204	Y	6.000000E+00	7.2024023E+04	5.814804E+00	3.18E+00	4.165277E-03
89Z 522 8ng/ml	ABA205	Y	8.000000E+00	9.9288414E+04	7.937458E+00	7.88E-01	4.028667E-03
89Z 522 10ng/ml	ABA206	Y	1.000000E+01	1.2729873E+05	1.011819E+01	-1.17E+00	3.927769E-03
89Z 522 2ng/ml	ABA218	N	2.000000E+00	2.7344855E+04	2.336333E+00	-1.44E+01	3.656995E-03
89Z 522 4ng/ml	ABA219	N	4.000000E+00	4.2077426E+04	3.483329E+00	1.48E+01	4.753142E-03
89Z 522 6ng/ml	ABA220	N	6.000000E+00	6.6609180E+04	5.393235E+00	1.13E+01	4.503884E-03
89Z 522 8ng/ml	ABA221	N	8.000000E+00	9.4375680E+04	7.554980E+00	5.89E+00	4.238380E-03
89Z 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.

B90-38A1.MIH

Standard Curve for avermectin B1a standards (2-10 ng/mL)
50 uL injections



B1a DERIVATIVE Conc * Inj Vol (= pg)

B1a DERIVATIVE Calibration Report

Printed: 21-FEB-1992 10:26:30 Method file: B9D-38X1.MTH

20

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

Rejection Tolerance: None
Weighting: None
Coef. of Determination (r^2): 0.9991290

Internal Standard: None
Forced Through Origin: No

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

These values are for the B1a derivative standard curve indicated by the valid points shown below.

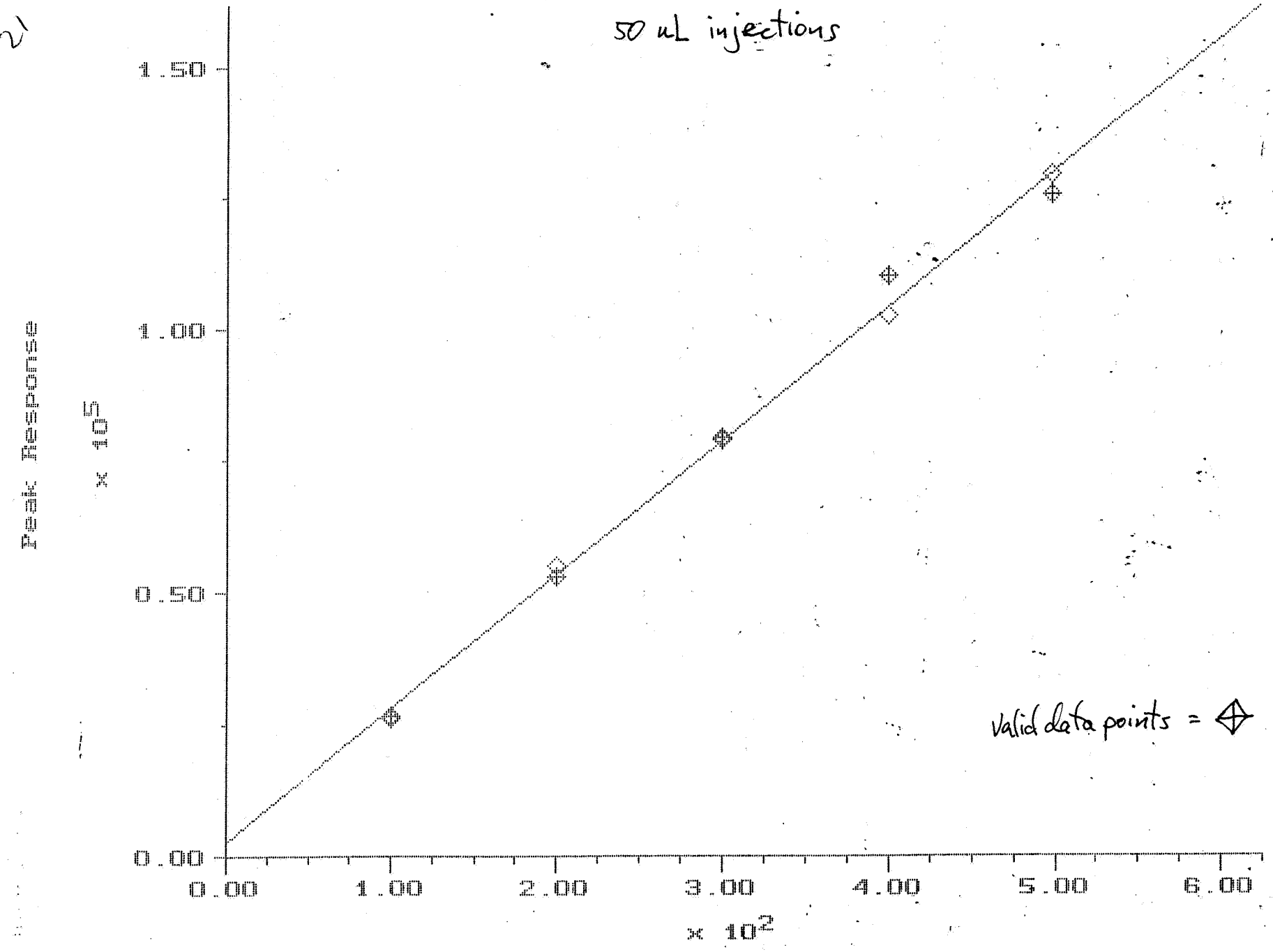
Sample	File Name	Valid	Concentration	Response	Calc'd Concentration	% Deviation	Response Facto
B1a 611 2ng/ml	ABA239	Y	2.000000E+00	2.4484279E+04	2.001114E+00	-5.57E-02	4.084253E-03
B1a 611 4ng/ml	ABA240	Y	4.000000E+00	4.5688215E+04	3.958572E+00	1.05E+00	4.377496E-03
B1a 611 6ng/ml	ABA241	Y	6.000000E+00	6.9136898E+04	6.123256E+00	-2.01E+00	4.339217E-03
B1a 611 8ng/ml	ABA242	Y	8.000000E+00	8.8094211E+04	7.873315E+00	1.61E+00	4.540593E-03
B1a 611 10ng/ml	ABA243	Y	1.000000E+01	1.1160512E+05	1.004374E+01	-4.36E-01	4.480081E-03
B1a 611 2ng/ml	ABA252	N	2.000000E+00	2.6641848E+04	2.200292E+00	-9.10E+00	3.753493E-03
B1a 611 4ng/ml	ABA253	N	4.000000E+00	4.9237047E+04	4.286185E+00	-6.68E+00	4.061982E-03
B1a 611 6ng/ml	ABA254	N	6.000000E+00	7.5542008E+04	6.714549E+00	-1.06E+01	3.971300E-03
B1a 611 8ng/ml	ABA255	N	8.000000E+00	1.0480035E+05	9.415556E+00	-1.50E+01	3.816781E-03
B1a 611 10ng/ml	ABA256	N	1.000000E+01	1.2155880E+05	1.096262E+01	-8.78E+00	4.113236E-03
B1a 611 2ng/ml	ABA265	N	2.000000E+00	2.4433318E+04	1.996410E+00	1.80E-01	4.092772E-03
B1a 611 4ng/ml	ABA266	N	4.000000E+00	4.5594070E+04	3.949881E+00	1.27E+00	4.386535E-03
B1a 611 6ng/ml	ABA267	N	6.000000E+00	7.2195148E+04	6.405581E+00	-6.33E+00	4.155404E-03
B1a 611 8ng/ml	ABA268	N	8.000000E+00	8.7540070E+04	7.822159E+00	2.27E+00	4.569336E-03
B1a 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 B1a standards (2-10 ng/mL) in the sample queue used for quantitation of B1a 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 B1a standard set. The pear samples and bracketing standards were run again between the second and third runs of the B1a standard set.

B90-38X2.MTH

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

50 uL injections



B1b DERIVATIVE Conc*Inj Vol (= pg)

B1b DERIVATIVE Calibration Report

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

Method file: B90-38X2.MTH

22

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

Rejection Tolerance: None
Weighting: None
Coef. of Determination (r^2): 0.9915346

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

Internal Standard: None
Forced Through Origin: No

These values are for the B1b derivati[illegible] 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

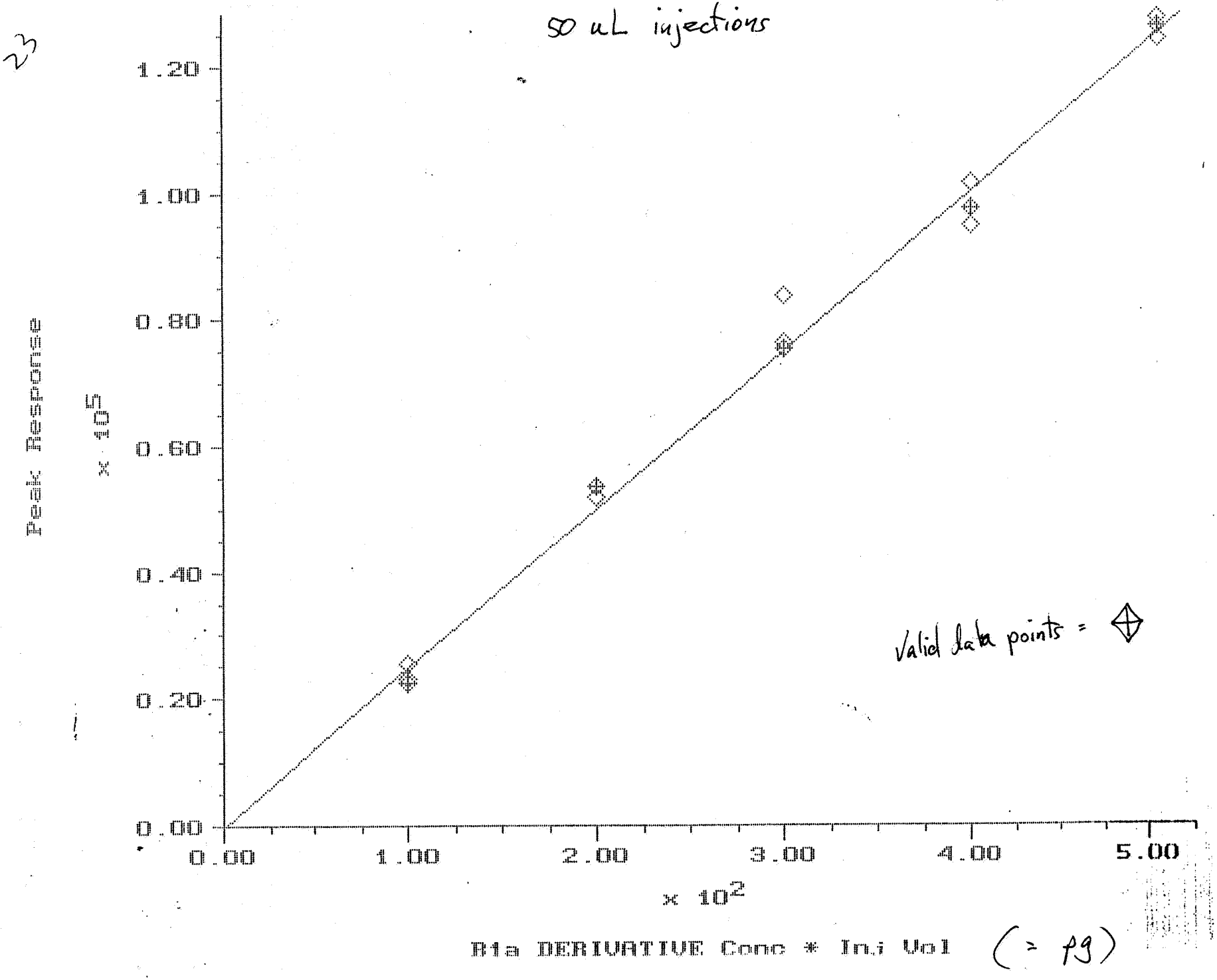
This calibration report is for the first ~~of~~ of two runs of a complete set of 5 B1b standards (2-10 ng/mL) in the sample queue used for quantitation of B1b in abamectin-fortified samples. Each B1b standard contained B1a at a concentration of 13.46 times that of B1b so that the set of B1b standards contained B1a at levels from ca 26.9 to 134.6 ng/mL. Besides these B1b standards containing high levels of B1a, the sample queue for this method file (B90-38X2.MTH) was arranged to include three runs of a complete set of 5 B1a standards at 2-10 ng/mL. A standard curve for one run of the 2-10 ng/mL B1a standard set and a standard curve for B1a in one run of the B1b standard set containing B1a 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 B1a abamectin standards, respectively, immediately after the first runs of the B1b standard set and immediately before the repeat runs of this set

B90-38X2.MTH

23

Standard Curve for avermectin B1a standards (2-10 ng/mL)
50 uL injections



(= pg)

20

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 (r^2): 0.9959060

Internal Standard: None
Forced Through Origin: No

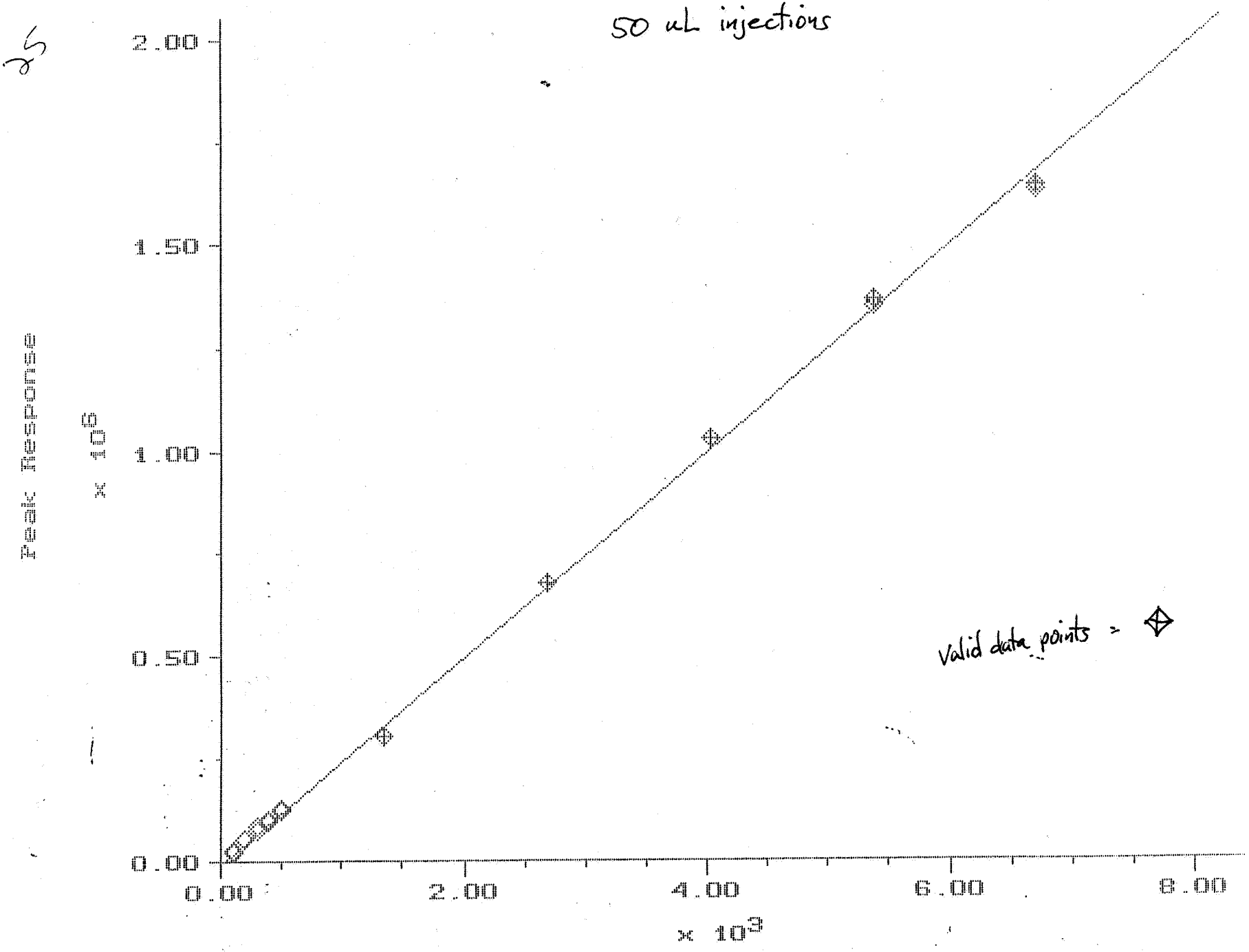
Equation: Conc*(Inj Vol) = 1.163702E+00 + 3.976333E-03 * R

These values are for the Blc 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 Facto
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 B1a standards (26.9-134.6 ng/mL)
50 uL injections



B1a DERIVATIVE Conc * Inj Vol (= pg)

B1a DERI ATIVE Calibra 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
Coef. of Determination (r^2): 0.9969664

Internal Standard: None
Forced Through Origin: No

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

These values are for the B1a standard curve given by the data points show as valid (Y) in the third column below. (This B1a standard curve is for the high concentrations of B1a in the B1b standard

Sample	File Name	Valid	Concentration	Response	Calc'd Concentration	% Deviation	Response Factor
B1a 617 2ng/ml	ABA270	N	2.000000E+00	2.2598955E+04	2.386745E+00	-1.62E+01	4.424983E-03
B1a 617 4ng/ml	ABA271	N	4.000000E+00	5.3642039E+04	4.864200E+00	-1.78E+01	3.728419E-03
B1a 617 6ng/ml	ABA272	N	6.000000E+00	7.5515992E+04	6.609894E+00	-9.23E+00	3.972668E-03
B1a 617 8ng/ml	ABA273	N	8.000000E+00	9.7695453E+04	8.379970E+00	-4.53E+00	4.094356E-03
B1a 617 10ng/ml	ABA274	N	1.000000E+01	1.2631623E+05	1.066411E+01	-6.23E+00	3.958319E-03
B1b 617 2ng/ml	ABA275	Y	2.692000E+01	3.0332112E+05	2.479034E+01	8.59E+00	4.437541E-03
B1b 617 4ng/ml	ABA276	Y	5.384000E+01	6.7561956E+05	5.450235E+01	-1.22E+00	3.984491E-03
B1b 617 6ng/ml	ABA277	Y	8.076000E+01	1.0328721E+06	8.301360E+01	-2.71E+00	3.909487E-03
B1b 617 8ng/ml	ABA278	Y	1.076800E+02	1.3673144E+06	1.097044E+02	-1.85E+00	3.937646E-03
B1b 617 10ng/ml	ABA279	Y	1.346000E+02	1.6440430E+06	1.317893E+02	2.13E+00	4.093567E-03
B1a 617 2ng/ml	ABA288	N	2.000000E+00	2.5460066E+04	2.615081E+00	-2.35E+01	3.927720E-03
B1a 617 4ng/ml	ABA289	N	4.000000E+00	5.1941520E+04	4.728486E+00	-1.54E+01	3.850484E-03
B1a 617 6ng/ml	ABA291	N	6.000000E+00	8.3730797E+04	7.265493E+00	-1.74E+01	3.582911E-03
B1a 617 8ng/ml	ABA292	N	8.000000E+00	1.0172520E+05	8.701572E+00	-8.06E+00	3.932162E-03
B1a 617 10ng/ml	ABA293	N	1.000000E+01	1.2789659E+05	1.079023E+01	-7.32E+00	3.909408E-03
B1b 617 2ng/ml	ABA302	N	2.692000E+01	3.0647781E+05	2.504226E+01	7.50E+00	4.391835E-03
B1b 617 4ng/ml	ABA303	N	5.384000E+01	6.7486287E+05	5.444196E+01	-1.11E+00	3.988959E-03
B1b 617 6ng/ml	ABA304	N	8.076000E+01	1.0326619E+06	8.299682E+01	-2.70E+00	3.910283E-03
B1b 617 8ng/ml	ABA305	N	1.076800E+02	1.3545125E+06	1.086828E+02	-9.23E-01	3.974862E-03
B1b 617 10ng/ml	ABA306	N	1.346000E+02	1.6291387E+06	1.305998E+02	3.06E+00	4.131017E-03
B1a 617 2ng/ml	ABA307	N	2.000000E+00	2.3028107E+04	2.420994E+00	-1.74E+01	4.342519E-03
B1a 617 4ng/ml	ABA308	N	4.000000E+00	5.2111840E+04	4.742079E+00	-1.56E+01	3.837899E-03
B1a 617 6ng/ml	ABA309	N	6.000000E+00	7.6571445E+04	6.694126E+00	-1.04E+01	3.917910E-03
B1a 617 8ng/ml	ABA310	N	8.000000E+00	9.4964422E+04	8.162014E+00	-1.98E+00	4.212104E-03
B1a 617 10ng/ml	ABA311	N	1.000000E+01	1.2437538E+05	1.050921E+01	-4.85E+00	4.020088E-03