

MRID 43755901

BENSULIDE

ANALYTICAL METHOD FOR THE DETERMINATION OF BENSULIDE AND BENSULIDE  
OXON IN VARIOUS CROPS BY GAS CHROMATOGRAPHY

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Method Number

CSI-009-00

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- I. Principle of Method: A subsample of the raw agricultural commodity is macerated with toluene in an appropriate container. The sample is then taken to dryness and redissolved in hexane. The residue is then partitioned into acetonitrile, extracted with a 2% citral in toluene solution, and washed with a saturated sodium chloride solution. Aliquots of the extract are injected into the gas chromatograph and the amount of bensulide and bensulide oxon is determined by the comparison of the peak heights of the samples and standards.
  
- II. Materials and Apparatus
  - A. Materials
    1. Bensulide, of known purity
    2. Bensulide Oxon, of known purity
    3. Hexane, reagent grade, distilled in glass
    4. Toluene, reagent grade, distilled in glass
    5. Acetonitrile, reagent grade, distilled in glass
    6. Citral 95% pure, mixture of cis and trans isomers
    7. Deionized water
    8. Sodium Chloride, granular AR grade
    9. Anhydrous Sodium Sulfate, granular AR grade
  
  - B. Standard Solutions
    1. Bensulide/Bensulide Oxon - Weigh accurately to the nearest 0.1 mg, about 25 mg of analytical standard into a clean 25 ml volumetric flask. Dilute to volume with toluene and mix thoroughly.
    2. Fortification solutions are made by making serial dilutions of II.B.1 in toluene.
    3. 2% Citral Solution (v/v) - Measure 20 ml of 95 % pure citral. Dilute to one liter with toluene and mix thoroughly.
    4. Reference standards are made by diluting II.B.2 with II.B.3 with concentrations as appropriate. Typical dilutions are 0.03 to 1 ug bensulide/ml and oxon/ml.

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C. Apparatus

1. Hewlett Packard 5890A Series II Gas Chromatograph with Nitrogen-Phosphorous Detector (or equivalent) and a Hewlett Packard 7673 Automatic Sampler (or equivalent)
2. 15 meter by 0.53 mm ID fused silica megabore column, with a 1.5 micron film thickness of cross-linked methyl silicone (DB-1, J&W Scientific)
3. Syringe: 10 ul or equivalent injection system to reproducibly introduce 3 ul of sample onto the GC column
4. Class A volumetric glassware shall be used throughout this method.

III. Preparation of Samples

- A. Accurately weigh 100 g of crop into an appropriate container.
- B. Accurately add 200 ml of toluene, reagent grade, and macerate with a Brinkmann Polytron (or equivalent).
- C. Centrifuge for at least 10 minutes at 2,000 rpm.
- D. Filter liquid phase through sodium sulfate and record change in volume.
- E. Transfer and concentrate to dryness.
- F. Transfer the residue to a separatory funnel using 50-100 ml of hexane.
- G. Extract 3 times with 10 ml portions of acetonitrile, using the first 10 ml to aid in the transfer. If needed, centrifuge partitions to break emulsions.
- H. Combine acetonitrile fractions in a 500 ml separatory funnel, add 200 ml of deionized water, 100 ml of saturated sodium chloride solution, and 50 ml 2% Citral in toluene and shake vigorously.
- I. Discard aqueous phase and wash the toluene layer 2 more times with 100 ml deionized water and 50 ml of saturated sodium chloride solution each time.
- J. Collect an aliquot of toluene layer in a culture tube over anhydrous sodium sulfate and shake.
- K. Transfer an adequate amount to an autosampler vial and proceed to step IV.A.3.

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IV. Quantitative Determination of the Active Ingredient

A. Instrumental analysis

1. Instrumental conditions should be set as follows: Injector temperature, 230°C, Detector temperature, 280°C; Initial Column Temperature 180°C with a one minute hold time, ramp at 10°C per minute to 250°C and hold at 250°C for eight minutes; Carrier gas, helium, about 30 ml per minute including make up; detector gases, air about 100 ml per minute, and hydrogen, about 3 ml per minute. (The gas flows, chart speed and attenuation should be set to obtain the optimum response and reproducibility for the instrument being used.)
2. Prepare a standard curve by injecting 3.0 uL aliquot of the solutions in II.B.4.
3. Inject a 3.0 uL aliquot of the solution from III.K. into the chromatograph.
4. The injection sequence should be duplicate sample injections followed by an injection of a standard solution. Monitor the response of the standards to determine that it has remained linear.
5. The number of injections and the sequence may be altered according to the needs and requirements of the analysis.
6. Determine the peak heights of the analytes and standard solutions by a suitable method (integrator or data acquisition system preferred).
7. Determine the amount of bensulide and bensulide oxon in the crop as shown below.

B. Calculations

1. Prepare a standard curve by plotting heights of the standard solutions from II.B.2. versus concentration.
2. Determine the slope and intercept of the standard curve by a suitable method.
3. Using the slope and intercept determined from the standard curve, the amount of bensulide (bensulide oxon) in the crop may be obtained by the following equation:

$$\text{ug bensulide} = \frac{(\text{Peak height bensulide}) - (\text{Intercept})}{(\text{Slope})} \times \text{dilution factor} \quad (\text{Equation 1})$$

4. To determine the concentration bensulide (bensulide oxon) in the crop, the following equation may be used:

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$$\text{ppm (ug bensulide/gram) crop} = \frac{\text{ug of bensulide}}{\text{gram of crop}} \quad (\text{Equation 2})$$

V. Applicability

This method has been successfully applied to the analysis of bensulide and oxon in cabbage and carrots. No other raw agricultural products have been analyzed at this time.

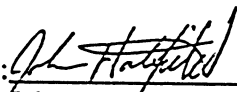
VI. Recoveries

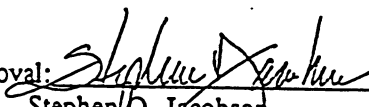
Recoveries of crop samples spiked with bensulide/oxon at approximately 0.05 to 0.5 ppm bensulide/g crop are shown in the Table below.

Crop	Fortification Level	Average % Recovery	
		Bensulide	Oxon
Cabbage	0.05 ppm	99.9	92.0
Cabbage	0.1 ppm	103.6	92.3
Cabbage	0.5 ppm	101.4	98.4
Carrots	0.05 ppm	93.7	110.1
Carrots	0.1 ppm	93.3	101.8
Carrots	0.5 ppm	95.1	98.9

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VI. Approval

Author:  Date: 9/28/93  
John Hatfield  
Residue Chemist, Compliance Services International

Approval:  Date: 9/28/93  
Stephen O. Jacobson  
Laboratory Manager, Compliance Services International

APPENDIX 3. Representative Chromatograms

Figure 1. Chromatogram of Bensulide and Bensulide Oxon standards

Chromatogram *GOWN 9201 11/22/93 GA*

Sample Name : STANDARD .3  
FileName : c:\2700\valoy\gown9201\cabval\NV19016.raw  
Method : VGWN.ins  
Start Time : 0.00 min  
Scale Factor: 1.0

Sample #: .3  
Date : 11/19/93 03:44 PM  
Time of Injection: 11/19/93 03:22 PM  
Low Point : -0.05 mV  
Plot Scale: 1.4 mV

Page 1 of 1  
High Point : 1.36 mV  
End Time : 22.50 min  
Plot Offset: -0 mV

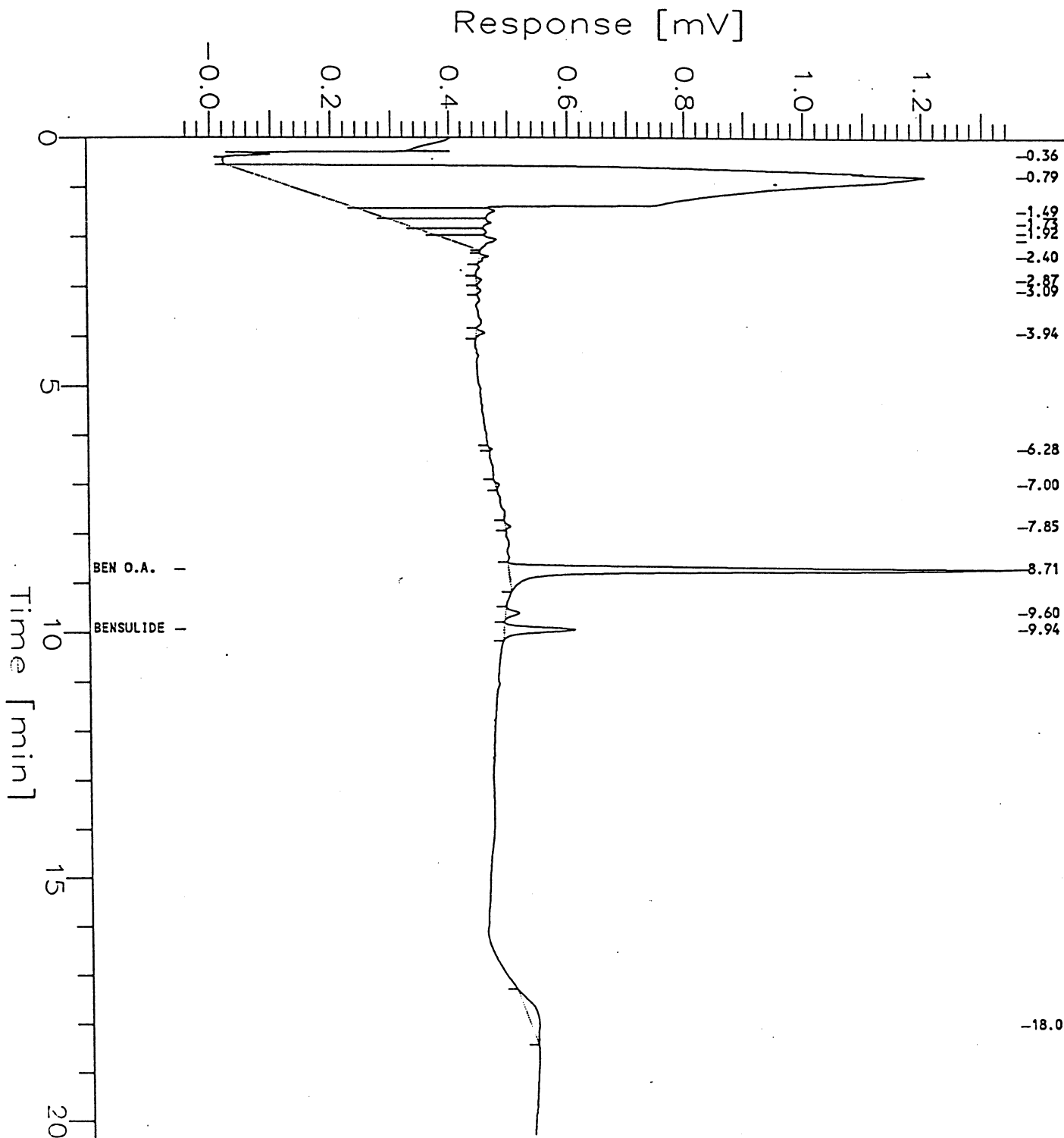


Figure 2. Chromatogram, Untreated Cabbage (Florida) with Wrapper Leaves - 42FL-91-634-1  
 Spiked 0.1 ppm each Bensulide and Oxon

(GOWN-9201 & 9203) 9202

Sample Name : CABBAGE  
 FileName : C:\2700\VALOY\GOWN9201\CABVAL\OCT1005.RAW  
 Method : VGWN  
 Start Time : 0.00 min  
 Scale Factor : 1.0

End Time : 15.99 min  
 Plot Offset : 0 mV

Sample #: GOWN156A-S  
 Date : 4/21/95 04:43 PM  
 Time of Injection: 10/2/93 03:44 AM  
 Low Point : 0.50 mV  
 High Point : 1.30 mV  
 Plot Scale: 0.8 mV

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*4/21/95 AW*

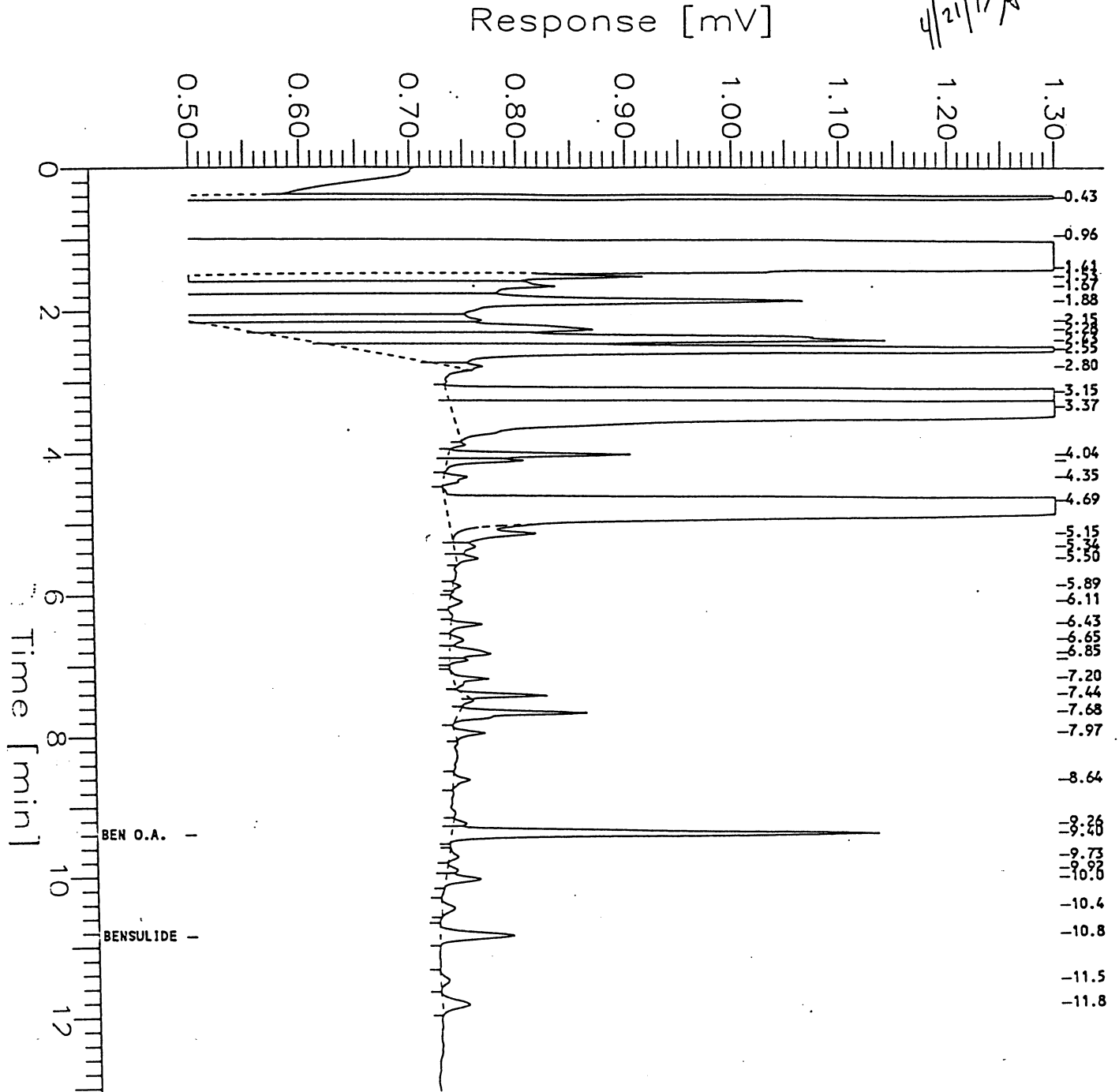


Figure 3. Chromatogram, Untreated Cabbage (Florida) without Wrapper Leaves - 42FL-91-634-3  
 Spiked 0.1 ppm each Bensulide and Oxon

(GOWN-9201 & 9203) 9202

Sample Name : CABBAGE  
 FileName : C:\2700\VALOY\GOWN9201\CABVAL\OCT1006.RAW  
 Method : VGNN  
 Start Time : 0.00 min  
 Scale Factor : 1.0

Sample #: GOWN160A-S  
 Date : 4/21/95 04:44 PM  
 Time of Injection: 10/2/93 04:08 AM  
 Low Point : 0.50 mV  
 Plot Scale: 0.8 mV  
 High Point : 1.30 mV

*4/21/95*

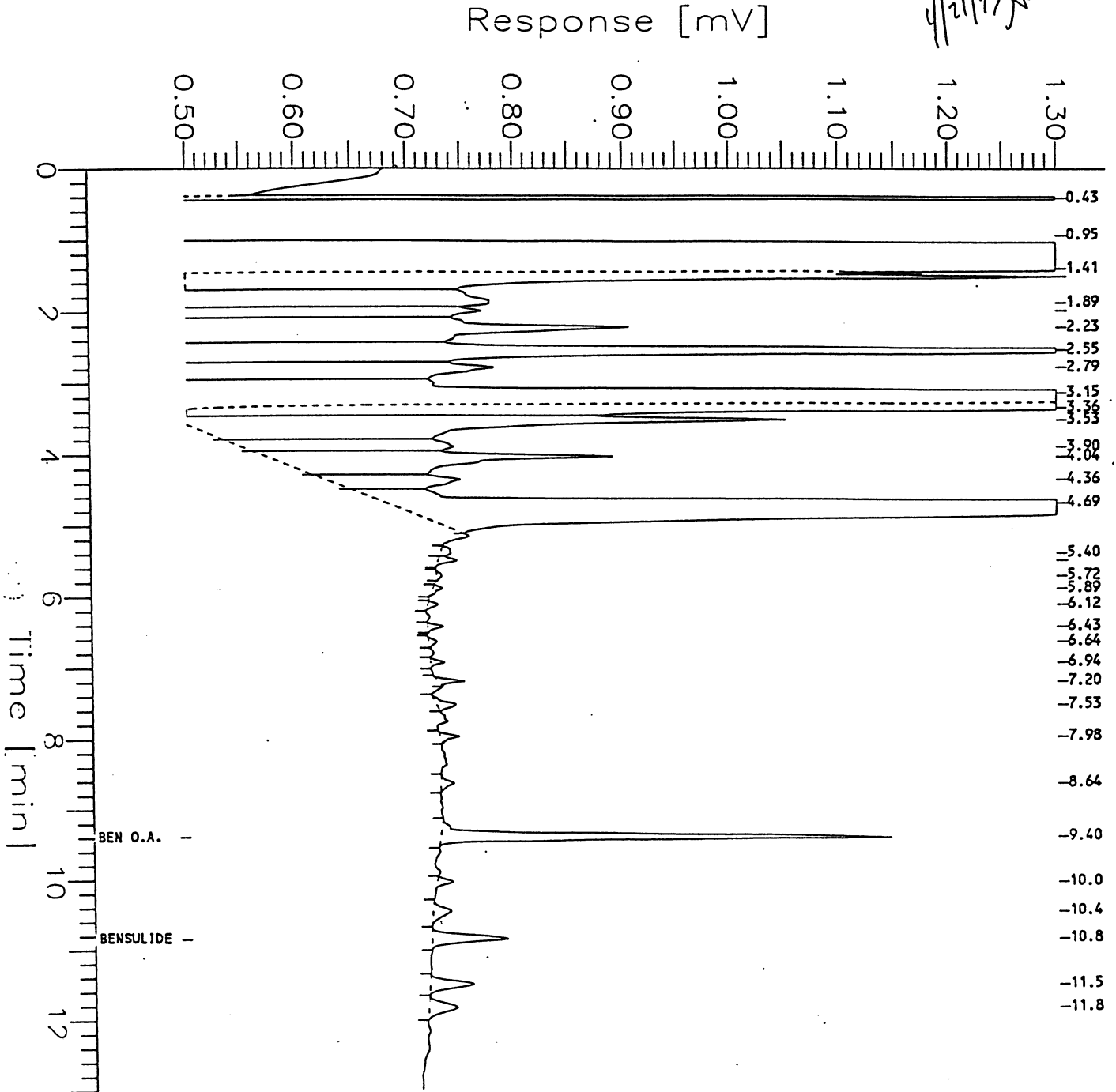


Figure 4. Chromatogram, Untreated Cabbage (Florida) with Wrapper Leaves - 42FL-91-634-1

(GOWN-9201 & 9203) 9202

Sample Name : CABBAGE  
 FileName : C:\2700\VALOY\GOWN9201\CABVAL\OCT1001.RAW  
 Method : VGWN  
 Start Time : 0.00 min  
 Scale Factor : 1.0

Sample #: GOWN156A  
 Date : 4/21/95 04:41 PM  
 Time of Injection: 10/2/93 01:18 AM  
 Low Point : 0.50 mV  
 Plot Scale: 0.8 mV  
 End Time : 15.99 min  
 Plot Offset: 0 mV  
 High Point : 1.30 mV

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*4/21/95 AW*

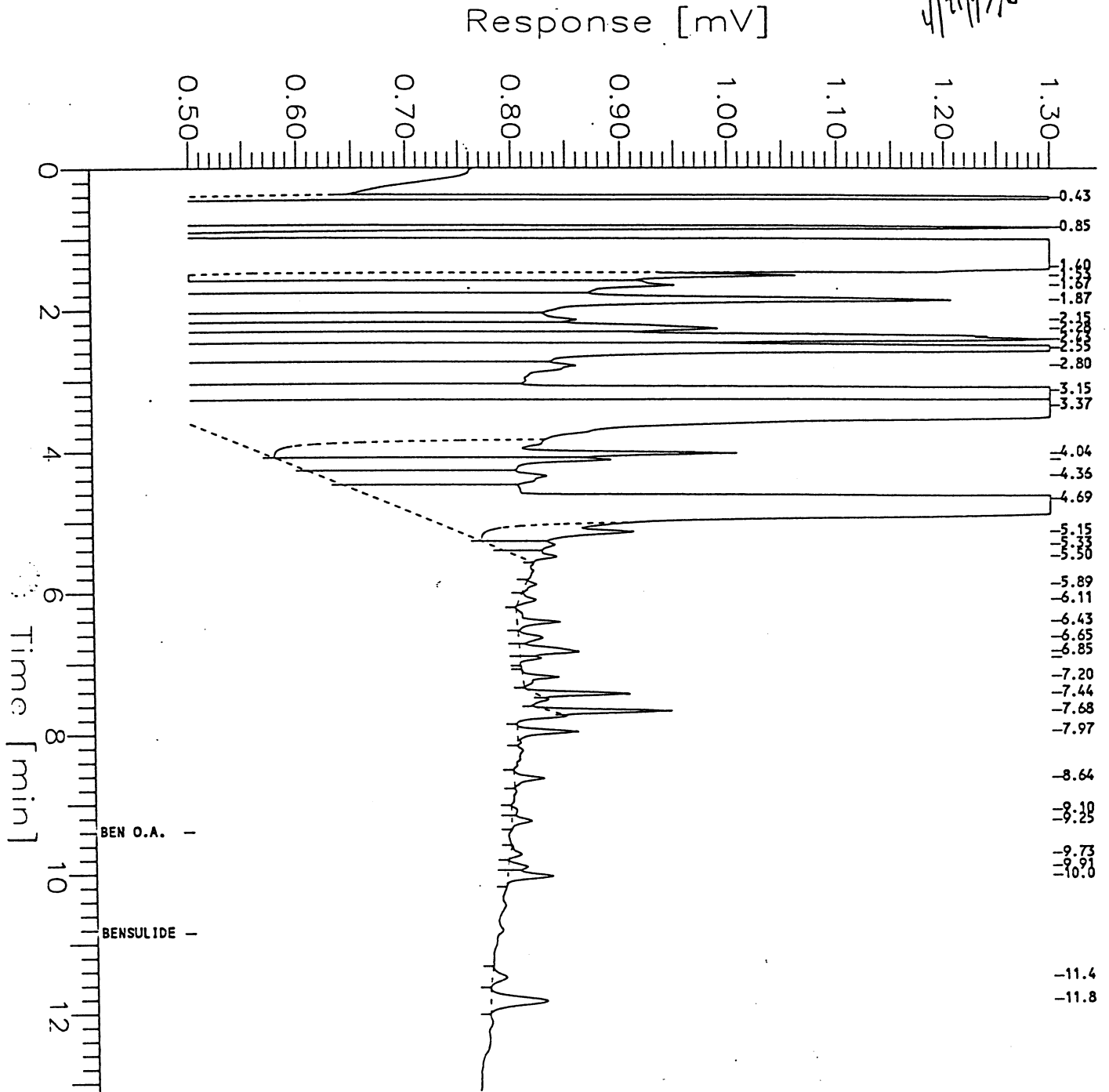


Figure 5. Chromatogram, Treated Cabbage (Florida) with Wrapper Leaves - 42FL-91-634-2

(GOWN-9201 & 9203), 4102

Sample Name : CABBAGE  
 FileName : C:\2700\VALOY\GOWN9201\CABVAL\OCT1002.RAW  
 Method : VGWN  
 Start Time : 0.00 min  
 Scale Factor: 1.0

Sample #: GOWN158A  
 Date : 4/21/95 04:42 PM  
 Time of Injection: 10/2/93 01:42 AM  
 Low Point : 0.50 mV  
 Plot Scale: 0.8 mV

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*4/21/95 AW*

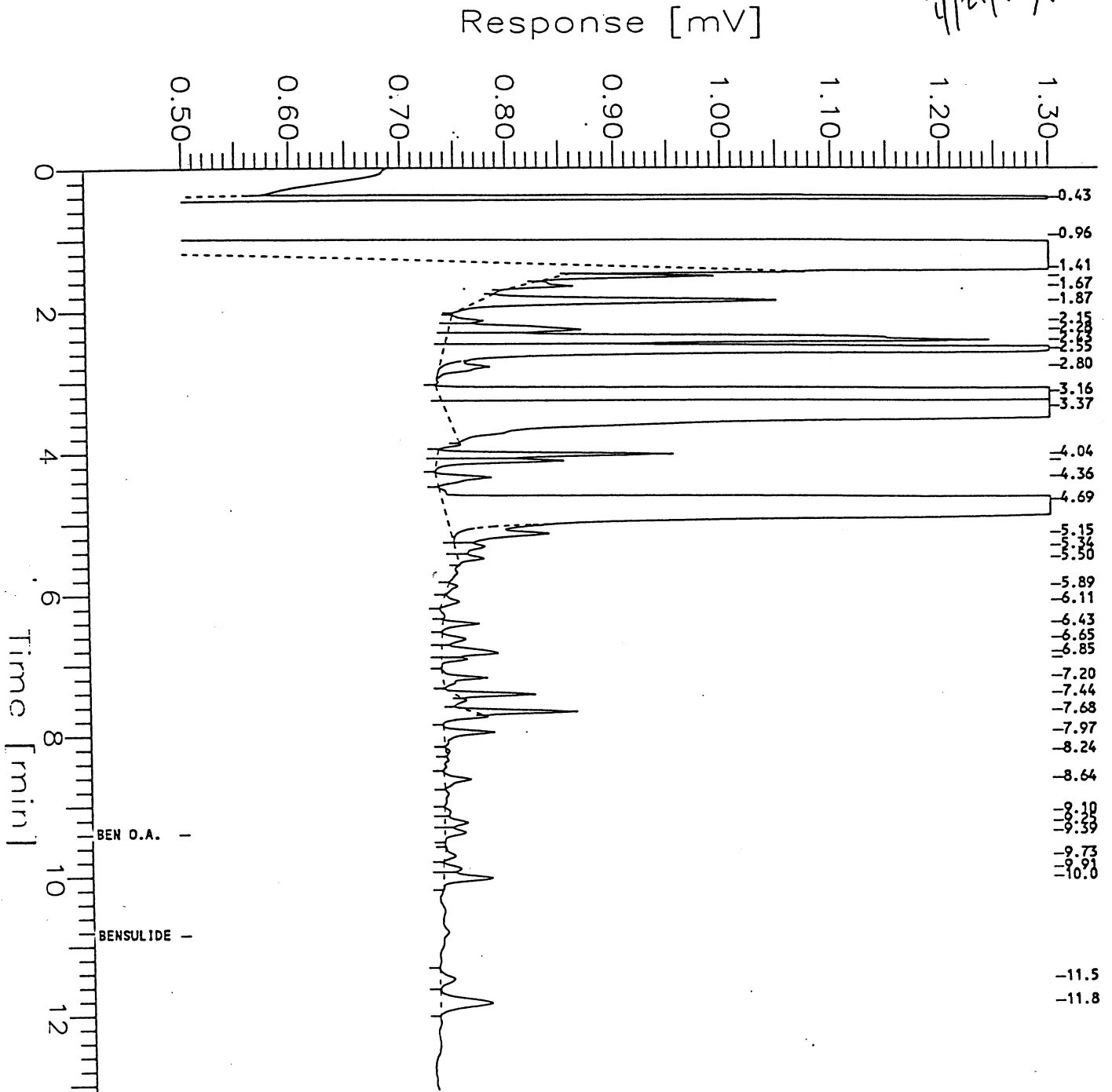


Figure 6. Chromatogram, Untreated Cabbage (Florida) without Wrapper Leaves - 42FL-91-634-3

(GOWN-9201 & 9203), 9202

Sample Name : CABBAGE  
 FileName : C:\2700\VALOY\GOWN9201\CABVAL\OCT1003.RAW  
 Method : VGWN  
 Start Time : 0.00 min  
 Scale Factor : 1.0

Sample #: GOWN160A  
 Date : 4/21/95 04:42 PM  
 Time of Injection: 10/2/93 02:31 AM  
 Low Point : 0.50 mV  
 Plot Scale: 0.8 mV

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*4/21/95 AW*

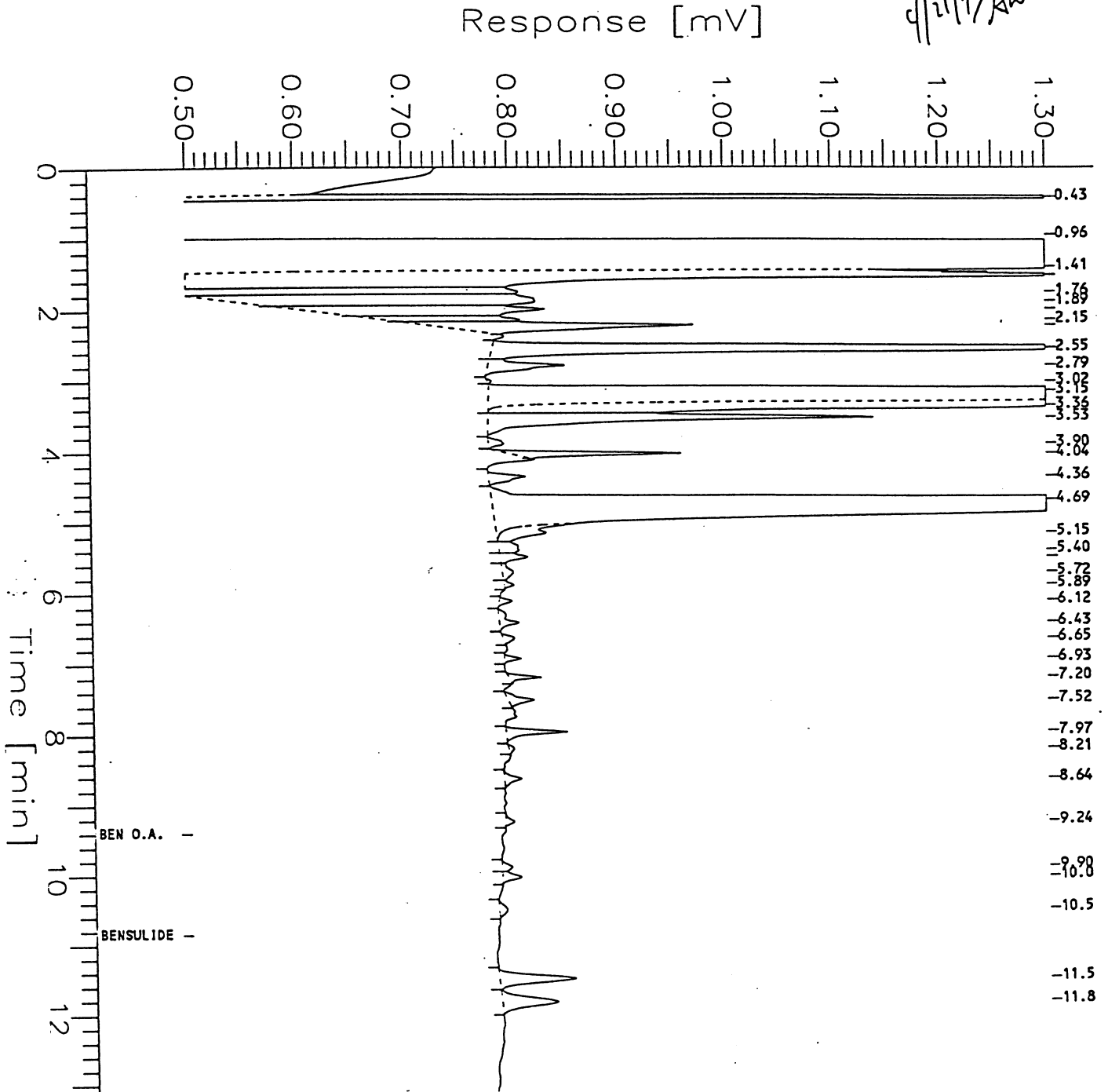


Figure 7. Chromatogram, Treated Cabbage (Florida) without Wrapper Leaves - 42FL-91-634-4

(GOWN-9201 & 9203) 9201

Sample Name : CABBAGE  
 FileName : C:\2700\VALOY\GOWN9201\CABVAL\OCT1004.RAW  
 Method : VGWN  
 Start Time : 0.00 min  
 Scale Factor : 1.0

End Time : 15.99 min  
 Plot Offset: 0 mV

Sample #: GOWN162A  
 Date : 4/21/95 04:43 PM  
 Time of Injection: 10/2/93 02:55 AM  
 Low Point : 0.50 mV  
 Plot Scale: 0.8 mV  
 High Point : 1.30 mV

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*4/21/95*

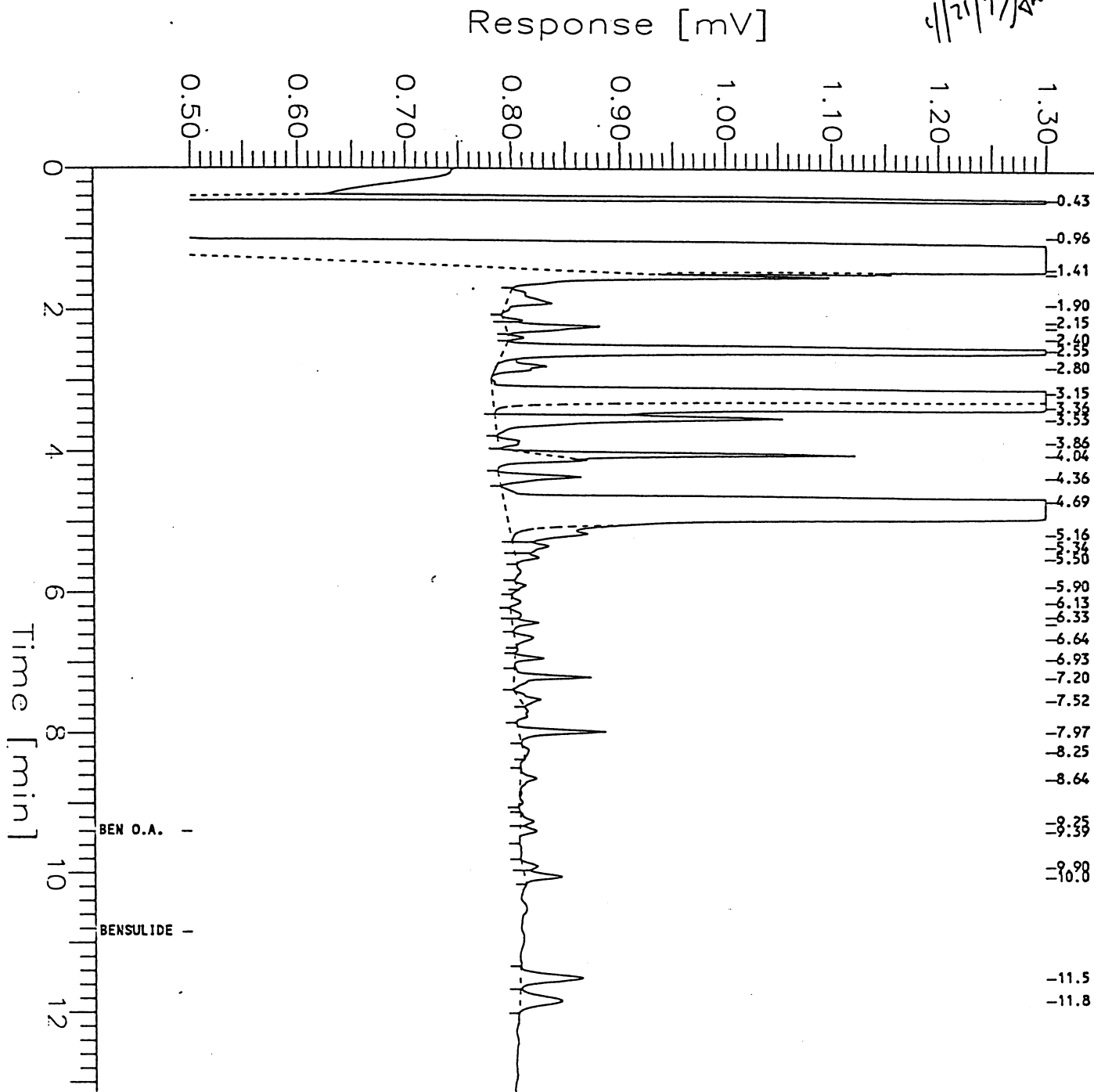


Figure 8. Chromatogram of Bensulide and Bensulide Oxon standards

Chromatogram *Gown 9201*

Sample Name : STANDARD .06  
 FileName : c:\2700\valoy\gown9201\cabval\NV19010.raw  
 Method : VGWN.ins  
 Start Time : 0.00 min  
 File Factor : 1.0

End Time : 22.50 min  
 Plot Offset: -0 mV

Sample #: .06  
 Date : 11/19/93 01:09 PM  
 Time of Injection: 11/19/93 12:47 PM  
 Low Point : -0.04 mV  
 High Point : 1.21 mV  
 Plot Scale: 1.3 mV

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*11/22/93 GH*

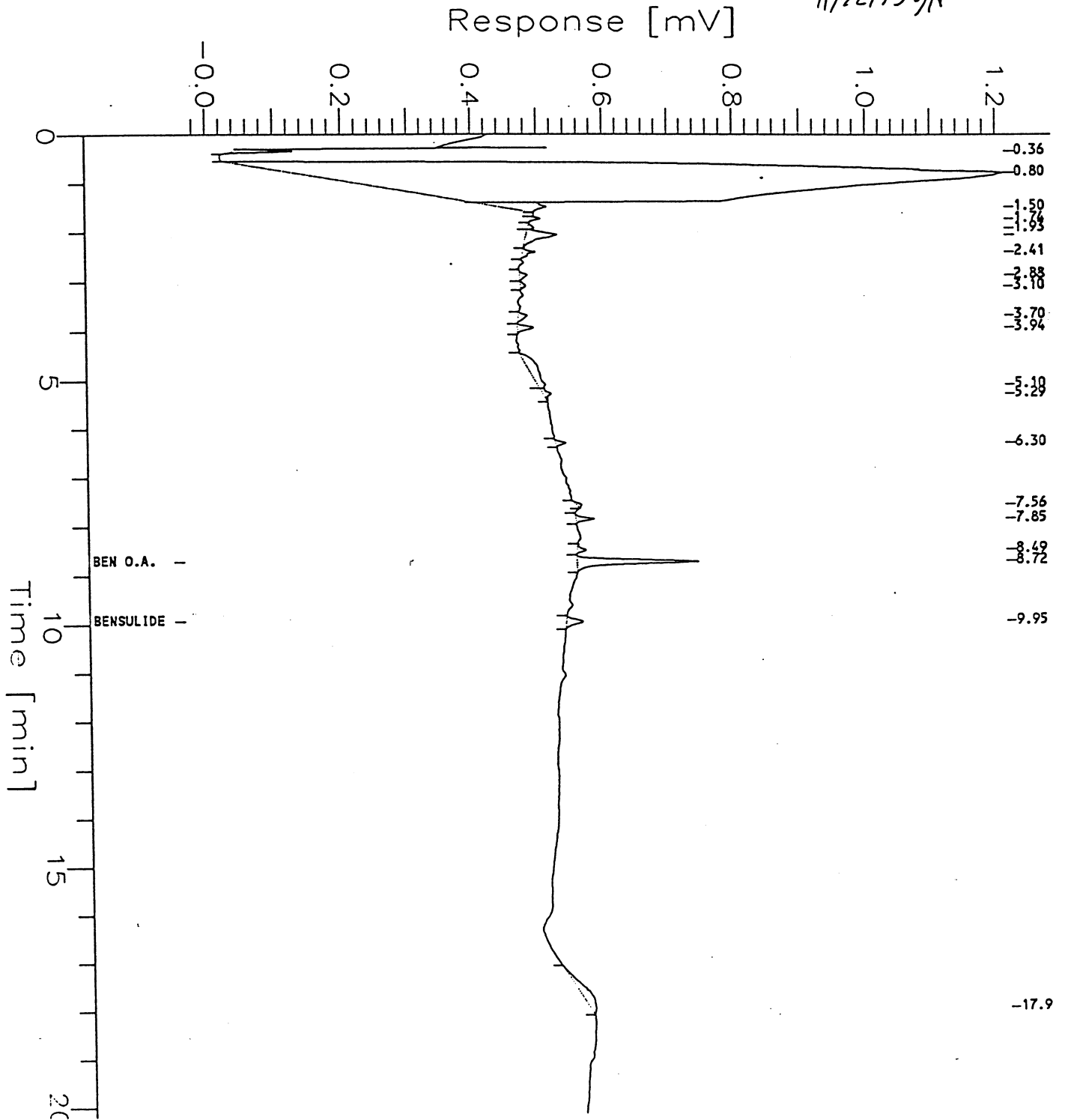


Figure 9. Chromatogram, Untreated Cabbage (Wisconsin) with Wrapper Leaves  
79WI-91-632, Sample 1  
Spiked 0.1 ppm each Bensulide and Oxon

Chromatogram (GOWN-9201&9203)

Sample Name : CABBAGE	Sample # : GOWN-164AS	Page 1 of 1
FileName : D:\BENSULIDE\GOWN9203\NV19008.RAW	Date : 4/19/95 01:03 PM	
Method : VGWN	Time of Injection: 11/19/93 11:55 AM	
Start Time : 0.00 min	End Time : 22.50 min	Low Point : -0.13 mV
Integrator : 1.0	Plot Offset: -0 mV	High Point : 2.87 mV
		Plot Scale: 3.0 mV

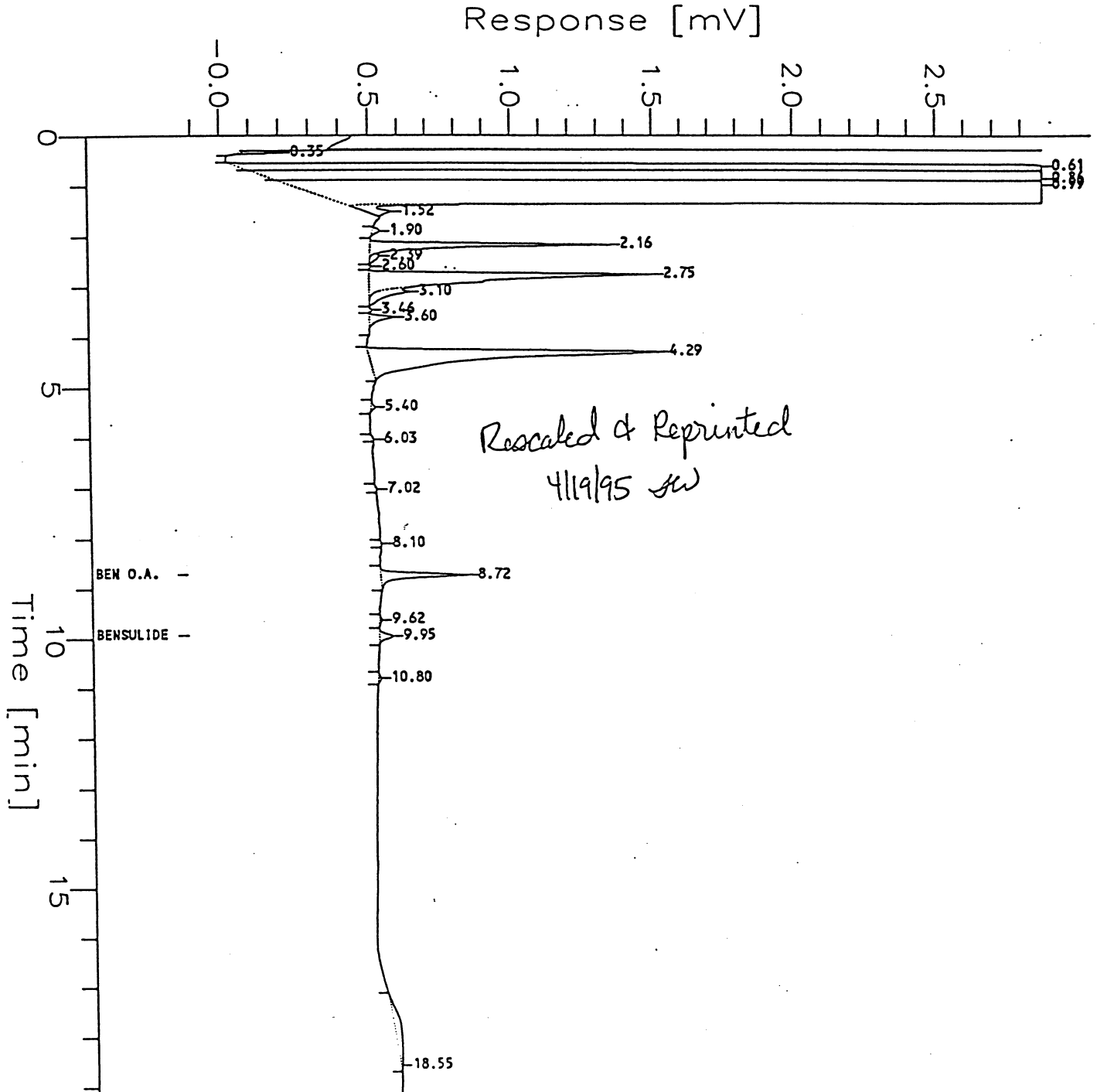


Figure 10. Chromatogram, Untreated Cabbage (Wisconsin) without Wrapper Leaves  
79WI-91-632, Sample 3  
Spiked 0.1 ppm each Bensulide and Oxon

Chromatogram (GOWN-9201&9203)

Sample Name : CABBAGE  
FileName : D:\BENSLIDE\GOWN9203\NV19009.RAW  
Method : VGW  
Start Time : 0.00 min  
End Time : 22.50 min  
Plot Offset: -0 mV  
le Factor: 1.0

Sample #: GOWN-168AS  
Date : 4/19/95 01:04 PM  
Time of Injection: 11/19/93 12:21 PM  
Low Point : -0.13 mV  
High Point : 2.87 mV  
Plot Scale: 3.0 mV

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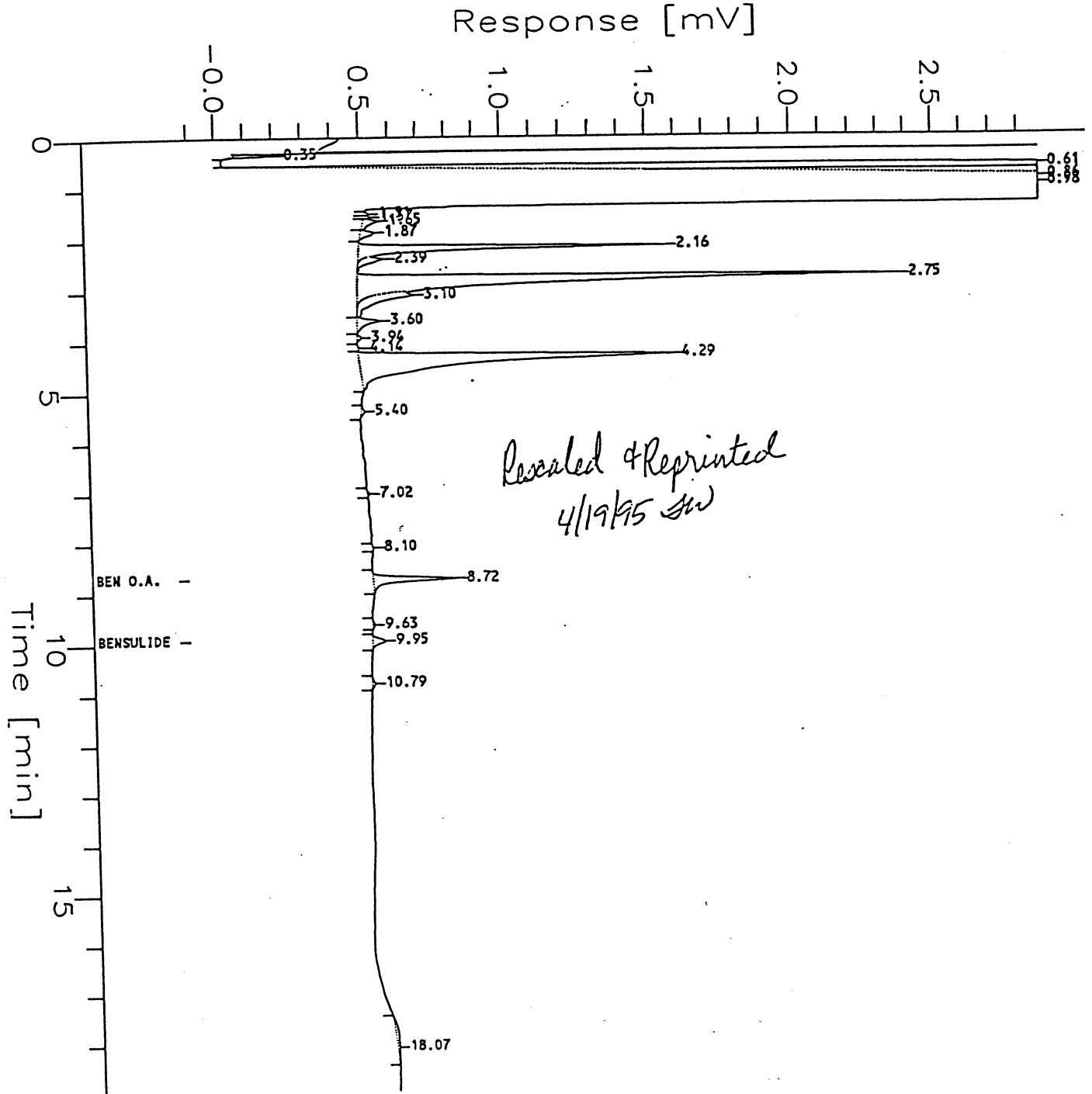


Figure 11. Chromatogram, Untreated Cabbage (Wisconsin) with Wrapper Leaves  
 79WI-91-632, Sample 1

Chromatogram (GOWN-9201&9203)

Sample Name : CABBAGE	Sample #: GOWN-164A	Page 1 of 1
FileName : D:\BENSLIDE\GOWN9203\NV19002.RAW	Date : 4/19/95 01:00 PM	
Method : VGWN	Time of Injection: 11/19/93 09:20 AM	
Start Time : 0.00 min	End Time : 22.50 min	Low Point : -0.13 mV
Le Factor: 1.0	Plot Offset: -0 mV	High Point : 2.87 mV
		Plot Scale: 3.0 mV

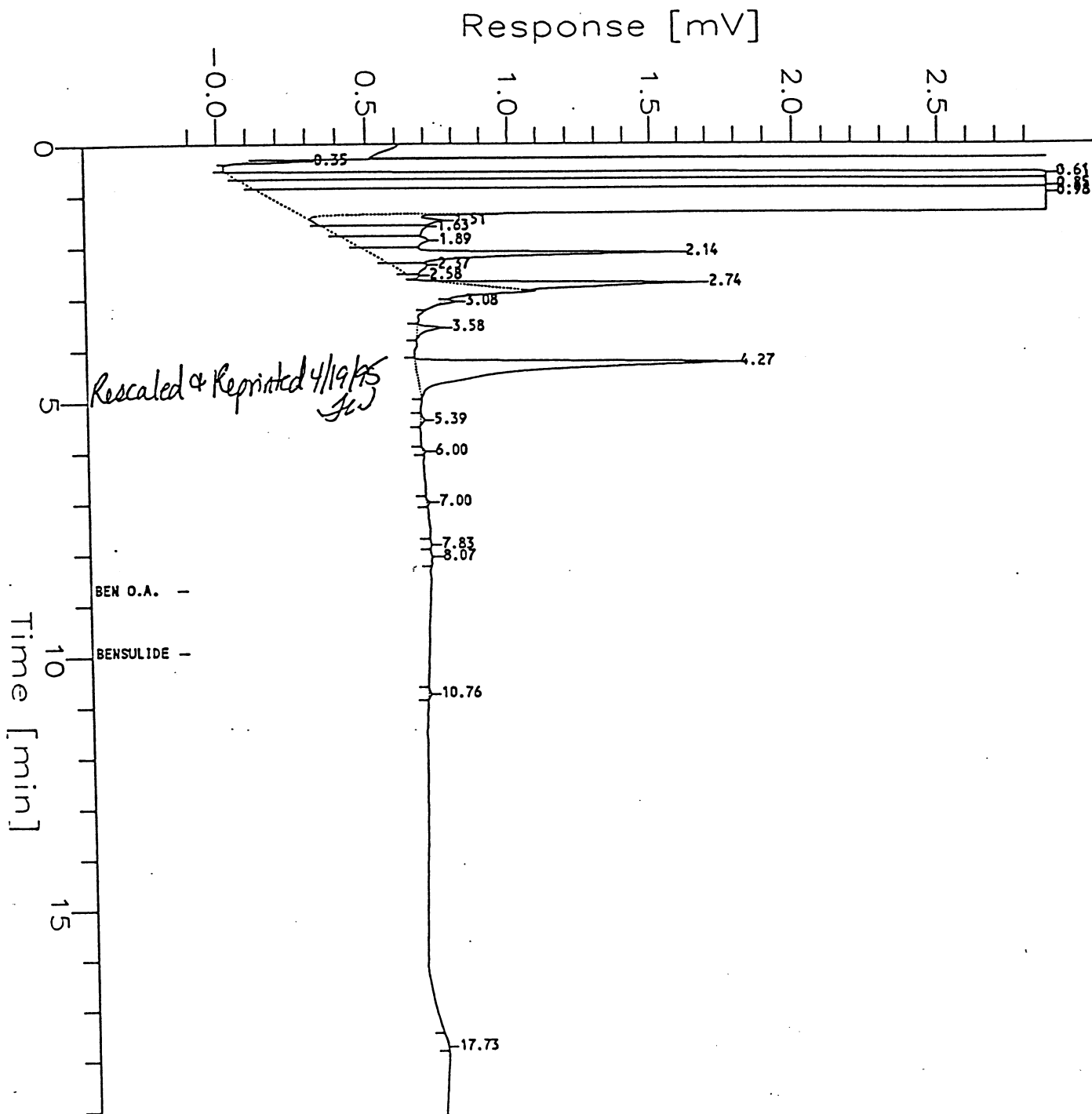


Figure 12. Chromatogram, Treated Cabbage (Wisconsin) with Wrapper Leaves  
79WI-91-632, Sample 2

Chromatogram (GOWN-9201&9203)

Sample Name : CABBAGE  
FileName : D:\BENSLIDE\GOWN9203\WV19003.RAW  
Method : VGWM  
Start Time : 0.00 min  
Gain Factor : 1.0

End Time : 22.50 min  
Plot Offset : -0 mV

Sample #: GOWN-166A  
Date : 4/19/95 01:01 PM  
Time of Injection: 11/19/93 09:45 AM  
Low Point : -0.13 mV  
Plot Scale: 3.0 mV  
Page 1 of 1  
High Point : 2.87 mV

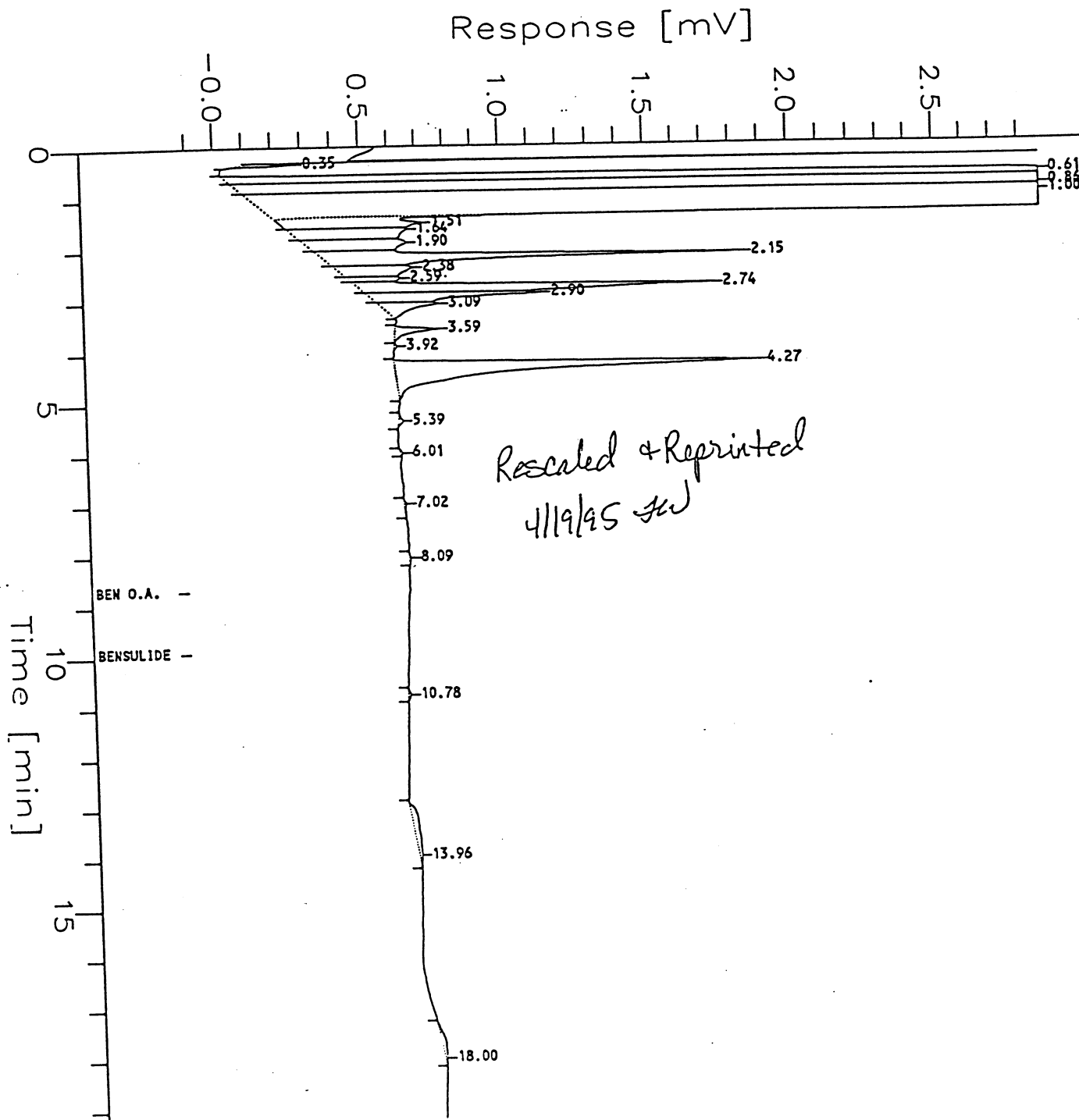


Figure 13. Chromatogram, Untreated Cabbage (Wisconsin) without Wrapper Leaves  
79WI-91-632, Sample 3

Chromatogram (GOWN-9201&9203)

Sample Name : CABBAGE  
FileName : D:\BENSLIDE\GOWN9203\NV19005.RAW  
Method : VGWN  
Start Time : 0.00 min  
File Factor : 1.0

End Time : 22.50 min  
Plot Offset: -0 mV

Sample #: GOWN-168A  
Date : 4/19/95 01:02 PM  
Time of Injection: 11/19/93 10:37 AM  
Low Point : -0.13 mV  
Plot Scale: 3.0 mV  
High Point : 2.87 mV

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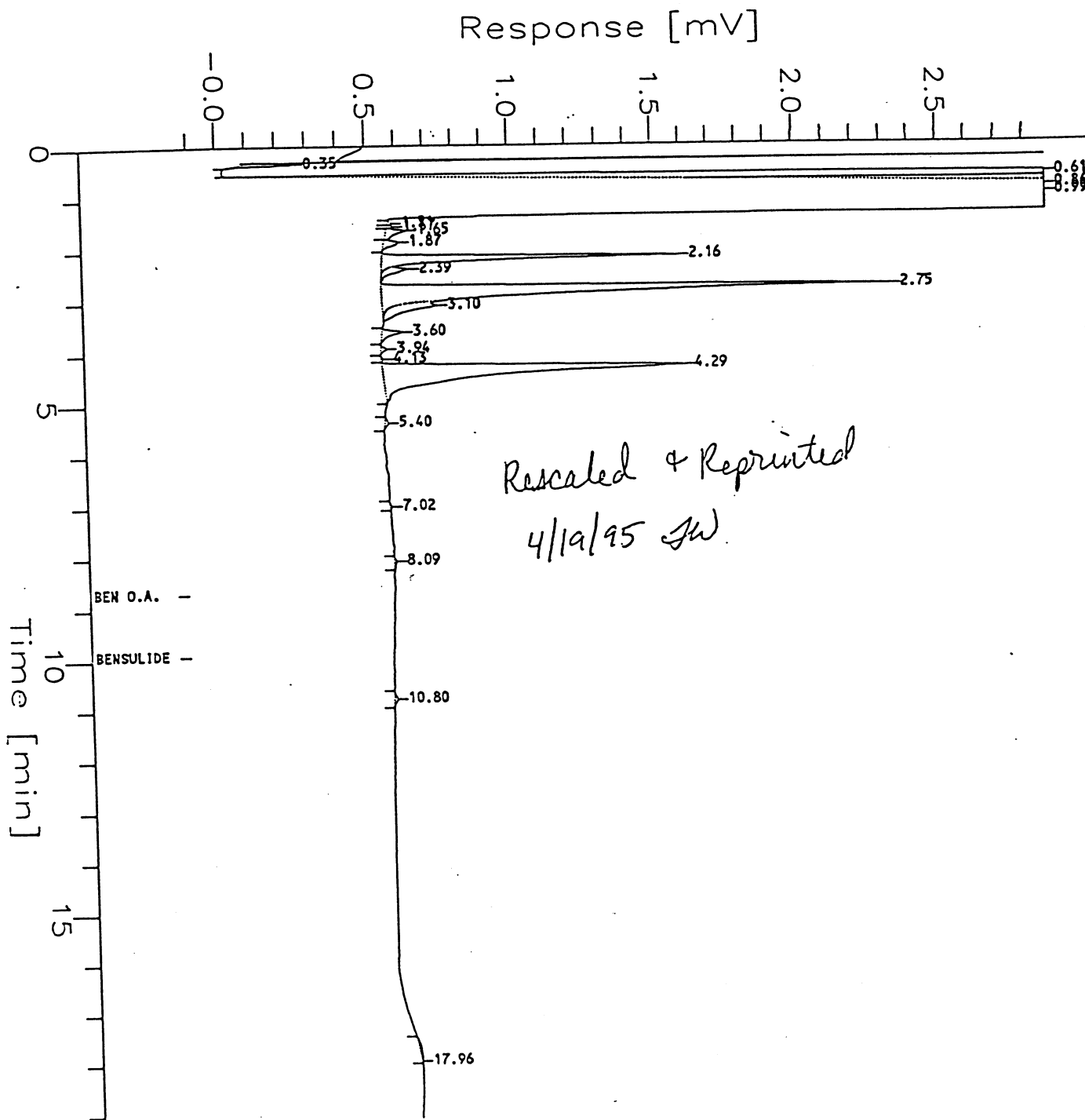


Figure 14. Chromatogram, Treated Cabbage (Wisconsin) without Wrapper Leaves  
79WI-91-632, Sample 4

Chromatogram (GOWN-9201&9203)

Sample Name : CABBAGE  
FileName : D:\BENSULIDE\GOWN9203\NV19006.RAW  
Method : VGWH  
Start Time : 0.00 min  
End Time : 22.50 min  
Plot Offset: -0 mV  
Sample #: GOWN-170A  
Date : 4/19/95 01:02 PM  
Time of Injection: 11/19/93 11:03 AM  
Low Point : -0.13 mV  
High Point : 2.87 mV  
Plot Scale: 3.0 mV  
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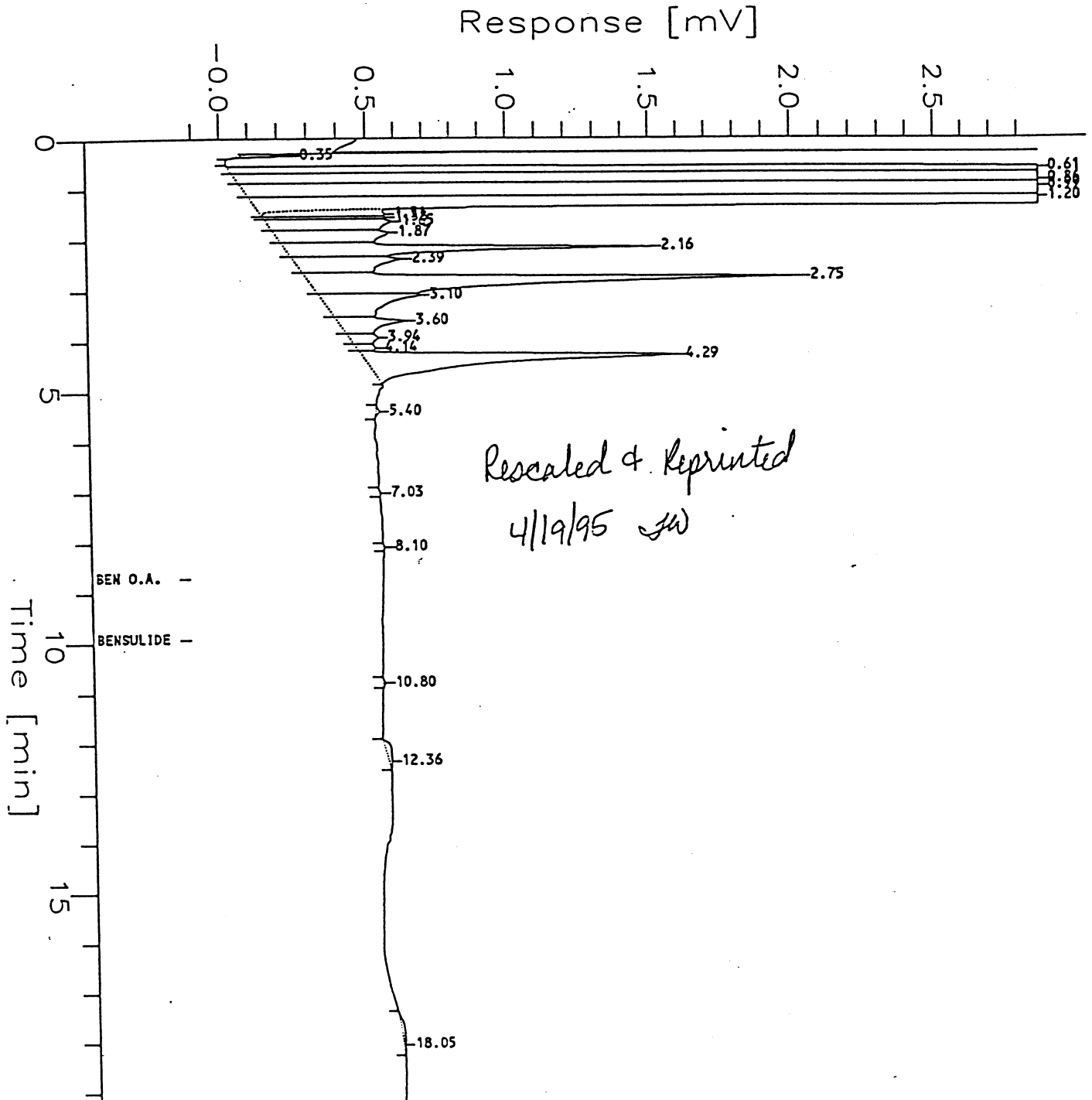


Figure 15. Chromatogram of Bensulide and Bensulide Oxon standards

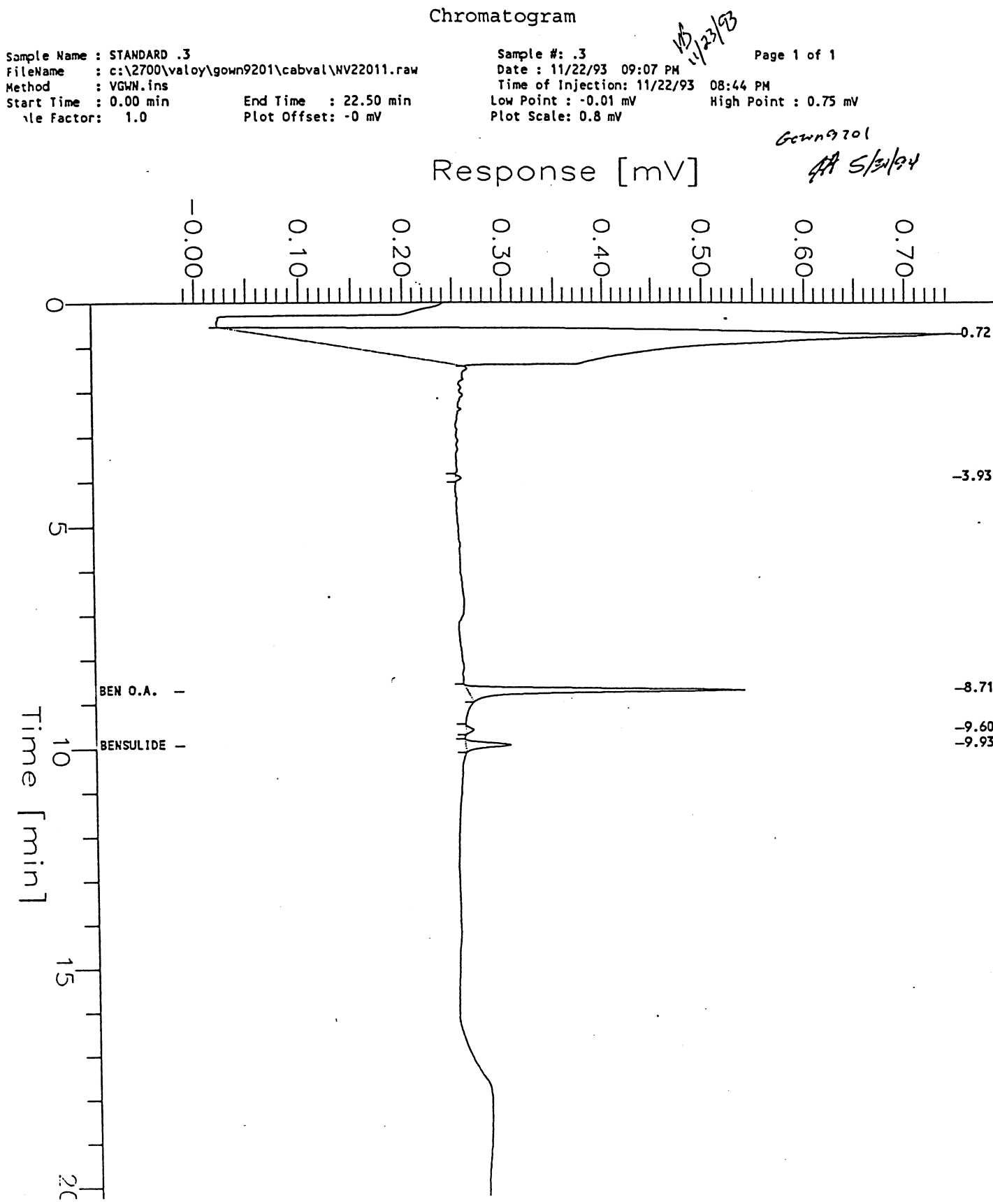


Figure 16. Chromatogram, Untreated Cabbage (New York) without Wrapper Leaves - 56NY-91-631-3  
Spiked 0.1 ppm each Bensulide and Oxon

Chromatogram (GOWN-9324)

Sample Name : CABBAGE  
FileName : D:\BENSLIDE\GOWN9201\NV22010.RAW  
Method : VGM.ins  
Start Time : 0.00 min  
Le Factor: 1.0

End Time : 22.50 min  
Plot Offset: 0 mV

Sample #: GOWN-176AS  
Date : 7/28/95 10:50 AM  
Time of Injection: 11/22/93  
Low Point : 0.20 mV  
Plot Scale: 0.2 mV

Page 1 of 1  
08:18 PM  
High Point : 0.45 mV

*7/28/95*

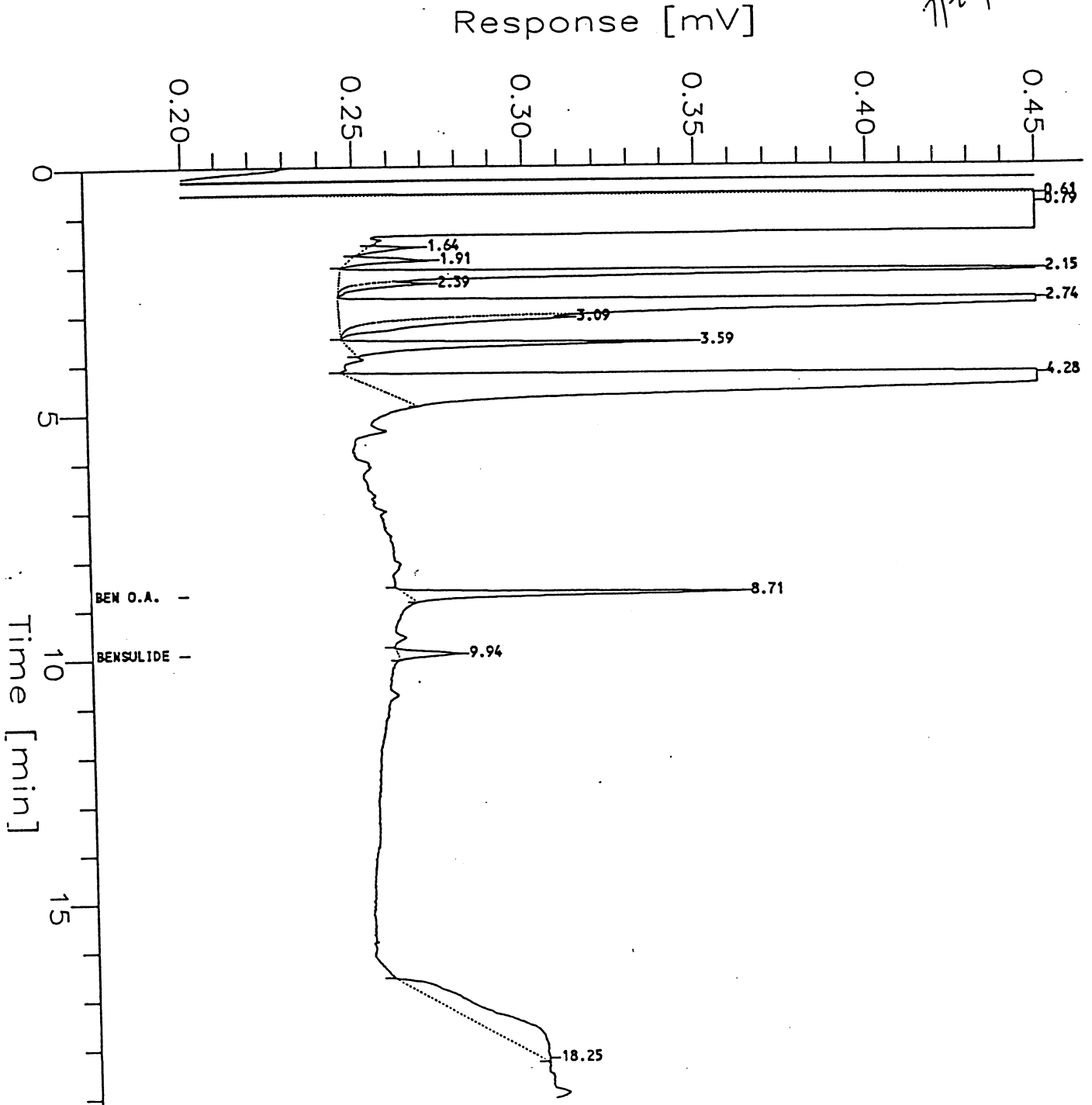


Figure 17: Chromatogram, Untreated Cabbage (New York) with Wrapper Leaves- 56NY-91-631-1

Chromatogram

Sample Name : CABBAGE  
FileName : c:\2700\valoy\gown9201\cabval\NV22002.raw  
Method : VGWN.ins  
Start Time : 0.00 min  
Gain Factor : 1.0

End Time : 22.50 min  
Plot Offset: -1 mV

Sample #: GOWN-172A  
Date : 11/22/93 05:14 PM  
Time of Injection: 11/22/93 04:51 PM  
Low Point : -1.26 mV  
Plot Scale: 26.7 mV

*VB 11/23/93*

*Gown9201  
SA 5/31/94*

Response [mV]

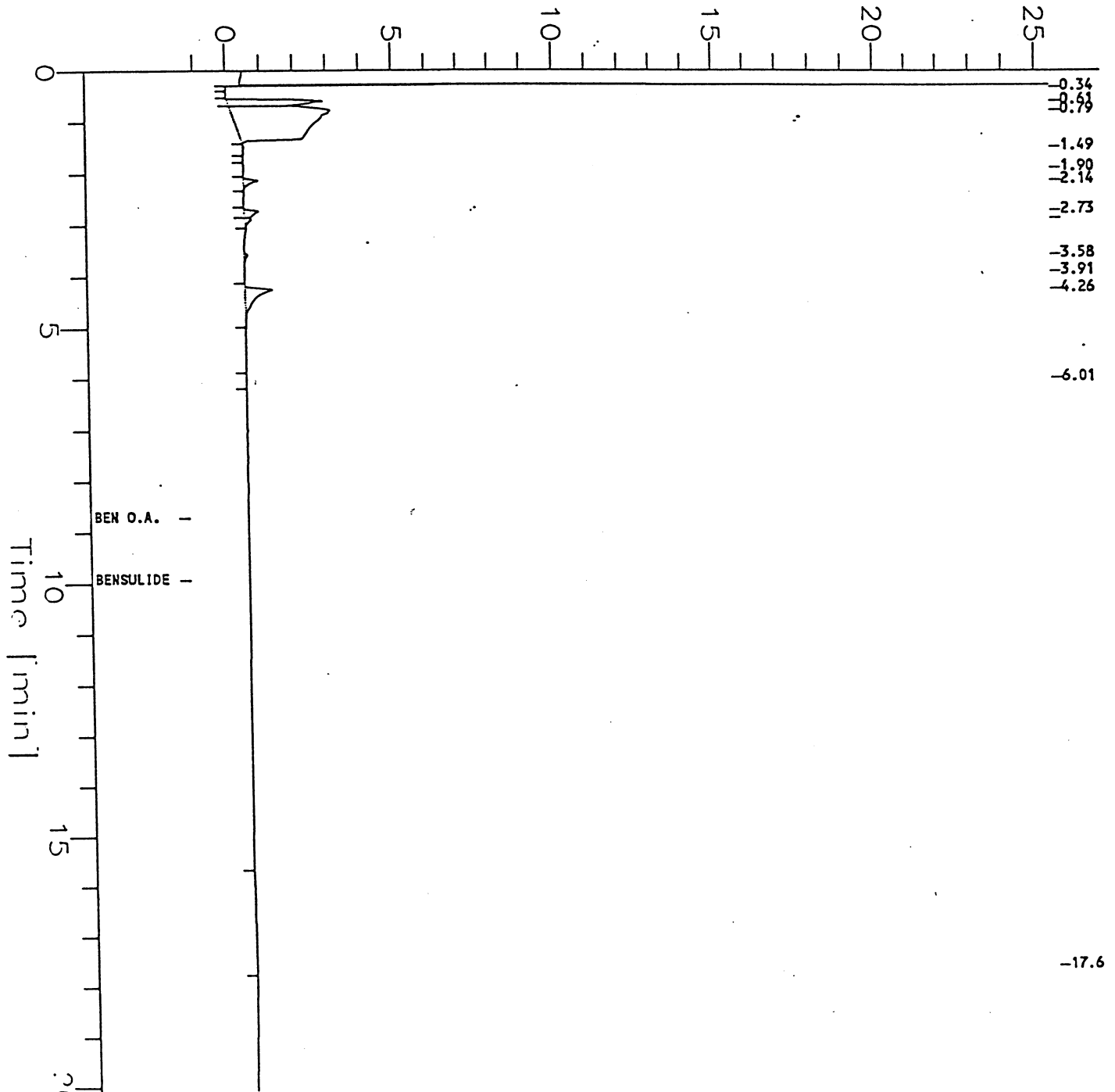


Figure 18. Chromatogram, Treated Cabbage (New York) with Wrapper Leaves - 56NY-91-631-2

Chromatogram

Sample Name : CABBAGE  
FileName : c:\2700\valoy\gown9201\cabval\NV22004.raw  
Method : VGWN.ins  
Start Time : 0.00 min  
Gain Factor : 1.0

Sample #: GOWN-174A  
Date : 11/22/93 06:06 PM  
Time of Injection: 11/22/93 05:43 PM  
Low Point : -1.29 mV  
Plot Scale: 27.3 mV

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*11/22/93*

*Gown 9201  
off 5/3/94*

Response [mV]

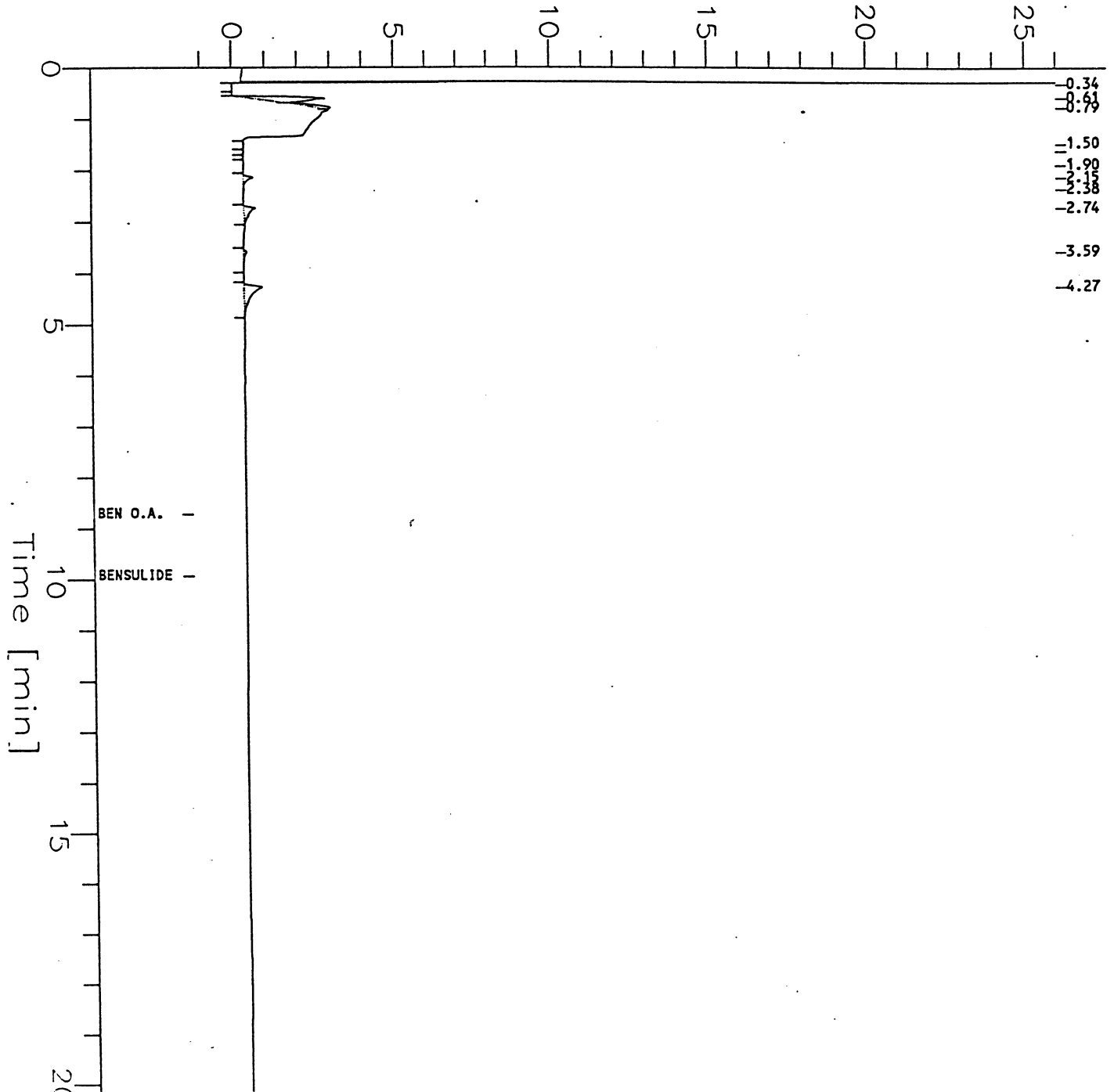


Figure 19. Chromatogram, Untreated Cabbage (New York) without Wrapper Leaves - 56NY-91-631-3

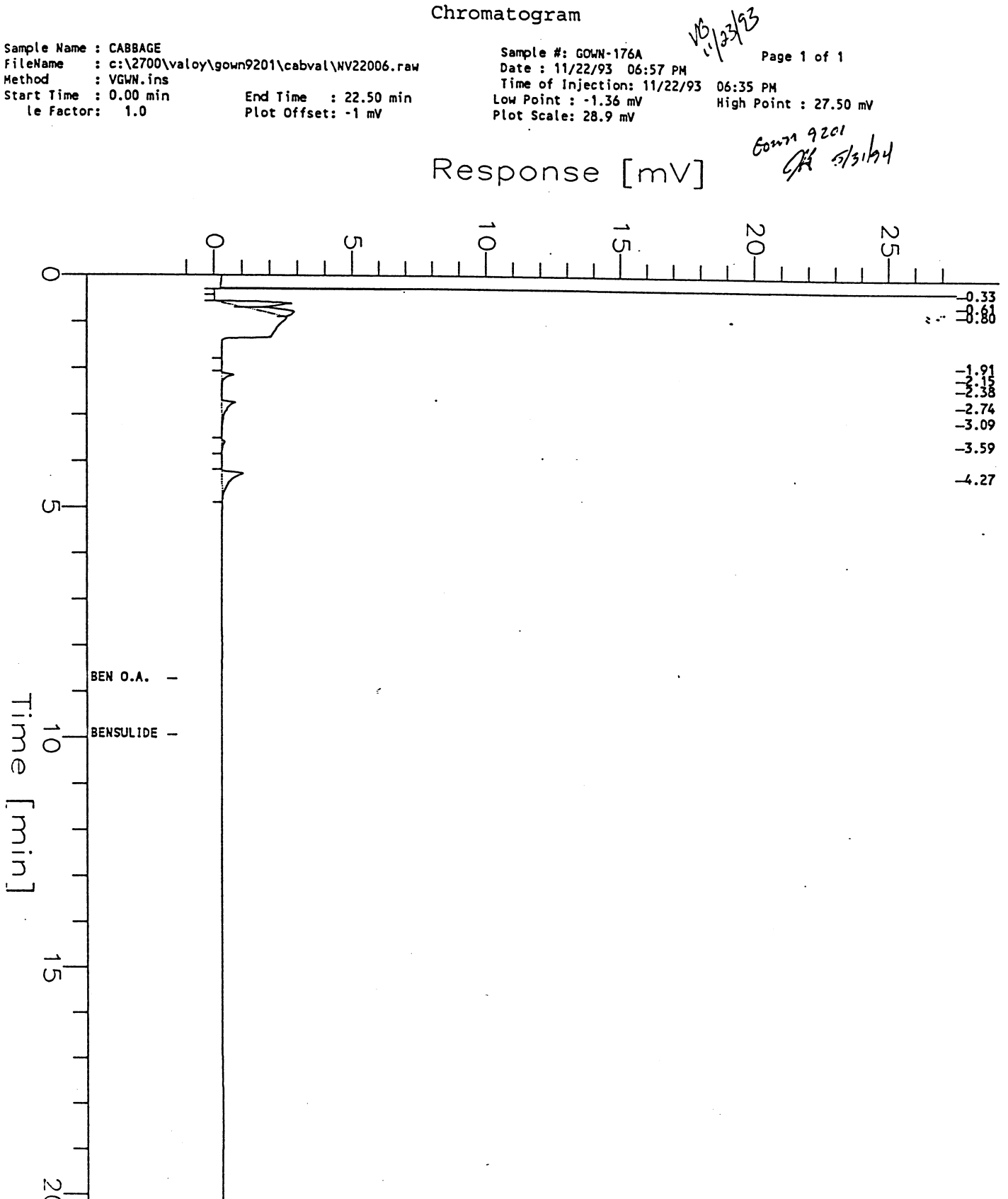


Figure 20. Chromatogram, Treated Cabbage (New York) without Wrapper Leaves - 56NY-91-631-4

Chromatogram

Sample Name : CABBAGE  
FileName : c:\2700\valoy\gown9201\cabval\NV22008.raw  
Method : VGWN.ins  
Start Time : 0.00 min  
Gain Factor: 1.0

Sample #: GOWN-178A  
Date : 11/22/93 07:49 PM  
Time of Injection: 11/22/93 07:27 PM  
Low Point : -1.48 mV  
Plot Scale: 31.6 mV

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*11/22/93*

*Gown 9201*

*at 5/31/94*

Response [mV]

