CYANAMID

M-1410
M. Roman/jh
03/20/84

AMERICAN CYANAMID COMPANY
AGRICULTURAL RESEARCH DIVISION
CHEMICAL DEVELOPMENT
P. O. Box 400
Princeton, New Jersey 08540

Recommended Method of Analysis

CL 252,214: GC Method for the Determination of CL 252,214 Residues in Soybean Plants, Straw, and Seed

A. Principle

Residues of CL 252,214 are extracted from finely chopped soybean tissues with acidic water-methanol. After filtration the extract is partitioned with methylene chloride. The methylene chloride is evaporated, the residue is dissolved in acetonitrile which is then washed three times with hexane. The acetonitrile is then evaporated and the residue is dissolved in acetone. An aqueous ammonium chloride-phosphoric acid solution is added to precipitate most of the impurities. After filtration the CL 252,214 is partitioned into methylene chloride. The methylene chloride is evaporated and further cleanup is achieved by a micro ion exchange column using 0.1N hydrochloric acid solution as eluant and then the CL 252,214 is partitioned into methylene chloride. After the methylene chloride is evaporated to dryness, the residue is dissolved in a measured amount of methanol. Trimethylanilinium hydroxide is added and quantitation of CL 252,214 is accomplished by gas chromatography (on-column methylation) using a 3% OV-17 column, a nitrogen-sensitive detector and the external standard technique. The validated sensitivity of the method is 0.05 ppm for each type of tissue.

B. Reagent

1. CL 252,214: [3-Quinolinecarboxylic acid, 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-]. Analytical standard obtainable from American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, New Jersey 08540.

2. Solvents: Distilled in Glass, Burdick and Jackson, Inc.

   a. Methanol
   b. Methylene Chloride
   c. Hexane
   d. Acetonitrile
   e. Acetone

3. Chemicals: Reagent Grade, J. T. Baker Chemical Company.

   a. Phosphoric Acid, 85%
   b. Ammonium Chloride
   c. Hydrochloric Acid, Concentrated

4. Diatomaceous Earth: Celite 545 AW, Johns Manville Company.

5. Solutions

   a. 1N Hydrochloric Acid: Add 90 ml of hydrochloric acid (concentrated, reagent grade) to approximately 500 ml of distilled water in a 1-L volumetric flask. Dilute to the mark with distilled water.

   b. 0.1N Hydrochloric Acid: Add 9 ml of hydrochloric acid to a 1-L volumetric flask. Dilute to the mark with distilled water and mix well.

   c. Extraction Solvent, 20% Water in Methanol: Add 200-ml of distilled water to a 1-L volumetric flask and dilute to the mark with methanol.

   d. Precipitation Solution: Dilute 2.5 ml of phosphoric acid and 1.25 g of ammonium chloride to 1-L with distilled water.

6. TMAH Methylation Reagent: Trimethylanilinium hydroxide, 0.2M in methanol, Cat. No. 3-3097, Supelco, Inc.

7. GC Packing: 3% OV-17 on 100/120 mesh Supelcoport, Cat. No. 1-1754 Supelco, Inc.

C. Apparatus

1. Balance, Analytical: Sartorius, precision of ± 0.05 mg.

2. Balance, Pan: Sartorius, Model 2254, precision of ± 5 mg.

3. Assorted Glassware: General laboratory.

4. Bottles: Wide-mouth, 500-ml capacity with polyethylene lined cap.

5. Horizontal Reciprocating Shaker: A. H. Thomas Company, No. 8291-510.

6. Flash Evaporator: Buchler Instruments Model PF-10DN or equivalent equipped with a heated water bath maintained at 35°C in which the evaporation flasks can be partially submerged.

7. Filtering Flasks: 500-, 250-, and 125-ml capacity, Corning Glass Works, Cat. No. 5340.

8. Filtering Funnels: Buchner, fritted disc, medium porosity, 600- and 150-ml capacity.

9. Filter Paper: 9- and 4-cm diameter, glass fiber filter, Whatman Inc.

10. Flasks: 24/40 T, 1000-, 500-, and 250-ml round bottom.

11. Separatory Funnels: Squibb type with Teflon stopcocks, 1-L and 250-ml capacity, Kontes Glass Co., Cat. No. K-636030.

12. Microliter Syringes

    a. Hamilton #701-N, 10-mcl capacity
    b. Hamilton #725-N, 200-mcl capacity

13. Disposable Syringe: Plastipak 30-ml capacity, Becton-Dickinson, Rutherford, New Jersey.

14. Ion Exchange Column: SAX quaternary amine Bond-Elut column, Analytichem International, Harbor City, California.

15. Gas Chromatograph: Tracor Model 560, or equivalent, equipped with a Model 702 nitrogen-phosphorus detector.

16. GC Column: 91 cm x 2 mm I.D., glass, packed with 3% OV-17 on 100/120 mesh Supelcoport. The column was packed using a slight vacuum. Silylated glass wool plugs were placed at each end. The column was then conditioned overnight at 275°C with a carrier gas flow of 30 ml/min.

D. Preparation of Standard Solutions

1. Stock Solution

Weigh accurately 10 ± 0.05 mg of analytical standard into a 100-ml volumetric flask. Dilute to the mark with methanol and mix well. Designate this solution which contains 100 mcg of CL 252,214/ml as Standard Solution A.

2. Standard Fortification Solutions

a. Transfer by pipet a 20-ml aliquot of Standard Solution A to a 100-ml volumetric flask. Dilute to the mark with methanol and mix. Designate this solution which contains 20 mcg of CL 252,214/ml as Standard Solution B.

b. Transfer by pipet a 10-ml aliquot of Standard Solution A to a 100-ml volumetric flask. Dilute to the mark with methanol and mix. Designate this solution which contains 10 mcg of CL 252,214/ml as Standard Solution C.

c. Transfer by pipet a 10-ml aliquot of Standard Solution C to a 100-ml volumetric flask. Dilute to the mark with methanol and mix. Designate this solution which contains 1 mcg of CL 252,214/ml as Standard Solution D.

NOTE: For a chromatographic standard, 2 ml of Standard Solution D must be mixed with 200 mcl of TMAH prior to gas chromatography.

E. Gas Chromatographic Conditions

1. Instrument: Tracor Model 560 gas chromatograph.

2. Detector: Model 702 N-P detector. Bead setting of 600-800 to give a peak height of approximately 30% FSD (full-scale deflection) for a 5-ng injection of standard CL 252,214.

3. Column: 91 cm x 2 mm I.D., glass, packed with 3% OV-17 on 100/120 mesh Supelcoport.

4. Instrument Conditions

Column Temperature:	240°C
Injection Port Temperature:	300°C
Detector Temperature:	290°C
Hydrogen Flow Rate:	2.5 ml/min
Helium Flow Rate:	30 ml/min
Air Flow Rate:	120 ml/min
Retention Time:	approximately 4 minutes

5. Recording Integrator: Hewlett-Packard Model 3380A

a.	Chart Speed	0.5 cm/min
b.	Area Reject	1000
c.	Slope Sensitivity	0.3 mV/min

6. Sensitivity: Electrometer sensitivity set to obtain a peak height of approximately 30% FSD (full-scale deflection) for a 5-ng injection of CL 252,214.

F. Linearity Check

1. Transfer 5-, 10-, and 20-ml aliquots of Standard Solution C into separate 100-ml volumetric flasks. Dilute to volume with methanol. These solutions will have concentrations of CL 252,214 of 0.5, 1.0 and 2.0 mcg/ml, respectively.

2. Add TMAH (see note in Section D.2) and inject 5-mcl aliquots of each solution.

3. Plot the height for each peak versus the nanograms injected to demonstrate the linearity of response. Significant departure from linearity over this range indicates instrumental difficulties which should be corrected before proceeding.

G. Sample Preparation

1. Pulverize sufficient dry ice in a food chopper to thoroughly chill the bowl and the blade.

2. Add the pre-frozen sample in portions of sufficiently small size to enable reduction to fine particle size. (It may be necessary to add small portions of dry ice during the chopping procedure to ensure that the samples remain in a frozen state).

3. Allow the sample to stand in a freezer overnight for the dry ice to dissipate completely.

4. Keep all samples frozen until ready for analysis.

H. Recovery Test

The validity of the procedure should always be demonstrated by recovery tests before analysis of unknown samples is attempted. A fortified sample should also be processed with each set of samples analyzed.

1. Weigh a 20-g subsample of control tissue into a wide-mouth bottle.

2. Add by pipet a volume of standard fortification solution appropriate to the fortification level to be tested.

3. Add the solution dropwise and mix the sample well before adding the extraction solvent.

4. Continue with the extraction and cleanup steps as described in the following sections.

I. Extraction

1. Seed and Plants

a. Weigh 20 g of the finely ground seed or plants into a wide-mouth bottle.

b. Add 200-ml of the extraction solvent and 2 ml of 1N hydrochloric acid solution and cap tightly. Shake on a horizontal reciprocating shaker for 30 minutes at high speed.

2. Straw

a. Weigh 20 g of finely ground straw into a wide-mouth bottle.

b. Add 80 ml of distilled water and allow to stand for 30 minutes.

c. Add 320 ml of methanol and 4 ml of 1N hydrochloric acid solution and cap tightly. Shake on a horizontal reciprocating shaker for 30 minutes at high speed.

3. Remove the sample (seed, plants or straw) from the shaker and filter with the aid of vacuum through a 600-ml medium porosity fritted glass funnel covered with glass-fiber filter paper.

4. Rinse the bottle and the sample on the filter pad with 100-ml of extraction solvent plus 1 ml of 1N hydrochloric acid.

5. Transfer the filtrate to a 1-L separatory funnel.

J. Methylene Chloride Extraction

1. For plant and seed samples add 50 ml of distilled water to the separatory funnel (I.5) and 200 ml of methylene chloride. Use 80 ml of distilled water and 250 ml of methylene chloride for straw samples. Stopper and shake for one minute.

2. Allow the phases to separate and draw off the lower methylene chloride layer into a 1-L round bottom flask.

3. Partition the aqueous layer twice more with 100-ml portions of fresh methylene chloride, shaking for one minute each time.

4. Allow the phases to separate and draw off the lower layers into the 1-L round bottom flask.

5. Evaporate the methylene chloride just to dryness using a rotary film evaporator with a water bath set at 35°C.

K. Hexane-Acetonitrile Partitioning

1. Dissolve the residue in the 1-L round bottom flask (J.5) in 5 ml of methanol, add 50-ml of hexane and transfer to a 250-ml separatory funnel.

2. Rinse the flask with 100-ml of acetonitrile and transfer to the 250-ml separatory funnel. Stopper and shake for 30 seconds.

3. Allow the phases to separate and draw off the lower (acetonitrile) layer into another 250-ml separatory funnel.

4. Partition the acetonitrile layer with two additional 50-ml portions of fresh hexane, shaking for 30 seconds each time.

5. Allow the phases to separate and draw off the lower acetonitrile layer into a 250-ml round bottom flask, discarding the hexane washes.

6. Evaporate the acetonitrile to dryness on a rotary film evaporator at 35°C.

L. Coagulation Cleanup

1. Dissolve the residue in 1 ml of acetone for seed and plant samples and in 10 ml of acetone for straw samples. Then add 50 ml of precipitating solution and mix well.

2. Add 2 g of Celite. Allow to stand for 30 minutes.

3. Filter the slurry through a 150-ml medium porosity fritted glass funnel precoated with 1 cm of Celite. Avoid decanting the Celite from the round bottom flask.

4. Wash the flask with 30 ml of precipitating solution. Pass the wash through the filter, retaining the Celite in the flask.

5. Transfer the filtrate to a 250-ml separatory funnel and add 100 ml of methylene chloride. Shake for 30 seconds.

6. Draw off the methylene chloride phase (lower layer) into a 500-ml round bottom flask.

7. Extract the aqueous phase with additional 100- and 50-ml portions of methylene chloride.

8. Combine the methylene chloride extracts in the 500-ml round bottom flask.

9. Evaporate the methylene chloride to dryness.

mk

10. Dissolve the residue in 2 ml of methanol and add 50 ml of distilled water.

11. To remove fines from straw samples, vacuum filter the solution through a 4-cm Buchner funnel fitted with glass-fiber filter paper and rinse the flask with an additional 5 ml of distilled water and pass through the funnel.

M. Ion Exchange Chromatography

1. Prepare an ion exchange BOND-ELUT column by attaching a 30-ml syringe and forcing in succession, 3 ml of hexane, 3 ml methanol, and 3 ml of water through the column. Do not allow the column to go dry.

2. Pour the solution from L.10 or L.11 into the syringe barrel and pull it through the column using a 125-ml filtering flask and vacuum.

3. Rinse the sample flask with an additional 5 ml of distilled water and pass through the column.

4. Discard the eluant and elute the column with 5 ml of 0.1N hydrochloric acid, collecting in a 100-ml beaker.

5. Add 40 ml of distilled water to the 100-ml beaker.

6. Pour the solution into a 250-ml separatory funnel and partition with 2 x 100 ml and 1 x 50 ml of methylene chloride, shaking for 30 seconds each time.

7. Combine the lower methylene chloride phases in a 500-ml round bottom flask and evaporate to dryness.

8. Add 25 ml of methanol to the residue and evaporate to dryness. Repeat with a second 25-ml portion of methanol to ensure complete removal of the methylene chloride.

9. Dissolve the residue in 2 ml of methanol.

10. Add 200-mcl of TMAH just prior to GC analysis.

NOTE: Use the same vial of TMAH for sample and GC standard.

N. Gas Chromatographic Analysis

1. After obtaining a stable GC response, inject a 5-mcl aliquot of the sample into a GC equipped with an N-P detector.

2. Compare the peak height with that obtained from a 5-mcl injection of the GC standard solution containing 1.0 mcg/ml of CL 252,214 (see Note in Section D.2).

3. If the sample peak goes off scale dilute to an appropriate volume with methanol. NOTE: All final (diluted) extracts prepared for GC should contain 100 mcl of TMAH/ml of methanol.

4. Make a standard injection after every sample and use the average peak height of the standards injected before and after the sample for the calculations.

O. Calculations

For each sample calculation, use the sample peak height and the average peak height measurement of the external standard obtained before and after the sample injection as follows:

$$ppm = \frac{R(SAMP) \times (V1) \times (V3) \times (C) \times (V5) \times (D.F.)}{R(STD) \times (W) \times (V2) \times (V4)}$$

Where:

R(SAMP) = Peak height of sample.

R(STD) = Average peak height of working standard.

W = Weight of sample taken for analysis in grams.

V1 = Volume of extracting solvent added to sample in milliliters.

V2 = Aliquot of extract taken for analysis in milliliters.

V3 = Volume of methanol added to dissolve the residue for chromatographic analysis in milliliters.

V4 = Volume of sample solution injected in microliters.

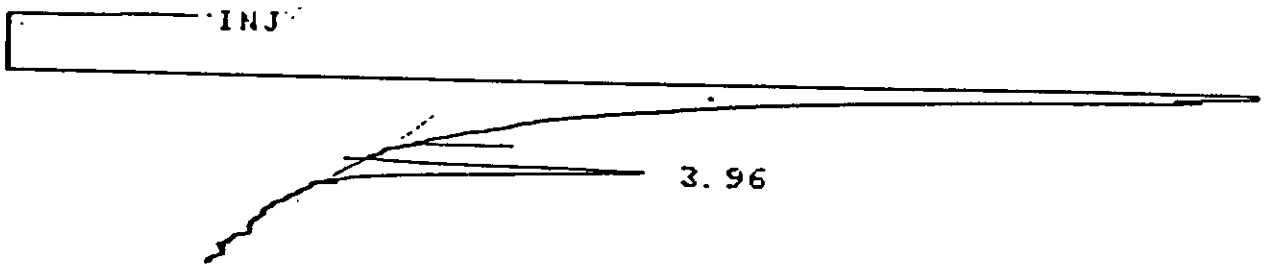
V5 = Volume of working standard solution injected in microliters.

C = Concentration of working standard solution in micrograms per milliliter.

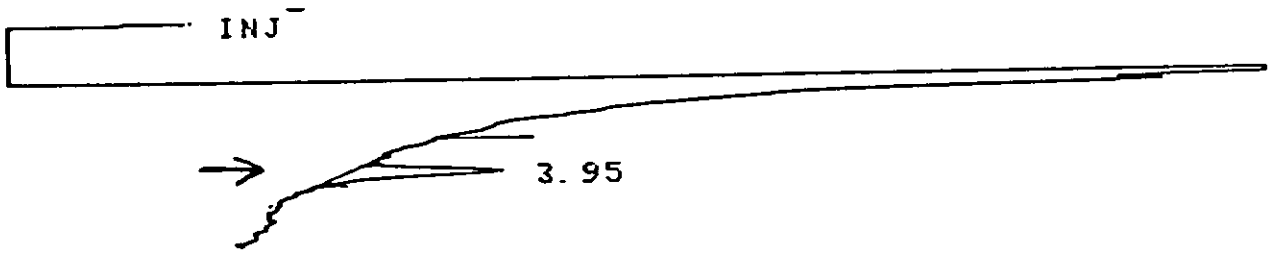
D.F. = Dilution factor.

Figures M-1410.A to M-1410.C show typical chromatograms for determining CL 252,214 residues in soybean plants, straw, and seed, respectively.

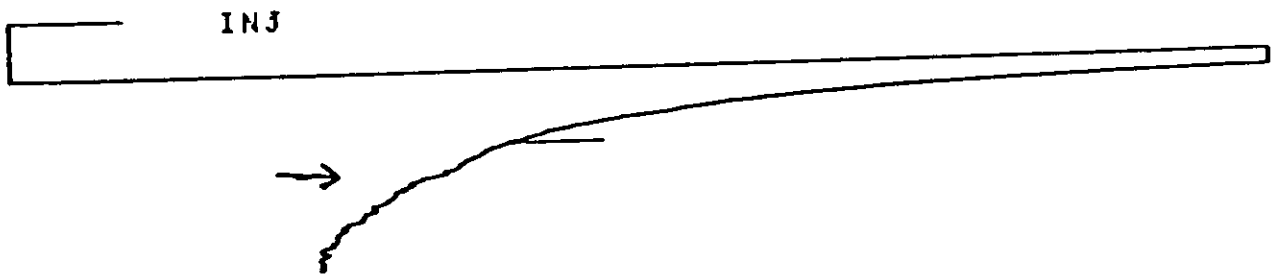
Figure M-1410.A: Typical Chromatograms for the Determination of CL 252,214 Residues in Soybean Plants



CL 252,214 Standard, 5 ng injected

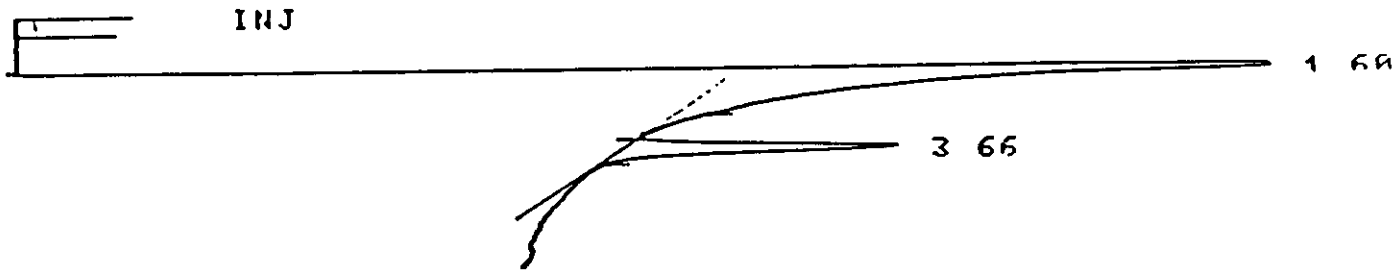


Fortified Soybean Plants at 0.05 ppm, equivalent of 50 mg injected

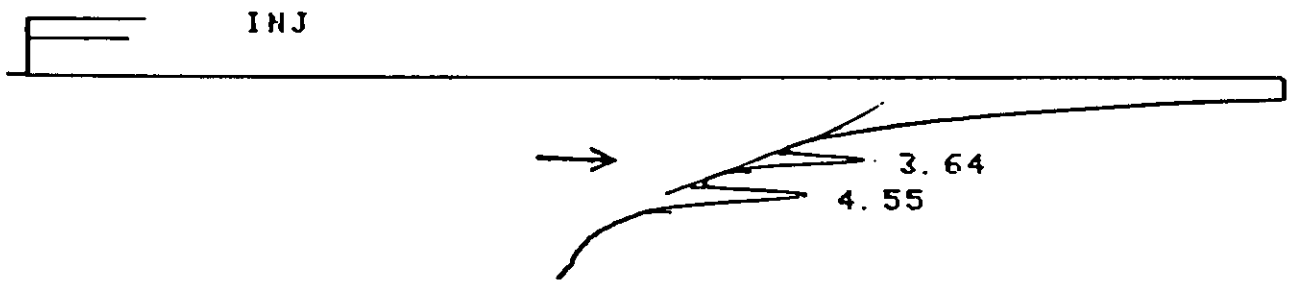


Control Soybean Plants, equivalent of 50 mg injected

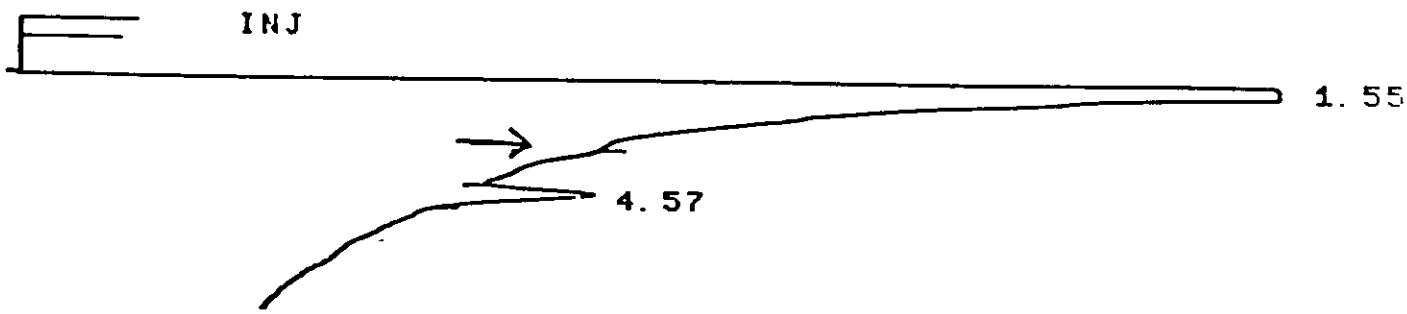
Figure M-1410.F: Typical Chromatograms for the Determination of CL 252,214 Residues in Soybean Straw



CL 252,214 Standard, 5 ng injected

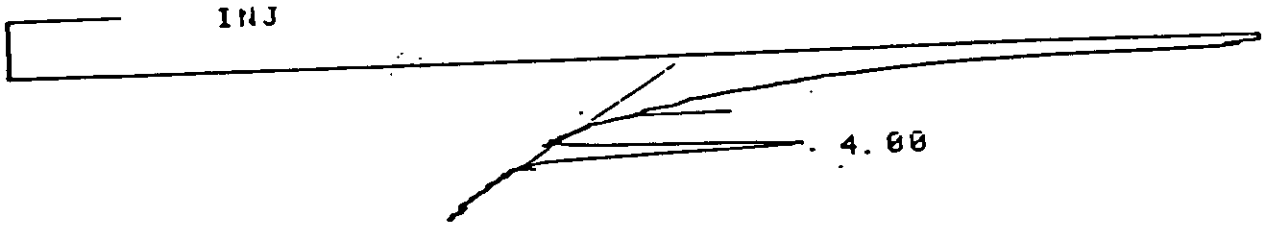


Fortified Soybean Straw at 0.05 ppm, equivalent of 50 mg injected

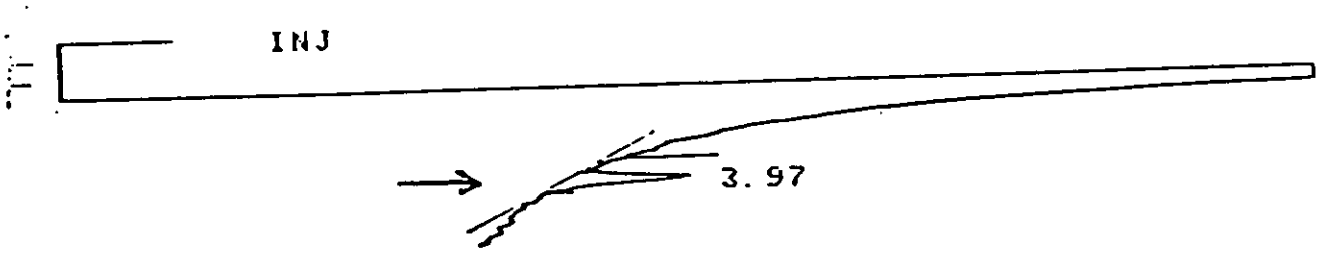


Control Soybean Straw, equivalent of 50 mg injected

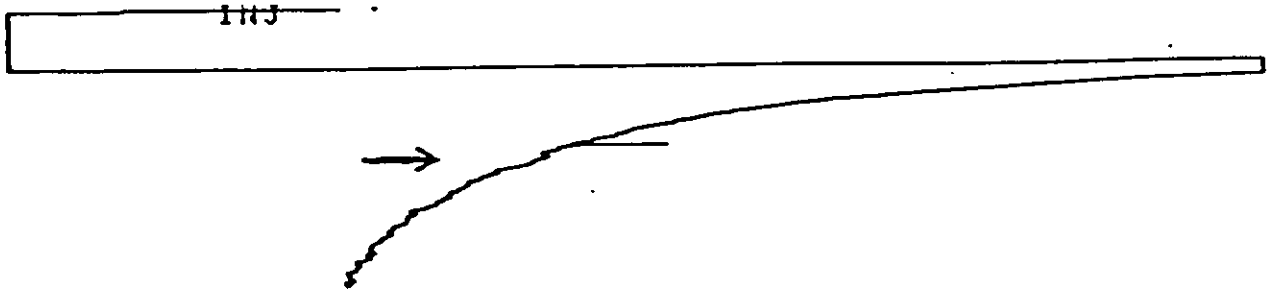
Figure M-1410.C: Typical Chromatograms for the Determination of CL 252,214 Residues in Soybeans



CL 252,214 Standard, 5 ng injected



Fortified Soybeans at 0.05 ppm, equivalent of 50 mg injected



Control Soybeans, equivalent of 50 mg injected

CYANAMID
American Cyanamid Company
Agricultural Research Division
Princeton, NJ 08540 USA

DEPARTMENT/STATION:	Chemical Development	REPORT NO.:	C-2404
GROUP/UNIT NO.:	09414	PAGES:	1-18
TYPE OF STUDY:	Residue, Plant	RETRIEVAL NO.:	936
PROJECT NUMBER:	0530	PERIOD COVERED From:	11/21/83
EXPERIMENT NUMBER:		To:	04/04/84
		REPORT ISSUED:	04/25/1984
REPORTED BY:	M.E.Roman	APPROVED BY:	J.H.Devine
WORK DONE BY:	M.E.Roman		
NOTEBOOK NUMBERS AND PAGES:	AC4505/135-138, 140-141; AC4688/20-22, 52-53, 57-59		

TITLE

SCEPTER* Herbicide, imazaquin (CL 252,214): Validation of GC Method M-1410 for the Determination of CL 252,214 Residues in Soybean Plants, Straw and Seed

PURPOSE

To validate Method M-1410 for the recovery of CL 252,214 residues in soybean seed, green plants and straw.

SUMMARY

Method M-1410 supersedes Methods M-1283 and M-1245 for determining CL 252,214 residues in soybean commodities. The latter methods were developed without extractability data. Initiation of freezer stability work with CL 252,214 residues showed that ethyl acetate (previous extraction solvent) extracted only about 50% of the residue. Hence, Method M-1410 was developed to overcome these extraction problems.

Method M-1410 has been found satisfactory for the determination of CL 252,214 residues in soybean seed, green plants and straw. The validated sensitivity of the method is 0.05 ppm. Detailed analytical data for the recoveries of CL 252,214 are presented as follows: soybean seed Table I, soybean green plants Table II and soybean straw Table III. Recoveries ranged from 83 to 100% in fortified soybean seed samples, from 80 to 97% in fortified soybean green plants, and from 83 to 102% in fortified soybean straw samples. The fortification levels ranged from 0.05 to 0.5 ppm for straw and seed, and from 0.05 to 50.0 ppm for green plants. Apparent CL 252,214 residues in control samples were <0.005 ppm.

* Trademark of American Cyanamid Company

Extractability of total carbon-14 from radiolabeled CL 252,214 treated soybean seed, straw, and green plants was also examined. The data are presented in Table IV and show adequate extraction of total carbon-14 employing the solvent used in Method M-1410.

M. Roman

MR/jh

TABLE I

TYPE OF EXPERIMENT: RECOVERY STUDY
SAMPLE TYPE: SOYBEAN SEED
ANALYZED FOR: CL 252,214
METHOD USED: M-1410
VALIDATED SENSITIVITY: 0.05 PPM
RESPONSE MEASURED AS: PEAK HEIGHT IN MM
V4 = V5 = : 5

$$\text{APP. PPM FOUND} = \frac{R(SAMP) * V1 * V3 * V5 * C(STD) * D.F.}{R(STD) * W * V2 * V4}$$

$$\text{\% RECOVERY} = \frac{\text{PPM FOUND} * 100}{\text{PPM ADDED}} = \frac{\text{PPM FOUND} * 100}{FV * FC / W}$$

	FORTIFICATION			SAMPLE PREPARATION					STAND. SOLN.	RESPONSES		APPARENT	
SAMPLE NUMBER	FV (mls)	FC mcg/ml	PPM added	W (gms)	V1 (mls)	V2 (mls)	V3 (mls)	DIL. fac.	C(Std) mcg/ml	R(SAMP) units	R(STD) units	PPM FOUND	PERCENT RECOVERY
1	0	0	0	20	300	300	2	1	1.00	-N.M.	40	<0.005	
2	0	0	0	20	300	300	2	1	1.00	-N.M.	25	<0.008	
										AVERAGES		0.007	
3	1	1	0.050	20	300	300	2	1	1.00	15	31	0.048	96.8
4	1	1	0.050	20	300	300	2	1	1.00	17	36	0.047	94.4
										AVERAGES		0.048	95.6
5	2	1	0.100	20	300	300	2	1	1.00	38	42	0.090	90.5
6	2	1	0.100	20	300	300	2	1	1.00	30	31	0.097	96.8
7	2	1	0.100	20	300	300	2	1	1.00	30	36	0.083	83.3
8	2	1	0.100	20	300	300	2	1	1.00	27	32	0.084	84.4
										AVERAGES		0.089	88.8
9	2	2	0.200	20	300	300	4	1	1.00	28	30	0.187	93.3
10	2	2	0.200	20	300	300	4	1	1.00	29	30	0.193	96.7
										AVERAGES		0.190	95.0
11	1	10	0.500	20	300	300	10	1	1.00	33	33	0.500	100.0
12	1	10	0.500	20	300	300	10	1	1.00	34	35	0.486	97.1
										AVERAGES		0.493	98.6

N.M.= not measurable (less than 2 units), corresponding, for control samples, to the limits given above in the APPARENT-residue column. These limits are calculated by insertion of the number 2 into the calculation as the value for R(SAMP) wherever N.M. is indicated. For treated samples, N.M. is expressed as less than the validated sensitivity of the method.

Control samples are indicated by a minus sign before the R(SAMP) value.

TABLE II

TYPE OF EXPERIMENT: RECOVERY STUDY
SAMPLE TYPE: SOYBEAN PLANTS
ANALYZED FOR: CL 252,214
METHOD USED: M-1410
VALIDATED SENSITIVITY: 0.05 PPM
RESPONSE MEASURED AS: PEAK HEIGHT INMM
V4 = V5 = : 5

$$\text{APP. PPM FOUND} = \frac{R(SAMP) * V1 * V3 * V5 * C(STD) * D.F.}{R(STD) * W * V2 * V4}$$

$$\% \text{ RECOVERY} = \frac{\text{PPM FOUND} * 100}{\text{PPM ADDED}} = \frac{\text{PPM FOUND} * 100}{FV * FC / W}$$

	FORTIFICATION			SAMPLE PREPARATION					STAND. SOLN.	RESPONSES		APPARENT	
SAMPLE NUMBER	FV (mls)	FC mcg/ml	PPM added	W (gms)	V1 (mls)	V2 (mls)	V3 (mls)	DIL. fac.	C(Std) mcg/ml	R(SAMP) units	R(STD) units	PPM FOUND	PERCENT RECOVERY
1	0	0	0	20	300	300	2	1	1.00	-N.M.	37	<0.005	
2	0	0	0	20	300	300	2	1	1.00	-N.M.	40	<0.005	
										AVERAGES		0.005	
3	1	1	0.050	20	300	300	2	1	1.00	19	40	0.048	95.0
4	1	1	0.050	20	300	300	2	1	1.00	18	40	0.045	90.0
										AVERAGES		0.047	92.5
5	2	1	0.100	20	300	300	2	1	1.00	35	38	0.092	92.1
6	2	1	0.100	20	300	300	2	1	1.00	33	36	0.092	91.7
7	2	1	0.100	20	300	300	2	1	1.00	32	37	0.086	86.5
										AVERAGES		0.090	90.1
8	2	2	0.200	20	300	300	4	1	1.00	33	36	0.183	91.7
9	2	2	0.200	20	300	300	4	1	1.00	36	39	0.185	92.3
										AVERAGES		0.184	92.0
10	1	10	0.500	20	300	300	10	1	1.00	35	36	0.486	97.2
11	1	10	0.500	20	300	300	10	1	1.00	36	38	0.474	94.7
										AVERAGES		0.480	96.0
12	1	100	5.000	20	300	300	100	1	1.00	45	56	4.018	80.4
13	1	100	5.000	20	300	300	100	1	1.00	42	52	4.038	80.8
										AVERAGES		4.028	80.6
14	2	500	50	20	300	300	1000	1	1.00	34	37	45.9	91.9
15	2	500	50	20	300	300	1000	1	1.00	34	40	42.5	85.0
										AVERAGES		44.2	88.5

N.M.= not measurable (less than 2 units), corresponding, for control samples, to the limits given above in the APPARENT-residue column. These limits are calculated by insertion of the number 2 into the calculation as the value for R(SAMP) wherever N.M. is indicated. For treated samples, N.M. is expressed as less than the validated sensitivity of the method.

Control samples are indicated by a minus sign before the R(SAMP) value.

TABLE III

TYPE OF EXPERIMENT: RECOVERY STUDY
SAMPLE TYPE: SOYBEAN STRAW
ANALYZED FOR: CL 252,214
METHOD USED: M-1410
VALIDATED SENSITIVITY: 0.05 PPM
RESPONSE MEASURED AS: PEAK HEIGHT IN MM
V4 = V5 = : 5

$$\text{APP. PPM FOUND} = \frac{R(SAMP) * V1 * V3 * V5 * C(STD) * D.F.}{R(STD) * W * V2 * V4}$$

$$\text{\% RECOVERY} = \frac{\text{PPM FOUND} * 100}{\text{PPM ADDED}} = \frac{\text{PPM FOUND} * 100}{FV * FC / W}$$

	FORTIFICATION			SAMPLE PREPARATION					STAND. SOLN.	RESPONSES		APPARENT	
SAMPLE NUMBER	FV (mls)	FC mcg/ml	PPM added	W (gms)	V1 (mls)	V2 (mls)	V3 (mls)	DIL. fac.	C(Std) mcg/ml	R(SAMP) units	R(STD) units	PPM FOUND	PERCENT RECOVERY
1	0	0	0	20	500	500	2	1	1.00	-N.M.	35	<0.006	
2	0	0	0	20	500	500	2	1	1.00	-N.M.	35	<0.006	
										AVERAGES		0.006	
3	1	1	0.050	20	500	500	2	1	1.00	15	35	0.043	85.7
4	1	1	0.050	20	500	500	2	1	1.00	16	38	0.042	84.2
										AVERAGES		0.043	85.0
5	2	1	0.100	20	500	500	2	1	1.00	32	36	0.089	88.9
6	2	1	0.100	20	500	500	2	1	1.00	35	40	0.088	87.5
7	2	1	0.100	20	500	500	2	1	1.00	47	46	0.102	102.2
										AVERAGES		0.093	92.9
8	2	2	0.200	20	500	500	4	1	1.00	45	53	0.170	84.9
9	2	2	0.200	20	500	500	4	1	1.00	20	24	0.167	83.3
										AVERAGES		0.169	84.1
10	1	10	0.500	20	500	500	10	1	1.00	35	40	0.438	87.5
11	1	10	0.500	20	500	500	10	1	1.00	29	34	0.426	85.3
										AVERAGES		0.432	86.4

N.M.= not measurable (less than 2 units), corresponding, for control samples, to the limits given above in the APPARENT-residue column. These limits are calculated by insertion of the number 2 into the calculation as the value for R(SAMP) wherever N.M. is indicated. For treated samples, N.M. is expressed as less than the validated sensitivity of the method.

Control samples are indicated by a minus sign before the R(SAMP) value.

Table IV: Extractability of Total Carbon-14 from Radiolabeled CL 252,214 Treated Soybean Plants (AC 4476-97)*

Commodity	Interval (months)	Total Carbon-14 (ppm)	% Carbon-14 Extracted
Green Plants	1.0	0.43	72
Straw	3.5	0.10	72
Seed	3.5	0.04	33

* Soybean plants were treated postemergence at 0.5 lb ai/A.