M-1754
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Approved by:

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Recommended Method of Analysis

COUNTER® terbufos (CL 92,100): GC Method for the Determination of Total CL 92,100-Related Residues in Field Corn Grain, Green and Dry Plants, Sweet Corn Kernels Plus Cob and Cannery Waste

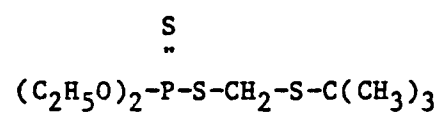
A. Principle

CL 92,100-related residues are extracted from finely ground corn tissue with 10% methanol-methylene chloride. After filtration, the extract is evaporated and the residue is dissolved in hexane which is then partitioned with acetonitrile. The acetonitrile is evaporated to dryness and the residue is dissolved in acetone and treated with activated charcoal. Following filtration and evaporation, the compounds are converted to terbufoxon sulfone (CL 94,302) by oxidation with m-chloroperbenzoic acid in methylene chloride. After washing the reaction mixture with sodium sulfite and bicarbonate solutions and with water, the methylene chloride is evaporated. The residue is dissolved in acetone and an aqueous ammonium chloride-phosphoric acid solution is added to precipitate most of the remaining oil impurities. After filtration, the CL 94,302 is extracted into methylene chloride. The methylene chloride is removed and the residue is dissolved in a measured volume of acetone. Quantitation is accomplished by gas chromatography with an instrument equipped with a flame photometric detector in the phosphorus mode. The results are calculated by comparison of the peak height to that of an oxidized external standard. The validated sensitivity of the method is 0.01 ppm in grain and kernels plus cob, and 0.05 ppm in green plants, dry plants and cannery waste.

B. Reagents

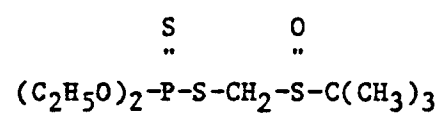
1. Analytical Standards: Obtained from American Cyanamid Company, Agricultural Research Division, P. O. Box 400, Princeton, NJ 08540.

a. Terbufos (CL 92,100)



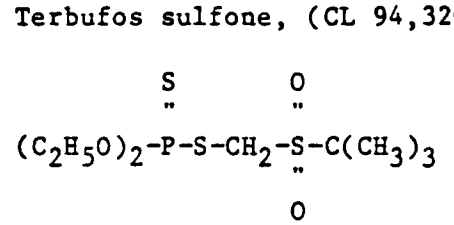
Phosphorodithioic Acid, S-(tert-butylthio) methyl O,O-diethyl ester

b. Terbufos sulfoxide (CL 94,301)



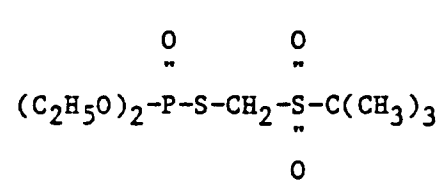
Phosphorodithioic Acid, S-(tert-butylsulfinyl) methyl O,O-diethyl ester

c. Terbufos sulfone, (CL 94,320)



Phosphordithioic acid, S-(tert-butylsulfonyl) methyl O,O-diethyl ester

d. Terbufoxon (CL 94,302)



Phosphorothioic acid, S-(tert-butylsulfonyl) methyl O,O-diethyl ester

2. Solvents: B & J Brand High Purity Solvents, American Burdick and Jackson, or equivalent.

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

3. Reagents: "Baker Analyzed" Reagents, J. T. Baker Chemical Company.

a. Ammonium Chloride
b. Sodium Sulfite
c. Sodium Bicarbonate
d. Polyethylene Glycol 400 (PEG 400), USP
e. Phosphoric Acid (85%)

4. Oxidant: m-Chloroperbenzoic acid, Aldrich Chemical Company.

5. Activated Charcoal: Nuchar C-190N, Eastman Kodak Company.

6. Diatomaceous Earth: Celite 545 AW, Manville Company

7. Solutions:

a. Extraction Solvent: 10% methanol in methylene chloride. Add 100 mL of methanol to a 1-L volumetric flask and dilute to the mark with methylene chloride and mix well.

b. 10% (Weight/Volume) m-Chloroperbenzoic Acid in Methylene Chloride: To be prepared immediately before oxidation step in the procedure. Transfer 1 gram of oxidant to a 10-mL volumetric flask and dilute to the mark with methylene chloride. Shake to dissolve. (Use of an ultrasonic water bath may be necessary to dissolve all of the oxidant).

c. Sodium Sulfite and Sodium Bicarbonate: Saturated solutions in distilled water.

d. Precipitation Solution: Dilute 2.5 mL of phosphoric acid and 1.25 gram of ammonium chloride in a 1-L volumetric flask with distilled water and mix well.

8. GC Column Packing: 3% OV-210 on 80/100 mesh Supelcoport, Cat. No. 1-1956, Supelco, Incorporated.

9. GC Column Packing: 5% Carbowax 20M on 100/120 mesh Supelcoport, Cat. No. 1793, Supelco, Incorporated.

C. Apparatus

1. Gas Chromatograph: Tracor Model 540 or equivalent instrument equipped with a flame photometric detector (phosphorus mode).

2. Recorder: Varian Model 9179 strip chart recorder.

3. Gas Chromatographic Column: 92 cm x 2 mm I.D., borosilicate glass column packed, using slight vacuum, with 3% OV-210 on 80/100 mesh Supelcoport. Leave 2-3 inches empty in the injection end of the column and place a small silylated glass-wool plug and fill with Carbowax 20M packing. At the end of the column add another silylated glass-wool plug. Condition the column overnight with column and inlet temperatures of 230°C and with a carrier flow of 30 mL/min. Remove the 3 inches of Carbowax packing and the small plug that separated the Carbowax from the OV-210 packing and add glass wool to replace the Carbowax. [Ives and Giuffrida, J. Assoc. Anal. Chem. 53, 1973 **(1970)**].

4. Glass Wool, Silane Treated: Catalog No. 14502, Applied Science Laboratories.

5. Microliter Syringe: 10-mcL capacity, Varian auto-sampler syringe.

6. Rotary Evaporator: Buchler Instruments or equivalent, equipped with a glass-evaporator trap (Number K-570200, Kontes Glass Company) between the concentration flask and the glass shaft of the evaporator. During evaporation, warm the flask in a water bath maintained at approximately 35°C.

7. Analytical Balance and Triple Beam Balance.

8. Waring Blendor: Or other suitable laboratory blendor with one-quart jar.

9. Assorted Glassware: General laboratory.

10. Filter Paper: 9- and 7-cm diameter, glass fiber; 934-AH for grain and GF/A for other commodities. Whatman, Incorporated.

11. Filtering Flasks: 500- and 250-mL capacity, Corning Glass Works, Cat. No. 5340.

12. Filtering Funnels: Buchner, fritted disc, medium porosity, 600- and 150-mL capacity.

13. Flasks: 24/40, 1000-, and 500-mL round bottom, and 250- and 500-mL flat bottom.

14. Funnels,Separatory: Squibb-type with teflon stopcocks, 500-, 250-, and 125-mL capacity, Kontes Class Company, Cat. No. K-636030.

15. Hobart Food Chopper: Model 84181D, Hobart Manufacturing Company, Troy, Ohio.

D. Preparation of Standard Solutions (These solutions are stable for at least 1 month if stored under refrigeration).

1. Stock Solution: (Equivalent for all compounds).

a. Weigh accurately 50.0 ± 0.5 mg of each analytical standard into separate, tared 10-mL volumetric flasks. Dilute to the mark with acetone and mix well. These solutions contain 5,000 mcg/mL of each compound. Label Standard Solution A.

b. Pipet 1-mL of each Standard Solution A into separate 100-mL volumetric flasks. Dilute to volume with acetone and mix well. These solutions contain 50 mcg/mL of each compound. Label Standard Solution B.

2. Standard Fortification Solutions

Pipet 20-, 10-, 5-, 2-, and 1-mL aliquots of each Standard Solution B into separate 100-mL volumetric flasks. Dilute each to the mark with acetone and mix well. These solutions contain 10, 5, 2.5, 1.0 and 0.5 mcg/mL, respectively, of each compound.

3. Preparation of Gas Chromatographic Standards (Oxidized)

   a. Pipet a 1-mL aliquot of the standard solution containing 5 mcg CL 92,100/mL into a 100-mL evaporation flask, add one drop of 10% PEG 400 solution and concentrate just to dryness.

   b. Oxidize the standard following the procedure described in Section L ("Oxidation"). Dissolve the CL 94,302 formed in exactly 5 mL of acetone. The concentration of terbufoxon sulfone is equivalent to 1 mcg/mL of the unoxidized standard compound and this solution is the external standard for gas chromatography. For recovery samples, the compound being tested is oxidized and used as a GC standard.

   NOTE: Before samples are analyzed, the oxidized standard should be compared with a 1 mcg/mL Standard Fortification Solution of CL 94,302 (the oxidation product of all compounds of interest) to show that the conversion is 50% or greater. A lower oxidation efficiency would indicate a problem with the conversion, probably due to degradation of the m-chloroperbenzoic acid.

E. Gas Chromatographic Conditions

1. Instrument: Bendix Model 2500

2. Detector: Flame photometric detector (phosphorus mode)

3. Column: 91-cm x 2-mm I.D., glass, packed with 3% OV-210 on 80/100 mesh Supelcoport, deactivated with Carbowax (see Section C.3).

4. Instrument Conditions

a. Column Temperature	165°C
b. Inlet Temperature	230°C
c. Detector Temperature	220°C
d. Helium Flow Rate	50 mL/min
e. Hydrogen Flow Rate	50 mL/min
f. Air Flow Rate	45 mL/min
g. Retention Time (approximate)	1.7 minutes

5. Strip Chart Recorder: 0.5 cm/min chart speed.

6. Sensitivity: Electrometer sensitivity and recorder attenuation set to obtain a peak height of approximately 30% FSD (full-scale deflection) for a 5-ng injection of oxidized standard CL 92,100. Several injections of the 100 mcg/mL standard solution (CL 94,302)

and sample extracts should stabilize the response.

F. Linearity Check The gas chromatograph should be checked for linearity at least weekly and whenever the column, new or used, is newly installed in the instrument.

1. Inject 5-mcL aliquots of CL 94,302 solutions prepared in Section D.2., containg 2.5, 1, and 0.5 mcg/mL.

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

G. 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 50-gram subsample of control field corn grain or control sweet corn kernels plus cob, or 20-gram subsample of green plant, dry plant or cannery waste and transfer it to a quart Waring blendor jar.

2. Add by pipette 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 extractant solvent.

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

5. For a GC standard, oxidize a quantity of the particular compound being tested, i.e., if recoveries are being run with CL 92,100, then a 5 mcg quantity of CL 92,100 should be oxidized along with the sample and should be used as the GC standard.

H. Sample Preparation

1. For corn grain, pulverize sufficient dry ice in a Waring blendor to aid in the preparation of the corn kernels.

2. Slowly add the kernels to the dry ice and grind until the sample is homogeneous.

3. For all other corn commodities, chill the blades of a Hobart food chopper by placing pulverized dry ice in the bowl and running it for several minutes.

4. Add the frozen sample in portions with additional dry ice and chop the entire sample.

5. Mix the chopped sample thoroughly and pack several subsamples in ice cream containers.

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

I. Extraction

1. Transfer a 50-g subsample of field corn grain or sweet corn kernels plus cob, or a 20-gram subsample of green plant, dry plant, or cannery waste to a quart blendor jar. Add 500 mL of extraction solvent to the 50-gram subsample or 350 mL to the 20-gram subsample and blend for 5 minutes with a Waring blendor at high speed.

2. Filter the mixture with a 600-mL sintered-glass funnel with vacuum.

3. Rinse the blendor jar with 50 mL of extraction solvent and rinse the filter cake in the funnel. Collect this rinse and combine with the filtrate.

4. Transfer the extract to a 1,000-mL evaporation flask and evaporate on a flash rotary evaporator at 35°C, until a viscous, oily residue remains in grain or a small water layer in green corn kernels plus cob. The residual water is removed by azeotroping with 25 mL of acetonitrile.

J. Hexane-Acetonitrile Partitioning

1. Transfer the residue from the evaporation flask to a 250-mL separatory funnel using two successive 50-mL portions of hexane followed by 100 mL of acetonitrile. Stopper and shake for 30 seconds.

2. Allow the phases to separate and draw off the lower (acetonitrile) layer into a 500-mL flat-bottom flask.

3. Add an additional 100 mL of acetonitrile to the separatory funnel, shake for 30 seconds, allow the phases to separate and combine the acetonitrile phase with the first extract in the 500-mL flask.

4. Evaporate the combined acetonitrile extracts to dryness on a rotary-film evaporator at 35°C.

K. Charcoal Cleanup

1. Dissolve the residue in 100 mL of acetone, add 1.5 gram of Nuchar C-190N and shake vigorously for 30 seconds. With the aid of a vacuum, filter the mixture through glass-fiber filter paper in a 150-mL capacity sintered-glass funnel.

2. Rinse the flask and funnel with 25 mL of acetone. Collect and transfer the filtrate to a 300-mL round-bottom flask.

3. Add 1 drop of 10% PEG 400 in acetone to the flask.

4. Evaporate the solvent just to dryness using a rotary-film evaporator.

L. Oxidation

1. Dissolve the residue in 20-mL of methylene chloride and add 3 mL of freshly prepared m-chloroperbenzoic acid reagent. Immediately transfer the solution to a 125-mL separatory funnel using two, 5-mL methylene chloride rinses. Let stand for 15 minutes.

2. Add 25 mL of saturated sodium sulfite solution to the funnel and mix gently for 15 seconds.

3. Allow the phases to separate and draw off the upper (aqueous) layer by suction with a disposable capillary pipet which is attached by tubing to a 1-liter suction flask.

4. Add 25 mL of saturated sodium bicarbonate solution and mix gently for 15 seconds. Allow the phases to separate and draw off the upper (aqueous) layer as descibed above.

5. Gently wash the methylene chloride phase with two 25-mL portions of distilled water. Again draw off the first water wash (upper layer) by suction.

6. Allow the methylene chloride and last water wash to separate and then draw off the lower (methylene chloride) phase into a 300-mL round-bottom flask.

7. Evaporate to dryness with a rotary evaporator at 35°C.

M. Coagulation Cleanup

1. Dissolve the residue in 5 mL of acetone and then add 50 mL of precipitation solution and mix well.

2. Add 2 grams of Celite and 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 300-mL round-bottom flask.

7. Extract the aqueous phase with an additional 50-mL portion of methylene chloride.

8. Combine the methylene chloride extracts in the 300-mL round-bottom flask.

9. Evaporate the methylene choride to dryness.

10. Dissolve the residue in 2.0 mL of acetone for gas chromatographic analysis with a flame photometric detector.

N. Gas Chromatographic Analysis

1. After obtaining a suitable GC response for a 5-ng injection of oxidized CL 92,100, compare the response to that of CL 94,302 to ensure acceptable conversion (see Note in Section D.3).

2. Inject a 5-mcL aliquot of the sample and compare the peak height obtained with that from a 5-mcL injection of oxidized standard (1 mcg/mL concentration).

3. If the sample peak goes off scale, dilute to an appropriate volume and reinject.

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

O. Calculations

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

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

Where:

R(SAMP) = Peak response of sample.

R(STD) = Average peak response of oxidized standard.

W = Weight of sample taken for analysis in grams.

V1 = Volume of extraction solvent added to sample in millimeters.

V2 = Aliquot of extract taken for analysis in millimeters.

V3 = Volume of acetone added to dissolve final residue for chromatographic analysis in millimeters.

V4 = Volume of sample solution injected in microliters.

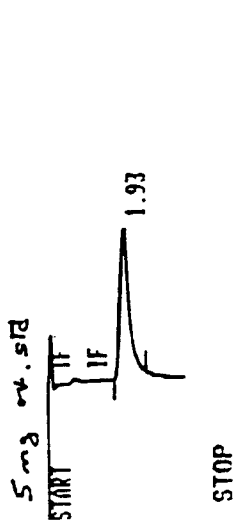
V5 = Volume of working standard solution injected in microliters.

C(STD) = Concentration of working standard solution in micrograms per millimeter.

D.F. = Dilution Factor

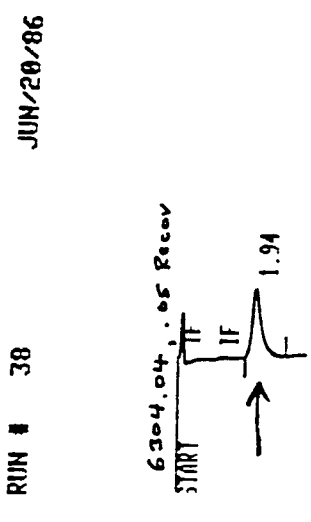
Figures M-1754.A to M-1754.C show typical chromatograms for determining total CL 92,100-related residues in corn commodities.

Figure M-1754.A: Typical Chromatograms for the Recovery of Total CL 92,100-Related Residues from Green and Dry Corn Plants

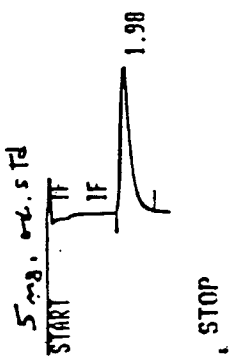


Oxidized Standard, 5 ng Injected

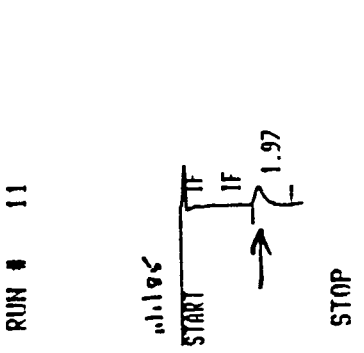
Control Green Corn Plants, 50 mg Injected, <0.001 ppm Apparent CL 92,100-Related Residue



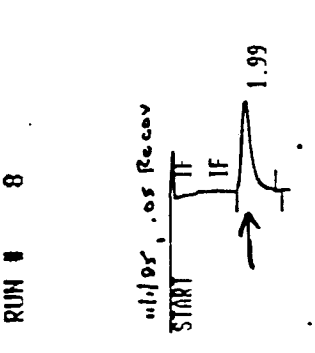
Green Corn Plants Fortified at 0.05 ppm, 50 mg Injected, 96% Recovered



Oxidized Standard, 5 ng Injected

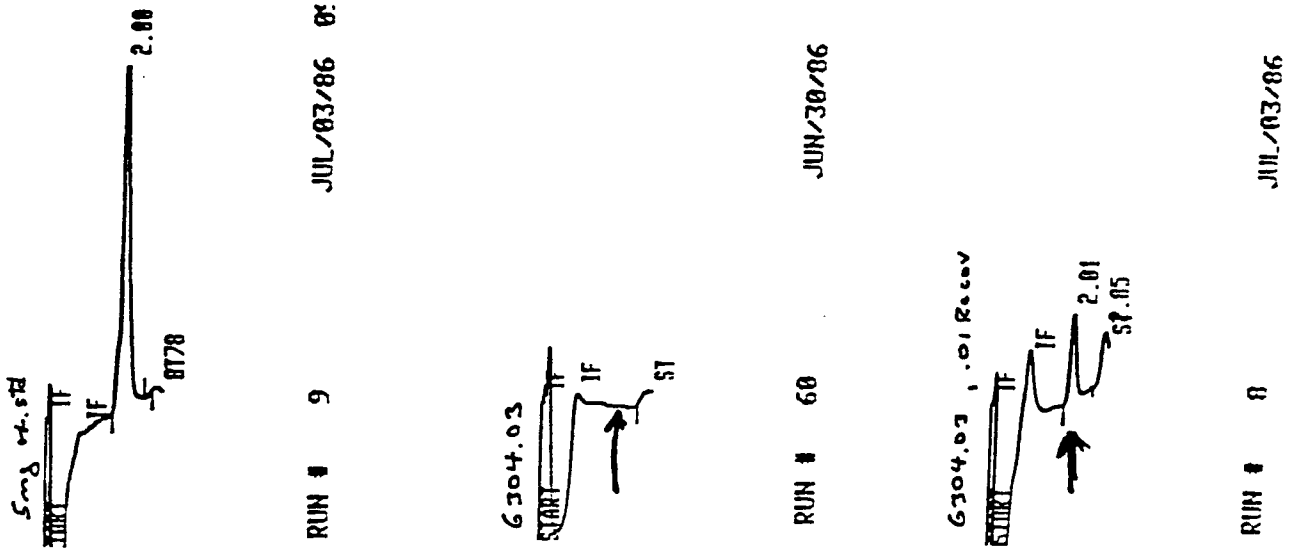


Control Dry Corn Plants, 50 mg Injected, 0.013 ppm Apparent CL 92,100-Related Residue



Dry Corn Plants Fortified at 0.05 ppm, 50 mg Injected, 96% Recovered

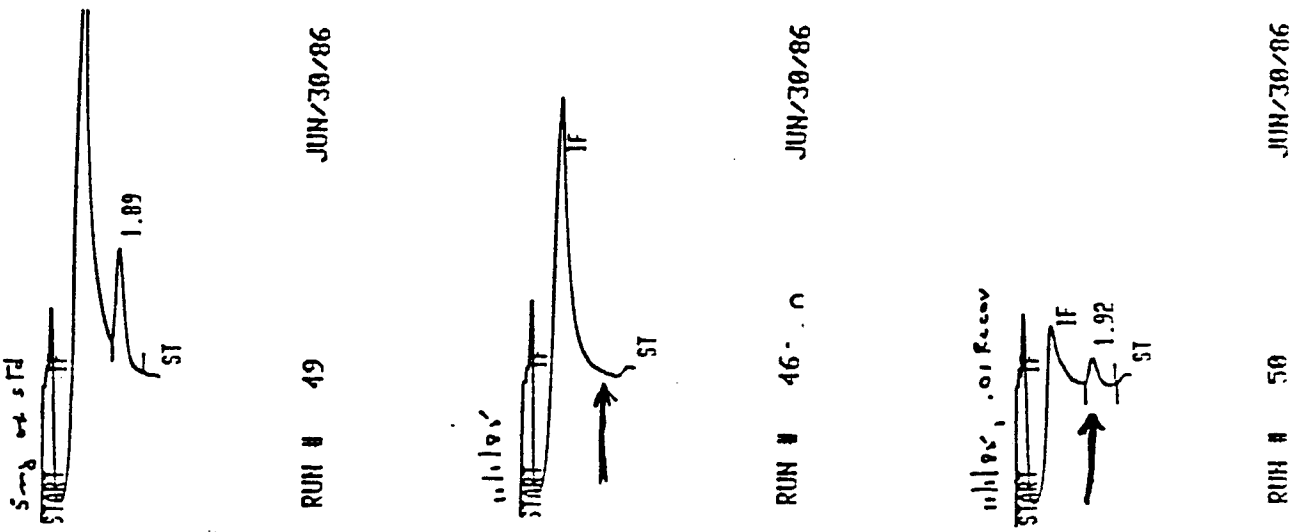
Figure M-1754.B: Typical Chromatograms for the Recovery of Total CL 92,100-Related Residues in Field Corn Grain and Sweet Corn Kernels plus Cob



Oxidized Standard, 5 ng Injected

Control Kernels plus Cob, 125 mg Injected, <0.001 ppm Apparent CL 92,100-Related Residue

Kernels plus Cob Fortified at 0.01 ppm, 125 mg Injected, 116% Recovered

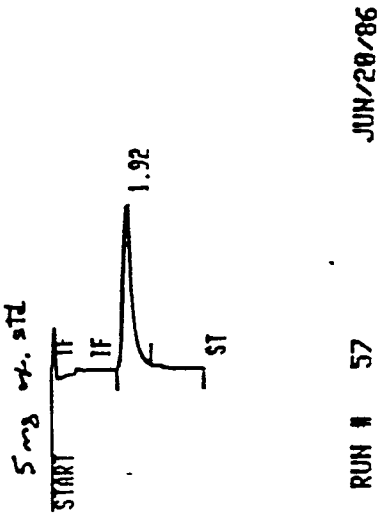


Oxidized Standard, 5 ng Injected

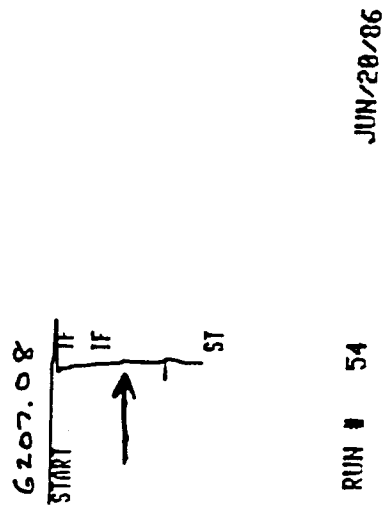
Control Corn Grain, 125 mg Injected, <0.001 ppm Apparent CL 92,100-Related Residue

Corn Grain Fortified at 0.01 ppm, 125 mg Injected, 105% Recovered

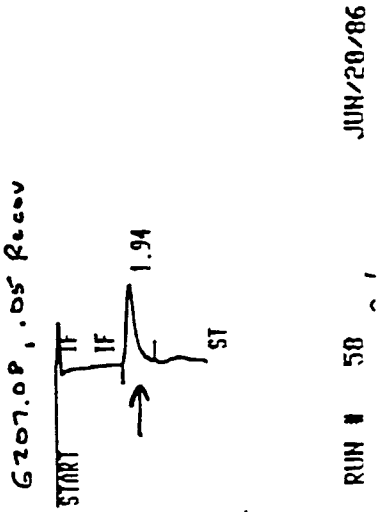
Figure M-1754.C: Typical Chromatograms for the Recovery of Total CL 92,100 Related Residues from Corn Cannery Waste



Oxidized Standard, 5 ng Injected



Control Cannery Waste 25 mg Injected, <0.001 ppm Apparent CL 92,100-Related Residue



Cannery Waste Fortified at 0.05 ppm, 25 mg Injecte 101% Recovered