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DETERMINATION OF SC-1029 (EPTC, R-29148
AND R-33865) RESIDUES IN CORN GRAIN AND
SILAGE BY GAS CHROMATOGRAPHY
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TITLE: DETERMINATION OF SC-1029 (EPTC, R-29148 AND R-33865) RESIDUES IN CORN GRAIN AND SILAGE BY GAS CHROMATOGRAPHY

I. SCOPE

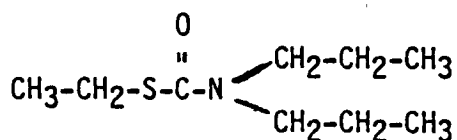
This method is intended for the analysis of corn grain or silage for residues of SC-1029 (EPTC, R-29148 and R-33865) at levels of 0.05 ppm or greater.

II. SUMMARY OF METHOD

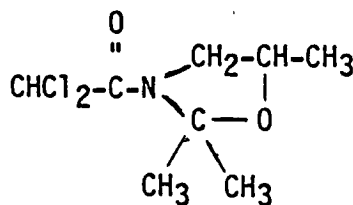
A sample of corn grain or silage is extracted with toluene. The extract is analyzed for SC-1029 (EPTC, R-29148 and R-33865) by gas chromatography with nitrogen-phosphorus detection.

III. INTRODUCTION

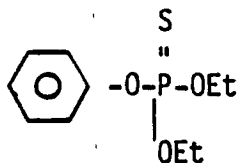
EPTC is S-ethyl N,N-dipropylthiocarbamate, R-29148 is 2,2,5-trimethyl-N-dichloroacetyl oxazolidine, R-33865 is O,O-diethyl O-phenyl phosphorothioate. The compounds have the following chemical structures:



EPTC



R-29148



R-33865

IV. EQUIPMENT AND REAGENTS

A. Equipment

1. Gas Chromatograph. Hewlett-Packard Model 5880 or equivalent, equipped with a nitrogen-phosphorus detector.

2. Gas Chromatographic Column. 120 cm x 2 mm i.d. silanized Pyrex®, packed with 10% SP-2401 on 100/120 mesh Supelcoport® (Supelco, State College, PA).
3. Waring Blendor. 1 pint capacity or equivalent.
4. Glass Funnels. 12.5 cm diameter.
5. Filter Paper. Whatman No. 2, 24 cm diameter.
6. Glass Bottles. 8 oz narrow mouth with Poly-Seal caps.
7. Syringe. 10 μ L, Hamilton No. 701 or equivalent.

B. Reagents

1. Toluene. Nanograde or equivalent.
2. Sodium Sulfate. Anhydrous, reagent grade.
3. EPTC, R-29148, R-33865. Analytical reference standards.
Available from Stauffer Chemical Company, 1200 So. 47th Street,
Richmond, California 94804, Attn: Manager, Analytical Section.

V. PROCEDURES

A. Extraction

Weigh 50 g of a thoroughly chopped and mixed sample into a 1 pt. Waring Blendor jar. Add 200 mL of toluene and blend the sample at moderate speed, 3 minutes for fodder and 5 minutes for dry corn grain. Set up a funnel that contains a filter paper and 25 to 30 g of sodium sulfate. Filter the extract and collect the filtrate in an 8 oz glass bottle containing a 0.5-cm layer of sodium sulfate.

B. Clean-up

The samples used in developing this method were analyzed without the need for a column clean-up. Previous work has indicated that some samples may contain compounds that interfere with the analysis of EPTC, R-29148 or R-33865. The clean-up procedure described in Appendix A has been used to remove interferences.

C. Analysis

1. Gas Chromatographic Conditions

Use the following conditions for determining EPTC, R-29148, and R-33865 with a Hewlett-Packard Model 5880 chromatograph:

Column temperature	140°C
Injector temperature	200°C
Detector temperature	300°C
Helium flow	30 mL/min
Air flow	80 mL/min
Hydrogen flow	3.0 mL/min
Quantitation	Peak height

Under these conditions, EPTC elutes in 2.2 min., R-29148 in 3.5 min. and R-33865 in 7.5 min.

2. Calibration

Inject 8.0 μ L of a calibration solution containing 0.012 μ g/mL each of EPTC, R-29148 and R-33865 in toluene. These concentrations correspond to a detection limit of 0.05 ppm for each analyte in crop samples. Record the retention times and peak heights for the analytes. Figures 1 through 4 illustrate chromatograms of typical calibration solutions.

3. Analysis of Extracts

Inject 8.0 μ L of the extract. Measure and record the heights of the peaks coincident in retention time to the calibration solution injected above. Reinject the calibration solution after every fourth or fifth injection of sample extract, and after all samples have been analyzed, to assure that instrument response is stable.

If a crop extract is found to contain one or more of the analytes at a concentration above the detection limit of the method, prepare a calibration curve using standard solutions that bracket the concentration range of the samples. Figures 2 and 4 illustrate chromatograms of extracts of fortified crop samples.



VI. CALCULATIONS

A. Calibration Factors

Calculate a calibration factor for each analyte for each injection of calibration solution as follows:

$$F \text{ (ng/cm analyte)} = \frac{C \times I}{H}$$

where C = concentration of analyte in calibration solution, $\mu\text{g/mL}$

I = volume of calibration solution injected, microliters

H = peak height in centimeters.

Alternatively, an on-line data system with appropriate software may be used for calibration.

B. Analyte in Sample

Choose the calibration factor calculated from the calibration solution whose peak most closely matches in height the peak for each analyte in the sample solution. Then calculate the concentration of analyte in the original sample as follows:

$$\text{Concentration (ppm)} = \frac{F \times B \times V_j}{I \times W}$$

where F = appropriate calibration factor, ng/cm

B = peak height from sample extract, cm

I = volume of extract injected, microliters

W = weight of crop extracted, g

V_j = volume of original extract, mL

In the procedures as given above, $V_j = 200 \text{ mL}$, $W = 50 \text{ g}$, and $I = 8.0 \text{ }\mu\text{L}$; using these values, the equation becomes:

$$\text{Concentration (ppm)} = 0.5 \times F \times B$$

Preferably, use an average calibration factor from calibration solutions whose responses bracket the response of the sample solution.

VII. DISCUSSION

A. Accuracy

Fortified crop samples were prepared by the addition of analytes to untreated samples. The samples were analyzed by the method specified above. Typical chromatograms of both fortified and unfortified

samples are shown in Figures 1 through 4. Recoveries from the temperature programmed analyses are listed in Table I. Average recoveries of EPTC, R-29148 and R-33865 from corn silage fortified at 0.05 ppm were 107%, 108%, and 104% respectively. From corn grain fortified with 0.05 ppm EPTC, R-29148 and R-33865 the average recoveries were 101%, 102%, and 102% respectively.

B. Method Precision

Precision was determined by analysis of three samples fortified at 0.05 ppm. The coefficient of variation (100 s/X) was 8%, 10%, and 8% for EPTC, R-29148 and R-33865 respectively in corn silage, and 14%, 11% and 8% for EPTC, R-29148 and R-33865 respectively in corn grain.

C. Interferences

Check samples (not containing analytes) must be extracted and analyzed with every sample set to ensure the absence of interfering compounds that could co-elute with the analytes during the GC analysis. At least one fortified check sample must be analyzed along with each set of samples. Recoveries from fortified check samples should be between 70 and 130%.

VIII. SAFETY PRECAUTIONS

A. Toluene

- Flammable.
- Use in well ventilated area.
- Avoid breathing vapor.
- Avoid skin contact.

B. R-33865

- Organophosphates
- Flammable.
- Use in well ventilated area.
- Avoid breathing vapor.
- Avoid skin contact.

IX. REFERENCES

WRC Notebook 8023-27 through 30.

13/14:M12:84-58



Table I. Recovery of EPTC, R-29148, and R-33865 from Fortified Crop Samples.

Sample Type	Fortification Level, ppm	Recoveries		
		% EPTC	% R-29148	% R-33865
Dry corn grain	0.05	110	106	110
" " "	0.05	94	100	98
" " "	0.05	84	86	94
" " "	1.0	92	90	96
Corn Silage	0.05	102	100	98
" "	0.05	118	120	112
" "	0.05	116	120	114
" "	1.0	84	92	87



STAUFFER CHEMICAL COMPANY

RICHMOND RESEARCH CENTER

1200 S. 47TH STREET, RICHMOND, CA 94804

Method No. RRC 84-58Page 7APPENDIX AClean-up

This clean-up has been used for corn extracts.

Add 3 g of a 19:1 w/w mixture of SilicAR CC4 and Nuchar carbon to a 11 mm i.d. x 15 cm chromatographic tube. Place a 1-2 cm layer of sodium sulfate on top of the mixture. Pipette a 20 mL aliquot of the toluene extract (5 g sample) onto the column and apply enough air pressure to deliver 1 mL/min. Interrupt pressurization when the solvent reaches the top of the sodium sulfate layer. Wash the column by adding an additional 20 mL toluene, then pressurizing as above. Elute the analytes from the column into a graduated centrifuge tube by adding 20 mL of a 95:5 v/v mixture of toluene and ethyl acetate then pressurize and collect the eluate. Carefully evaporate the eluate to near dryness on a Buchler Rotary Evapo-Mix, maintaining the water bath at 45°C. Reduction of final volume to dryness may cause loss of analytes and is to be avoided. Dilute to 0.5 mL with toluene prior to GC analysis.

Reference RRC Method 79-33.

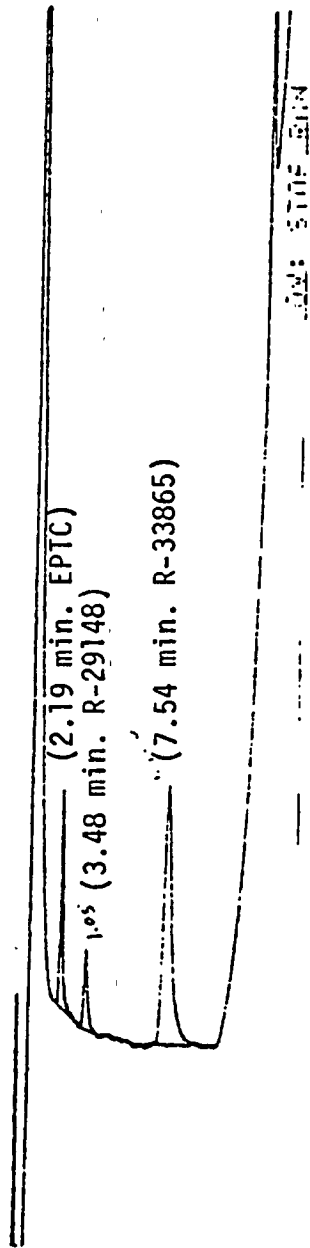
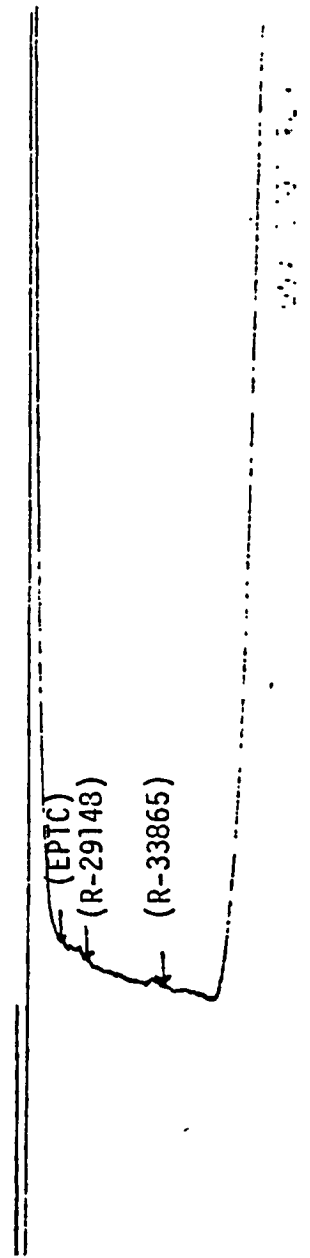


Figure 1.

8 μ L of a 0.012 μ g/mL
Standard of EPTC, R-29148,
and R-33865



8 μ L of an untreated
corn grain control
sample extracted at
0.25 g/mL

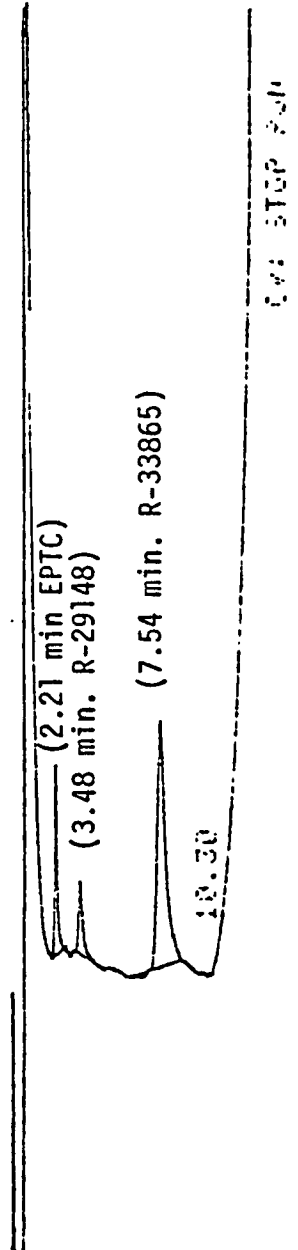
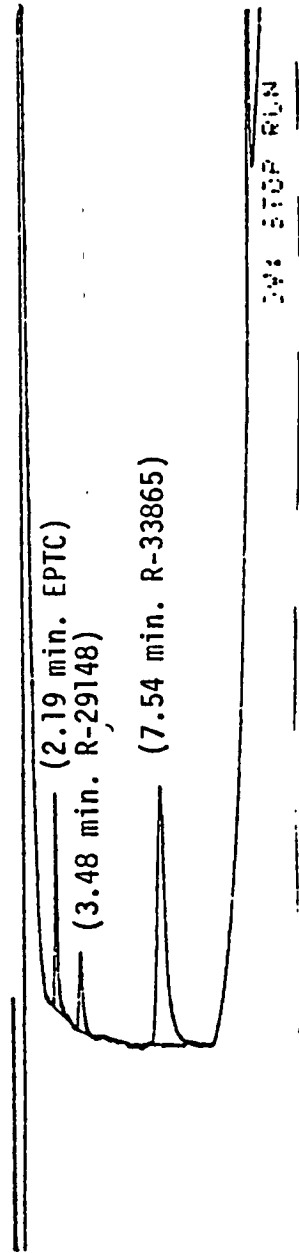


Figure 2.

8 μ L of a 0.012 μ g/mL
Standard of EPTC,
R-29148, R-33865

8 μ g of an untreated
corn grain sample
fortified at 0.05 ppm
and extracted at 0.25 g/mL

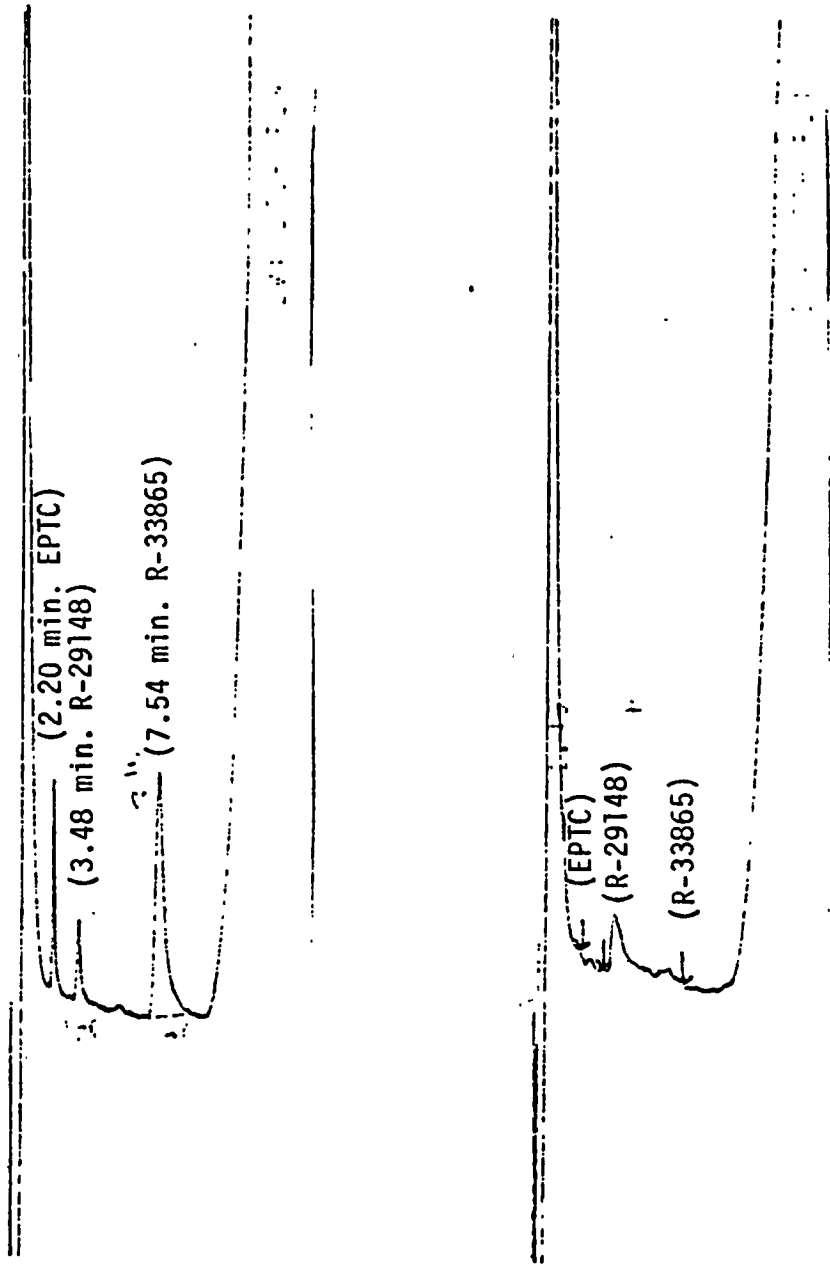


Figure 3.

8 μ L of a 0.012 μ g/mL
 Standard of EPTC, R-29148,
 R-33865 -

8 μ L of an untreated
 silage control sample
 extracted at 0.25 g/mL

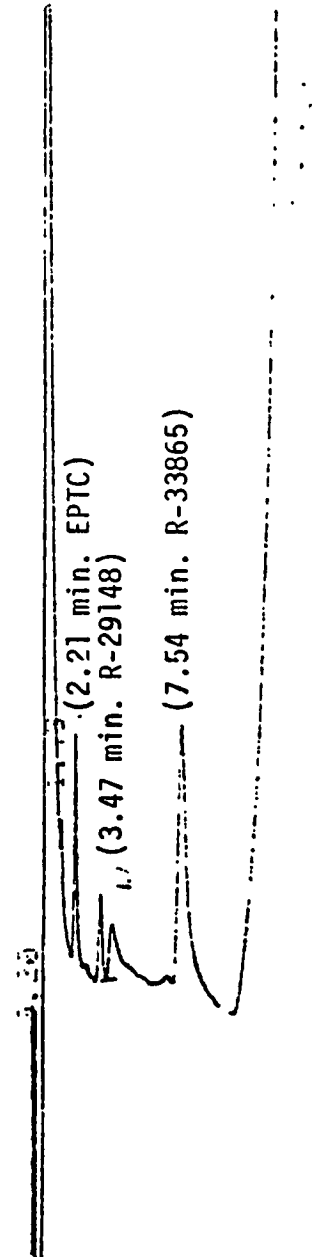
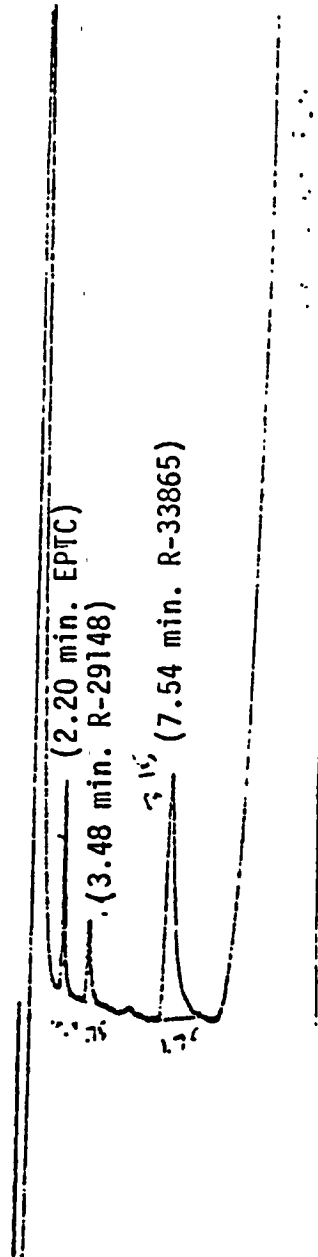


Figure 4

8 μ L of a 0.012 μ g/mL
Standard of EPTC,
R-29148, R-33865

8 μ L of an untreated
silage control sample
fortified at 0.05 ppm
and extracted at
0.25 g/mL