Determination of Ethyl-*p*-nitrophenylthionobenzene Phosphonate (EPN) Residues by Electron-Capture Gas Chromatography

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An electron-capture gas chromatographic procedure has been devised for the determination of ethyl-*p*-nitrophenylthionobenzene phosphonate (EPN) residues on a variety of plant tissues. A simple isolation procedure of surface residues produces an extract which can be directly chromatographed without cleanup. Excellent recoveries were obtained throughout a range of 0.02 to 2.1 p.p.m. of EPN added to a number of different crops. Several other phosphorus- and/or sulfur-containing pesticides tested do not interfere with the EPN determination.

Various analytical procedures for determining ethyl-*p*-nitrophenylthionobenzene phosphonate (EPN) residues are in the literature (*1*, *6–9*). However, these methods usually involve extensive cleanup procedures during the isolation of the residue, and often do not afford the desired selectivity or sensitivity.

Gas chromatography with selective detection is clearly a superior approach for the determination of EPN residues. Several qualitative studies involving the gas chromatography of EPN have been reported, but a quantitative method suitable for routine application on a variety of crops is not available. The qualitative gas chromatography of EPN with electron-capture detection has been reported, but no quantitative analyses were described (*2*, *3*, *14*). The detection of EPN with microcoulometric titration (*4*, *5*) and sodium thermionic detection (*10*) has also been demonstrated, but no data were given regarding quantitative analysis. Nelson (*12*) has reported a brief study on the determination of EPN residues by gas chromatography. Using microcoulometric detection, he obtained EPN recoveries of 78% at the 0.2- to 1.5-p.p.m. level in peaches and lettuce.

This paper describes an electron-capture gas chromatographic procedure for quantitatively determining EPN residues on the surface of a wide variety of plant tissues. The simple procedure is more sensitive than previously reported methods, and is highly selective.

Experimental

Apparatus and Reagents. GAS CHROMATOGRAPHY INSTRUMENT. The electron-capture gas chromatograph used throughout this study was constructed in this laboratory. The electron-capture detector (100-mc. titanium tritide source) was obtained from the Jarrell-Ash Co., Waltham, Mass. This unit is housed in an insulated compartment in which the temperature is controlled by a Minneapolis-Honeywell Versatrans, Model R-7079.

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A high velocity air bath is heated with a 1500-watt resistive element and is capable of circulating air at 1500 linear feet per minute by means of a centrifugal blower. The temperature of the air bath is controlled with an F&M Scientific Corp. Model 220 power-proportioning temperature controller.

An electrometer from a Wilkins Instrument Co. Model 500 flame ionization unit is used to measure the output from the detector. Detector d.c. voltage is monitored with a RCA Voltohmyst, Jr., and the electrometer output is fed into a Bristol Dynamaster 1-mv. recorder. A General Radio Co. Model 1217-B unit pulser, Model 1203-B unit power supply, and Model 1219-A unit pulse amplifier are used in conjunction with the electron-capture cell in order to obtain a pulsed potential operation as suggested by Lovelock (*11*).

A carrier gas of 5% methane in argon was used for pulsed potential detector operation. Nitrogen was employed for d.c. potential studies.

The instrument is equipped with a flash vaporizer containing a quartz liner. Vaporizer temperature is monitored with a thermocouple.

GAS CHROMATOGRAPHIC COLUMN. The column was made from 17.7-inch, 0.25-inch o.d., 0.188-inch i.d. stainless steel tubing filled with 2% Epon 1001 epoxy resin on 80- to 100-mesh Diatoport S (F&M Scientific Corp.). The packing was coated by dissolving the resin in acetone, mixing it with the support, and removing the solvent by gently stirring the mixture over a steam bath. The packing was dried in a vacuum oven for about 4 hours at 100° C. The column was filled with this packing by conventional vibration techniques, placed in the instrument, and conditioned at 210° C. for 72 hours with a carrier flow rate of 250 cc. per minute before use.

EPN, standard reference material, E. I. du Pont de Nemours & Co., Industrial & Biochemicals Department, Agrichemicals Sales Division.

MICROSYRINGE, Model 705, 50-μl. capacity, Hamilton Co., Whittier, Calif.

Benzene, reagent grade, redistilled.

Procedure. ISOLATION OF EPN. Weigh a suitable-size aliquot of coarsely diced fruit or vegetable, randomly selected to give a representative sample

(100 to 500 grams, dependent upon the bulk of the material), into a large screw-cap jar. Line the cap of the jar with aluminum foil to minimize leaks and prevent direct contact of the solvent with the plastic cap liner. Add 300 ml. of redistilled benzene to the jar and close tightly. Extract the EPN from the crop by alternately shaking, rolling, and inverting the jar for 2 to 3 minutes. Filter the benzene extract through No. 40, 12.5-cm. Whatman filter paper into a 1000-ml. volumetric flask. Repeat the extraction two more times with 300 ml. of benzene, combining the filtered solvent in the volumetric flask. Dilute to the mark with benzene and mix thoroughly.

GAS CHROMATOGRAPHIC ANALYSIS. Calibration. Equilibrate the gas chromatograph as follows: column temperature, 210° C.; vaporizer temperature, 285° C.; detector temperature, 215° C.; carrier flow, 250 cc. per minute of 5% methane in argon; detector pulse operation, 30 kc., 0.7 μsec., 35 volts; electrometer input, 10^9 ohms. (Background current should be stable at about 2 to 4 $\times$ 10^{-9} ampere before quantitative data are obtained.)

Prepare fresh stock solutions containing 10, 20, 50, 100, and 200 ng. of EPN per ml. of benzene. Inject a 10-μl. aliquot of each of these calibration solutions evenly over a 10-second period into the gas chromatograph. Determine the height of the EPN peak and prepare a calibration plot of EPN peak height *vs.* nanograms of EPN injected.

ANALYSIS OF UNKNOWN. Inject a 10-μl. aliquot of unknown sample extract prepared as described in "Isolation." Determine the EPN peak height and refer to the calibration plot to determine the nanograms of EPN in the aliquot.

CALCULATIONS.

$$\frac{\text{Ng. EPN in aliquot} \times 100}{\text{sample wt., grams}} = \text{p.p.m. EPN in sample}$$

Results

Analyses of untreated samples of oranges, peaches, apples, tomatoes, string beans, cut corn, and raw and finished silage fortified with EPN are summarized in Table I. Known amounts of EPN in solution were added to the diced crops, the fortified sample was allowed to stand for at least an hour, and the extraction and analysis were carried out as described in "Procedure." EPN recoveries averaged 104% throughout a range of 0.02 to 2.1 p.p.m. All crops tested except peaches showed a background interference of less than 0.01 p.p.m., based on a 300-gram sample or larger. Figure 1 shows the chromatograms obtained on the crude extracts of untreated tomatoes, and tomatoes fortified with 0.024 p.p.m. of EPN.

The tomato control curve in Figure 1 has a minor background peak which is not present in the chromatogram of fortified tomato sample, although it is completely separated from EPN and represents no interference. This minor variation in background can be attributed to the fact that the tomato control and the tomatoes which were fortified represent two different samples. At the very high instrumental sensitivity used, minor variations in background for different samples of the same crops are normal. Even at the limit of sensitivity, these minor background variations offer no difficulty as long as it is demonstrated that insignificant changes occur at the retention time of the peak to be measured.

Table I. EPN Recovery Studies

Sample	Sample Wt., G.	P.P.M. EPN Added	P.P.M. EPN Found[a]	Recovery, %
Oranges	447	0	N.D.	...
	450	0.022	0.03	136
	480	0.10	0.11, 0.10	105
	480	0.21	0.19	93
Peaches	213	0	N.D.	...
	211	0.095	0.08	84
	220	0.45	0.41, 0.45	96
	208	0.72	0.43	60
Apples	420	0	N.D.	...
	346	0.058	0.07	122
	360	0.22	0.19	85
	420	0.30	0.26	88
Tomatoes	323	0	N.D.	...
	415	0.024	0.03	125
	395	0.13	0.13	100
	317	0.47	0.47	100
String beans	100	0	N.D.	...
		0.10	0.09	90
		0.40	0.39	98
		0.80	0.88	109
Raw silage	100	0	N.D.	...
		1.2	1.3	108
	25	2.1	2.4	114
		0.14	0.21	150
Finished silage	100	0	N.D.	...
		1.2	1.3	108
	25	2.1	2.4	114
		0.14	0.12	86
Cut corn	100	0	N.D.	...
		1.2	1.3	108
	25	2.1	2.4	114
		0.14	0.12	86

[a] N.D. Not detected.

To demonstrate the accuracy of the procedure at levels down to the limit of detection, recoveries were attempted on samples fortified at the lowest levels (Table I). Obviously, the precision of recoveries at the limit of detection will necessarily be poor, and recovery data obtained at this level unduly bias the average per cent recovery for all samples analyzed, which was found to be 104%. The value in carrying out recovery studies at the limit of detection is to demonstrate clearly that accurate analyses can actually be obtained at this level with various crops.

To demonstrate the applicability of the electron-capture gas chromatographic method for EPN residue

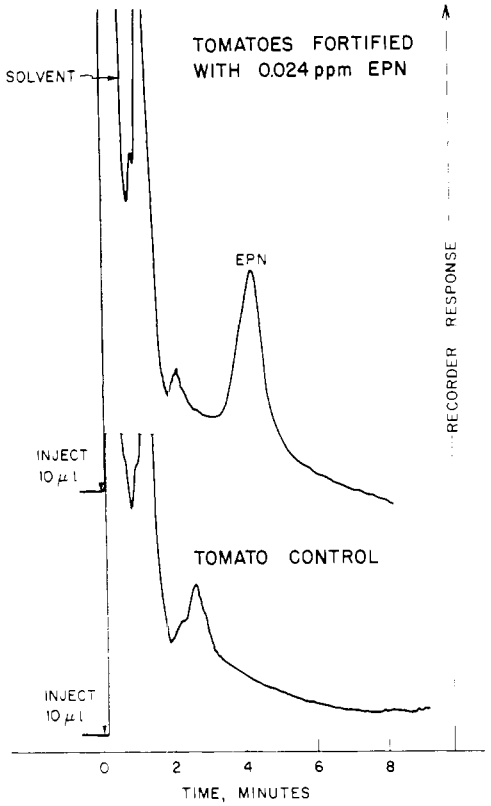

Figure 1. Gas chromatograms of tomato extracts

Lower. 323 grams of untreated tomatoes (control)
Upper. 415 grams of tomatoes fortified with 0.024 p.p.m. EPN
Sensitivity. $\frac{1}{2}$ maximum

determination, a series of corn and silage samples was analyzed by the electron-capture GC procedure and a frequently used colorimetric method for EPN (*1, 13*). For colorimetric analyses, the crude extracts were concentrated to 100 ml. by solvent evaporation, so that a sufficient concentration of EPN in the final solution could be obtained for measurement. (These concentrated extracts were appropriately diluted where necessary for electron-capture gas chromatography.) Data obtained by the colorimetric and gas chromatographic procedures show good agreement (Table II). Average recoveries were theoretical for the gas chromatographic procedure and somewhat higher than theoretical for the colorimetric method. The positive bias indicated for the colorimetric procedure probably is due to the inability to maintain a constant blank.

Discussion

To ensure optimum quantitative analyses, data were obtained with the electron-capture detector in both the d.c. and pulsed potential modes. Attempts to quantitate the d.c. detector mode were disappointing. Significant variability in the detector response was found, and considerable difficulty was experienced in maintaining accurate calibrations. This variability was partially due to the continuous change in the standing current, influenced by inconstant column liquid bleed, continuous

Table II. Comparison of EPN Methods

	EPN, P.P.M.				
		Colorimetric		Gas Chromatographic	
Sample	Added	Found	Recovery, %	Found	Recovery, %
Raw silage	0	0.14	...	N.D.	...
	0.59	0.70	119	0.69	117
	1.18	1.24	105	0.96	81
Finished silage	0	0.17	...	N.D.	...
	0.59	0.64	118	0.60	102
Cut corn	0	0.16	...	N.D.	...
	0.59	0.65	110	0.64	108

elution of very high boiling unknowns from extracts, etc.

Significantly improved reproducibility was obtained by employing the pulsed potential detector mode in conjunction with using a carrier gas of 5% methane in argon, as suggested by Lovelock (*11*). With the pulsed mode, replicate samples analyzed during a single day have shown a 2δ variation of about 4%. Long-term detector response has varied as much as 15 to 20% because of standing current changes resulting from changes in column bleed characteristics. Short-term variations are adjusted by running a daily standard.

Use of the pulsed potential detector operation virtually eliminates negative peaks which sometimes occur when a d.c. detector mode operation is used. This is in keeping with the proposal by Lovelock (*11*) that pulsed operation, in conjunction with a carrier gas such as 5% methane in argon, desensitizes the electron-capture detector to other effects such as electron mobility, space charges, and metastable states.

Even with the pulsed mode of detector operation, negative peaks can occasionally occur. These are most likely when a major nonelectron-capturing species elutes from a column having some partitioning liquid bleed with slight electron-capturing properties. In effect, the eluting nonelectron-capturing peak dilutes the slight electron-capturing column bleed sufficiently so that a momentary increase in standing current occurs. This increase produces a negative peak at the retention time of the eluting nonelectron-capturing compound.

A range of pulse characteristics was investigated to ensure optimum performance of the detector. A pulse frequency of 30 kc. was found to be optimum throughout the 10- to 100-kc. region covered. A study of pulse duration throughout the range of 0.5 to 15 μsec. showed that pulses of 0.7 μsec. produced the highest response for the chromatographic process employed. The effect of the intensity of the voltage pulses is shown in Figure 2. Although not producing the highest response, a potential of 35 volts was selected since this setting occurs in a response plateau. Minor changes in excitation conditions result in a minimum change in detector response.

Base line peak height measurements were used for obtaining quantitative EPN data. This method of

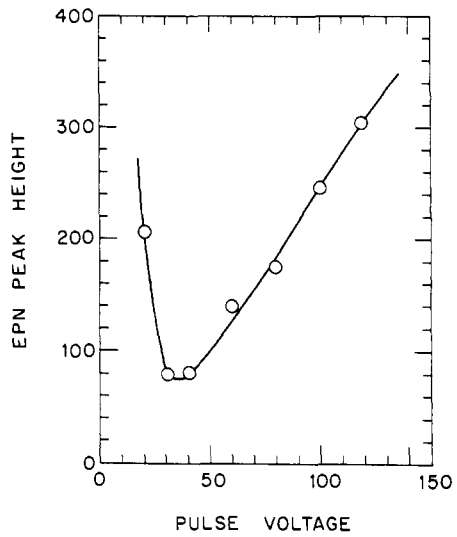

Figure 2. Effect of detector pulse voltage

Pulse. 0.7 μsec., 30 kc.

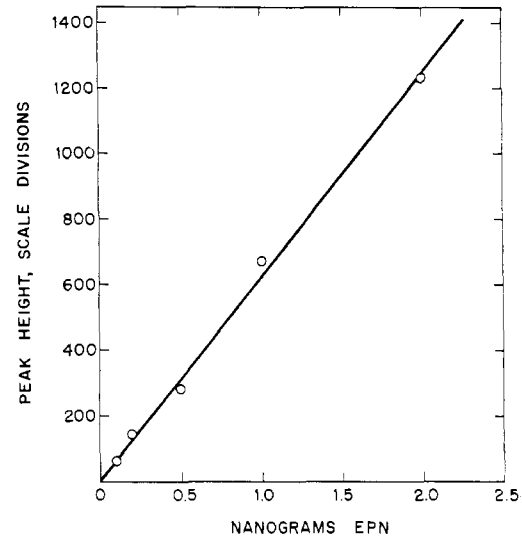

Figure 3. EPN calibration curve

10-μl. sample aliquot

measuring concentration affords maximum freedom of interference from neighboring unidentified peaks occurring in some plant extracts. Using the conditions described in "Experimental," linear calibration curves were obtained for EPN in amounts up to 2 ng., as shown by the calibration plot in Figure 3. Log-log plots of EPN peak areas *vs.* concentration are linear for amounts up to 50 ng. of EPN.

Unidentified materials extracted from peaches represented the only measurable interferences encountered during this study. However, by making use of peak height measurement and estimating the true base line of the EPN peak, the interference from an overlapping unknown was maintained below 0.02 p.p.m., which is the claimed limit of sensitivity for EPN. Figure 4 shows the chromatogram of the extract from about 200 grams of peaches which had been fortified with 0.095 p.p.m. of EPN.

Impurities in some lots of chemically pure benzene interfere with the measurement of the EPN peak. While not all samples of benzene exhibit this interference, a simple distillation completely removes this difficulty. Each lot of benzene should be checked for this interference before use.

The extraction-isolation step used during this study was primarily developed to permit the determination of EPN on the surface of various plant tissues. The isolation method is an adaptation of a published procedure for isolating residues (*1*). A single extraction with benzene is capable of removing better than 85% of the EPN from fortified crop samples; hence, three extractions are employed to ensure quantitative removal.

With appropriate modifications, the electron-capture GC procedure herein described could also probably be used in conjunction with other EPN extraction procedures (*6, 7, 12*).

Benzene is particularly desirable for the extraction, since EPN has a high solubility in this solvent. Another advantage is that only small amounts of other

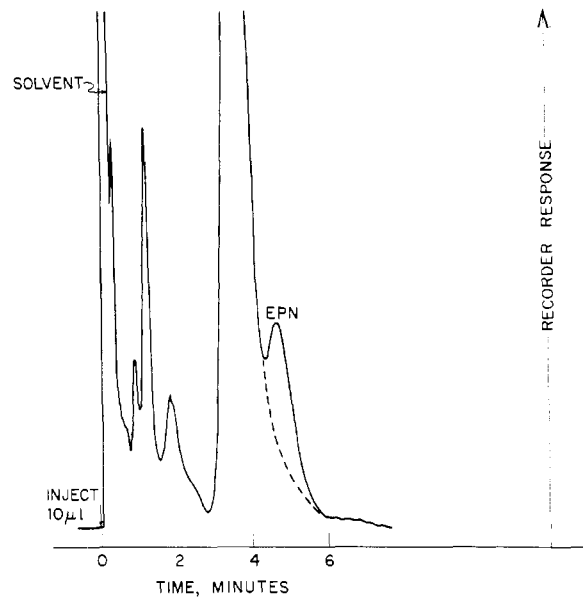

Figure 4. Gas chromatogram of peach extract

211 grams of peaches fortified with 0.095 p.p.m. of EPN
Sensitivity. 1/4 maximum

electron-capturing species are extracted from most of the plant tissues investigated. The high selectivity of the benzene extraction process, combined with selectivity and very high sensitivity of the EPN electron-capture detection, provides for sensitive and accurate analytical methods.

Epon 1001 resin was selected as the column stationary liquid, although Dow-Corning DC-200 silicone oil (12,500 cs.) column liquid also produces satisfactory chromatograms. The Epon 1001 column is preferred because of the superior symmetry of the EPN peaks it affords. The EPN calibration plot obtained with the Epon column (Figure 3) intercepts zero, indicating

Table III. Relative Retention of Some Other Pesticides

Compound	Formula	Relative Retention
EPN	$C_{14}H_{14}NO_4PS$	1.00[a]
Sulfenone	$C_{12}H_9ClO_2S$	0.45
Parathion	$C_{10}H_{14}NO_5PS$	0.36[b]
Demeton	$C_8H_{19}O_3PS_2$	0.36[c]
Ethion	$C_9H_{22}O_4P_2S_4$	0.23
Chlorobenside	$C_{13}H_{10}Cl_2S$	0.20
Malathion	$C_{10}H_9O_6PS_2$	0.12
VC-13	$C_{10}H_{13}Cl_2O_3PS$	[d]

[a] Absolute retention time; 4.2 min.
[b] In acetone; much weaker peak at 0.16 in benzene.
[c] In acetone; in benzene, no peak after solvent
[d] No peak after 45 min. following solvent.

insignificant loss of EPN during the chromatographic process. The quantitative aspects of a DC-200 column were not investigated.

To determine possible interference of certain other pesticides, several phosphorus- and/or sulfur-containing compounds of agricultural importance were gas-chromatographed by the EPN procedure, using 10-μl. aliquots of 200 ng. per ml. solutions (Table III). No interference with EPN was found for any of the materials tested. Apparently, EPN can be determined in the presence of any of these materials.

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