A Method for Determining Ethylenebis(dithiocarbamate) Residues on Food Crops as Bis(trifluoroacetamido)ethane

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Ethylenebis(dithiocarbamate) added to food crops was measured as ethylenediamine after hydrolysis with acid containing stannous chloride. The ethylenediamine was isolated by ion exchange chromatography and quantitated by gas–liquid chromatography of its bis(trifluoroacetate). Overall recoveries in the 0.16–1.3-ppm range were greater than 80%. Free ethylenediamine was determined after acid extraction but without prior hydrolysis. Storage of lettuce containing maneb for 4 days did not affect the yield of ethylenediamine obtained after hydrolysis.

The most widely used approach to the analysis of dithiocarbamates has been that of decomposition with acid and measurement of the released carbon disulfide. Various procedures and their modifications have been reviewed by Thorn and Ludwig (1962), and more recently by Raizman and Thompson (1972). While the determination of acid-released carbon disulfide is rapid and useful for the simultaneous measurement of dialkyl dithiocarbamates, thiuram sulfides, and ethylenebis(dithiocarbamates), it lacks the specificity afforded by determination of the amine. Because of their different chemical nature, fate on plants (Vonk and Kaars Sijpesteijn, 1970), and potential to form ethylenethiourea in foods (Newsome and Laver, 1973), it was considered necessary to distinguish ethylenebis(dithiocarbamates) from dialkyl dithiocarbamates and thiuram sulfides.

Several methods have been described for the determination of diamines in tissues, including colorimetry (Morris, 1971), fluorimetry (Pasarela and Waldron, 1967), and gas-liquid chromatography (Smith, 1970). Of these, gas–liquid chromatography appears the most selective and offers the possibility of mass spectral confirmation. However, direct gas-liquid chromatography of the free amine is difficult and does not possess the required sensitivity, necessitating derivitization to a compound suitable for electron capture gas-liquid chromatography. Various derivatives were examined including the tosyl, benzyl, and 2,4-dinitrophenyl, and the bis(trifluoroacetate) was found the most useful.

EXPERIMENTAL SECTION

Materials. Zineb was supplied as Dithane Z-78 by the Rohm and Haas Co. of Canada, West Hill, Ontario, and was labeled as containing 82.9% zineb. Maneb was obtained from DuPont of Canada Limited, Toronto, Ontario, as Manzate Maneb Fungicide and had a guaranteed ingredient of 80% maneb. Polyram was supplied by Niagara Chemicals, Division of FMC Corporation, Burlington, Ontario, and was labeled as assaying 39.0% carbon disulfide. Aqueous suspensions of the ethylenebis(dithiocarbamates) (EBDC) were prepared immediately before fortification of the samples. The levels of fortification were calculated in terms of the amount of active ingredient present in the formulation.

Dowex 50W-X8, 100–200 mesh cation exchange resin, was purchased from Sigma Chemical Co., St. Louis, Mo. The resin was washed by suspension in sufficient 2 *N* NaOH to produce a strongly alkaline pH. The bulk of the resin was allowed to settle and the fines and supernatant liquid were removed by aspiration. The procedure was repeated with 1 *N* NaOH and distilled water. The resin was then converted to the H^+ form with 1 *N* HCl. After aspiration of the HCl, the resin was converted to the Na^+ form with NaOH and permitted to stand overnight. The alkali was aspirated and the resin washed with distilled water and finally twice with 0.2 *N* NaOH. The settled volume of the resin was measured and an equal volume of 0.2 *N* NaOH added.

Ion Exchange Columns. Glass columns, 18 × 1.5 cm i.d., were fitted with 2-mm Teflon stopcocks and a plug of glass wool above the stopcock of such a size as to support the resin and provide a minimum void volume. The outlet was tapered and cut to a 2-cm length to further reduce the void volume. Columns were packed by adding 6.0 ml of the 50% (v/v) resin suspension in 0.2 *N* NaOH to the columns approximately half filled with distilled water and allowing the resin to settle. The columns were eluted before use at a flow rate of 30 ml/hr with 1 *N* HCl (15 ml), 1 *N* NaOH (15 ml), 1 *N* HCl (15 ml), and finally distilled water until the effluent was neutral. The resin was discarded after each run, since regeneration was found impractical.

ANALYTICAL PROCEDURE

Sample Preparation and Digestion. Samples were cut into small pieces and portions (10.0 g) weighed into 125-ml flasks. Aqueous suspensions of EBDC were pipetted onto the samples in volumes of 0.10–1.6 ml. Freshly prepared $SnCl_2 \cdot 2H_2O$ in 1 *N* HCl (1.0 mg/ml; 25 ml) was added to the flasks which were heated to reflux for 60 min. The flasks were cooled and the digest filtered, using gentle vacuum, through a disk of Whatman No. 41 paper placed on Whatman No. 1 paper in a Buchner funnel.

Ion Exchange Chromatography. The digest filtrate was added to an ion exchange column and the ethylenediamine adsorbed at a flow rate of 25–30 ml/hr. The column was then eluted with 1.0 *N* NaCl (15 ml). When the surface of the NaCl eluent reached the top of the resin, the flow was stopped and a 5-ml volumetric flask placed to collect the effluent. Saturated aqueous $NaHCO_3$ (1–2 ml) was added to the column and elution of the ethylenediamine commenced. Further saturated $NaHCO_3$ was added as required and elution continued until 5.0 ml of effluent was collected.

Trifluoroacetylation. Two hundred microliters of $NaHCO_3$ eluate was added to 5-ml vials containing concentrated HCl (50 μl). The acidified solution was evaporated to dryness in an evacuated dessicator over NaOH pellets. A reference standard consisting of ethylenediamine dihydrochloride dissolved in saturated $NaHCO_3$ was also acidified and dried. Redistilled trifluoroacetic anhydride (0.1 ml) was added and the vials capped with Teflon-lined tops. After 1 hr at room temperature, the excess trifluoroacetic anhydride was evaporated in an evacuated dessicator and saturated $NaHCO_3$ (50 μl) added. The

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Table I. Recoveries of Zineb, Maneb, and Polyram from Apple

Added, ppm			Found, ppm			% recovery		
Zineb	Maneb	Polyram[a]	Zineb[b]	Maneb[c]	Polyram[a,b]	Zineb	Maneb	Polyram
0.162	0.171	0.174	0.160	0.185	0.172	98.8	108	98.9
0.324	0.342	0.348	0.273	0.300	0.289	85.3	87.7	83.0
0.648	0.684	0.696	0.593	0.612	0.535	91.5	89.5	76.9
1.30	1.37	1.39	1.32	1.36	1.17	102	99.3	84.1

[a] Calculated as zineb. [b] Corrected for 0.021 ppm background. [c] Corrected for 0.038 ppm background.

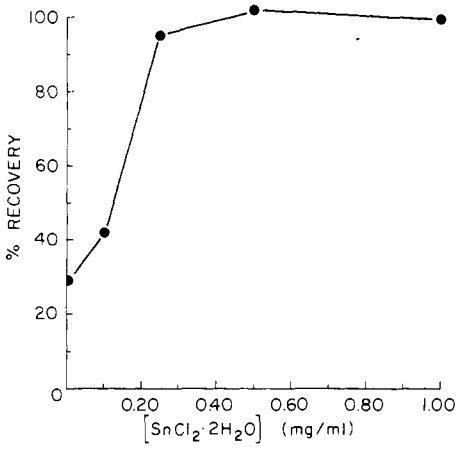

Figure 1. Effect of the concentration of $SnCl_2 \cdot 2H_2O$ in refluxing 1 *N* HCl on the yield of acid-released ethylenediamine obtained from zineb added to apple. The zineb was added at a level of 0.64 ppm.

Table II. Recovery of Zineb from Lettuce and Tomato

Added, ppm		Found, ppm		% recovery	
Lettuce	Tomato	Lettuce[a]	Tomato[b]	Lettuce	Tomato
0.167	0.185	0.145	0.156	86.8	84.3
0.334	0.370	0.301	0.362	90.1	97.8
0.668	0.740	0.626	0.680	93.7	91.9
1.34	1.48	1.35	1.42	101	95.9

[a] Corrected for 0.019 ppm background. [b] Corrected for 0.087 ppm background.

contents of the vial were mixed and the trifluoroacetylated ethylenediamine extracted into benzene (400 μl) by agitation on a Vortex mixer. Aliquots (2 μl) were taken for analysis by gas–liquid chromatography.

Gas–Liquid Chromatography. An Aerograph 705 fitted with a glass injection insert and tritium foil electron capture detector was used for routine quantitative determinations. The 6 ft × 4 mm i.d. glass column was packed with 5% butanediol succinate on 100–120 mesh Chromosorb W, HP, and conditioned for 48 hr at 190°. Operating parameters were: injection port, 190°; column, 180°; nitrogen flow rate, 90 ml/min. Under these conditions, bis(trifluoroacetamido)ethane had a retention time of 7.5 min. The peak area was measured by triangulation and quantitated by comparison to that of the trifluoroacetylated ethylenediamine reference. The recovery was calculated in terms of the parent EBDC used to fortify the sample.

Determination of Free Ethylenediamine. Ethylenediamine was extracted from samples (10.0 g) by maceration at low speed on a Sorvall homogenizer with 0.1 *N* HCl (30 ml). The macerate was filtered immediately, as previously described, and the filtrate applied to an ion exchange column. The remainder of the procedure was as described for EBDC hydrolysates.

RESULTS

The treatment of zineb with cold 1 *N* HCl for 1 hr yielded approximately 60% ethylenediamine in the absence of sample material, and no detectable ethylenediamine in the presence of sample. Using hot 1 *N* HCl for 1 hr, a 30% yield of ethylenediamine was obtained in the presence of sample (*cf.* Figure 1). The addition of $SnCl_2$ under the conditions of refluxing 1 *N* HCl further improved the yield as shown in Figure 1. A time course experiment with maneb in apple, using the standard reaction conditions, showed virtually no differences in recovery for reflux times from 30 to 120 min (mean 87.8%, range 84.7–91.3%).

The recoveries of some EBDC's added to apple at levels from 0.16 to 1.3 ppm are presented in Table I. Similar recoveries were obtained from lettuce and tomato as shown in Table II. Typical gas–liquid chromatograms of bis(trifluoroacetamido)ethane obtained from apple fortified with various levels of zineb are shown in Figure 2. Similar patterns were found using other commodities. Background interference imposes a lower limit of quantitative detection of 0.1 ppm in terms of EBDC. This was sufficient for our purposes and further reduction was not attempted. Ethylenethiourea and 5,6-dihydroimidazo[2,1-*c*]-1,2,4-dithiazole-3-thione (ETM) added to apple yielded 12 and 5%, respectively, of their theoretical ethylenediamine by the standard procedure.

It was found necessary to include an ethylenediamine standard in the trifluoroacetylation step since, under the conditions employed, the yield of trifluoroacetate was not quantitative, but a consistent 70–80% relative to pure bis(trifluoroacetamido)ethane. As a check on the possible interference from other diamines, equal weights of the dihydrochlorides of ethylenediamine, 1,2-diaminopropane, 1,3-diaminopropane, and 1,4-diaminobutane were trifluoroacetylated and subjected to gas–liquid chromatography. All were separated with retention times relative to ethylenediamine as follows: 1,2-diaminopropane, 0.57; ethylenediamine, 1.00; 1,3-diaminopropane, 1.18; 1,4-diaminobutane, 2.11. The peak areas of trifluoroacetylated 1,3-diaminopropane and 1,4-diaminobutane dihydrochlorides were 36 and 18%, respectively, of that obtained for ethylenediamine indicating low yields of the trifluoroacetate and/or low electron capture responses.

By extracting with 0.1 *N* HCl without prior hydrolysis, it was found possible to determine free ethylenediamine in the presence of EBDC. The recoveries from apple fortified with both ethylenediamine and maneb are presented in Table III. Low recoveries (50%) of ethylenediamine resulted when water was used as the extractant.

The applicability of the method to determining residues of EBDC or possible degradation products on crop material after storage was examined with chopped lettuce maintained at 5°. As the results of Table IV indicate, aging does not affect the recovery obtained after 4 days. A slight decrease in recovery was found after 9 days. Negligible amounts of free ethylenediamine were found.

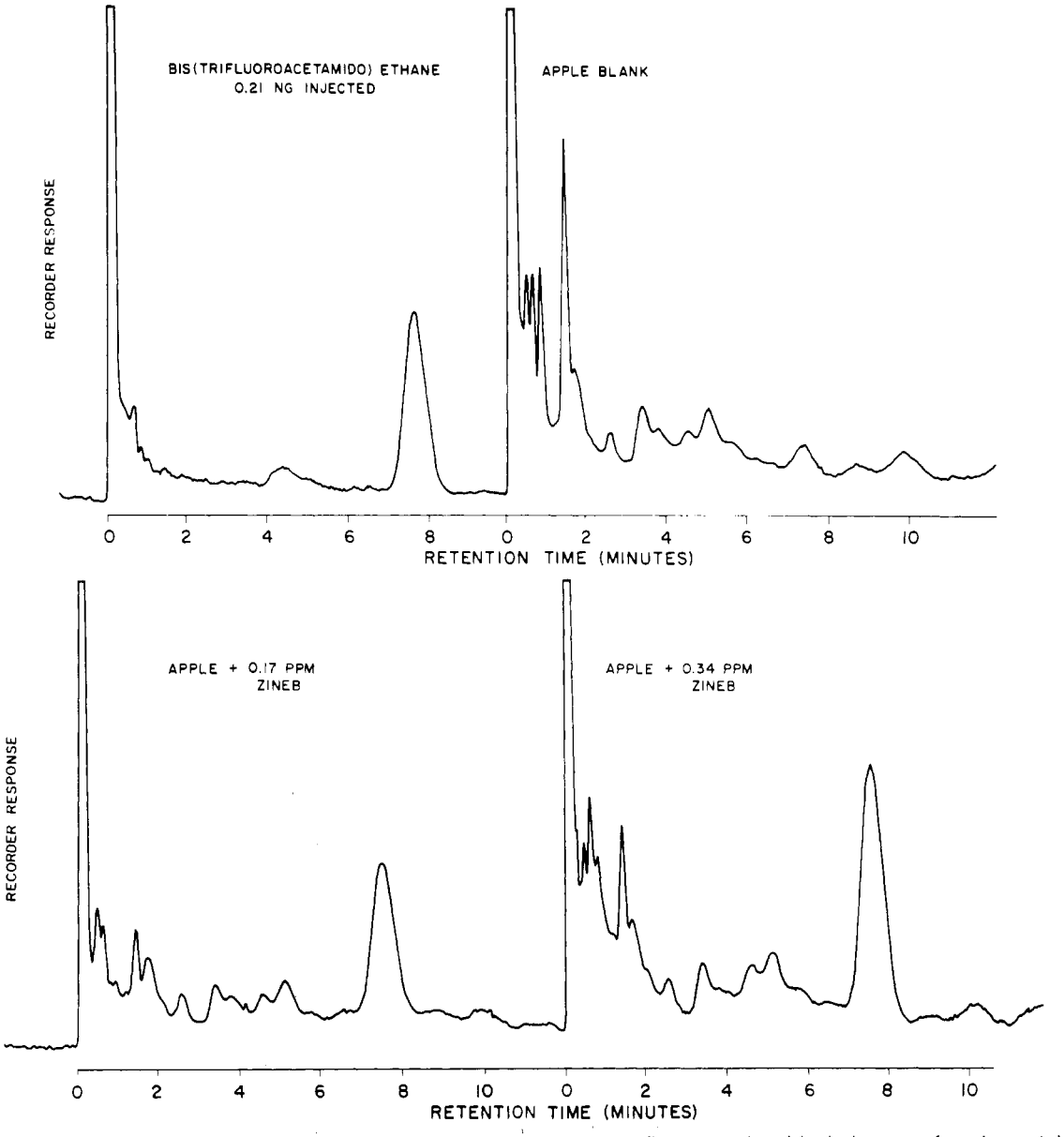

Figure 2. Gas-liquid chromatograms of bis(trifluoroacetamido)ethane and of trifluoroacetylated hydrolysates of apple containing various levels of zineb. Samples were processed as described in the text. Each injection represents the equivalent of 2 mg of apple.

Table III. Determination of Ethylenediamine in Apple in the Presence of Maneb (4.7 ppm)

Ethylene-diamine–2HCl added, ppm	Ethylene-diamine–2HCl found, ppm	% recovery[a]
0	0.007	
0.118	0.119	94.9
0.235	0.220	90.6
0.470	0.464	97.2
0.941	0.957	101

[a] Corrected for 0.007 ppm background.

Table IV. Stability of Maneb (0.954 ppm) in Chopped Lettuce at 5°

Elapsed time, days	Maneb found, ppm	Mean recovery, %
0	0.855	85.3
	0.772	±4.4
1	0.785	83.7
	0.812	±1.4
2	0.785	81.4
	0.767	±0.95
3	0.855	83.5
	0.737	±6.2
4	0.873	89.6
	0.837	±1.9
9	0.734	76.6
	0.727	±0.35

DISCUSSION

With the present method, recoveries of EBDC added to various foods are comparable to those reported using the carbon disulfide method (Keppel, 1971; Pease, 1957). Although more time consuming, the measurement of acid-released ethylenediamine provides greater specificity since it is unaffected by dialkyl dithiocarbamates, thiuram sulfides, or carbon disulfide. The fact that EBDC in plant tissue does not produce ethylenediamine on extraction

with cold acid permits the determination of free ethylenediamine and enables EBDC to be determined by difference.

The use of $SnCl_2$ as suggested by Keppel (1969) facilitates the decomposition of EBDC to ethylenediamine and, although the mechanism is as yet obscure, its action may be unrelated to the enhanced hydrolysis observed with the addition of EDTA (Pease, 1957).

Two decomposition products of EBDC, ethylenethiourea and ETM (Czeglédi-Janko, 1967; Thorn and Ludwig, 1962), produce small yields of ethylenediamine under the conditions used for hydrolysis and could, if present in large amounts, give rise to erroneously high results. However, ethylenethiourea may be determined by independent methods (Newsome, 1972; Haines and Adler, 1973) while ETM has been determined in foods polarigraphically (Engst and Schnaak, 1970). A gas–liquid chromatographic method for ETM is being developed in this laboratory.

From a study of the stability of EBDC's in kale (Howard and Yip, 1971) it was concluded that significant decomposition occurred during 3 days of refrigeration. The results of the present experiment with lettuce indicate that if maneb reacts upon storage, the reaction products yield the theoretical amount of ethylenediamine after hydrolysis.

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Total ^{14}C Residues and Dieldrin Residues in Milk and Tissues of Cows Fed Dieldrin-^{14}C

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Two dieldrin-^{14}C cow feeding trials have been carried out. In the first trial two guernsey cows were fed dieldrin-^{14}C at a level equivalent to 0.11 ppm in their total ration for 3 weeks. The pooled mean residues of ^{14}C and dieldrin determined by a specific glpc method in milk collected at 15, 17, 19, and 21 days were 0.019 ppm equivalents and 0.017 ppm, respectively. In the second trial three guernsey cows were fed dieldrin-^{14}C at a level equivalent to 0.21–0.36 ppm in their ration for 42 days. The pooled mean residues of ^{14}C and dieldrin determined by a specific glpc method in milk collected at 28, 34, 39, and 41 days were 0.036 ppm equivalents and 0.036 ppm, respectively. The mean fat-feed ratios in the mesenteric fat, subcutaneous fat, and butter fat are 1.8, 1.3, and 3.4, respectively. None of the known or hypothetic dieldrin metabolites were detected in the milk or tissues of the cows.

The presence of dieldrin (Technical dieldrin contains 85% 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8α-octahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene (HEOD) and 15% related compounds; following Chemical Abstracts usage we have used the name dieldrin for 100% HEOD) in the milk and fat of dairy cattle and other farm animals fed aldrin and dieldrin has been reported by Bann *et al.* (1956). Subsequently, metabolic fate studies of dieldrin in nonruminant animals have led to the identification of three metabolites which are (see Table I for chemical name) aldrin-*trans*-diol (Korte and Arent, 1965), the pentachloro ketone (Richardson *et al.*, 1968; Klein *et al.*, 1968), and 9-hydroxydieldrin (Richardson *et al.*, 1968; Baldwin *et al.*, 1970). Hedde *et al.* (1970) and Feil *et al.* (1970) have found aldrin-*trans*-diol and 9-hydroxydieldrin in the urine of sheep fed dieldrin. Because the sheep were not lactating the concentration of dieldrin and its metabolites in their milk was not determined.

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In order to determine whether these or other metabolites are excreted in milk or stored in the tissues of cows, two feeding experiments with lactating dairy cattle have been made. This article describes the experiments and the results.

EXPERIMENTAL SECTION

Dieldrin Synthesis. Dieldrin-^{14}C was synthesized by the Mallinckrodt/Nuclear Corporation (St. Louis, Mo. 63145) from bicycloheptadiene and uniformly labeled hexachlorocyclopentadiene-^{14}C by the method of Burton *et al.* (1957).

The radiochemical purities of the dieldrin-^{14}C preparations were determined by cochromatographing the dieldrin-^{14}C and an authentic sample of dieldrin on thin layer plates of silica gel. Both Eastman thin layer sheets (Eastman Chemicals, Eastman Kodak Co. catalog 6060, 100 μ thick with poly(vinyl alcohol) binder and lead–manganese activated calcium silicate fluorescence indicator) and Brinkman tlc plates (E. Merck, A. G. Dormstold, 250 μ thick with organic binder and manganese activated zinc