Determination of Polychlorinated Benzoic Acid Herbicide Residues by Gas Chromatography

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Residues of trichloro- and polychlorobenzoic acids can be determined in soils and plant tissues by a routine method involving programmed temperature microcoulometric gas chromatography. The chloro- acids are isolated by an extractive procedure and converted to their methyl esters for subsequent chromatographic analysis. Relatively large sample extract aliquots are analyzed using a column concentration technique. Recoveries of about 80% for the various chlorobenzoic acids have been demonstrated with a large number of fortified samples. Satisfactory analyses have been carried out at the 0.04-p.p.m. level. No interferences have been seen in a wide variety of materials analyzed.

Selective methods for the routine determination of Trysben (2,3,6-trichlorobenzoic acid) and Zobar (polychlorinated benzoic acid) herbicides (trademarks, E. I. du Pont de Nemours & Co., Wilmington, Del.) residues have not previously been reported. While unpublished methods based on a colorimetric procedure (*12*) and on ultraviolet spectrophotometry (*3*) show the total amount of polychlorobenzoic acids present, they do not permit the determination of each specific isomer.

An earlier study has shown that complex mixtures of polychlorinated benzoic acids can be readily analyzed by gas chromatography after conversion to the methyl esters (*7*). This approach has been extended to permit the quantitative determination of trace residues of the subject compounds in soils and plant tissues. Use of gas chromatography in the analysis of residues of other agriculturally important acidic compounds has been reported recently (*1, 4–6, 9, 13*).

To obtain good reproducibility and freedom from the many possible interferences, a selective isolation procedure is used in combination with an efficient gas chromatographic column attached to a microcoulometric titration detector. After extractive isolation of the chlorinated benzoic acid residues, a liquid-liquid partition cleanup step is employed, and the acids are converted to the methyl esters with diazomethane (*7*). The extract is then chromatographed, using a column concentration technique (*8*) in conjunction with a programmed temperature microcoulometric gas chromatograph.

The procedure herein described can be used interchangeably for the determination of both trichlorobenzoic acids (TBA) and polychlorinated benzoic acids (PBA) without modification. Satisfactory recoveries in the 0.04-p.p.m. range have been demonstrated. Average recoveries of about 80% have been obtained for TBA and PBA in a variety of sample types.

Experimental

Apparatus and Reagents. Gas Chromatography Instrument. Model G-100, Dohrmann Instruments Co., San Carlos, Calif., with a Model T-200 titration cell, a 304 stainless steel vaporizer block, and a quartz liner injection system.

The Model G-100 Dohrmann microcoulometric gas chromatograph is limited to isothermal column operation. The column oven supplied with the instrument lacks a temperature controller and has a very large heat capacity, making it unsuitable for rapid changes in column temperature. To carry out programmed temperature operation, the original instrument was modified with a special air bath, a programmer-controller, and a differential carrier gas flow controller.

The construction of the air bath attached to the existing Dohrmann instrument is detailed in Figure 1. The bath is 7 inches deep, 10 inches wide, and 14 1/2 inches high (outside dimensions), and is constructed of 1/16-inch aluminum sheet metal, insulated with a 1/2-inch layer of Fiberglas (Owens-Corning PF-105). The exterior is covered with 1/16-inch expanded aluminum metal. The resultant oven has an extremely low mass and adequate insulation properties, enabling rapid heating and cooling.

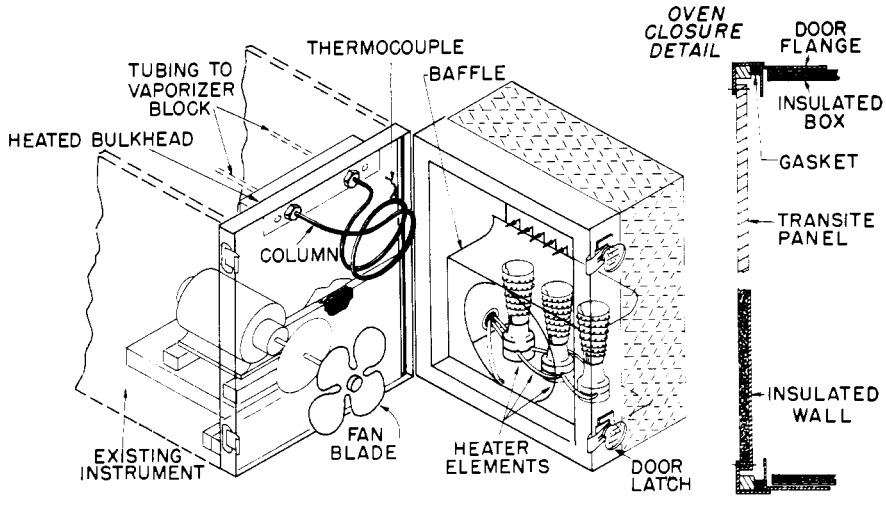

Figure 1. Air bath for programmed temperature operation

Air is rapidly circulated inside the bath by means of a 5½-inch four-bladed fan, driven with a 1/15th h.p., 3450 r.p.m. motor (Bodine Electric Co.). The motor is remotely mounted from the air bath, underneath the Dohrmann vaporizer block compartment. Heat is furnished to the air bath by three 650-watt radiant heater elements, connected in parallel. The air bath is capable of programmed temperature operation up to 30° C. per minute with no significant temperature lag.

The column, formed in a 5-inch i.d. loop, is attached by means of Swagelok fittings to a heated bulkhead which connects to the Dohrmann vaporizer block by means of ¼-inch o.d. stainless steel tubing.

The temperature of the column is monitored by an iron-constantan thermocouple which is silver-soldered inside the jaws of a microalligator clip, modified with a spring unaffected by high temperature. During initial work, the thermocouple was connected into a Model 40 programmed temperature controller (F & M Scientific Corp., Avondale, Pa.), which supplied the power to heaters via a 25-ampere mercury relay. It was subsequently found, however, that improved temperature control could be obtained using an F & M Model 240 power proportionating temperature controller, and this device was used for most of the measurements described in this report.

Carrier gas was regulated to the column by means of a Model 63BU-L differential flow regulator (Moore Products Co., Philadelphia, Pa.) and a Nupro needle valve (Nuclear Products Co., El Monte, Calif.)

Gas Chromatographic Column. Six-foot, ¼-inch o.d. stainless steel tubing filled with 20% Apiezon L grease on acid-washed Chromosorb W, 60 to 80 mesh. The packing was coated by dissolving the grease in benzene, 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., and sieved to obtain the desired mesh fraction. The column was packed using conventional vibration techniques, placed in the instrument, and conditioned at 275° C. for 24 hours with a helium flow rate of 50 cc. per minute, before use.

Before quantitative work was attempted, the chromatographic system was "conditioned" with polychlorobenzoic acid, methyl esters. Several successive 2- to 5-mg. samples were injected into the column, making sure the sample was vented before it reached the combustion furnace. Presumably this treatment eliminates certain active sites within the vaporizer or column which tend to decompose, or irreversibly adsorb, small amounts of TBA and PBA. This treatment needs to be carried out only when a new column is placed in operation.

Polychlorinated Benzoic Acids. Technical grade, minimum of 92% polychloro acids as standard reference material, E. I. du Pont de Nemours & Co., Industrial & Biochemicals Dept., Biochemicals Sales Division.

Ethereal Diazomethane Solution. Prepared as described in (7).

Sargent Mill Assembly. Centrifugal, wet, size No. 2, catalog No. F-61690, E. H. Sargent & Co., Chicago, Ill.

Vigreaux Column and Condenser. 12-Inch column, ¾-inch diameter.

Centrifuge. International, size 1, type SB, or equivalent, with 250-ml. centrifuge bottles.

Agla Micrometer Syringe. Burroughs-Wellcome Co., Tuckahoe, N. Y.

Procedure. Isolation of Acids. Preliminary handling of a sample depends upon the nature of the material to be analyzed. If the sample is a grain, weigh 50 grams in a 400-ml. tall beaker, add 150 ml. of methyl ethyl ketone (MEK) (reagent grade), 1.5 ml. of reagent grade 85% phosphoric acid, and 5 ml. of distilled water; grind with a Sargent wet mill for 30 minutes.

If the sample is a fruit or vegetable tissue, place up to 50 grams of the chopped material in a Waring Blendor jar, add 200 ml. of MEK and 2 ml. of reagent grade 85% phosphoric acid, cover, and blend at high speed for 3 to 5 minutes.

Should the sample be a foliar material such as corn fodder or wheat straw, place 25 grams of the air-dried material in a Waring Blendor jar, add 200 ml. of MEK, 2 ml. of reagent grade 85% phosphoric acid, and 5 ml. of distilled water, cover, and blend at high speed for 5 to 10 minutes.

If a soil is to be analyzed, place a 25-gram sample in a glass-stoppered Erlenmeyer flask, add 150 ml. of MEK, 1.5 ml. of 85% phosphoric acid, and 5 ml. of water, and shake on a wrist-action shaker (or equivalent) for 15 minutes.

After the preliminary treatment, transfer the blended sample quantitatively to a 250-ml. centrifuge bottle, with several small portions of MEK as wash. Centrifuge at 2000 r.p.m. for 5 to 10 minutes, and then carefully decant the MEK phase through absorbent cotton into a 500-ml. separatory funnel. Add 150 ml. of MEK and 1.5 ml. of 85% phosphoric acid to the substrate remaining in the centrifuge bottle, stopper with a cork plug, and shake vigorously for 2 to 3 minutes. Centrifuge as before, and combine the MEK phase with the contents of the separatory funnel. Repeat this extraction operation and combine the MEK phases.

Wash the combined extracts twice with 60-ml. portions of a saturated sodium sulfate solution by shaking for 2 minutes and allowing at least 10 minutes for

Table I. Recoveries from Soil

Keyport Silt Loam, Newark, Del.; 25-gram samples

Trichlorobenzoic Acids								
2,3,6-Isomer			*2,4,5- + 2,3,4-Isomers*			*Total*		
P.P.M. added	*P.P.M. found*	*% Recovery*	*P.P.M. added*	*P.P.M. found*	*% Recovery*	*P.P.M. added*	*P.P.M. found*	*% Recovery*
0.70	0.55	79	0.29	0.22	76	0.99	0.77	78
7.0	6.2	89	2.9	2.3	79	9.9	8.5	86
35	28	80	15	10	67	50	38	76
70	72	103	29	22	76	99	94	95
350	337	96	145	104	72	495	441	89
		Av. 89%			Av. 74%			Av. 85%

Polychlorinated Benzoic Acids																				
2,5-Isomer			*2,3,6-Isomer*			*2,3,5- + 2,4,5-Isomers*			*2,3,5,6-Isomer*			*2,3,4,5-Isomer*			*Total*				
P.P.M. added	*P.P.M. found*	*% Recovery*	*P.P.M. added*	*P.P.M. found*	*% Recovery*	*P.P.M. added*	*P.P.M. found*	*% Recovery*	*P.P.M. added*	*P.P.M. found*	*% Recovery*	*P.P.M. added*	*P.P.M. found*	*% Recovery*	*P.P.M. added*	*P.P.M. found*	*% Recovery*			
0.12	0.09	75	0.07	0.05	71	0.23	0.20	87	0.27	0.25	93	0.15	0.10	67	0.84	0.69	82			
1.2	1.0	83	0.68	0.61	90	2.3	2.2	96	2.7	2.5	93	1.5	1.1	73	8.4	7.4	88			
11.8	9.8	83	6.8	4.8	71	23	21	91	27	26	96	15	9.8	65	84	71	85			
	Av.	80%		Av.	77%		Av.	91%		Av.	94%		Av.	68%		Av.	85%			

complete phase separation. Discard the lower sulfate layers. Decant the MEK layer into a 500-ml. round-bottomed flask, add a boiling chip, and concentrate the solution to approximately 35 ml. on a steam bath, using the Vigreaux column and condenser. Cool to room temperature and add 200 ml. of a water solution containing 2.5% sodium carbonate and 6.25% sodium chloride. Transfer the mixture to a 500-ml. separatory funnel using small portions of distilled water as wash. Extract this solution with 50 ml. of *n*-hexane (reagent grade or redistilled), shaking for 2 minutes and allowing at least 5 minutes for the layers to separate. Drain the water layer into a second 500-ml. separatory funnel and discard the hexane layer. Repeat the wash with an additional 50 ml. of *n*-hexane. Drain the water layer into a 1-liter round-bottomed flask, add a boiling chip, and concentrate the solution, with frequent swirling to avoid excess foaming, to approximately 75 ml., using a burner flame.

While the solution is still hot, add dropwise from a buret, very carefully and with swirling, 10 ml. of 1:1 H_2SO_4. Cool the acid solution to room temperature and transfer to a 125-ml. separatory funnel using 25 ml. of hexane as wash. Extract the chlorobenzoic acids into the hexane by shaking the flask for 2 minutes, allowing 5 minutes for phase separation. Draw the lower water layer back into the round-bottomed flask and carefully decant the hexane layer into a 100-ml. beaker. Repeat this extraction procedure four more times, each time using a 25-ml. portion of hexane. Combine the hexane extracts and concentrate to about 10 ml. by evaporation in a hood or with a stream of filtered nitrogen.

Emulsions may be encountered during this extraction step and may necessitate centrifuging to obtain complete recovery of the solvent. If this is so, the extraction is conducted using a 250-ml. centrifuge bottle substituted for the 125-ml. separatory funnel. The 2-minute shaking period is still necessary, but the mixture is now centrifuged for 5 to 10 minutes at 2000 r.p.m. Draw off the hexane phase using a pipet and mild suction, being careful not to disturb the lower aqueous layer, and transfer the solvent to a 100-ml. beaker. Repeat the extraction, as before, using four additional 25-ml. portions of hexane and combining the hexane extracts in the 100 ml. beakers.

ESTERIFICATION. Quantitatively transfer the hexane extract from above to a 30-ml. beaker using 1- to 2-ml. washes of hexane. Carefully evaporate the sample to about 2 ml. with a stream of filtered nitrogen. Add about 0.25 ml. of fresh ethereal diazomethane solution, swirl the beaker contents to mix, and allow to stand for 5 minutes on a steam bath. Concentrate the solution to about 0.5 ml., using a stream of filtered nitrogen. Transfer the solution quantitatively into a 1-ml. volumetric flask, using a finely drawn-out dropper and several small washes of hexane. Dilute to the mark with hexane and mix.

GAS CHROMATOGRAPHIC ANALYSIS. Equilibrate the gas chromatograph as follows: furnace temperature, 820° C.; vaporizer block temperature, 280° C.; vaporizer block oven, 280° C.; column temperature, 100° C.; damping, 4; time constant, 3; carrier flow, helium 80 cc. per minute; purge flow, helium 180 cc. per minute; oxygen flow, 50 cc. per minute. Inject up to 0.50 ml. of sample evenly over a 2-minute period,

Table II. Recoveries from Plant Tissues

50-gram samples

Trichlorobenzoic Acids

2,3,6-Isomer			2,4,5- + 2,3,4-Isomers			Total Chlorobenzoic Acids			2,3,6-Isomer			2,4,5- + 2,3,4-Isomers			Total Chlorobenzoic Acids		
P.P.M. added	P.P.M. found	% Recovery	P.P.M. added	P.P.M. found	% Recovery	P.P.M. added	P.P.M. found	% Recovery	P.P.M. added	P.P.M. found	% Recovery	P.P.M. added	P.P.M. found	% Recovery	P.P.M. added	P.P.M. found	% Recovery
SORGHUM									BARLEY								
0.04	0.03	75	...	...	...	0.04	0.03	75	0.07	0.06	86	...	...	...	0.07	0.06	86
0.07	0.04	57	...	...	...	0.07	0.04	57	0.14	0.11	79	0.06	0.06	100	0.20	0.17	85
0.14	0.08	57	0.05	0.03	60	0.19	0.11	58	0.27	0.19	70	0.11	0.08	73	0.38	0.27	71
0.28	0.23	82	0.10	0.08	80	0.38	0.31	82	0.41	0.38	93	0.17	0.18	106	0.58	0.56	97
0.71	0.53	75	0.24	0.22	92	0.95	0.75	79	0.68	0.57	84	0.28	0.26	93	0.96	0.83	86
0.14	0.11	79	0.06	0.05	83	0.20	0.16	80		Av.	82%		Av.	94%		Av.	86%
0.41	0.30	73	0.17	0.16	94	0.58	0.36	62									
	Av.	71%		Av.	82%		Av.	70%	PINEAPPLE								
WHEAT									0.04	0.04	100	...	...	...	0.04	0.04	100
									0.07	0.04	57	...	...	...	0.07	0.04	57
0.04	0.03	75	...	...	...	0.04	0.03	75	0.14	0.10	71	0.05	0.03	60	0.19	0.13	68
0.07	0.05	71	...	...	...	0.07	0.05	71	0.28	0.27	96	0.10	0.09	90	0.38	0.36	95
0.14	0.10	71	0.05	0.04	80	0.19	0.15	79	0.71	0.62	87	0.24	0.17	71	0.95	0.78	82
0.28	0.27	96	0.10	0.10	100	0.38	0.37	97		Av.	82%		Av.	74%		Av.	81%
0.71	0.56	79	0.24	0.20	83	0.95	0.76	80									
0.14	0.13	93	0.06	0.06	100	0.20	0.19	95	SUGAR CANE								
0.41	0.32	78	0.17	0.16	94	0.58	0.48	83	0.04	0.03	75	...	...	...	0.04	0.03	75
5.6	4.6	82	2.3	1.8	78	7.9	6.4	81	0.07	0.05	71	...	...	...	0.07	0.05	71
	Av.	81%		Av.	89%		Av.	83%	0.14	0.11	79	0.05	0.04	80	0.19	0.15	79
									0.28	0.22	78	0.10	0.08	80	0.38	0.30	79
									0.71	0.62	87	0.24	0.22	92	0.95	0.84	88
										Av.	78%		Av.	84%		Av.	78%

Polychlorinated Benzoic Acids

2,5-Isomer			2,3,6-Isomer			2,3,5- + 2,4,5-Isomers			2,3,5,6-Isomer			2,3,4,5-Isomer			Total Chlorobenzoic Acids		
P.P.M. added	P.P.M. found	% Recovery	P.P.M. added	P.P.M. found	% Recovery	P.P.M. added	P.P.M. found	% Recovery	P.P.M. added	P.P.M. found	% Recovery	P.P.M. added	P.P.M. found	% Recovery	P.P.M. added	P.P.M. found	% Recovery
WHEAT																	
0.08	0.06	75	0.04	0.03	75	0.15	0.14	93	0.18	0.15	83	0.10	0.07	70	0.55	0.45	82
0.27	0.25	93	0.15	0.13	87	0.51	0.44	86	0.62	0.49	79	0.32	0.22	69	1.9	1.5	79
0.65	0.52	80	0.37	0.29	78	1.2	1.0	83	1.5	1.1	73	0.76	0.56	74	4.5	3.5	78
	Av.	83%		Av.	80%		Av.	87%		Av.	78%		Av.	71%		Av.	80%

using a micrometer syringe. Two minutes after injection, start column temperature programming at 10° C. per minute. Program to 300° C. and hold.

CALCULATIONS.

Micrograms of individual isomer in aliquot $= \frac{A \times 4.42 \times 10^3}{\Omega \times (\%\ \mathrm{Cl})}$

where A = area in sq. inches and Ω = recorder input resistance. (For recorder sensitivity of 1 mv. per inch and a chart speed of 0.5 inch per minute.)

p.p.m. TBA or PBA in crop = $\frac{\mu g.\ \text{in aliquot} \times \text{aliquot factor}}{\text{sample weight}}$

Results

Recovery studies of trichlorobenzoic and polychlorobenzoic acids added to untreated samples of Keyport silt loam were made and are summarized in Table I. The main components are recovered at about 83% of theory. This figure actually represents extraction efficiency rather than chromatographic efficiency, since the chlorine yield of a known amount of chlorinated acids injected into the chromatograph is essentially quantitative. Only the major isomers of the mixtures are calculated, since the minor isomers are present at insignificantly low concentration levels. No significant difference between the level of recovery for the various isomers is evident.

Gas chromatograms of extracts from control soil and soil fortified with Trysben and Zobar are shown in Figure 2. The control soil extract curve shows two minor unknown components; however, these peaks do not interfere with the determination of the major components of either chloro-acid mixture.

The present method has been used successfully for the analysis of TBA and PBA in various plant tissues. Table II shows recoveries obtained on sorghum, wheat, barley, pineapple, and sugar cane samples fortified with various levels of TBA and PBA. Average total recoveries of about 81% were obtained. No significant variation in the recovery of the various isomers of either mixture is apparent.

Discussion

Programmed temperature gas chromatography has been shown to be an effective tool for measuring trace quantities of high boiling organic compounds, such as pesticides (*2, 8*). This procedure is superior to conventional isothermal column operation, since it generally offers higher sensitivity and provides more optimum conditions for measuring complex mixtures of varying boiling points. Use of selective detectors, such as microcoulometric titration, introduces still another area of improvement. Where applicable, the microcoulometric de-

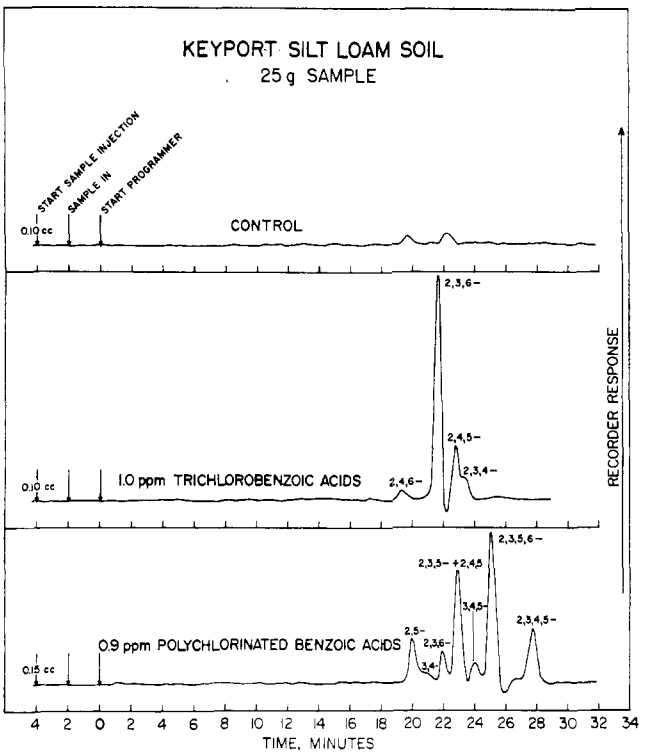

Figure 2. Gas chromatograms of Keyport silt loam soil control and fortified with trichlorobenzoic and polychlorinated benzoic acids

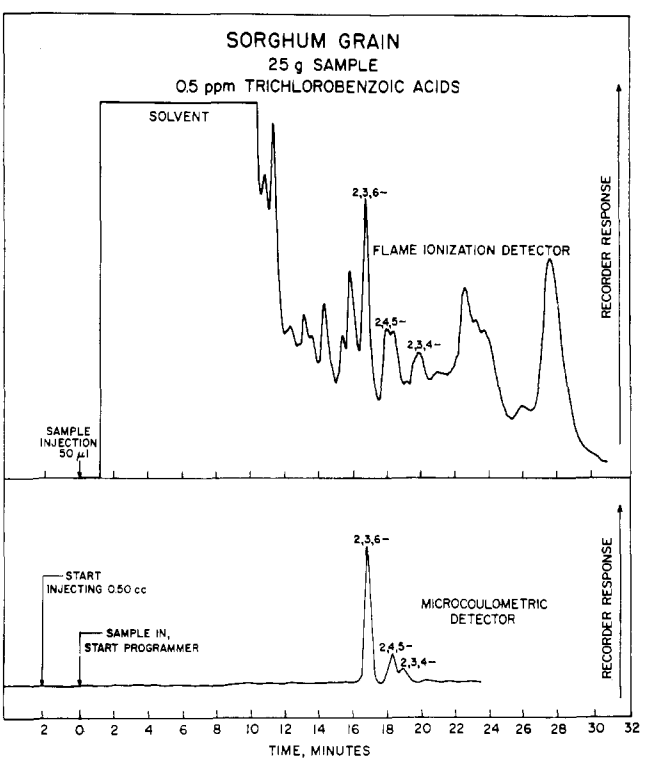

Figure 3. Comparison of flame ionization and microcoulometric titration detectors on fortified sorghum grain extract samples

Column temperature program, 9° C. per minute

tector is capable of measuring desired compounds at very low levels, with little or no response from background materials. The advantage of this selective detector is demonstrated in the chromatograms shown in Figure 3. The upper curve was obtained on a fortified sorghum grain extract using a nonselective flame ionization detector (*8*) operated at high sensitivity. Extraneous peaks occurring in close proximity to the various trichlorobenzoic acid peaks offer some interference in accurately measuring the desired components. Because of the presence of these neighboring peaks, the flame ionization method is limited to about 0.2-p.p.m. sensitivity for the various TBA isomers in this particular matrix. The lower curve in Figure 3 shows a fortified sorghum extract analyzed with the microcoulometric titration detector. Because interfering peaks are usually not found when this selective detector is employed, the practical limit of sensitivity for the chlorinated acids is materially higher. In the lower scan, the 2,4,5-peak, which is about 20% of full scale, represents 0.1 p.p.m. of this isomer in the 25-gram grain sample used.

Preliminary studies using the selective electron affinity detector (*10*, *11*) have indicated that trichlorobenzoic acids and polychlorinated benzoic acids can also be determined successfully by this approach. Although programmed temperature column operation is not feasible when using this detector at high sensitivity, selective measurement of TBA and PBA can be made in many types of extracts. However, the microcoulometric detector has the advantage of better selectivity and, since its response is based on Faraday's law, it does not require custom calibration for the individual components as does the electron affinity detector.

An important advantage of the programmed temperature column operation for the trace analysis of higher-boiling compounds is that it permits the analysis of very large sample aliquots (*8*). This results in improved over-all sensitivity, and in many instances, reduces the need for concentrating extracts. By maintaining the initial column temperature just above the solvent boiling point, a relatively large sample aliquot can be injected over a period of several minutes, if desired. The solvent rapidly elutes from the column and is vented, so that it does not reach the combustion furnace. Since the initial column temperature is considerably below the boiling of the residue, this high-boiling material is effectively retained at the beginning of the column in a sharp band. This procedure permits the sampling of relatively large sample aliquots without noticeably broadening the width of the peak in the resultant chromatogram. Aliquots up to 2.5 ml. have been chromatographed by this procedure with no difficulties in the analysis. The slow injection technique cannot be used effectively with isothermal gas chromatographic separations, since excessive broadening of the residue peaks will occur.

Programmed temperature column operation also offers improved peak sharpness and shorter analysis time. The sharper peaks produced by the programmed temperature operation sometimes improve over-all sensitivity, since these peaks are more easily detected and integrated.

The extraction procedure described previously produces final extracts sufficiently devoid of extraneous materials, so that relatively large sample aliquots can be injected into the gas chromatograph without unduly contaminating the vaporizer, combustion tube, or detector. Attempts to eliminate certain of the steps in the extraction procedure produced extracts which were less suited for chromatographic analysis. No interfering peaks have occurred with any of the sample types analyzed, with the recommended extraction procedure.

As previously described (*7*), methylation of polychlorinated benzoic acids is quantitative when carried out with diazomethane. Attempts to esterify these highly sterically-hindered chlorinated acids by other reactions, such as alcohol with mineral acid and BF_3–methanol, gave less than quantitative results.

The column used in this study has been adequate for separating the desired constituents in these complex mixtures. In some cases, certain of the isomer pairs are incompletely separated. These are generally minor components and are added together to obtain the analysis. These incompletely resolved components can be separated, if desired (*7*). However, to make as rapid analyses as possible, some sacrifice in resolution was deemed desirable.

More recent studies have indicated that considerably less rigorous conditioning is required if a dimethylchlorosilane- or hexamethyldisilazane-treated, acid-washed Chromosorb W support is employed. The silanized supports show considerably lower surface activity, and might be generally more useful for the analysis of polychlorinated benzoic acids.

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Received for review December 5, 1963. Accepted February 14, 1964.