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Simultaneous gas chromatographic determination of 2,4-D and dicamba in human blood and urine

The compounds 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methoxy-3,6-dichlorobenzoic acid (dicamba) are herbicides commonly used to control a variety of broadleaf plants. An acute poisoning case was recently investigated by the Hawaii Community Study on Pesticides in which the victim intentionally ingested a formulation containing 2,4-D and dicamba. The occurrence of this attempted suicide created a need for a rapid and sensitive method for detecting these compounds in small amounts of human blood and urine.

Several methods for detecting 2,4-D residues in a variety of substrates were considered for the analysis of 2,4-D and dicamba in human blood and urine[1–3]. While generally sensitive, these methods were neither rapid nor applicable to the relatively small samples available.

The Hawaii Community Study on Pesticides uses the method of BEVENUE *et al.*[4] to determine pentachlorophenol (PCP) residues in human blood. In an evaluation done by this laboratory[5], the procedure was shown to be rapid, accurate, sensitive and reproducible. Because 2,4-D and dicamba display chemical characteristics similar to those of PCP, these compounds are also detected by the procedure.

This note describes the application of the method of BEVENUE *et al.*[4] to the simultaneous determination of 2,4-D and dicamba in human blood and urine.

Experimental

Solvents and reagents. The following solvents and reagents were used: benzene, hexane, isooctane (Mallinckrodt Nanograde Reagents), used as received; 0.1 *N* H_2SO_4, benzene extracted; 2,4-dichlorophenoxyacetic acid; 2-methoxy-3,6-dichlorobenzoic acid; N-methyl-N'-nitro-N-nitrosoguanidine, supplied by Aldrich Chemical Co., Milwaukee, Wisc.

The diazomethane solution[6] was prepared as follows: hexane (25 ml) and 2 ml of 20% aqueous NaOH were combined in a 50-ml erlenmeyer flask and 0.5 g of N-methyl-N'-nitro-N-nitrosoguanidine was added in small increments. When the reaction was complete, the hexane layer was decanted into a small glass bottle equipped with a Teflon-lined screw cap. The diazomethane solution was kept in a freezer for up to one week. Because diazomethane is a skin irritant and a carcinogen, its preparation and use was confined to a well-ventilated hood.

Standard solutions. Stock solutions of 2,4-D and dicamba in benzene were prepared, each containing 1 mg of compound per ml of solvent. In separate 100-ml volumetric flasks 1.0 ml of each stock solution was treated with 0.5 ml of diazomethane solution and allowed to stand for 15 min. The solutions were then diluted to 100 ml with isooctane, thus giving solutions which contained 10 μg/ml of each compound as its methyl ester. From these solutions, working standards were prepared in appropriate concentrations.

Apparatus. Gas chromatographic determinations were made on a MicroTek MT 220 gas chromatograph equipped with a ^3H electron capture detector and a 0.32 cm I.D. × 180 cm borosilicate glass column packed with a mixture of 4%

SE-30 and 6% QF-1 on Chromosorb W (AW-DMCS), high-performance grade, 80–100 mesh.

The instrument conditions were: inlet temperature 220°, detector temperature 208°, column temperature 190°; nitrogen flow rate 85 ml/min.

The 25-ml evaporative concentrator tube was Kontes No. 570050-2526, the evaporative concentrator column Kontes No. 569251-0324.

Extraction. One milliliter of blood serum or 5 ml of urine were combined with 20 ml of 0.1 *N* H_2SO_4 and 12 ml of benzene in a 125-ml glass-stoppered erlenmeyer flask equipped with a 1-in. Teflon-coated magnetic stirring bar and mixed for 20 min at 50° on a heated stirplate. After cooling, the contents of the flask were transferred to a 35-ml centrifuge tube and centrifuged for 15 min to effect phase separation. The benzene (top) layer was transferred to a 25-ml evaporative concentrator tube, and the aqueous phase was re-extracted three times with 5-ml portions of benzene. With each wash the mixture was shaken vigorously for 1 min, and the phases were separated by centrifugation. The benzene extracts were combined in the 25-ml evaporative concentrator tube, a boiling chip was added, an evaporative concentrator column was attached, and the combined extract was concentrated to 2 ml in a water-bath at 100°.

Methylation. Diazomethane solution (0.2 ml) was added to the concentrated extract, and the mixture was allowed to stand for 15 min. Excess diazomethane was then removed by gently bubbling dry nitrogen through the extract until the yellow color disappeared, and the volume was adjusted to 10.0 ml with isooctane.

Gas chromatography. The methylated extract was diluted as necessary, and 2 to 8 μl were injected into the gas chromatograph. Following each extract injection, an amount of standard which gave a response of similar peak height ($\pm$ 10%) was injected.

Results and discussion

The 2,4-D and dicamba levels in the poisoning case samples are given in Table I. The results of a recovery study to determine the applicability of the procedure to the analysis of human blood and urine for 2,4-D and dicamba residues are given in Table II. The samples were fortified, prior to extraction, with 2,4-D and dicamba acids at levels which compared generally with the wide range of concentrations found in the poisoning case samples.

In all cases responses to 2,4-D and dicamba (as their methyl esters) were quantitated by comparison with standard responses in terms of peak height. By comparing responses with peak heights differing by 10% or less, quantitation problems associated with lack of linearity and changing sensitivity, sometimes encountered with electron capture detectors, were minimized.

The limits of detectability in 1 ml of blood serum were 0.03 p.p.m. for dicamba and 0.05 p.p.m. for 2,4-D. In 5 ml of urine the limits of detectability were 0.01 and 0.02 p.p.m. for dicamba and 2,4-D, respectively.

Under the conditions given, dicamba and 2,4-D methyl esters had retention times of 1.2 and 1.9 min, respectively; PCP methyl ether had a retention time of 2.2 min. Because the three compounds are well separated on the gas chromatographic column, the procedure is useful for detecting any or all of the compounds in the same sample.

TABLE I

2,4-D AND DICAMBA LEVELS IN POISONING CASE SAMPLES

Sampling	*Blood serum*		*Urine*					*2,4-D (p. p. m.)*		*Dicamba (p. p. m.)*	
	Date[a]	*Time*	*Date*[a]	*Time*	*to Date*[a]	*Time*	*Vol. (ml)*	*Serum*	*Urine*	*Serum*	*Urine*
1	10/14	7:00 a.m.	10/14	11:00 a.m.	10/14	3:00 p.m.	740	1006	145.2	46.4	215.0
2	10/14	3:00 p.m.	10/14	3:00 p.m.	10/14	11:00 p.m.	375	1031	274.0	22.3	280.3
3	No sample taken		10/14	11:00 p.m.	10/15	9:00 a.m.	1230	—	151.5	—	47.5
4	10/15	9:00 a.m.	10/15	9:00 a.m.	10/15	4:00 p.m.	—	911.9	762.0	8.52	12.8
5	10/15	4:00 p.m.	10/15	4:00 p.m.	10/15	11:00 p.m.	775	931.2	599.8	8.12	1.95
6	10/15	11:15 p.m.	10/15	11:00 p.m.	10/16	7:45 a.m.	1170	736.4	784.3	6.99	4.22
7	10/16	7:50 a.m.	10/16	7:45 a.m.	10/16	4:15 p.m.	720	648.6	408.9	4.47	1.08
8	10/16	4:15 p.m.	10/16	4:15 p.m.	10/16	11:00 p.m.	835	461.0	1333	3.66	0.95
9	10/16	11:00 p.m.	10/16	11:00 p.m.	10/17	9:00 a.m.	740	383.1	1900	2.54	1.35
10	10/17	9:00 a.m.	10/17	9:00 a.m.	10/17	5:00 p.m.	310	364.3	1442	1.97	1.06
11	10/17	5:00 p.m.	10/17	5:00 p.m.	10/18	10:00 a.m.	1640	72.4	409.6	1.30	0.80
12	10/18	10:00 a.m.	10/18	10:00 a.m.	10/18	4:30 p.m.	910	5.23	77.9	0.47	0.54
13	10/18	4:30 p.m.	10/18	4:30 p.m.	10/19	9:45 a.m.	1400	1.69	8.60	0.23	0.40
14	10/19	9:45 a.m.	10/19	9:45 a.m.	10/19	3:00 p.m.	450	1.78	3.88	0.15	0.25
15	No sample taken		10/19	3:00 p.m.	10/20	9:00 a.m.	2150	—	4.51	—	0.12
16	10/20	9:00 a.m.	10/20	9:00 a.m.	10/21	8:30 a.m.	2420	1.26	2.90	0.15	0.06
17	10/21	8:30 a.m.	10/21	8:30 a.m.	10/22	9:30 a.m.	3710	0.38	1.58	0.15	<0.01
18	10/22	9:30 a.m.	10/22	9:30 a.m.	10/23	8:00 a.m.	2600	0.16	1.74	0.03	<0.01
19	10/23	7:45 a.m.	10/23	8:00 a.m.	10/24	8:45 a.m.	4465	0.30	0.54	0.07	<0.01
20	10/24	8:45 a.m.	10/24	8:45 a.m.	10/25	1:00 p.m.	2960	0.88	0.71	0.06	0.03
21	10/25	1:00 p.m.	10/25	1:00 p.m.	10/27	10:45 a.m.	3800	0.38	0.25	<0.03	0.04
22	10/27	10:45 a.m.	10/27	10:45 a.m.	10/29	8:00 a.m.	2110	0.28	0.42	<0.03	<0.01
23	No sample taken		10/29	8:00 a.m.	10/31	1:00 p.m.	3030	—	1.15	—	<0.01
24	10/31	1:00 p.m.	10/31	1:30 p.m.	11/3	7:30 a.m.	1360	0.33	1.13	<0.03	<0.01
25	11/7	9:00 a.m.	11/7	6:00 a.m.	11/8	6:00 a.m.	1300	0.56	0.84	<0.03	<0.01

[a] 1969

TABLE II

RECOVERY OF 2,4-D AND DICAMBA ADDED TO HUMAN BLOOD SERUM AND URINE

Sample[a]	*Fortification level (p. p. m)*		*Found (p. p. m.)*		*% recovery*	
	Dicamba	*2,4-D*	*Dicamba*[b]	*2,4-D*[b]	*Dicamba*	*2,4-D*
Urine	—	—	< 0.01[c]	< 0.02[c]	—	—
	65.0	685	68.8	779	106	114
	13.0	137	12.5	132	96	96
	1.30	13.7	1.15	11.6	88	85
	0.13	1.37	0.11	1.35	85	98
	0.013	0.137	0.01	0.15	77	109
	0.006	0.068	< 0.01[c]	0.07	—	103
Blood serum	—	—	< 0.03[c]	< 0.05[c]	—	—
	65.0	685	61.0	636	94	93
	6.50	68.5	5.91	68.9	91	101
	0.65	6.85	0.55	5.96	85	87
	0.065	0.685	0.07	0.60	108	88
	0.006	0.068	< 0.03[c]	0.06	—	88

[a] Each sample a single analysis.
[b] Detected as their methyl esters.
[c] Limit of detectability.

No interfering responses were encountered in any of the samples analyzed; however, the use of the diazomethane solution in amounts greater than those indicated in the procedure can lead to interferences due to impurities in the reagent.

Using this procedure, an experienced analyst can process approximately sixteen samples per day.

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