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A GAS CHROMATOGRAPHIC METHOD OF QUANTITATING DDT IN THE PRESENCE OF INTERFERING POLYCHLORINATED BIPHENYL

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SUMMARY

Beroza and co-workers have developed a method of identifying pesticides on the basis of *p*-values. This paper extends the use of *p*-values to quantitating the components of a two-component system which cannot be resolved on a given gas chromatographic column.

INTRODUCTION

The qualitative and quantitative analysis of pesticide residues is currently being intensively investigated. Beroza and co-workers[1–3] have developed the use of *p*-values as a method of identifying pesticides and as a practical guide to sample clean-up at the nanogram level. The *p*-value is defined[1] as "the fraction of total solute that distributes itself in the non-polar phase of an equivolume solvent pair". They have shown that by a judicious choice of solvent systems, one can make a positive identification even when one could not make such a choice based on gas chromatographic (GC) retention times alone. Our particular interest in the problem derives from the fact that the analysis of DDT may be complicated by PCB components. A rapid method of quantitating the DDT concentration in such analyses would be beneficial.

At present, the problem of separating PCBs from halogenated pesticides to permit reliable quantitation has been approached in several ways. One common method utilizes the separation of PCBs and halogenated pesticides by column chromatography. Methods using Florisil[4], silicic acid[5,6] and alumina[7] have been developed. Each class of compounds can then be quantitated by GC. A second general approach is to structurally modify one of the interfering components. An example of such a procedure is the conversion of DDT derivatives into benzophenones, leaving the PCB to be analysed by GC[8].

These methods add an additional clean-up step or chemical reaction to the

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analysis and it would seem that they would add substantially to the time required for analysis and could conceivably introduce some error due to the extra handling required. As most laboratories involved in pesticide or PCB analysis routinely analyze large numbers of samples, a more rapid method of quantitation would be extremely useful. This paper describes a procedure which seems to fit such a requirement.

THEORETICAL

When one uses *p*-values, certain assumptions are made: (a) *p*-values are independent of concentration; (b) *p*-values are not affected by co-extraction of other components of a mixture; and (c) the GC detector response is linear. Within the concentration range normally expected for pesticides, Beroza *et al.*[1] have shown these assumptions to be valid. When one is analyzing mixtures, two additional considerations are important: (a) differences in detector response and (b) additivity of peak heights when two compounds have the same retention time.

Our initial assumption was that peak heights would be additive and that a correction could be made for detector response. The following derivation reflects these considerations. Let

$$P_A = \frac{Q_A}{Q^\circ_A}$$

where P_A=*p*-value for component A, Q°_A=initial amount of A (pg) in non-polar solvent and Q_A=final amount of A (pg) in non-polar solvent.

Similarly, for component B

$$P_B = \frac{Q_B}{Q^\circ_B}$$

Taking into account the detector response, we obtain for the GC peaks:

$$\text{Initial peak height} = Q^\circ_A R_A + Q^\circ_B R_B$$

where R_A and R_B = detector response for A and B (mm/pg).

$$\text{Final peak height} = Q_A R_A + Q_B R_B$$
$$= Q^\circ_A P_A R_A + Q^\circ_B P_B R_B$$

The observed *p*-value (P_m) then will be defined as:

$$P_m = \frac{\text{Final peak height}}{\text{Initial peak height}} = \frac{Q^\circ_A P_A R_A + Q^\circ_B P_B R_B}{Q^\circ_A R_A + Q^\circ_B R_B}$$

This can be converted into a linear equation of the following form:

$$P_m = \frac{Q_A R_A}{Q^\circ_A R_A + Q^\circ_B R_B} \cdot (P_A - P_B) + P_B$$

with slope=$P_A - P_B$ and intercept=P_B.

For a given observed *p*-value, P_m, we can then determine the quantity

$$\frac{Q^{\circ}_{A}R_{A}}{Q^{\circ}_{A}R_{A}+Q^{\circ}_{B}R_{B}}$$

As R_A is a measurable quantity and $Q^{\circ}_{A}R_{A}+Q^{\circ}_{B}R_{B}$ is the initial peak height, then

$$Q^{\circ}_{A}=\frac{\dfrac{Q^{\circ}_{A}R_{A}}{Q^{\circ}_{A}R_{A}+Q^{\circ}_{B}R_{B}}(\text{Initial peak height})}{R_{A}} \quad (1)$$

It should be noted that the detector response is apt to be variable and must be measured regularly when using eqn. 1 to solve for Q°_{A}. However, once the curve in Fig. 1 is generated it is independent of detector response.

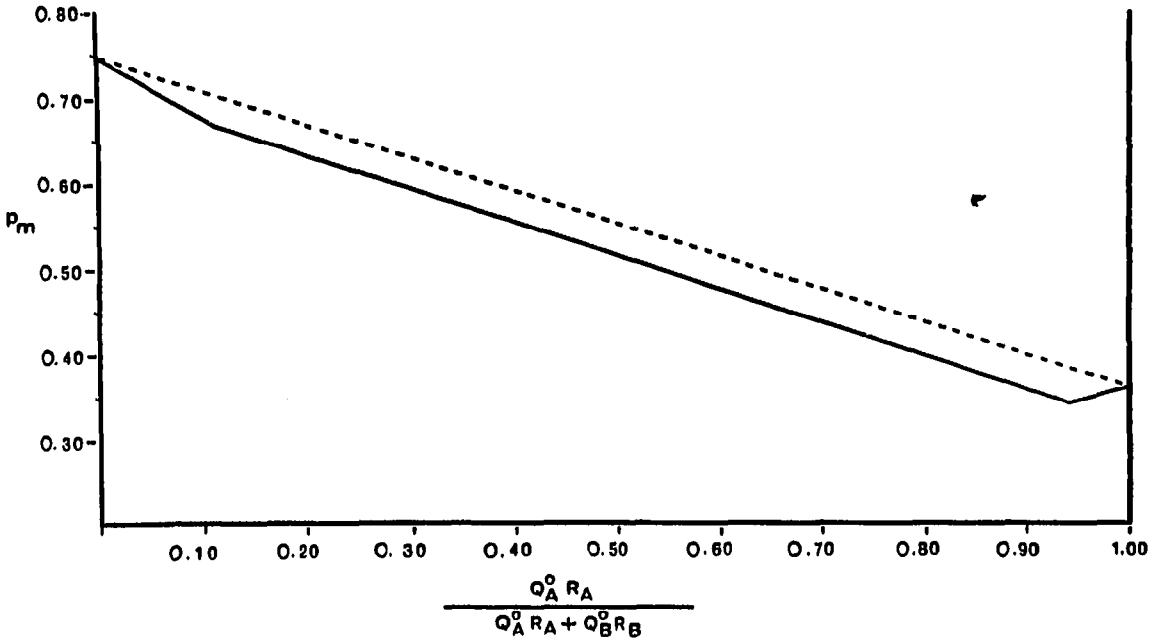

Fig. 1. P_m *versus* $Q^{\circ}_{A}R_{A}/(Q^{\circ}_{A}R_{A}+Q^{\circ}_{B}R_{B})$ for *p,p'*-DDT (A) and HCB-IX (B). Broken line, theoretical; solid line, empirical.

EXPERIMENTAL AND RESULTS

Mallinckrodt nanograde solvents were used throughout this work. All solvents used in the extractions were pre-equilibrated as cautioned by Beroza and co-workers[1]. The *p,p'*-DDT was obtained from Polyscience Corp. and HCB-IX was obtained by preparative GC. All GC analyses were performed on a Varian Model 1200 gas chromatograph equipped with a ^{3}H electron capture detector under the conditions specified.

Preparative GC of Aroclor 1254

Preparative GC was carried out on a Varian Model 1200 gas chromatograph using a 2 m × 4 mm I.D. Pyrex column filled with 3% SE-30 on Chromosorb W/HP.

The oven temperature was 180° and the injector and detector were maintained at 225°, with a nitrogen flow-rate of 26 ml/min. HCB-IX, the ninth peak eluting from the mixture, which corresponds to *p,p'*-DDT in retention time, was collected. Re-injection proved it to be not less than 99% pure.

Preparation of mixtures

Stock solutions of *p,p'*-DDT and HCB-IX were prepared in light petroleum. The concentration of *p,p'*-DDT was 0.074 p.p.m. (74 pg/μl) and that of HCB-IX was 0.125 p.p.m. (125 pg/μl). Both known and unknown mixtures were analyzed on a 2 m × 2 mm I.D. Pyrex column filled with 4% SE-30–6% QF-1 on Chromosorb W/HP. The oven temperature was 202° and the injector and detector were maintained at 225°, with a nitrogen flow-rate of 32 ml/min. The *p*-values were determined according to the following procedure. A 5-ml volume of the mixture was placed in a 60-ml glass-stoppered centrifuge tube. A sample large enough that the signal-to-noise ratio exceeded 50:1 (ref. 1) was injected into the gas chromatograph and the peak height recorded (mm/μl). A 5-ml volume of acetonitrile was added and the mixture partitioned by shaking on an Adams Cyclo-Mixer for 30 sec. After allowing the sample to equilibrate for 5–10 min, an aliquot of the non-polar (upper) phase was injected into the gas chromatograph and the peak height again recorded. The *p*-value

TABLE I

EMPIRICAL DETERMINATION OF P_m *vs.* $(Q^0_A R_A/Q^0_A R_A + Q^0_B R_B)$ FOR *p,p'*-DDT (A) AND HCB-IX (B)

Sample No.	Q^0_A (pg/μl)	R_A (mm/μl)	Q^0_B (pg/μl)	R_B (mm/μl)	$\frac{Q^0_A R_A}{Q^0_A R_A + Q^0_B R_B}$	P_m
1	74	0.116	0.00	–	1.000	0.360
2*	51.8	0.242	3.75	0.174	0.955	0.348
3*	46.3	0.242	3.90	0.174	0.945	0.340
3*	46.3	0.242	3.90	0.174	0.945	0.340
4	53.2	0.0832	16.8	0.0808	0.765	0.420
5	43.7	0.0832	24.2	0.0808	0.650	0.418
6	41.0	0.105	33.7	0.0784	0.620	0.485
7	34.4	0.0832	31.0	0.0808	0.533	0.543
8	32.0	0.0832	33.0	0.0808	0.500	0.505
9	28.5	0.105	48.6	0.0784	0.440	0.510
10	21.4	0.0832	39.2	0.0808	0.360	0.550
11	19.4	0.0832	49.4	0.0808	0.288	0.635
12	10.5	0.0832	52.8	0.0808	0.170	0.655
13	6.4	0.105	68.0	0.0784	0.115	0.670
13	6.4	0.105	68.0	0.0784	0.115	0.670
14	3.1	0.105	55.2	0.0784	0.070	0.685
15	1.1	0.105	57.5	0.0784	0.025	0.726
16	0.00	–	12.5	0.0916	0.00	0.750

* In order to obtain reasonable peak heights, it was necessary to decrease the attenuation on the detector.

TABLE II

ANALYSIS OF "UNKNOWN" MIXTURES OF *p,p'*-DDT (A) AND HCB-IX (B)

Sample No.	P_m	$\frac{Q^0_A R_A}{Q^0_A R_A + Q^0_B R_B}$	*Initial peak height (mm/μl)*	R_A	Q^0_A *(calc.)*	Q^0_A *(actual)*	*Deviation (%)*
1	0.486	0.575	7.46	0.196	41.5	44.4	− 6.5
2	0.638	0.200	8.42	0.106	15.9	14.8	+ 7.4
3	0.649	0.173	9.09	0.110	14.3	15.1	− 5.3
4	0.594	0.309	9.10	0.110	25.5	26.0	− 1.9
5	0.608	0.272	8.04	0.110	19.9	17.9	+11.2
6	0.588	0.323	7.35	0.110	22.1	23.5	− 6.0
7	0.481	0.574	7.17	0.103	40.0	41.9	− 4.5
8	0.598	0.299	8.11	0.103	23.5	22.2	+ 5.9
9	0.548	0.422	6.25	0.103	25.6	26.5	− 3.4
10	0.533	0.459	7.41	0.103	33.0	34.1	− 3.2
11	0.560	0.393	8.30	0.103	31.6	33.6	− 6.0
12	0.572	0.363	10.7	0.103	37.7	39.5	− 4.6
13	0.515	0.504	7.64	0.102	37.7	40.0	− 5.5
14	0.592	0.314	9.71	0.102	29.9	32.0	− 7.5
15	0.641	0.193	8.11	0.102	15.3	15.1	+ 1.3
16	0.706	0.0583	9.31	0.102	5.32	5.92	−10.1
17*	0.348	0.925	11.2	*0.242	42.8	43.8	− 2.3
	0.348	0.968	11.2	0.242	44.6	43.8	+ 1.8

* This mixture represents a sample where duplicate values are obtained. In order to obtain reasonable peak heights, it was necessary to decrease the attenuation on the detector.

was calculated and the amount of *p,p'*-DDT determined according to eqn. 1. The results for the mixtures used to generate the empirical curve are given in Table I. The results and percentage deviations for "unknowns" are presented in Table II.

Determination of non-additivity of peak heights

Known mixtures of *p,p'*-DDT and HCB-IX were directly analyzed by GC.

TABLE III

^{3}H ELECTRON CAPTURE DETECTOR RESPONSE FOR KNOWN *p,p'*-DDT (A) AND HCB-IX (B) MIXTURES

Theoretical peak height based on $R_A = 0.128$ mm/pg and $R_B = 0.094$ mm/pg.

Mixture	Q_A *(pg/μl)*	Q_B *(pg/μl)*	*Theoretical* $Q_A R_A + Q_B R_B$ *(mm/μl)*	*Observed peak height (mm/μl)*	*Deviation (%)*
1	74	0	9.47	9.47	0
2	59.2	25	9.94	9.39	− 5.5
3	44.4	50	10.4	9.07	−12.8
4	29.6	75	10.9	9.00	−17.4
5	14.8	100	11.3	10.3	− 8.8
6	0	125	11.8	11.8	0

The observed peak height was compared with a theoretical peak height based on the detector response for the individual components. The results are given in Table III.

DISCUSSION

Eqn. 1 was applied to a mixture of *p,p'*-DDT and HCB-IX (the ninth peak measured from an Aroclor 1254 sample), both of which had the same retention time on the 4% SE-30–6% QF-1 column.

Letting *p,p'*-DDT be component A and HCB-IX be component B, the following values were experimentally observed: $R_A = 0.116$ mm/pg; $R_B = 0.080$ mm/pg; $P_A = 0.36$; and $P_B = 0.75$.

Using these values, a straight line (Fig. 1, dashed line) was generated. Three mixtures known to contain 10.3, 30.3 and 14.8 pg/μl of *p,p'*-DDT admixed with HCB-IX were analyzed and values of 15.9, 37.9 and 19.7 pg/μl of *p,p'*-DDT were obtained. These values represent errors of 54, 25 and 33%, respectively. We attribute the large errors to the fact that the peak heights were not additive. This was confirmed when mixtures of *p,p'*-DDT and HCB-IX were directly analyzed by GC (Table III). In all mixtures, the observed peak heights were smaller than values based on contributions from the individual components. The observed values deviated by as much as 17% from the calculated values. The reason for the non-additivity was not established.

Rather than attempting to derive the actual effect of non-additivity, an empirical curve was generated by analyzing a number of mixtures of known composition, and the data are given in Table I.

The data could be treated in several ways. One could assume a straight-line relationship with the realization that pure *p,p'*-DDT and HCB-IX would give values that would indicate that they were not pure. We chose instead to generate a curve made of three linear components covering the ranges of $Q^0_A R_A/(Q^0_A R_A + Q^0_B R_B)$ of 0.00–0.115, 0.115–0.945 and 0.945–1.00 (Fig. 1, solid line). The following equations, derived by the least-squares method, were used to generate the three components.

$Q^0_A R_A/Q^0_A R_A + Q^0_B R_B$ *range*	*Equation*
0.00–0.115	$P_m = -9.686[Q^0_A R_A/(Q^0_A R_A + Q^0_B R_B)] + 0.746$
0.115–0.945	$P_m = 0.405[Q^0_A R_A/(Q^0_A R_A + Q^0_B R_B)] + 0.719$
0.945–1.00	$P_m = 0.334[Q^0_A R_A/(Q^0_A R_A + Q^0_B R_B)] + 0.026$

It can be seen from Fig. 1 that mixtures containing more than about 90% of *p,p'*-DDT cannot be analyzed because duplicate values would result. For any other mixture, one can use the empirical curve to estimate the amount of *p,p'*-DDT in the sample. A total of 17 "unknowns" were prepared and analyzed, and the results are given in Table II. All of the values now fall within $\pm 11\%$ of the actual values. One determination was made on a mixture which was in the range where our curve would give duplicate values for $Q^0_A R_A/(Q^0_A R_A + Q^0_B R_B)$ (sample No. 17). Even here the results fell well within the range of our other values. One would expect a maximum error in $Q^0_A R_A/(Q^0_A R_A + Q^0_B R_B)$ of about 10% in this region of the curve.

We consider the results that we have obtained to be favorable compared with methods of quantitation used at present, especially in view of the rapidity and ease of the method once the empirical curve is derived. The non-additivity of peak heights certainly warrants further investigation as it could introduce significant errors into experiments in which quantitative estimates are made on the basis of differences in peak heights.

CONCLUSION

The use of *p*-values in pesticide analysis as developed by Beroza and co-workers has been extended to the quantitative determination of the components of a binary mixture which is not resolved on a given GC column. When applied to *p,p'*-DDT–HCB-IX mixtures, the quantitation of *p,p'*-DDT deviated by no more than ±11% from the actual value. The results are comparable with the methods used at present and the method has the advantage of being reasonably rapid and simple.

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