Electron Capture Gas Chromatographic Analysis of DDA: Utilization of 2-Chloroethanol Derivative

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The electron capture gas chromatographic analysis of DDA, the carboxylic acid metabolite of DDT, requires the formation of a suitable volatile derivative. The methyl ester has been utilized but had the unsatisfactory characteristic of poor electron capture response (1). This paper reports the utilization of the 2-chloroethanol derivative in the electron capture gas chromatographic analysis of DDA.

MATERIALS AND METHODS

Apparatus and Equipment: The gas chromatograph used was the Tracor Model MT-220 fitted with an electron capture detector system operated under the following conditions: a glass column, 6' x 1/4", packed with 1.95% QF-1/1.5% OV-17 on Supelcoport, 80/100 mesh; nitrogen carrier gas with a flow rate of 60 ml/min; column temperature, 200°C; injection port, 225°C; and detector temperature, 210°C.

Chromatographic Columns: Size 22, i.d. 7 mm, length 200 mm, Kontes No. 420100.

Standards: Analytical standard of 2,2-Bis(p-chlorophenyl) acetic acid (p,p'-DDA) 99+% was obtained from the pesticide repository of the Perrine Primate Laboratory, Perrine, Florida 33157.

Reagents: Hexane and Benzene, Mallinckrodt Nanograde solvents or equivalent. Boron trichloride-Methanol 10% BCl_3 (w/v) and Boron trichloride-2-chloroethanol 10% BCl_3 (w/v) were obtained from Applied Science Laboratory, State College, Pennsylvania.

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Silica gel, Woelm, Activity Grade I, Waters Associates, Inc., Framington, Mass. The adsorbent was dried for 48 hours at 170°C and stored in a dessicator. On the day of use, 1 gm of silica gel was deactivated by adding 15 microliters of water and 1 gm of silica gel to a 125 ml Erlenmeyer flask. The flask was stoppered and rotated until the water was evenly distributed throughout the adsorbent. The adsorbent was allowed to equilibrate for 2-3 hours with periodic shaking. The chromatographic column was prepared just before use (2).

Distilled water, sodium sulfate and glass wool, extracted with Nanograde hexane, were used throughout the experiment.

Esterification Procedure: To approximately 5 mg of p,p'-DDA in a test tube was added 2.0 ml of BCl_3-Methanol 10% w/v, or 2.0 ml of BCl_3-2-chloroethanol 10% w/v. Placed in a steam bath for thirty minutes, removed and quenched the reaction with 5 ml of cold distilled water. Extracted three times with 3 ml portions of hexane. Combined the hexane extracts and filtered through anhydrous sodium sulfate. Adjusted to appropriate volume for analysis by electron capture gas chromatography.

Preparation of Standard Solutions: A stock solution of p,p'-DDA in benzene was prepared by weighing 52 mg into a 50 ml volumetric flask. Diluted to volume with benzene. The methyl ester and the 2-chloroethanol derivatives was prepared from the stock solution by esterification with BCl_3-Methanol and BCl_3-2-chloroethanol, respectively. The concentration of the resulting working standards was 211 pg/μl and 64.4 pg/μl of the methyl ester and 2-chloroethanol derivatives, respectively, calculated as DDA.

Efficiency of Conversion of p,p'-DDA to the 2-Chloroethanol Derivative: To check the efficiency of conversion of p,p'-DDA to the 2-chloroethanol derivative, approximately 20 mg portions of p,p'-DDA were accurately weighed into each of six 50 ml test tubes (glass stoppered). The samples were esterified with 10 ml of BCl_3-2-chloroethanol. After esterification procedure, the hexane extracts, in previously tared concentrator tubes, were taken to dryness over a gentle stream of nitrogen. Concentrator tubes

were then placed in dessicator overnight to assure dryness and allow time to equilibrate. The concentrator tubes were then accurately reweighed to determine the weight of the 2-chloroethanol derivative of DDA. The efficiency of conversion of DDA to the 2-chloroethanol derivative was calculated.

Elution Pattern and Recovery of 2-Chloroethanol Derivative of DDA from the Silica Gel Column: Occasionally it is necessary to clean-up urine samples from the "general human population" prior to gas chromatographic determination utilizing the electron capture detector to remove interfering substances. The silica gel column chromatographic clean-up as described by Shafik, et al., (2) provided adequate clean-up of the 2-chloroethanol derivative of DDA prior to electron capture detection.

Duplicate samples of the 2-chloroethanol derivative of DDA (0.64 micrograms) were placed on silica gel columns and eluted with hexane followed by 10, 20, 30, 40, 50, 60, 70, and 80 percent benzene in hexane. Each sample was analyzed gas chromatographically to determine the elution pattern and percent recovery of the 2-chloroethanol derivative.

Response of 2-Chloroethanol and Methyl Ester Derivatives of DDA to Electron Capture Detection: To determine the sensitivity of the 2-chloroethanol derivative of DDA as compared with the sensitivity of the methyl ester derivative of DDA to electron capture detection; five injections, each containing 1.28 ng of the 2-chloroethanol derivative and 2.11 ng of the methyl ester derivative, were made into the gas chromatograph. The response was calculated for each derivative in terms of area under the curve and expressed as square millimeters per nanogram. The response ratio of 2-chloroethanol to the methyl ester was calculated.

RESULTS AND DISCUSSION

The efficiency of conversion of p,p'-DDA to the 2-chloroethanol derivative is shown in Table 1.

TABLE 1

EFFICIENCY OF CONVERSION OF p,p'-DDA TO THE 2-CHLOROETHANOL DERIVATIVE

No.	Weight of p,p'-DDA (mg.)	Weight of 2-Chloroethanol Derivative (mg)	% Conversion[a]
1	19.0	25.2	92.1
2	22.5	29.4	93.5
3	18.0	24.6	89.4
4	22.7	30.9	89.7
5	22.4	28.7	95.3
6	18.1	24.8	89.1
		Mean ± S.E. = 91.5 ± 1.0%	

[a]

$$\% \text{ Conversion} = \frac{342 \times \text{Weight of p,p'-DDA}}{280 \times \text{Wt. of 2-Chloroethanol Derivative}} \times 100.$$

The silica gel column chromatographic clean-up of the 2-chloroethanol derivative of p,p'-DDA was sufficient for the gas chromatographic analysis utilizing the electron capture detector.

The percent recovery of two samples, each containing 0.644 micrograms of p,p'-DDA after formation of the 2-chloroethanol derivative with BCl_3-2-chloroethanol 10% w/v from the silica gel column is given in Table 2.

TABLE 2

PERCENT RECOVERY OF 2-CHLOROETHANOL DERIVATIVE OF DDA FROM SILICA GEL COLUMN

Sample Number	2-Chloroethanol Derivative (as DDA) (µg)	% Recovery
1	0.624	96.9
2	0.629	97.7
	Mean ± S.E. = 97.3 ± 0.4%	

As shown in Table 2, the mean (±S.E.) for the recovery of the 2-chloroethanol derivative of DDA from the silica gel column was 97.3 ± 0.4%. The column was eluted with hexane followed by 10, 20, 30, 40, 50, 60, 70, and 80 percent benzene in hexane. The 2-chloroethanol derivative was eluted in the 50% benzene in hexane fraction.

For future clean-up of the 2-chloroethanol derivative of DDA on the silica gel column it would be sufficient to elute with 40% benzene, 60% benzene, and 80% benzene in hexane with the 2-chloroethanol derivative appearing in the 60% benzene in hexane fraction.

The response of 2-chloroethanol and the methyl ester derivatives of DDA to the electron capture detector, as well as, the response ratio of 2-chloroethanol to the methyl ester is given in Table 3.

TABLE 3

RESPONSE OF 2-CHLOROETHANOL AND METHYL ESTER DERIVATIVES OF DDA TO ELECTRON CAPTURE DETECTION

Injection Number	Electron Capture Response (Peak Area)		Response Ratio 2-Chloroethanol Derivative / Methyl Ester Derivative
	2-Chloroethanol Derivative (mm^2/ng)	Methyl ester Derivative (mm^2/ng)	
1	293.65	72.96	3.69:1
2	278.52	76.78	3.63:1
3	293.75	75.59	3.89:1
4	266.41	75.35	3.54:1
5	284.18	74.64	3.81:1

The average electron capture response ratio of 2-chloroethanol derivative to the methyl ester derivative of DDA for the five determinations was 3.7 ± 0.1:1.

Another advantage of using the 2-chloroethanol derivative of DDA instead of the methyl ester derivative of DDA for the gas chromatographic analysis of DDA is that the retention time (Figure 1) of the 2-chloroethanol derivative is such that the DDA will be separated from other chlorinated hydrocarbons which might interfere with the analysis.

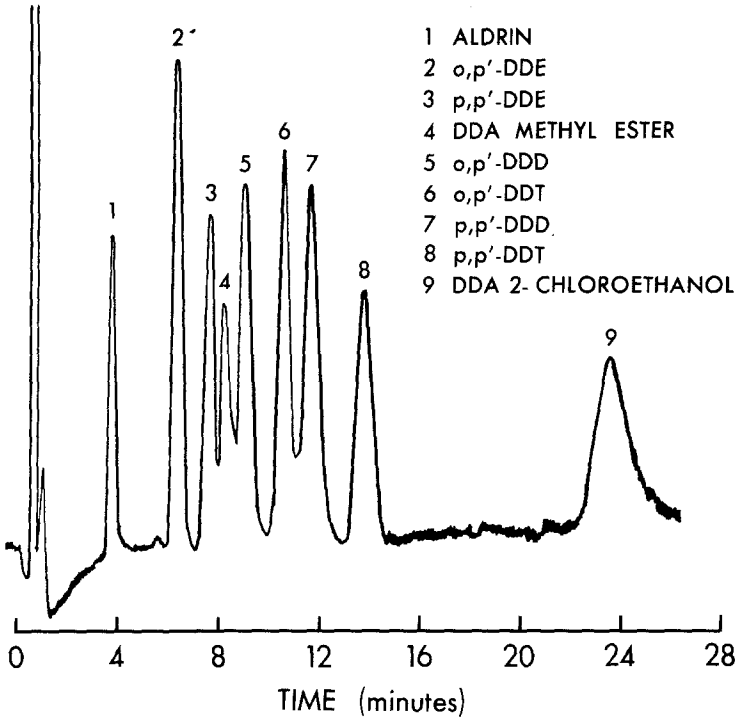

FIGURE 1 - Typical chromatogram of 9 organochlorine pesticides obtained using a 6' x 1/4" glass column packed with 1.95% QF-1/1.5% OV-17 on Supelcoport 80/100 mesh.

SUMMARY

This paper reports the use of the 2-chloroethanol derivative in the electron capture gas chromatographic analysis of DDA. The efficiency of conversion of DDA to the 2-chloroethanol derivative was 91.5 ± 1.0%. The utilization of a silica gel column for clean up prior to electron capture analysis is discussed and the elution pattern for the 2-chloroethanol derivative is given with a recovery of 97.3 ± 0.4%. The 2-chloroethanol derivative is 3.7 ± 0.1 times more responsive to electron capture detection than the methyl ester of DDA and produces a retention time which separates the DDA from other chlorinated hydrocarbon pesticides which might interfere with the analysis.

REFERENCES

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