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COMPARISON OF ELECTRON-CAPTURE AND ELECTROLYTIC-CONDUCTIVITY DETECTION FOR THE GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF HEPTAFLUOROBUTYRYL DERIVATIVES OF SOME AGRICULTURAL CHEMICALS

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SUMMARY

The gas–liquid chromatographic characteristics of the heptafluorobutyryl (HFB) derivatives of several agricultural chemicals have been compared. Electron-capture detection was found to measure smaller amounts of the derivatives at a working level than electrolytic-conductivity detection in the halogen (reductive) mode (40–400 pg *versus* 1–5 ng). However, for sample analysis in foods, the detection methods were similar owing to the selectivity of the electrolytic-conductivity detector which permitted a greater quantity of sample to be injected. As low as 1–2 ppb* of diethylstilbstrol-HFB in beef liver and 30–50 ppb of both carbofuran and 3-keto-carbofuran in turnips could be detected by either detector. Response of both detectors was found to increase with the number of heptafluorobutyryl groups added. Thus 3-hydroxycarbofuran was twice as sensitive as carbofuran by electrolytic-conductivity detection and 2–3 times more sensitive by electron-capture detection.

INTRODUCTION

One of the most versatile and frequently used methods for detecting low levels ($<$ 1 ppm) of agricultural chemicals in food samples is gas–liquid chromatography (GLC). Separation of various compounds can be effected by many stationary phases, but detection of small amounts is usually limited to either electron-capture (ECD) or selective detectors such as the electrolytic-conductivity detector. Since these two detection modes are commonly used, it was felt worthwhile to compare their detection properties for measuring agricultural chemicals.

In order for many of these compounds to be detected with both sensitivity and high resolution, recourse is often made to derivative formation[1] to increase volatility, minimize adsorption, and allow small amounts to be measured. To this end, fluorinated derivatives of polar compounds are extensively used because of their excellent

* Throughout the article the American billion (10^9) is meant.

electron-capture properties. In addition, it has been shown recently[2] that these same fluorinated derivatives can be measured by a electrolytic-conductivity detector in the halide mode at low levels owing to their high fluorine content. This paper compares the detection limits of HFB derivatives of some agricultural chemicals using both ECD and electrolytic-conductivity detection and applies this comparison to both pure standards and extracts of beef and turnip samples.

EXPERIMENTAL

Reagents and chemicals

All solvents were residue-free glass-distilled grade. Trimethylamine (anhydrous; Eastman, Rochester, N.Y., U.S.A.) in glass ampoules was cooled in ice water and added with stirring to cool tared benzene to give 0.1 *M*. Heptafluorobutyric anhydride was supplied by PCR (Gainesville, Fla., U.S.A.) and used as received.

The agricultural chemicals were analytical crystalline grade from commercial sources and gave single peaks by GLC on OV-1 after derivatization. Those compounds examined were DES (4,4′-dihydroxy-α,β-diethylstilbene), hexestrol (dihydrodiethylstilbestrol), β-estradiol (estra-1,3,5(10)-triene,3,17β-diol), clopidol (2,6-dimethyl-3,5-dichloropyridin-4-ol), lindane (hexachlorocyclohexane), carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl-N-methylcarbamate), carbofuran-3-OH (2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuran-7-yl-N-methylcarbamate), carbofuran-3-keto (2,3-dihydro-2,2-dimethyl-3-ketobenzofuran-7-yl-N-methylcarbamate), baygon (2-isopropoxyphenyl-N-methylcarbamate), and linuron (N-methyl-N-methoxy-N′-(3,4-dichlorophenylurea)).

Standard solutions of the compounds were prepared in either benzene, ethyl acetate or acetonitrile (depending on solubility) at a level of 100 ppm (10 mg in 100 ml). Clopidol, however, was very insoluble and only a 20 ppm solution could be made using acetonitrile.

Gas–liquid chromatography

All columns were of borosilicate glass and were packed with 3% OV-1 on Chromosorb W HP (80–100 mesh) or 3% OV-17 on Chromosorb W (80–100 mesh). Length and internal diameter are stated for specific examples.

Electron-capture detection

The instrument used was a Hewlett-Packard 5713 gas chromatograph equipped with a ^{63}Ni pulsed detector operated at 250° at a flow-rate of 60 ml/min of argon–methane (95:5). The injection port temperature was equal to or slightly higher than that of the column. The latter was varied to give a retention time of 3–6 min. The attenuation used routinely was 32 on a 1.0-mV recorder.

Electrolytic-conductivity detection

A Microtek MT-220 (Tracor) gas chromatograph was fitted with a Coulson electrolytic-conductivity detector set up for halogen analysis (reductive mode). Operating conditions were: transfer unit, 210°; pyrolysis furnace 800°; helium carrier and sweep, 40 ml/min; hydrogen flow, 40 ml/min; d.c. bridge potential, 30 V. A stainless-steel wire (diameter 0.004-in.) was inserted into the capillary water entrance to the

mixing chamber[3]. The water was cooled with tap water by a coiled glass tube immersed in the reservoir[4].

Mass spectrum

An Hitachi RMS-4 mass spectrometer was used with ionization voltage of 80 eV and probe maintained either at 20° or 100°.

Preparation of derivatives

The compound (1–10 μg) in standard solution solvent (0.1–0.4 ml) was added to a 15-ml centrifuge tube with glass-stopper or a centrifuge tube with PTFE-lined screw cap. To this was added 0.1–0.4 ml of 0.1 *M* trimethylamine (TMA) in benzene followed by 20 μl of the heptafluorobutyric anhydride (final volume 0.5 ml). After 1 h at room temperature or 65°, 0.5 ml of benzene was added plus 5 ml of water and the vessel shaken vigorously for 30 sec. After centrifugation, the upper benzene layer was analysed by GLC either directly or after dilution with benzene.

RESULTS AND DISCUSSION

The derivatization reaction was found to proceed more readily with the phenols than with the carbamates or ureas. The former went to completion at room temperature while the latter two required heat. Also derivatization of the insecticides following earlier work published on these compounds[5,6] was not satisfactory because of the long reaction times, high temperatures and incomplete yields. However, the addition of a catalyst such as TMA to the reaction mixture greatly facilitated derivative formation under milder conditions[2]. The method chosen was essentially that described by Ehrsson *et al.*[7]. After the reaction, the excess reagent anhydride was hydrolysed to the free acid which was removed by partitioning with water.

TABLE I

GLC CHARACTERISTICS OF FLUORINATED STANDARD COMPOUNDS BY ECD

Column (182 cm × 4 mm I.D.) packed with 3% OV-1 on Chromosorb W HP (80–100 mesh); flow-rate, 60 ml/min.

Compound	*Derivative**	*Column temp. (°C)*	*Retention-time (min)*	*Response*** *(pg)*
DES	2	200	3.3, 5.1	250, 100***
Hexestrol	2	200	5.1	126
β-Estradiol	2	230	4.1	170
Clopidol	1	125	4.0	40
Lindane	—	160	6.0	141
Carbofuran	1	160	3.9	230
Carbofuran-3-keto	1	180	4.6	403
Carbofuran-3-OH	2	170	4.6	88
Baygon	1	150	3.9	155
Linuron	1	170	5.8	175

* Indicates number of heptafluorobutyryl groups per molecule of parent.

** 1.0-mV recorder. Amount of parent compound required to produce 50% full scale deflection at attenuation 32×.

*** *Cis* and *trans* peaks, respectively.

Since the primary interest was residue levels of chemicals, derivatization was performed with small amounts (< 10 μg) and not carried out on milligram quantities. The reaction conditions chosen are believed to be essentially quantitative. The OV-1 and OV-17 liquid phases gave very good resolution, no tailing and low background noise over a wide temperature range (120–240°) for both detectors.

Table I shows the GLC characteristics by ECD of some HFB derivatives. Lindane was included for comparison purposes. Generally, it appeared that the electron-capture response depended significantly upon the number of fluorine atoms present in the derivative but the response was not linear. The mono-HFB derivatives were less sensitive than similar di-HFB derivatives. Carbofuran-3-OH, for example, was 2–3 times more sensitive than carbofuran because of the addition of the second HFB group. A similar effect was shown earlier for trifluoroacetyl derivatives[8]. The electron-capture response was also affected by structure of the parent compound. Clopidol-HFB, which has only one HFB group, was 2–3 times more sensitive than hexestrol or β-estradiol, which have two HFB groups in the derivatives. This strong response was due both to the electron-capturing chlorine atoms on the aromatic system and to the lower molecular weight of the parent compound (response in Table I is measured as pg of equivalent parent injected which produces a 50% full scale deflection, not pg of derivative). Generally, 40–400 pg of equivalent parent were required to produce 50% full scale response as mentioned in Table I. Since peak height was used to quantitate, the results vary within this range with retention time, peak shape and the GLC stability of the derivatives. However, all compounds could be measured at < 10 pg as standards by ECD.

Table II presents the GLC characteristics by electrolytic-conductivity detection of the same derivatives mentioned in Table I. It was found that response was related to the molecular weight of the parent and to the number of halogens in the derivative.

TABLE II

GLC CHARACTERISTICS OF FLUORINATED STANDARD COMPOUNDS BY ELECTROLYTIC-CONDUCTIVITY DETECTION

Column (120 cm × 4 mm I.D.) packed with 3% OV-1 on Chromosorb W HP (80–100 mesh); flow-rate, 60 ml/min.

Compound	*Derivative**	*Column temp. (°C)*	*Retention time (min)*	*Response*** *(ng)*
DES	2	200	2.7, 3.9	7.5, 3.0***
Hexestrol	2	210	3.9	2.0
β-Estradiol	2	233	5.4	2.0
Clopidol	1	124	3.9	1.2
Lindane	—	180	3.2	2.5
Carbofuran	1	170	4.5	2.0
Carbofuran-3-keto	1	190	5.4	5.0
Carbofuran-3-OH	2	175	5.4	1.0
Baygon	1	170	2.5	2.0
Linuron	1	180	3.8	5.0

* Indicates number of heptafluorobutyryl groups per molecule of parent.

** 1.0-mV recorder. Amount of parent compound required to produce 50% full scale deflection at 30 V; attenuation, 4×.

*** *Cis* and *trans* peaks, respectively.

Thus, sensitivities for all derivatives were of the same order of magnitude although varying somewhat owing to retention time and chromatographic stability. Carbofuran-3-keto and linuron, for example, were significantly less sensitive than carbofuran by both detection methods even though they should be similar based on halogen content. This, perhaps, reflects poorer chromatography evident by broader peaks which would require a greater quantity of sample to produce 50% full scale response. Generally, 1–5 ng of the compounds were required to produce a 50% recorder deflection at 4 × attenuation. The addition of a second HFB group increased sensitivity only 2-fold compared to a greater increase for ECD (*e.g.* carbofuran and carbofuran-3-OH). The response characteristics of the electrolytic-conductivity detector to halogens is more linear than with the ECD owing to the nature of the detection mechanism. Thus, it is easier to predict response of organo-halogen compounds to the electrolytic-conductivity than to the electron-capture detector.

The identity of some of these derivatives was supported by mass spectrometry (MS) and GLC–MS. Thus, DES-HFB gave a peak at *m/e* 660, either on the probe or by GLC and this value corresponded to the addition of two fluorinated moieties to the molecule. Similarly, clopidol-HFB showed peaks at *m/e* 389 and 387 (one chlorine substituent) for the simple monoacyl compound.

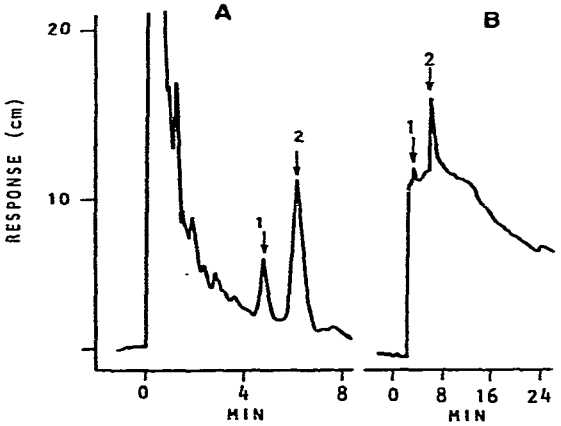

Fig. 1. Chromatograms of beef liver extract containing 2.0 ppb DES; peaks, 1 = *cis* DES-HFB, 2 = *trans* DES-HFB. (A) Measured by ECD, column: 3% OV-17, 182 cm × 2 mm I.D.; temperature, 170°; argon–methane (95:5) flow-rate, 60 ml/min; 37.5-mg sample injected. (B) Measured by electrolytic-conductivity detection, column: 5% OV-17, 120 cm × 4 mm I.D.; temperature, 195°; helium flow-rate, 40 ml/min; 500-mg sample injected. Attenuation: (A), 32×; (B), 4×.

The two detection systems were compared for analysis of samples of food extracts where interferences from background and secondary peaks were significant. Fig. 1 is a GLC tracing of an extract of beef liver (50 g) which was spiked with 2 ppb DES, cleaned up by the method of Coffin and Pilon[9], and measured as the HFB derivative. It can be seen that both detection modes produced similar results. With the ECD, less material needed to be injected because of the high sensitivity of the derivative, resulting in a low background. With the electrolytic-conductivity detector, even though the product was less sensitive to detection, more could be injected on

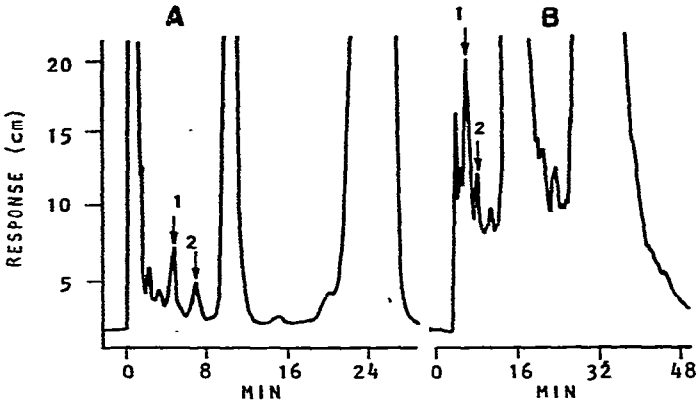

Fig. 2. Chromatograms of turnip containing 0.1 ppm carbofuran and 0.1 ppm carbofuran-3-keto. Peaks, 1 = Carbofuran; 2 = carbofuran-3-keto. (A) Measured by ECD, column conditions as in Fig. 1A; 10-mg sample injected; attenuation, 256×. (B) Measured by electrolytic-conductivity detection, column conditions as in Fig. 1B, temperature, 175°; 250-mg sample injected; attenuation, 4×.

the column due to the greater selectivity of the halogen detector. Fig. 2 shows the same format as in Fig. 1 for the HFB derivative of carbofuran and carbofuran-3-keto (0.1 ppm) in turnip which had been extracted and cleaned up by the method of Luke *et al.*[10]. Turnip was chosen as a real test for the method. It can be seen that much coextractive material is present in the chromatograms. However, samples such as potato, corn or cabbage have much less background response enabling these compounds to be analysed at much lower levels. The results and conclusions are similar to those of DES in the liver samples, indicating that the two detection modes are complementary in their virtues and can both be used either singly or together (for confirmation) to detect low levels of these chemicals in food samples.

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