CHROM. 3937

Identification of organophosphate pesticides by gas chromatography with the flame photometric detector*

Determination of retention times on gas chromatographic columns of different polarity is one technique for compound identification[1,2]. The combination of this technique with the use of a specific detector increases the reliability of the identification. Detectors which have been used for detection of organophosphate pesticides include the electron affinity detector[3], the microcoulometric detector[2], the thermionic detector[2], and the flame photometric detector[4]. The flame photometric detector provides the best combination of sensitivity and selectivity. Since the flame photometric detector will give a response for compounds containing both phosphorus and sulfur, it is especially well suited for the detection and identification of pesticides which contain these two elements.

In a recent study of pesticides in air, details of which will be published elsewhere, gas chromatography was selected for the analysis of samples primarily because the levels in most cases were too low to permit detection and identification by other methods. Both chlorinated pesticides and organophosphate pesticides were specifically sought; the latter included methyl parathion, malathion, and parathion. In addition, if any unknown organophosphate pesticides were detected, they had to be identified.

The approach selected for the detection and identification of organophosphate pesticides in this study consisted of measurement of retention times on two columns of different polarity plus detection of the compounds with the flame photometric detector in both the phosphorus and sulfur modes. This approach was especially useful for identifying organophosphates in three different types of samples. A description of the approach and of the applications follows.

Gas chromatographic conditions

A Micro-Tek GC 2500R gas chromatograph containing a Micro-Tek flame photometric detector equipped for use in either the phosphorus or the sulfur mode

TABLE I

RELATIVE RETENTION TIMES FOR ORGANOPHOSPHATE COMPOUNDS

Temperature, 140°; nitrogen carrier gas flow rate 100 ml/min. Methyl parathion retention times·
12 min on QF-1, 10 min on OV-1.

Compound	Relative retention time	
	QF-1	OV-1
Phorate	0.17	0.42
Tri-n-butyl phosphate	0.27	0.39
Methyl parathion	1.00	1.00
Malathion	1.10	1.58
Parathion	1.42	1.58
S,S,S-Tributyl phosphorotrithioate	1.15	3.18

was employed. Glass columns 1 m long by 6 mm O.D. were used; one column was packed with 5% QF-1 (Chemical Research Services, Inc.) on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories, Inc.) and the other column was packed with 5% OV-1 (Supelco, Inc.) on 100–120 mesh Gas-Chrom Q. Relative retention times for several organophosphate compounds on these columns are given in Table I. The column oven was set at temperatures from 140° to 170°; the higher temperatures were used in confirming runs for S,S,S-tributyl phosphorotrithioate (DEF, Chemagro Corp.) to reduce the necessary time of analysis. In all cases, samples were compared with standards that were analyzed within a few hours.

Results and discussion

Organophosphate pesticides were identified in the samples by combining the measurement of retention times on the two columns with the specific response of the flame photometric detector. Three examples of the application of this approach are shown. Methyl parathion, parathion, and DEF were identified by comparing the retention times of peaks in the samples with those of knowns on two columns; the pesticides were confirmed by the presence of peaks at the right retention times with the detector in the sulfur mode. A peak in some samples was shown to be tri-*n*-butyl phosphate, a solvent, rather than phorate by the use of the detector in the sulfur mode.

Differentiation between phorate and tri-n-butyl phosphate. An early peak in a number of samples could have been either phorate or tri-*n*-butyl phosphate from retention times. A typical chromatogram of a sample, recorded with the detector in the phosphorus mode, is shown in Fig. 1a; chromatograms of standards are shown in Fig. 1b and c. Chromatograms obtained on the QF-1 column gave similar agreement
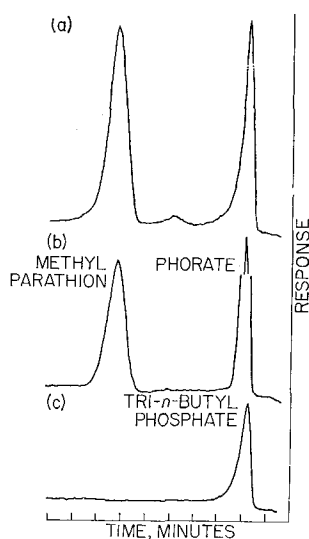

Fig. 1. Chromatograms of pesticides with flame photometric detector in phosphorus mode. (a) Sample, 5.0 μl. (b) Standard containing 4.1 ng each of methyl parathion and phorate. (c) Standard containing 4.2 ng of tri-*n*-butyl phosphate. Column, OV-1; temperature, 150°; amplifier sensitivity, 3.2 × 10⁻⁸ a.f.s.

of retention times, except that the retention time of the unknown peak was closer to that of tri-*n*-butyl phosphate than it was to that of phorate. The peak shape, which is often a useful parameter for identifying compounds, more closely resembled the peak for tri-*n*-butyl phosphate than for phorate.

Because the phorate molecule contains two sulfur atoms while tri-*n*-butyl phosphate contains none, the use of the detector in the sulfur mode permitted positive differentiation between the two compounds.

Fig. 2a is a chromatogram of the sample with the detector in the sulfur mode and Fig. 2b is a chromatogram of the standard. The absence of a peak in Fig. 2a at the retention time for phorate shows that the peak in Fig. 1a cannot be phorate but is probably tri-*n*-butyl phosphate.

Identification of methyl parathion. The later peak shown in Fig. 1a was identified as methyl parathion by comparison of its retention times on the two columns with a standard. This identification was confirmed by the use of the detector in the sulfur mode, as shown in Fig. 2.
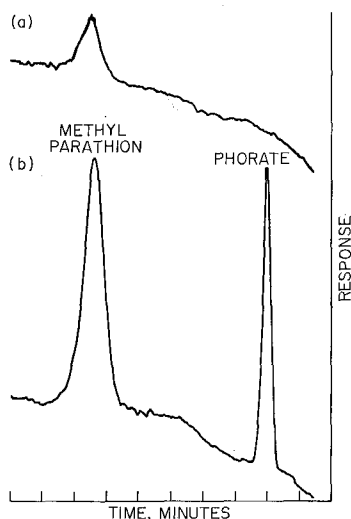

Fig. 2. Chromatograms of pesticides with flame photometric detector in sulfur mode. (a) Sample, 8.5 μl. (b) Standard containing 20 ng of methyl parathion and 4.0 ng of phorate. Column, OV-1; temperature, 150°; amplifier sensitivity, 3.2 × 10^{-8} a.f.s.

Identification of parathion and of DEF. Parathion and DEF were identified in other samples by a comparison of the retention times on the two columns and confirmed by the use of the detector in the sulfur mode.

Typical chromatograms are shown for a sample containing parathion in Fig. 3. Fig. 3a shows a chromatogram of the sample with the detector in the phosphorus mode; for comparison, a chromatogram of a standard containing parathion is given in Fig. 3b Fig. 3c shows a chromatogram of the sample with the detector in the sulfur mode; for comparison, a chromatogram of a standard containing parathion is given in Fig. 3d. Parathion had the same retention time as malathion on the OV-1 column.
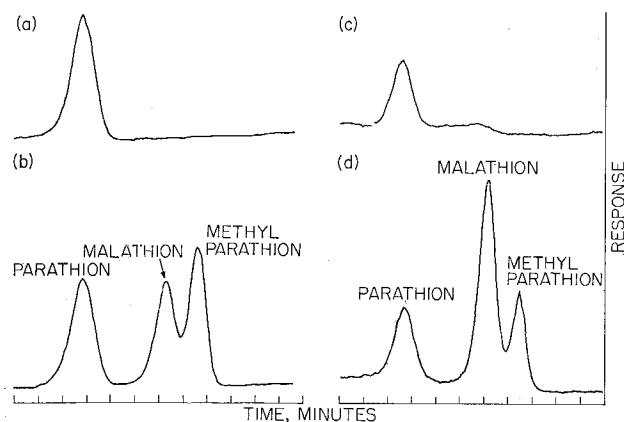
Fig. 3. Identification of parathion. (a) Sample, 5.2 μl; detector in phosphorus mode. (b) Standard containing 5.3 ng each of parathion, malathion and methyl parathion; detector in phosphorus mode. (c) Sample, 14.3 μl; detector in sulfur mode. (d) Standard containing 18.8 ng each of parathion, malathion, methyl parathion; detector in sulfur mode. Column, QF-1; temperature, 140°; amplifier sensitivity, 3.2 × 10⁻⁸ a.f.s.

Since the retention times of the sample on both columns agree with those of parathion, the unknown peak was identified as parathion.

Fig. 4a shows a chromatogram of the sample with the detector in the phosphorus mode. Fig. 4b shows a chromatogram of a standard containing DEF with the detector in the phosphorus mode. Fig. 4c shows a chromatogram of the sample with the detector in the sulfur mode. Fig. 4d shows a chromatogram of the standard containing DEF with the detector in the sulfur mode. DEF has essentially the same retention time as malathion on the QF-1 column but has a different retention time on the OV-1 column. The unknown peak was identified by a comparison of the retention times of the peak in the sample with those of a standard on the two columns, and confirmed by the
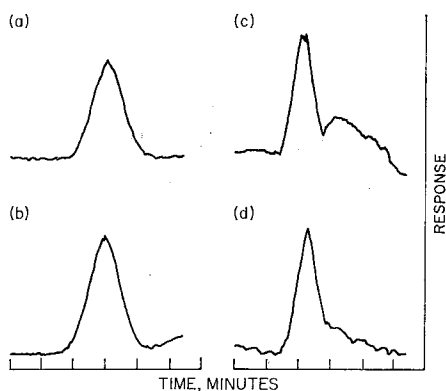

Fig. 4. Identification of DEF. (a) Sample, 5.0 μl; detector in phosphorus mode. (b) Standard containing 5.6 ng of DEF; detector in phosphorus mode. (c) Sample, 16.9 μl; detector in sulfur mode. (d) Standard containing 17.4 ng of DEF; detector in sulfur mode. Column, OV-1; temperature, 158°; amplifier sensitivity, 3.2 × 10⁻⁸ a.f.s.

presence of a peak in the sample at the right retention time with the detector in the sulfur mode.

Figs. 3b and d also illustrate the difference in response of the detector in the phosphorus and the sulfur modes. In the phosphorus mode, the detector has roughly equal responses for the three pesticides, methyl parathion, malathion, and parathion; in the sulfur mode, the detector has a greater response for malathion than for methyl parathion or parathion, since malathion contains two sulfur atoms as compared to one sulfur atom in either of the other two pesticides. For compounds which contain one phosphorus and one sulfur atom, at the nanogram level, the response in the phosphorus mode is approximately three times the response in the sulfur mode. Since the response is linear for the detector in the phosphorus mode while it is logarithmic in the sulfur mode[4], all quantitation was done with the detector in the phosphorus mode.

Conclusions

Organophosphate pesticides may be identified by comparing the peak retention times of the unknown sample with those of a known sample on two columns of different polarity and by using the flame photometric detector, which is a specific detector. Since most common organophosphorus pesticides also contain sulfur, the use of the flame photometric detector in the sulfur as well as the phosphorus mode gives further evidence as to the identification.

Acknowledgements

Midwest Research Institute, CHARLES W. STANLEY
425 Volker Boulevard, JOHN I. MORRISON*
Kansas City, Mo. 64110 (U.S.A.)

1 A. BEVENUE, in G. ZWEIG (Editor), *Analytical Methods for Pesticides, Plant Growth Regulators, and Food Additives*, Vol. 1, Academic Press, New York, 1963.
2 J. A. BURKE, *J. Assoc. Offic. Agr. Chemists*, 48 (1965) 1037.
3 C. E. COOK, C. W. STANLEY AND J. E. BARNEY, *Anal. Chem.*, 36 (1964) 2354.
4 S. S. BRODY AND J. E. CHANEY, *J. Gas Chromatog.*, 4 (1966) 42.

* To whom reprint requests should be addressed.