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Note

Coupling high-pressure liquid chromatography with a cholinesterase inhibition AutoAnalyzer for the determination of organophosphate and carbamate insecticide residues*

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Within the last few years, high-pressure liquid chromatography (HPLC) has become a generally accepted analytical technique. For most purposes, the sensitivity of commercially available detectors is sufficient. For residue analysis, however, only the UV detector has sufficient sensitivity and some degree of specificity. Therefore, this type of detector has been used by several workers dealing with residue determination by HPLC[1–3]. The fluorescence detector, which would offer high sensitivity, is limited to fluorescent compounds or needs fluorigenic labelling as a preliminary step[4]. Recently, two novel types of detector were described by Juvet and Mowery[5], but so far nothing is known about their performance. The aim of this paper is to show the possibility of coupling a liquid chromatograph with an AutoAnalyzer system sensitive to cholinesterase-inhibiting compounds and to present some preliminary results obtained with this combination.

EXPERIMENTAL

A high-pressure liquid chromatograph constructed in our workshop from commercially available parts was used. This chromatograph consisted of an Orlita DMP-SK 1513 reciprocating membrane pump, dual Bourdon tubes for pulsation damping, a dual septum injector according to Pearce and Thomas[6], a 50 cm × 3 mm I.D. stainless-steel column and a Zeiss PM 2A spectrophotometer. The AutoAnalyzer assembly as described by Voss and Geissbühler[7] was used, with the modification that the outlet tube of the spectrophotometer cell was substituted for the liquid sampler of the AutoAnalyzer and the proportioning pump was by-passed by the sampling tube. The principle of this assembly is shown in Fig. 1.

The performance of the system was checked with different compounds comprising the four organophosphates CGA 18809, which is an experimental insecticide, phosphamidon (Dimecron), monocrotophos (Nuvacron) and dicrotophos (Carbicron), and the two carbamates dioxacarb (Elocron) and dimetilan (Snip). The structures of these compounds are shown in Fig. 2.

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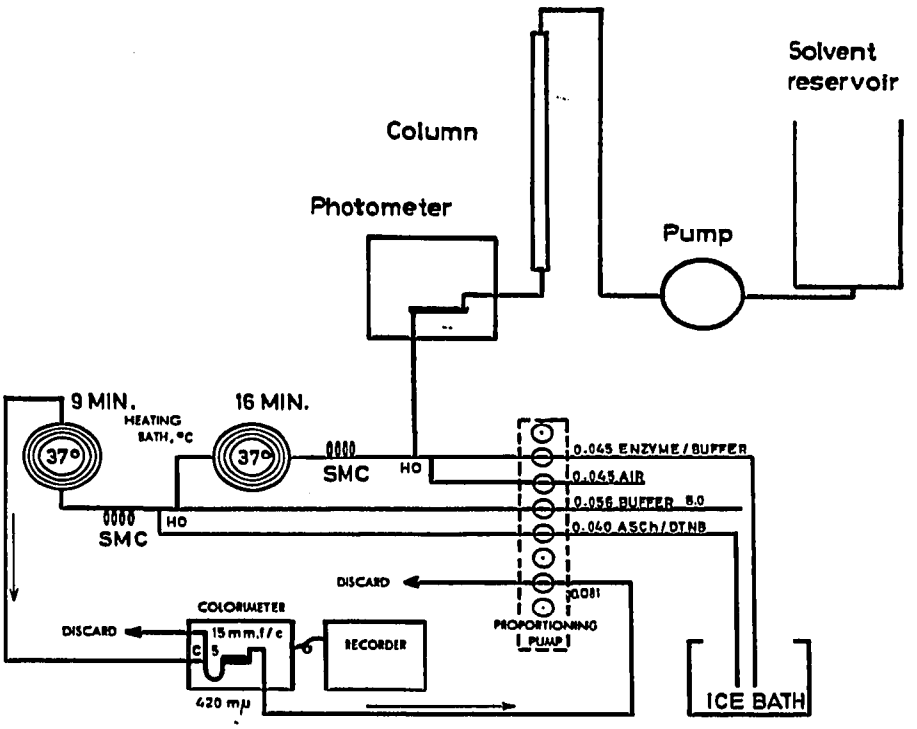

Fig. 1. Liquid chromatograph–AutoAnalyzer assembly. Sample is injected on the column, where separation takes place. The sample passes the photometer and enters the AutoAnalyzer system, where cholinesterase-inhibiting compounds are detected.

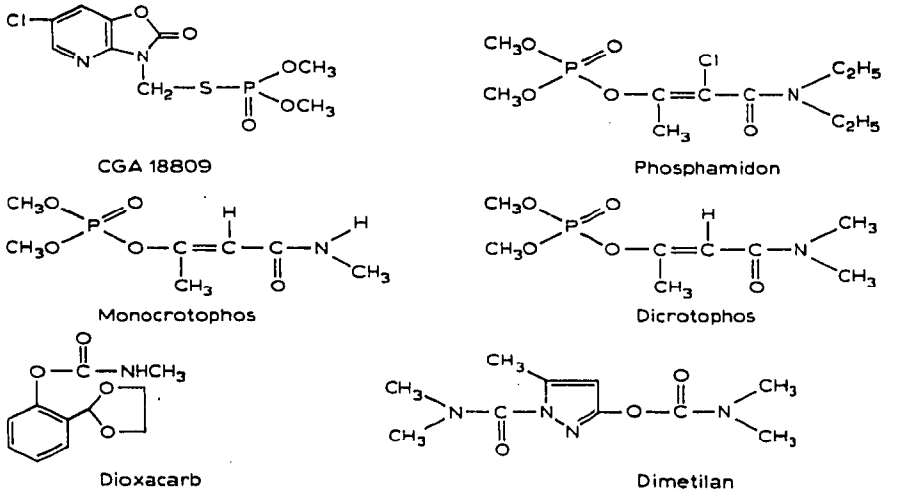

Fig. 2. Structures of the four organophosphate and two carbamate insecticides used in this study.

RESULTS AND DISCUSSION

From 12 duplicate injections of different cholinesterase inhibitors in amounts ranging from 50 to 500 ng, a relative standard deviation of the peak height of 6% was calculated. This value, caused by a 30-min passage of the compounds through the AutoAnalyzer system, is about four times higher than the relative standard deviation calculated for HPLC with spectrophotometric detection.

This relatively high standard deviation, however, is still tolerable in residue analysis and it is compensated by the high specificity of the method: first, only cholinesterase-inhibiting compounds are detected and secondly, compounds are separated according to their retention times in the liquid chromatographic column used. The combination considerably increases the validity and specificity of the former AutoAnalyzer cholinesterase inhibitor method. This method detected only the sum of cholinesterase inhibitors present in an aqueous sample extract, whereas the above combination can separate and simultaneously measure individual cholinesterase inhibitors. Fig. 3 shows the detection of 250 ng each of dicrotophos and CGA 18809. This chromatogram clearly shows the separating power of the system.

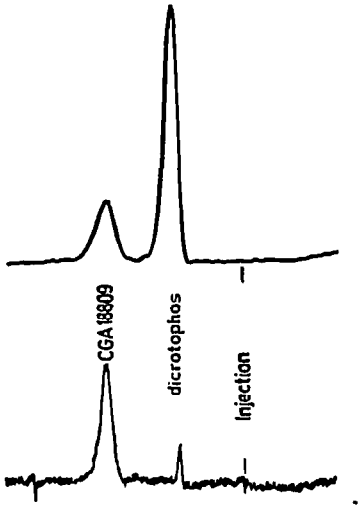

Fig. 3. Separation of dicrotophos and CGA 18809, 250 ng each. Conditions: column, stainless steel, 50 cm × 3 mm I.D.; stationary phase, Permaphase ETH; mobile phase, water; flow-rate, 0.7 ml/min; wavelength for UV detector, 297 nm. Upper trace, cholinesterase-inhibitor detection; lower trace, UV detection.

The detection of CGA 18809 in the presence of co-extracts is also possible. Fig. 4 shows the separation of this compound from a 1-mg aliquot of plum leaves without preliminary clean-up. This example was chosen simply to show the capability of the method; for routine analysis, a preliminary clean-up step would be preferable.

The limit of detection (three times the background) was determined to be 20 ng for CGA 18809. From the listing of relative cholinesterase-inhibiting powers of different compounds given in Table I, the approximate limits of detection for other

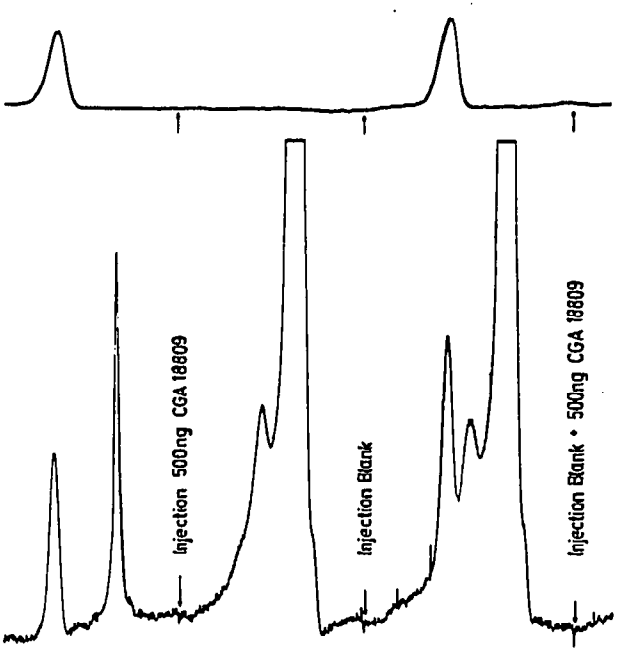

Fig. 4. Determination of CGA 18809 in plum leaf extract. Conditions and traces as in Fig. 3.

compounds can be calculated, and range between 10 pg for diazoxon and 200 ng for dioxacarb. In most instances, these detection limits are satisfactory for residue analysis, considering the fact that no interferences occur from non-cholinesterase-inhibiting co-extracts. It should be borne in mind that the equipment described is suitable only for polar compounds that prefer the water over the oil phase; otherwise, absorption in the connecting tubes will occur. This absorption, however, can be overcome by using an all-glass AutoAnalyzer system for all parts that come into contact with the compound to be analyzed.

The method described has the advantage over existing methods for the deter-

TABLE I

RELATIVE CHOLINESTERASE INHIBITION OF SOME ORGANOPHOSPHATES AND CARBAMATES USING BUTYRYLTHIOCHOLINE AND HUMAN PLASMA, ACCORDING TO VOSS AND GEISSBÜHLER[7].

Common name or code no.	*Relative cholinesterase inhibition**
Diazoxon	2000
Dichlorovos	20
Dicrotophos	2
CGA 18809	1
Monocrotophos	1
Phosphamidon	1
Dimetilan	1
Dioxacarb	0.1

* Relative to CGA 18809 = 1.

mination of carbamate residues[4,8] that no preliminary hydrolysis and/or derivatization are needed.

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