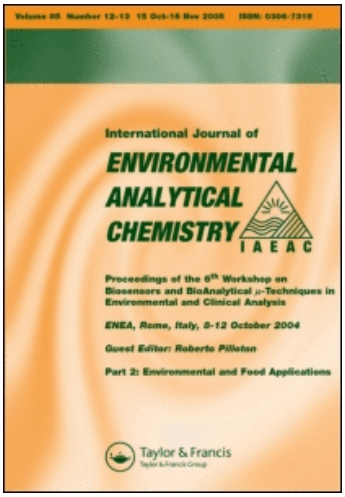

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Analytical Methodology for Organophosphorus Pesticides Used in Canada†

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An overview of analytical methodology for the determination of organophosphate pesticides residues in foods is presented. Sample extraction is carried out with acetone followed by a dichloromethane–hexane partition. The organic extract is purified by automated gel permeation chromatography and analysed by capillary gas chromatography with flame photometric or thermionic detection. Confirmation can be carried out by a variety of chemical derivatization techniques including hydrolysis followed by reaction of the phosphate or phenol moiety, direct alkylation or trifluoracetylation. Thin-layer chromatography with enzyme inhibition detection can be used as a rapid screening technique or to confirm results obtained by gas chromatography. Liquid chromatography has not been used much for the determination of organophosphorus compounds in foods.

KEY WORDS: Organophosphorus pesticides, insecticides, capillary gas chromatography, thin-layer chromatography, chemical derivatization, solvent extraction, gel permeation, flame photometric detector, thermionic detector.

INFORMATION

In Canada there are some thirty-eight organophosphorus pesticides registered for use on food crops or food producing animals. They are

†Presented at the Workshop on Chemistry and Fate of Organophosphorus compounds, Amsterdam, June 18–20, 1986.

registered individually for specific uses and cannot be used on crops for which they are not registered. Table I lists twenty-two compounds registered for use on fruits and vegetables with specific maximum residue limits. The limits are determined by taking into account the toxicity of the organophosphate as well as the rate and quantity of consumption of the food item. Table II lists those compounds registered for food crops on a "negligible residue" basis only. For analytical purposes this can be considered as those pesticides which yield residues below 0.1 μg/g in the crop at harvest. A number of organophosphates listed in Table I are also permitted for use on some crops on a negligible residue basis. Table III lists those organophosphates which are registered for use on animals and their maximum residue limits in the meat or meat by-products. In

Table I Organophosphorus pesticides registered for use on fruits and vegetables

Compound	Max. residue limit
Azinphos-methyl[a] (Guthion)	0.2–5.0 ppm[b]
Bromophos	1.5
Carbophenothion (Trithion)	0.5–0.8
Demeton (Systox)	0.2–0.75
Diazinon[a]	0.2–0.75
Dichlorovos[a] (Vapona)	0.25–2.0
Dimethoate[a] (Cygon)	0.5–2.0
Dioxathion (Delnav)	2.0–5.0
Disulfoton[a] (Disyston)	0.2–0.5
Ethephon[a] (Etherel)	0.5–20
Ethion[a]	0.5–2.5
Malathion[a]	0.5–8.0
Methamidophos[a] (monitor)	0.5–1.0
Methidathion[a]	0.2–2.0
Mevinphos[a] (Phosdrin)	0.2–0.25
Monocrotophos (Azodrin)	0.5–1.0
Naled (Dibrom)	0.5–3.0
Parathion	0.7–1.0
Phosalone[a] (Zolone)	1.5–15.0
Phosmet[a] (Imidan)	1.0–10.0
Tetrachlorvinphos (Gardona)	10.0
Tetradifon (Tedion)	1.0–100.0

[a]Also registered on a negligible residue basis.
[b]ppm = μg/g.

Table II Organophosphorus pesticides registered for use on a negligible residue basis only

Compound
Chlorfenvinphos
Crotoxyphos
2,4-DEP
Fensulfothion
Fenthion
Fonofos
Glyphosate
Isofenphos
Menazon
Oxy-demeton-methyl
Phorate
Terbuphos

Table III Organophosphorus compounds registered for use on animals

Compound	Max. residue limit (meat and meat by-products)
Chlorpyrifos (Dursban)	1.0 ppm
Coumaphos (Co-Ral)	0.5 ppm
Crufomate (Ruelene)	1.0 ppm
Dioxathion	1.0 ppm
Ethion	2.5 ppm
Ronnel	3–7.5 ppm
Tetrachlorvinphos (Gardona)	0.75–1.5 ppm

addition to those organophosphates registered in Canada we need to have methodology for those compounds which may appear as residues in imported foods. The total lists of organophosphorus compounds which must be considered is over one hundred.

SAMPLE PREPARATION

The main routine methodology for organophosphate determinations at the Canadian Department of Health and Welfare presently

includes capillary gas chromatography with phosphorus selective detection with either flame photometric or thermionic detectors. Sample cleanup is predominantly done by gel permeation[1] (size exclusion) chromatography. Figure 1 shows an overall schematic of the procedure for fruits and vegetables. It is designed to fit into our general multi-pesticide residue screening methodology. Acetone is used as a universial extractant and this is partitioned with dichloromethane/hexane, then the organic extracts are evaporated to a small volume and dissolved in acetone or dichloromethane/cyclohexane (1+1) for gel permeation cleanup for the determination of the

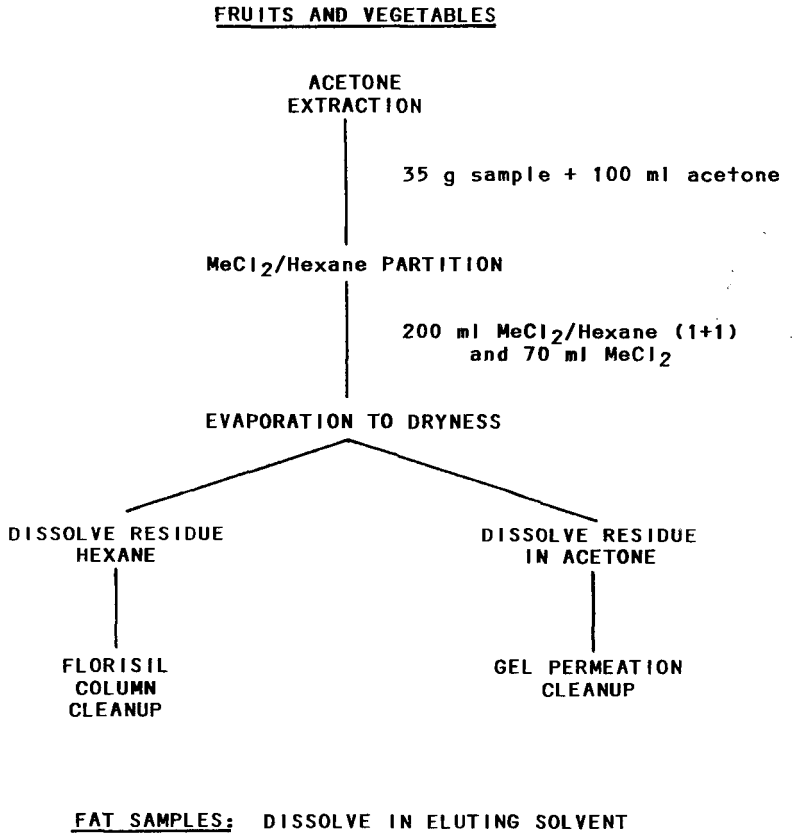

Figure 1 Schematic for sample extraction and cleanup for organophosphate insecticides in fruits and vegetables.

organophosphorus compounds. The extracts are further cleaned by Florisil or other type of open column chromatography for the determination of other classes of pesticides. Fat samples are directly diluted with acetone or dichloromethane/cyclohexane for gel permeation cleanup.

The gel permeation apparatus (Autoprep 1002, ABC Labs) is automated and capable of handling 23 samples in a single run. The system normally employs 60 g of Biobeads SX-3 with eluting solvents of either dichloromethane/cyclohexane (1 + 1) as mentioned above or dichloromethane/acetone (7 + 3) at a flowrate of 5 mL/min. Table IV lists the approximate elution volumes for a variety of substances. It can be seen that pesticides including organophosphates, elute in about 150–200 mL while lipid material such as fish oil elutes in less than 100 mL. The main function of gel permeation is to remove the higher molecular weight lipid material which so often interferes in gas chromatographic analyses of pesticides. After this cleanup the extracts are generally clean enough to be concentrated and analysed directly by gas chromatography with selective detection. Table V shows the recoveries obtained for fourteen organophosphates spiked in vegetable oil and lettuce at levels of 0.1–0.25 ppm (μg/g). It can be seen that with the exception of fenthion and perhaps ethion, recoveries are very good. It is possible that the elution pattern for these two pesticides is different from the rest resulting in losses.

Table IV Approximate elution volumes of compounds from Biobeads SX-3 with dichloromethane/cyclohexane (1 + 1)

Substance	Elution volume (mL)
Fish oil	40–100
Aliphatics	100–160
Stearic acid	110–150
Phthalates	115–150
Pesticides	150–200
PCB's	170–210
Phenols	175–240
Polycyclic aromatics	190–260
Nitrophenols	240–315

Table V Organophosphates evaluated for GPC cleanup

Compound	Veg. oil[a]		Lettuce[b]	
	Level added (ppm)	Recovery (%)	Level added (ppm)	Recovery (%)
Diazinon	0.25	82	0.10	96
Parathion	0.25	95	0.10	88
Ethion	0.25	68	0.10	91
Fenthion	0.10	46	0.10	109
Ronnel	0.10	107	—	—
Malathion	0.15	100	—	—
Chlorpyrifos	—	—	0.10	110
Carbophenothion	—	—	0.10	99
Dimethoate	—	—	0.10	79
Dimethoxon	—	—	0.10	94
Fonofos	—	—	0.10	103
Methamidophos	—	—	0.10	81
Fensulfothion	—	—	0.10	104
Phosphamidon	—	—	0.10	106

[a]Veg. oil: $MeCl_2$/cyclohexane (1 + 1).
[b]Lettuce: $MeCl_2$/acetone (7 + 3).

GAS CHROMATOGRAPHY

Figure 2 shows a capillary gas chromatographic separation of 38 organophosphorus pesticides and related compounds with a DB-17 column and flame-photometric detection. This detector was found to be generally more selective than the thermionic detector. Figures 3 and 4 compare the two detectors for extracts of green peppers (containing 0.37 ppm methamidophos and 1.3 ppm acephate) and oranges (containing approximately 0.1 ppm ethion oxon and 1.0 ppm ethion). In both cases the flame photometric results show fewer peaks than the thermionic ones, although both detectors easily made the determination of the indicated organophosphates possible. However, in monitoring unknown samples for a large number of organophosphates, the flame photometric detector would be preferred because of the fewer peaks observed. In the case of the thermionic detector, the additional peaks observed would have to be matched to the mixed standards (as shown in Figure 2, for example)

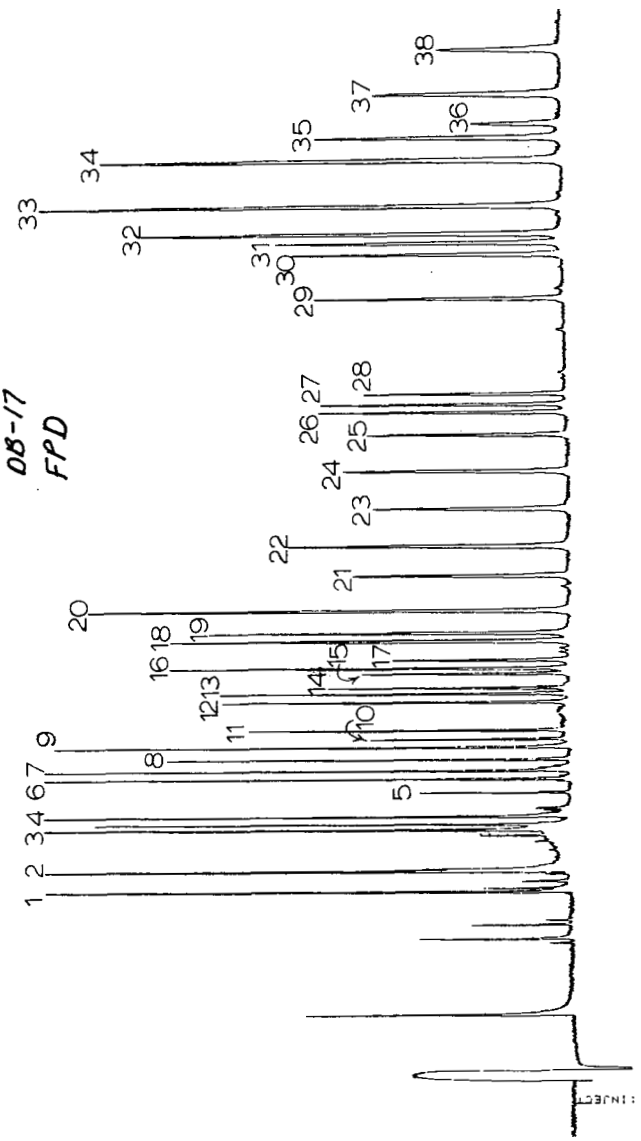

Figure 2 Chromatogram of 38 organophosphates on a DB-17 column with flame-photometric detection. 1. trichlorofon, 2. methamidophos, 3. phosdrin, 4. acephate, 5. phorate oxon, 6. phorate, 7. dimethoate oxon, 8. diazinon, 9. monocrotophos, 10. dioxathion, 11. dimethoate, 12. methyl chlorpyrifos, 13. malaoxon, 14. methyl parathion, 15. chlorpyrifos, 16. malathion, 17. parathion, 18. fenthion, 19. ruelene, 20. chlorfenvinphos, 21. phenthoate, 22. tetrachlorvinphos, 23. methadithion, 24. ethion oxon, 25. ethion, 26. sulprofos, 27. carbophenothion, 28. fensulfothion, 29. EPN, 30. leptophos, 31. phosolone, 32. phosmet, 33, azinphos methyl oxon, 34. menazon, 35. azinphos methyl, 36. dialifos, 37. azinphos ethyl, 38, coumaphos.

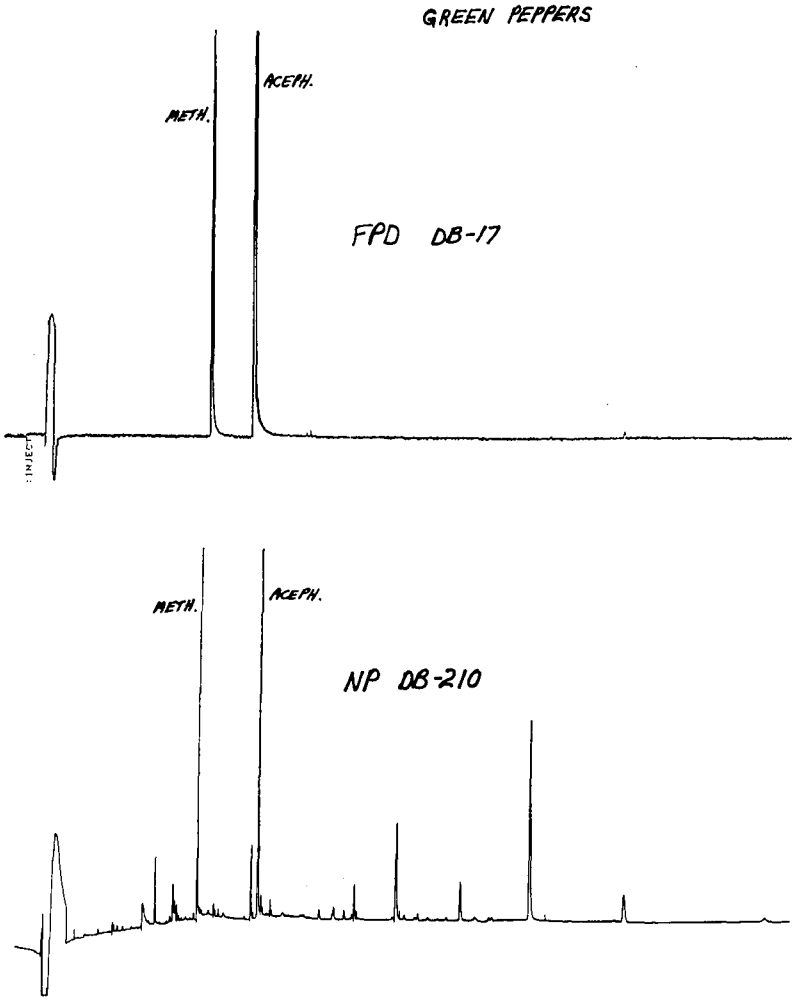

Figure 3 Chromatograms of an extract of green pepper containing methamidophos and acephate with flame photometric (FPD) and thermionic (NP) detection using a DB-17 and a DB-210 column respectively.

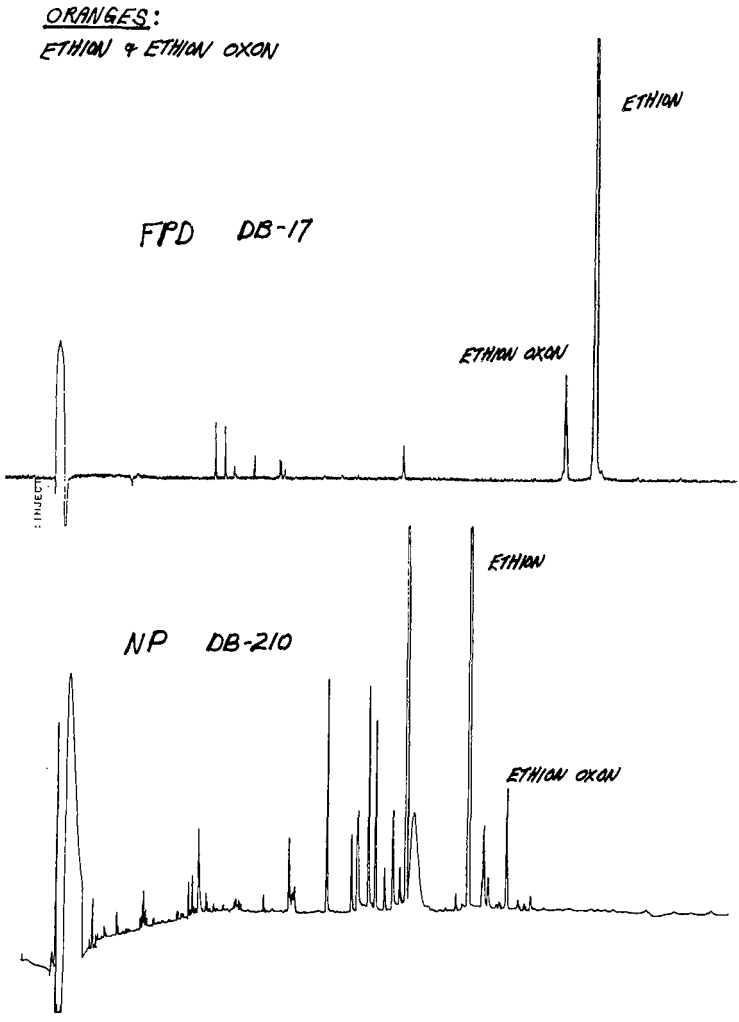

Figure 4 Chromatogram of an extract of oranges containing ethion and ethion oxon with flame photometric (FPD) and thermionic (NP) detection using a DB-17 and a DB-210 column respectively.

for confirmation of identity. This takes time and there is a possibility that a wrong identification could be made. In order to avoid this, confirmation of the peaks by some other means (different columns or detectors, or chemical derivatization) would need to be carried out.

CHEMICAL DERIVATIZATION

Chemical derivatization is a very useful means for confirming the identity of an unknown peak. By carrying out a chemical reaction on an unknown and matching the chromatographic properties of the product to that obtained from authentic material, an analyst can obtain useful information to help unequivocally identify the unknown. Figures 5 and 6 show two approaches for forming derivatives of parathion. In Figure 5 parathion is hydrolysed under basic conditions to yield the thiophosphate moiety and *p*-nitrophenol.[2] The phenol is

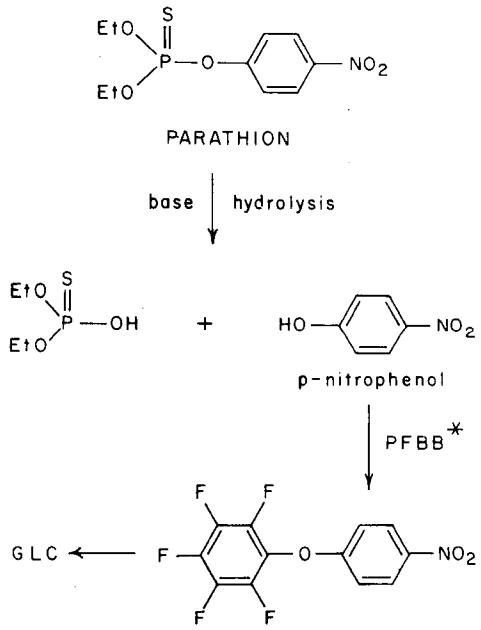

Figure 5 Hydrolysis of parathion and derivatization with pentafluorobenzyl bromide (PFBB).

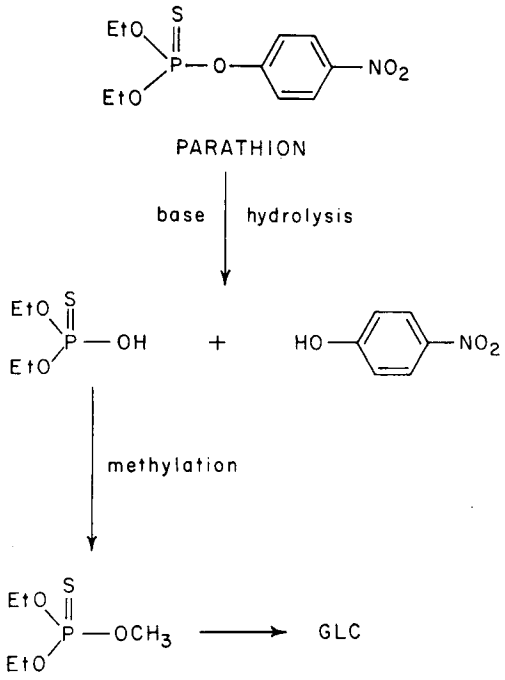

Figure 6 Hydrolysis of parathion and derivatization with diazomethane.

then derivatized with pentafluorobenzyl bromide (PFBB) to form the ether derivative which is very sensitive to electron capture detection. In Figure 6 the same hydrolysis procedure is used, however the phosphorus moiety is methylated for determination by gas chromatography with flame photometric detection.[3] In most cases a single step derivatization is preferred but can only be carried out when the pesticide structure permits it. Figure 7 shows the direct alkylation of dimethoate using methyl iodide with sodium hydride as a base.[4] This reaction simply adds a methyl group to the molecule which results in a retention time shift. Since the phosphorus atom is still present in the derivative, the selective flame photometric detector can still be used for detection.

In some cases basic conditions cannot be used. For example although Azodrin (monocrotophos) has a free N-H proton similar to dimethoate, the methyl iodide/sodium hybride alkylation reaction

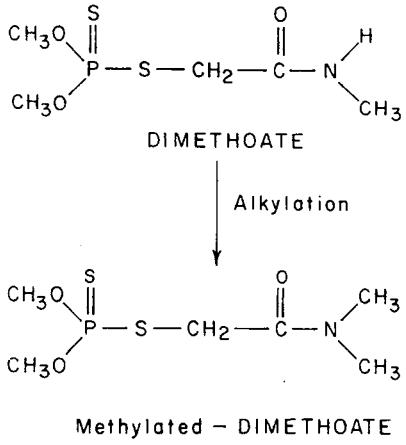

Figure 7 Alkylation of dimethoate with methyl iodide/sodium hydride.

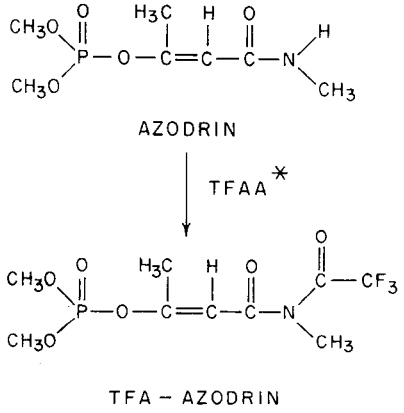

Figure 8 Reaction of Azodrin with trifluoroacetic anhydride (TFAA).

was not successful due to decomposition of the insecticide under the conditions used. However, if acidic conditions such as reaction with trifluoroacetic anhydride (TFAA) are used, a well defined product can be obtained.[5] Figure 8 illustrates the reaction scheme using TFAA. The product is more volatile than the parent so elutes earlier under the same conditions. Figure 9 shows examples of the detection

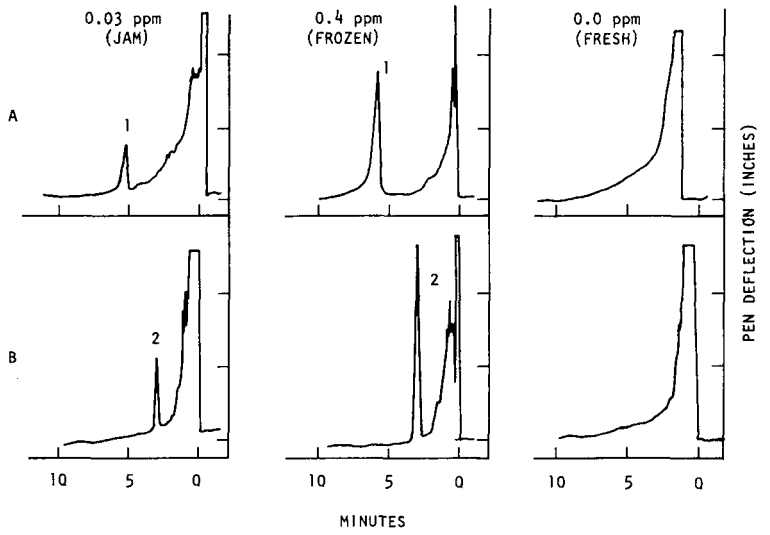

Figure 9 Determination of Azodrin in fresh and frozen strawberries and strawberry jam. A. Upper chromatograms, direct analyses. B. Lower chromatograms, after TFAA reaction. 1 = azodrin. 2 = TFA-azodrin.

and confirmation of Azodrin in strawberries and strawberry jam using packed column gas chromatography and flame photometric detection.

THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography has also been used both as a means of confirmation of organophosphate identity and as a rapid screening technique.[6-8] The detection method employed is enzyme inhibition normally using a spray solution consisting of mixtures of beef liver esterases and the substrate, 5-bromoindoxyl acetate. The approach is very selective as well as sensitive, detecting many organophosphates at low nanogram levels. Compounds such as azinphos-methyl, carbophenothion, diazinon, ethion, malathion, mevinphos, parathion and others have been detected in spiked extracts of fruits and vegetables at levels of 0.1–0.2 ppm. Figure 10 shows a thin-layer

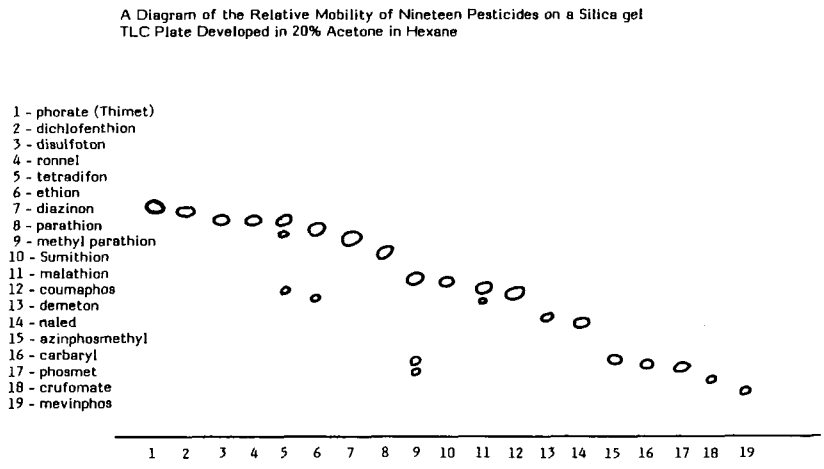

Figure 10 Thin-layer chromatogram of 18 organophosphates and carbaryl. Small extra spots represent impurities.

separation of 18 organophosphates and carbaryl which are detectable using the enzyme inhibition technique.

LIQUID CHROMATOGRAPHY

Although, liquid chromatography has been evaluated for some organophosphates either directly or as derivatives,[9,10] the method has not found widespread use for routine determination. This is mainly because capillary gas chromatography with flame photometric (or thermionic) detection is ideally suited for the direct quantitation of most organophosphates in foods at levels down to the low ppb (ng/g) range with minimal sample cleanup.

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