Liquid Chromatography of Parathion and Paraoxon with a Relatively Specific Colorimetric AutoAnalyzer® System On-stream as Secondary Detector to an Ultraviolet Absorption Detector

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Ultraviolet absorption (UV) detectors for liquid chromatography (LC) are presently much in use, indeed also with increasing usage for the determination of pesticides and pesticide residues as has been variously reviewed (HORGAN 1973, MOYE 1975, OTT 1975). However, these detectors offer little specificity. Some increase in specificity can be obtained with a dual simultaneous UV detector capable of operation at two wavelengths, usually 254 and 280 nm. The degree of specificity relative to a given analyte is highest for a single UV detector if a spectrophotometric (variable wavelength) detector is chosen. A greater increase in specificity can be obtained by simultaneous operation with one or more other detectors. This type of operation is easily accomplished because high-performance UV detectors are essentially nondestructive to their analytes and also produce insignificant band broadening. Thus, direct connection to another detector is permitted. For example, RAMSTEINER and HÖRMANN (1975) have described the coupling of a spectrophotometric detector liquid chromatograph to an AutoAnalyzer system which responds to cholinesterase-inhibiting agents.

To be presented is another example of a spectrophotometric detector liquid chromatograph coupled to an AutoAnalyzer system. However, in this example the AutoAnalyzer system responds to aromatic nitrogroup-containing compounds. Results obtained from some pesticide and pesticide residue samples examined by this combination system are discussed. Since the results are of preliminary nature, major emphasis at this time is placed upon the secondary detector as a confirmatory detector. It has been used to help confirm the presence of paraoxon[1/] and parathion residues in soil surface-dust samples collected from a parathion-treated orange grove.

With simple substitution of one of the reagents pumped into the AutoAnalyzer colorimetric detector it is able to respond to azinphosmethyl and azinphosmethyl-oxon, after separation on the LC column, even though they do not contain nitro groups.

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1/Converted from parathion in these samples under field conditions in the surface soil; additional details published separately (KVALVÅG *et al.* 1977).

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MATERIALS AND METHODS

Samples. Soil surface-dust samples were some of those prepared for LC analysis as described by KVALVÅG et al. (1977) which had been first collected by vacuuming into bags of a household vacuum cleaner through a 100 mesh screen at the drip-line of orange trees sprayed several days earlier with parathion.

Liquid chromatograph. The spectrophotometric liquid chromatograph and operating conditions were as described by KVALVÅG et al. (1977) for soil-dust samples.

AutoAnalyzer system. This detector system (Fig. 1) consisted basically of the simplest version of the two AutoAnalyzer systems reported by FRIESTAD (1971). Minor modifications were made to improve the minimum detectable response for parathion. Also, for this application it was operated without a sampler module and was connected, without a sample pump-tube in the proportioning pump, through a 4-way valve directly to the effluent stream from the UV detector liquid chromatograph. The valve permitted equilibrium flow of about 1 ml/min of wash 60% acetonitrile (ACN) in water into the AutoAnalyzer system, as pumped in by an acidflex pump-tube[2/] in the proportioning pump, when the valve was switched to bypass this detector. Examples of bypass conditions were when the LC column was purged with eluant at more than 1.0 ml/min or when the column effluent was stopped for any reason such as in a stopped-flow injection technique which was used. However, at any time operation might be conducted at constant effluent flow rate of 1.0 ml/min, then the valve and associated plumbing can be completely removed permitting direct connection from the UV detector to the AutoAnalyzer system.

AutoAnalyzer reagents. Borate buffer, pH 8. - Dissolve 2.2 g sodium hydroxide and 6.9 g boric acid in water, add 22 ml of 2N hydrochloric acid, and dilute with water to one liter. Before direct use into the AutoAnalyzer add Brij 35 (Technicon) to a concentration of 1 ml/L.

Thiourea dioxide (TUD). - Daily prepare an approximate 0.25% solution of TUD in borate buffer, pH 8: in a few minutes time dissolve as much as possible of 0.5 g of TUD in 200 ml of the borate buffer solution which has no Brij 35 added. Before use, filter this TUD solution and keep it in a flask immersed in ice-cold water during use.

Sodium nitrite. - Daily prepare a 0.25% solution in water.

[2/] Acidflex must be used here or else plasticizers leached from a Tygon pump-tube by ACN will seriously interfere downstream in the colorimetric system.

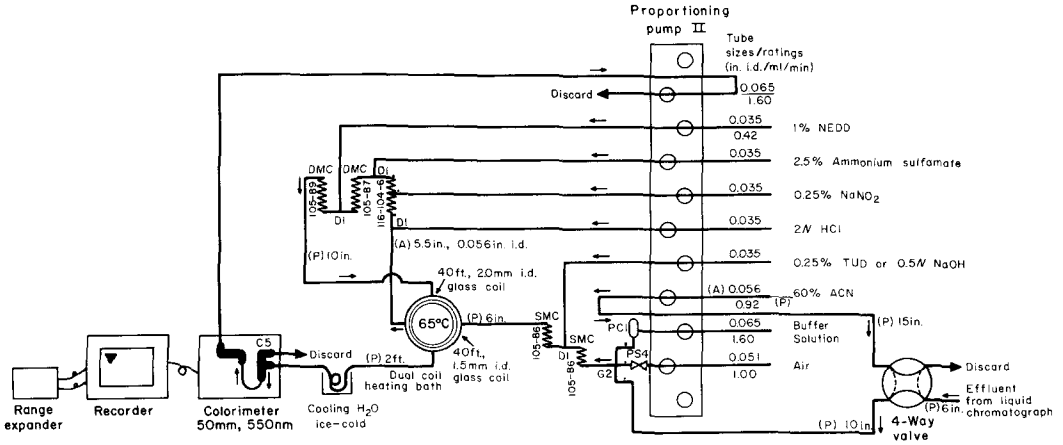

Fig. 1 - AutoAnalyzer system as a colorimetric detector for a liquid chromatograph; SMC = single mixing coil; DMC = double mixing coil; (A) = acidflex tubing with N9 nipples at each end; (P) = polyethylene tubing, 0.034 in. id. The 4-way valve was from Hamilton Co., Reno, NV 89510 (Part No. 4MMMM4); Technicon N5 nipples were butted against the four male luer ports with acidflex sleevings permitting butting of 0.034 in. id polyethylene tubing against the tips of those nipples with 0.040 in. id acidflex as sleevings. The end of the sample stream inlet polyethylene tubing is similarly butted against the end of a 1/16 in. od stainless steel tube coming from the UV detector of the liquid chromatograph.

Ammonium sulfamate. - Weekly prepare a 2.5% solution in water. Refrigerate when not in use.

N-(1-naphthyl)-ethylenediamine dihydrochloride (NEDD). - Weekly prepare a 1% solution in water. Refrigerate when not in use and filter just before use.

RESULTS AND DISCUSSION

Some of the last soil-dust sample extracts which were subjected to liquid chromatographic assay with UV detection at 272 nm by KVALVÅG et al. (1977) were additionally examined concurrently on-stream by the described rather specific colorimetric AutoAnalyzer system as a confirmatory detector. For these samples the main advantage of this detector when coupled with the UV detector was to confirm that the peaks emerging at the given retention times for paraoxon and parathion were indeed aromatic nitrogroup-containing materials rather than some other UV-absorbing materials. All of the samples thus examined and which gave apparent detectable levels of paraoxon and parathion by UV detection also responded at the appropriate times by on-stream colorimetric detection. Therefore the sought confirmation was obtained.

The chart records for both detectors from a single injection from one sample and from a single injection of a standard mixture of paraoxon and parathion are shown superimposed in Figure 2. The chart speeds were not identical but the time elapsed between peak maxima between apparent paraoxon and parathion peaks from residue samples on both records were identical. Also, the times at which these peak maxima were recorded on each recorder after the injection point corresponded with those after injections of standard solution mixtures of paraoxon and parathion. Sample component residence time in the colorimetric detector is about 14 min. Note that for this detector there is an essential absence of interference from sample co-extractives. Also, no response is seen due to the 25 µl of 90% acetone extractant which passed through the column after injection of this aliquot of the sample extract because the acetone was allowed to bypass this detector before the valve was switched to bring in the rest of the sample components. Ordinarily acetone is avoided in a method dependent upon UVLC, but for these samples 90% acetone-10% water was chosen as the extraction solution prior to a final decision on all end analytical considerations. Thus, for simplicity in the method, no attempt was made to eliminate the acetone, rather the separating power of the reversed-phase LC mode of operation was relied upon to separate the components of interest from each other and from the acetone and other UV-absorbing interferences.

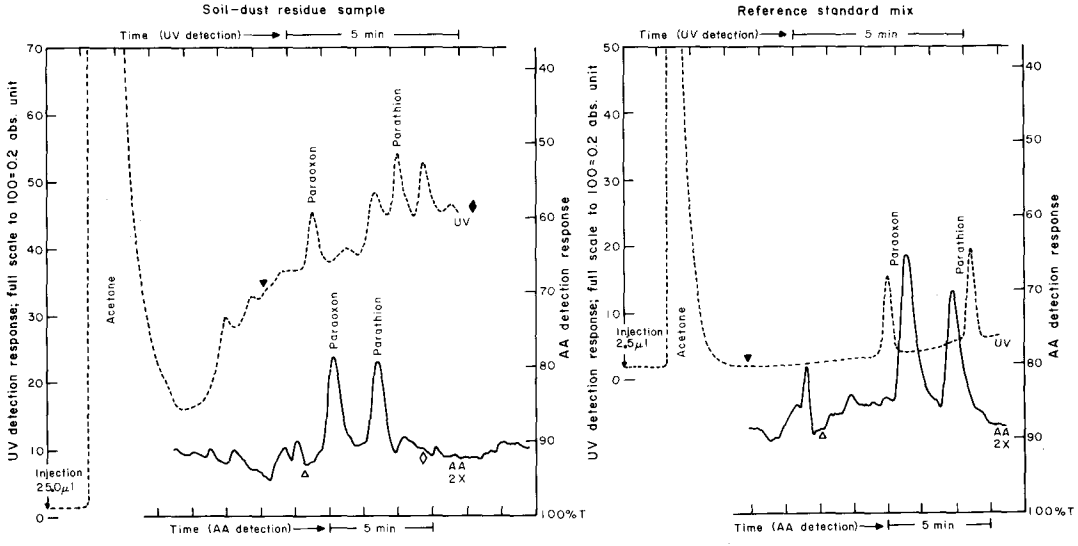

Fig. 2 - Superimposed chart records from the two detectors with separate recorders; UV = ultraviolet absorption detection at 272 nm; AA 2X = AutoAnalyzer colorimetric detection with 2X scale expansion; ▾ = point of switching the UVLC effluent stream into the AutoAnalyzer, ▵ = the corresponding effective point; ♦ = point of switching the UVLC effluent stream away from the AutoAnalyzer, ◊ = the corresponding effective point; the standard mix injection contained 0.30 and 0.35 µg of paraoxon and parathion, respectively.

Best quantitation with the colorimetric detector can be done with no scale expansion and with use of chart paper marked in absorbance units; then peak heights measured in absorbance units from unknowns are compared to those from corresponding standards. If the Technicon scale expander is used, follow the manufacturer's set of instructions for that module regarding quantitative purposes.

For this colorimetric detector under the present conditions, including no scale expansion of response, the minimum detectable levels of paraoxon and parathion are approximately equal, around 0.1 µg. A study of precision at these levels was not conducted but it may be inferred based upon the work of RAMSTEINER and HÖRMANN (1975) that an AutoAnalyzer colorimetric system used as a liquid chromatographic detector offers less quantitative reproducibility than a UV detector. However, the lower precision of the colorimetric detector is largely compensated by that detector's greater specificity compared to the UV detector.

As found by FRIESTAD (1971) in his development of the basic AutoAnalyzer flow scheme used here, the system can be easily made to determine azinphosmethyl by simple substitution of 0.5N NaOH solution for the TUD reducing reagent. It has been further established here that azinphosmethyl-oxon is determinable in this mode of operation. Very preliminarily this presents a new instrumental approach for the determination of azinphosmethyl-oxon and is a topic to be included in detail in another place (KVALVÅG et al. 1976).

FRIESTAD (1971) recommended use of ethanol in his AutoAnalyzer system which is an automated modification of the well known AVERELL and NORRIS (1948) colorimetric method for determining parathion. In the present procedure ACN proved to be very useful in the liquid chromatographic conditions and to be also compatible with the AutoAnalyzer colorimetric detector. Therefore, most any other liquid chromatographic conditions using ACN-water or ethanol-water should also be compatible. Ideal conditions for this detector are 1.0 ml/min LC column effluent and use of constant concentration of ACN (or of ethanol) in water for least baseline disturbance. However, solvent programming from 10% ACN in water to 100% ACN had been already selected in the LC conditions related to the soil-dust samples. This variation from a constant solvent concentration mode proved to give tolerable levels of baseline disturbance if the output of the Technicon colorimeter was not expanded with the scale expander more than two-fold (2X). Isocratic LC operation at constant flow of 1.0 ml/min should permit 4X and possibly even 10X scale expansion, although as yet no isocratic conditions with ACN-water mixtures in the column used have been found to effect a workable separation of paraoxon from parathion. Very broad parathion peaks at unreasonable retention times or with complete retention of parathion on the column were the results of a limited search

for acceptable isocratic conditions. Thus, the ability to produce sharp peaks in a reasonable time is one of the great merits of the solvent programming capability of the liquid chromatographic instrumentation used. Another definite merit of this capability was found in the savings in solvents and time in preparing and changing to various solvent concentrations which would otherwise have been required to be done manually.

The choice for use of 60% ACN in water as the wash liquid (see MATERIALS AND METHODS) for the AutoAnalyzer colorimetric detector system, when bypassed from the LC column, was based upon an estimation that paraoxon and parathion are eluted from the column by concentrations of ACN between 45 and 75% ACN in the linear solvent program, so 60% ACN is the calculated average and the arbitrarily selected wash solution. No significant difference was observed when either 50 or 70% solutions were used.

In addition to being a confirmatory detector as presented here, this colorimetric LC detector may also be of interest as a primary detector, for certain pesticides, in those pesticide analytical laboratories equipped with Technicon AutoAnalyzer modules but without high-performance UV detectors for LC. Low cost chromatographic systems to connect to the AutoAnalyzer colorimetric detector can be obtained or constructed (STILLMAN and MA, 1973) without detectors. A limitation of AutoAnalyzer systems used for LC detectors is that they generally cause considerably more band spreading than UV detectors. Thus, multicomponent samples which may be inadequately separated on the LC column, perhaps a frequent occurrence with simple LC apparatus, cannot be expected to be analyzed efficiently with these detectors.

If there should be a need to simultaneously determine parathion and paraoxon in the presence of azinphosmethyl and azinphosmethyl-oxon and if concentration levels permit, then the LC effluent could be split to go into two identical AutoAnalyzer systems as singly reported here, but with one operating with TUD reagent while operating the other with the alkaline solution with output of both colorimeters connected to a dual pen recorder. If simultaneous determination would not be necessary, then separate aliquots could be analyzed first one way and later the other way on a single AutoAnalyzer system. This approach would improve the minimum detectability in each mode since there would be no requirement for stream splitting.

Parathion, azinphosmethyl, and methyl parathion are presently much used pesticides so it is additionally noted here that this colorimetric LC detector is also potentially applicable for the determination of methyl parathion and methyl paraoxon.

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