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Note

Use of 214-nm and 229-nm discrete-line sources for the UV absorbance detection of some pesticides separated by high-performance liquid chromatography

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It is generally accepted that fixed-wavelength filter photometers, especially at 254 nm, are more stable, exhibit less background noise and are less expensive than variable-wavelength detectors operating with a continuum source. The major disadvantage of most fixed-wavelength detectors up to now has been their inability to monitor high-performance liquid chromatography (HPLC) eluents at wavelengths below 254 nm. The region below 254 nm down to about 200 nm is an area where most pesticides have their absorbance maxima and thus these are not effectively detected at 254 nm. As a result, the variable-wavelength detector has been most useful for analyses in this region even though absolute detector sensitivity is less than that possible with the fixed-wavelength detector. Recently there became available two discrete-line sources (214 and 229 nm) which have much potential for application in the region below 254 nm. This work describes an evaluation of these as applied to the detection of some pesticides separated by HPLC. It compares the two sources with 254 nm (mercury lamp) and with variable-wavelength detection.

EXPERIMENTAL

Chemicals

The pesticides studied in this work were carbaryl (1-naphthylmethylcarbamate), carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), barban (4-chlorobut-2-ynyl-3-chlorophenylcarbamate), atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine), metobromuron (3-(*p*-bromophenyl)-1-methoxy-1-methylurea), methabenzthiazuron (1-(2-benzothiazolyl)-1,3-dimethylurea) and pentachlorophenol. These were prepared in acetonitrile and diluted as required with mobile phase. All solvents were distilled-in-glass grade.

Chromatography

The chromatography system consisted of a Waters Model 6000A solvent delivery pump, a Valco syringe-loop injection port with a 25-μl loop and a μBondapak C_{18} column (4.6 mm × 30 cm). A prototype Waters Model 441 fixed-wavelength detector was connected in series with a Pye Model LC-3 variable-wavelength detec-

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tor. The Model 441 was operated with a vacuum jacketed zinc lamp for 214-nm absorption measurements and a cadmium lamp for 229 nm, with the respective filters. The detector outputs were connected to a two-pen linear recorder with 10-mV or 1-mV spans. Mobile phase consisted of combinations of acetonitrile and water at a flow-rate of 1.0 ml/min.

RESULTS AND DISCUSSION

Comparison with 254 nm

Table I lists relative absorbances (approximate) at various wavelengths for a number of important pesticides representing several classes of compounds. It can be seen that with the exception of the urea and uracil classes, the vast majority absorb more strongly at the lower wavelengths than at 254 nm by factors from 4–280. At the selected wavelengths (214 nm and 229 nm), the relative absorbances were 4–56 fold greater than 254 nm. As a consequence of this, the wavelength region below 254 nm is, in general, the preferred area for absorbance detection of pesticides. This has also been illustrated through the work of Gore *et al.*[1] where about 90% of the pesticides they examined absorbed more strongly below 254 nm than above it.

TABLE I

RELATIVE ABSORBANCE OF SOME PESTICIDES AT SELECTED WAVELENGTHS

Values estimated in part from Refs. 1–3.

Pesticide	*Relative absorbance (254 nm = 1)*		
	$\lambda_{max.}$ *(nm)*	*214 nm*	*229 nm*
Carbamates			
Carbaryl	40 (222 nm)	29	9
Carbofuran	120 (200 nm)	15	10
Barban	60 (206 nm)	30	17
Ureas			
Linuron	1 (248 nm)	0.1	0.5
Metobromuron	1 (247 nm)	0.2	0.6
Methabenzthiazuron	1.7 (226 nm)	0.25	1.6
Triazines			
Atrazine	8 (229 nm)	6	8
Simazine	5 (223 nm)	5	5
Uracil			
Terbacil	1.2 (247 nm)	0.8	0.6
Phenoxy acids			
2,4-D	280 (201 nm)	56	56
2,4,5-T	93 (207 nm)	50	20
Other			
Pentachlorophenol	50 (214 nm)	50	8

Although sensitivity is a very important criterion for determining pesticide residues environmental samples, selectivity is of equal importance. This may be described as the ability of the detector to discriminate between the compound of interest and any coeluted material. In general, for UV absorbance detection, the use of

lower wavelengths usually results in lower selectivity, that is, more interferences are likely to appear in the analysis. Thus, optimum conditions are obtained by taking into account the gain in sensitivity achieved for the pesticide at a lower wavelength compared to the loss in selectivity. The former may be calculated while the determination of loss of selectivity must be done empirically by analysing the sample extract at the different wavelengths. Fig. 1 compares results at 214 nm and 200 nm for a plum extract containing 1.0 ppm of carbofuran which had been cleaned up by a method described elsehwere[4] that included an organic solvent extraction, liquid–liquid partition and a column chromatographic purification. Although from Table I, carbofuran is about eight times more sensitive at 200 nm than at 214 nm, the selectivity at 200 nm is proportionately worse resulting in no advantage to the analysis. The estimated detection limits are essentially the same. When one considers that this extract has been extensively cleaned up, it is likely that for "dirtier" samples, 200 nm would be even less useful even compared to 214 nm.

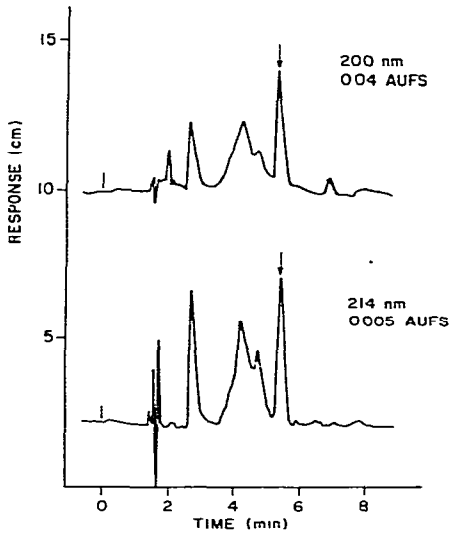

Fig. 1. Chromatograms of a plum extract containing 1.0 ppm carbofuran detected at 200 nm (Pye LC-3) at 0.04 absorbance units full scale (AUFS), and 214 nm (Waters 441) at 0.005 AUFS. Mobile phase, 50% acetonitrile–water (1:1).

In a comparison of results for the pesticide, carbaryl, spiked in an apple extract, also cleaned up in the same manner as the plum extract, the decrease in selectivity from 254 to 214 nm was insignificant compared to the almost 30-fold increase in sensitivity. Generally it was found that over the region of 210–260 nm, selectivity seldom changed by more than a factor of 2 (as estimated by the increased total peak areas due to the absorbance of unknown coextractives in the sample extract) for fruits, vegetables or grains. Thus, if increases in sensitivity significantly greater than 2-fold can be achieved in the region below 254 nm (down to about 210 nm) then it would likely be advantageous for the analyst to operate in that region. As mentioned earlier, this may apply to perhaps 90% of pesticides.

Comparison to variable-wavelength detection

Before comparison studies were carried out, new lamps were installed in the detectors and they were tested to ensure that they operated within the manufacturer's specifications. The comparisons were for the most part carried out with a degassed mobile phase of acetonitrile–water (1:1). Fig. 2 shows background noise levels using 10× expanded recorder span (*i.e.*, 1 mV as opposed to 10 mV full scale) and 0.02 absorbance units (AU) setting on each detector which resulted in a recorder full scale of 0.002 AU. The fixed-wavelength sources were about four times better than the Pye.

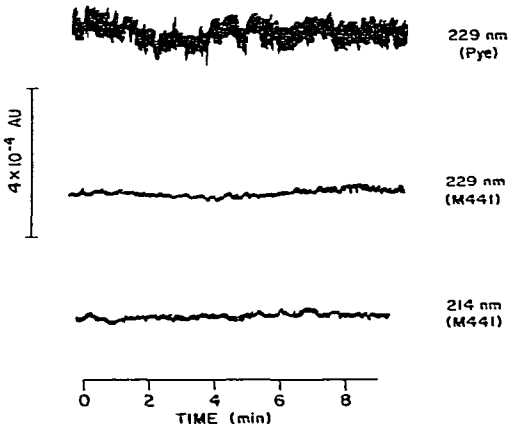

Fig. 2. Noise levels for the detectors studied. The Pye LC-3 produced the same noise level over the range 200–260 nm. Mobile phase as in Fig. 1.

Baseline stability from initial warmup for the fixed-wavelength sources was attained in about one-half to one-third the time as that required for the Pye detector. Also, the long-term stability (over a 6-h period) was superior for the fixed-wavelength sources. Both of these facts have been demonstrated before and they have been generally accepted as advantages of fixed-wavelength detection particularly with the mercury lamp at a wavelength of 254 nm.

The major advantage of variable-wavelength detection has always been that the exact absorbance maximum of a particular compound could be used for an analysis. Figs. 3 and 4 show results of analyses carried out with the detectors comparing responses of the two fixed-wavelength sources with those of the variable-wavelength detector set to the absorbance maxima. Fig. 3 shows results for barban. The detector responses correlate well with the estimated relative response as shown in Table I based on absorption spectra. Although 206 nm is the most sensitive in terms of absorbance units, the fixed-wavelength source, 214 nm, proved to be superior in terms of signal-to-noise ratio (see Fig. 2) and therefore in detection limits. The 229-nm source was found to be similar to the Pye at 206 nm on a signal-to-noise basis. However, for sample analysis, the two higher wavelengths would be preferred for barban analysis because of the likelihood of a further gain in detectability as the result of slightly better selectivity when analysing actual samples.

Fig. 4 compares results for carbaryl. In terms of signal-to-noise ratio, the 214-nm fixed-wavelength proved to be the best by a factor of 2–3 over the variable-wavelength detector. In all studies with other carbamate pesticides, the fixed-wave-

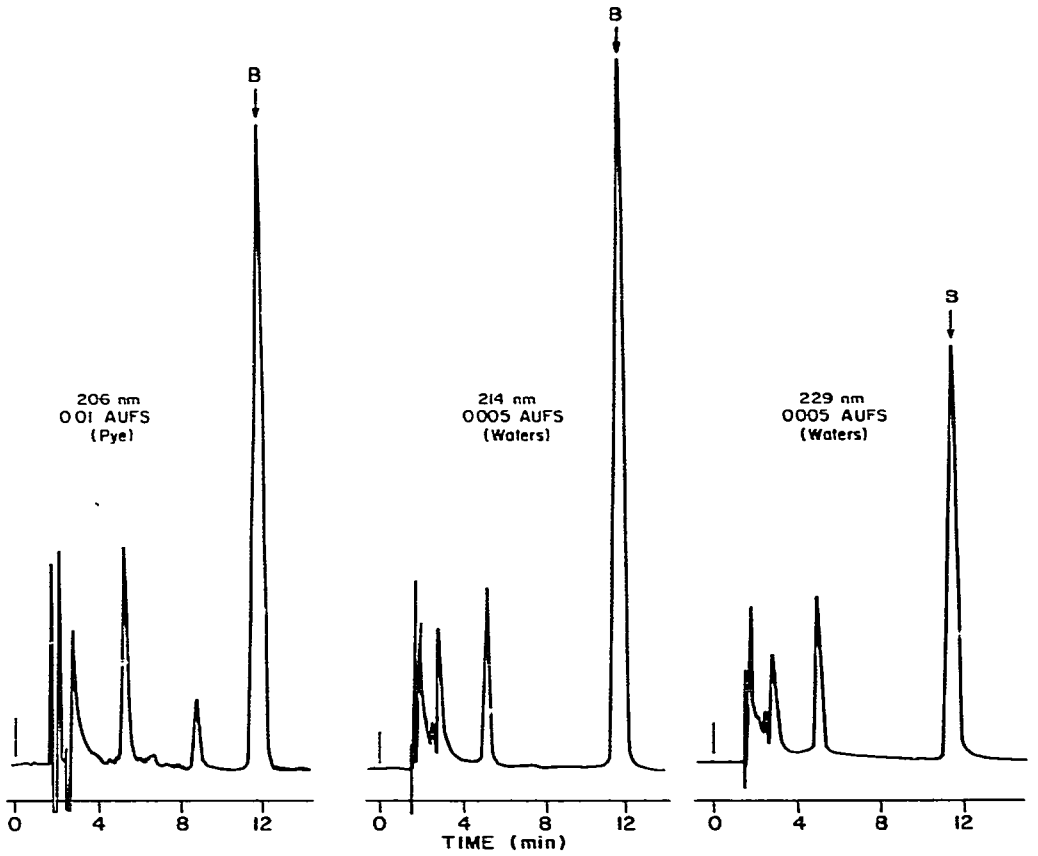

Fig. 3. Chromatographic results of 25 ng barban (B) detected at different wavelengths; 206 nm is the absorbance maximum. Mobile phase as in Fig. 1.

length detector, particularly 214 nm, proved to be superior to the variable wavelength detector. For other compounds such as atrazine, 2,4-D and methabenzthiazuron, the 229-nm fixed-wavelength source proved to be superior to both 254-nm detection and to the variable-wavelength detector. It should be pointed out that where two wavelengths provide similar sensitivities, the higher one should be chosen first for sample analysis because of potentially better selectivity. This would be the case for 2,4-D and the triazines as shown in Table I.

CONCLUSION

The two line sources, 214 nm and 229 nm of the Waters Model 441, evaluated herein, typically demonstrated about four times lower background noise than the Pye LC-3 variable-wavelength detector. As a result, the two fixed wavelengths generally produced better results on a signal-to-noise basis over most of the region <254–206 nm for the compounds studied. This is particularly impressive considering that the Pye detector is already one of the best variable-wavelength detectors available. In

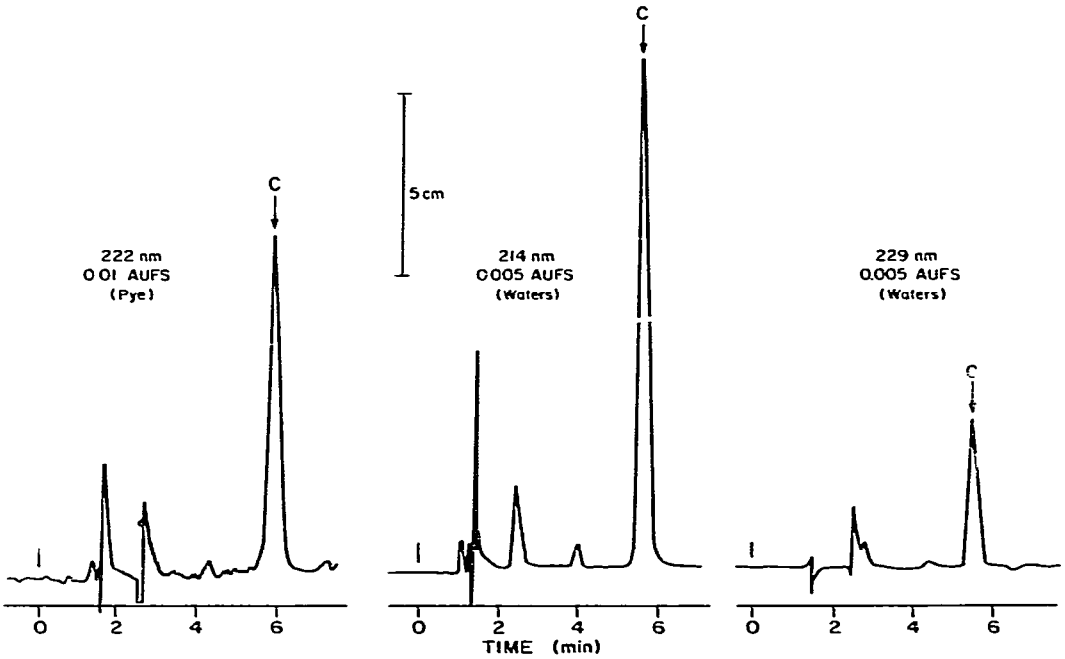

Fig. 4. Chromatographic results for 2.5 ng of carbaryl (C) detected at different wavelengths; 222 nm is the absorbance maximum. Mobile phase as in Fig. 1.

addition, a mercury lamp is available as a standard accessory for the Model 441 enabling the sensitive detection of pesticides such as ureas, uracils and others which absorb more strongly near 254 nm or higher.

Since fixed-wavelength filter detectors are by their design more stable and sensitive than variable-wavelength detectors, the Model 441 with its two line sources in the <254 nm region, should be particularly attractive to pesticide residue analysts especially since this type of detector is also normally less expensive than the variable-wavelength variety.

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