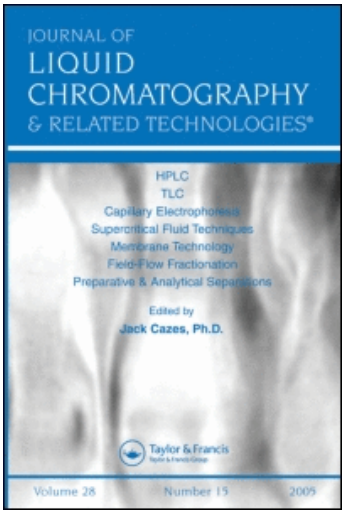

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Use of Derivative Spectrophotometry in the Resolution of Overlapped Peaks in Liquid Chromatography and Its Application in the Analysis of Active Components in Insecticide Formulations

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USE OF DERIVATIVE SPECTROPHOTOMETRY IN THE RESOLUTION OF OVERLAPPED PEAKS IN LIQUID CHROMATOGRAPHY AND ITS APPLICATION IN THE ANALYSIS OF ACTIVE COMPONENTS IN INSECTICIDE FORMULATIONS

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A procedure for the determination of active components in insecticide formulations showing overlapped peaks in liquid chromatography has been devised. The procedure is based in the use of derivative spectra of the components obtained by a diode-array spectrophotometer around the maxima signal of the chromatographic peak, and has been applied to the analysis of mixtures of piperonyl butoxide, neopynamine and fenitrothion with satisfactory results.

One of the most serious problems with which chromatography workers are confronted is the occurrence of only partially resolved peaks arising from coelution of solutes in the sample or from similarities between their retention times. Traditionally, this type of

problem was addressed by modifying the experimental conditions by trial and error until the aforesaid errors were minimized. Thus, different mobile or stationary phases (columns) or even working techniques (e.g., isocratic or gradient elution) were tested, which was time-consuming and involved consumption of expensive solvents.

The advent of multi-dimensional detection systems and the affordability of personal computers provides with software that allows storage and subsequent processing of chromatographic data has fostered developments of new experimental procedure for the characterization of unresolved peaks. There are three basic alternatives to computer-assisted resolution of chromatographic peaks, namely:

(a) Fitting the chromatogram peak to known functions. There are some precedents to the use of this alternative in gas chromatography ranging from the use of Gaussian and non-Gaussian models, to convoluted Gaussian curves with exponential decay and fast Fourier transform techniques. Solutions involving comparison of logarithmic spectra [1] or the use of recent chemometric methods [2-6] have been tested in liquid chromatography as implemented with diode-array detectors.

(b) Integration by tracing a line perpendicular to the baseline from the valley between two peaks or one joining the valley and the end of the second peak (skimming) by computing the area of each separately from the two zones thus established.

(c) Use of derivative techniques. As far as practical applications, the information supplied by derivative spectroscopy used as detection system in liquid chromatography has been exploited in two different

ways, namely: i) By using the first derivative of the elution profile obtained at the wavelength of the absorption maximum. In theory, the derivative should be zero at this point and therefore the dissapearance of the main peak may reveals the presence of other constituents with different absorption features. This procedure is called "null spectral derivative technique" [7]; ii) By using the complete derivative obtained by recording the elution profile. This procedure, known as "spectral derivative mapping technique" was studied theoretically by Grant et al. [8], who discussed specific cases where the spectral curves of potential impurities lay within the spectral band of the major components. This procedure has been applied in the resolution of diverse mixtures [9,10]

Other procedures have been described also. Thus, Hayashi et al. [11] proposed a one-dimensional Kalman filter, run in real time, to resolve partially overlapped chromatographic peaks using a one-dimensional empirical model based on prior measurements of peak shape and location. A Kalmen filter, based on repetitive filtering of diode-array spectra obtained accross a chromatogram, has been developed recently [12]. Campins [13] developed a procedure, named "H-point standard additions method" in which the analytical signal absorbance values (peak-heights) registered at the retention time of the analyte are used.

In the present work, a new procedure has been developed by using the second derivative spectra of the components obtained around the maxima signal of the overlapped chromatographic peaks. A diode-array spectrophotometer is used with this purpose. This procedure, which has been applied to the analysis of active components in

insecticide formulations, make possible the easy transformation of a chromatographic problem in a spectrophotometric problem.

EXPERIMENTAL SECTION

Chemicals

All the reagents were of analytical-reagent grade and acetonitrile (mobile phase) were of LC grade. Standard solutions of insecticides were prepared by dissolving in acetonitrile pure piperonyl butoxide, neopynamine and fenitrothion (Chem Service Inc.) at different concentrations.

Apparatus

Spectra were recorded on a Hewlett-Packard 8452A diode-array spectrophotometer linked to a data system (Lab Calc from Galactic Ind. Co.) for data acquisition and storage. The system was coupled to a quaternary pump (Hewlett-Packard, 1050 series). The analytical column was a 10μm Lichrosorb RP-8 (25 cm x 4.6 mm i.d.) from Merck.

Procedure

The solvent flow-rate was set at 2 ml min^{-1} and 20 μl of each sample were injected. The detector was set to collect a spectrum every 1 s (over the range 190-310 nm). Spectrophotometric measurements were performed between the maximum (peak) and minimum (valley) of the second-derivative spectrum, viz. between 226-230, 230-234, 234-238 and 238-242 nm for neopynamine, 206-214 nm for piperonyl butoxide and between 200-210 nm for fenitrothion. A calibration graph was

previously constructed for each component from solutions of known concentrations of each. The concentration of neopynamine should be between 2.8-5.2 x 10^{-5} M, while that of piperonyl butoxide should lie in the range 1.4-2.6 x 10^{-4} M and the fenitrothion one between 6-14 x 10^{-5} M.

RESULTS AND DISCUSSION

The mixtures of fenitrothion, neopynamine and piperonyl butoxide show peaks with a great overlapping in liquid chromatography under the experimental conditions habitually used. The use of the second-order derivative spectra obtained around the maximum signal of the chromatographic peak make possible the discrimination of the spectral bands of each component of the mixture. Zero-order uv-visible spectrophotometry cannot be used with this purpose owing to extensive spectral overlap of the absorption bands of these compounds, but the second-derivative spectra show differents peaks (Fig. 1) which make possible the resolution of the mixtures.

The second-order derivative spectra show different peaks which allow to realize measures to various wavelengths, whereas that piperonyl butoxide and fenitrothion show a peak only, both to lower wavelengths. In all cases, the measurements of the derivative signal is made between a maximum (peak) and a minimum (valley), the intensity of which is dependent on the concentration of the corresponding compound. Table 1 list the figures of merit of the calibration graphs run for the three substances assayed.

The procedure was compared with other methods: conventional absorption spectrophotometry (without chromatographic separation) (1),

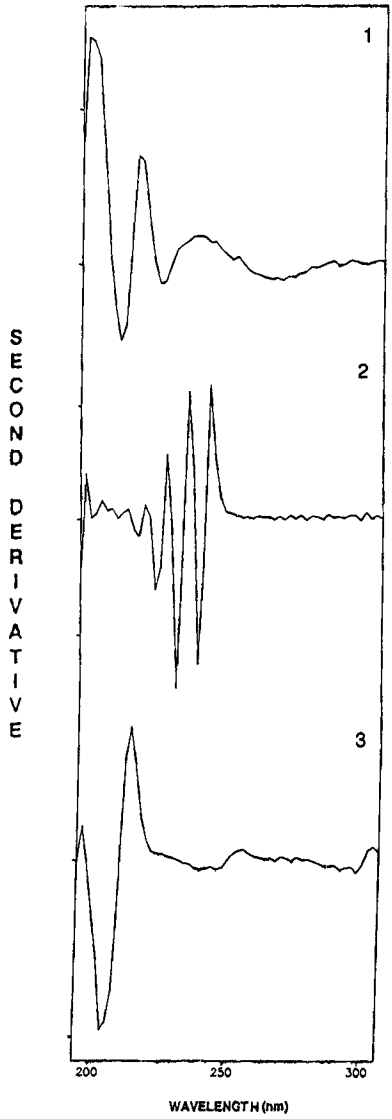

Figure 1. Second-order derivative spectra of piperonyl butoxide (1), neopynamine (2) and fenitrothion (3).

Table 1. Figures of merit of the calibration graphs.

Insecticide	Calibration equation	Wavelength range (nm)	Coef. of correlation	%RSD (n=6)
Neopynamine	h = 0.0661 C + 0.0008	226 - 230	0.997	0.97
	h = 0.0834 C + 0.0063	230 - 234	0.994	0.86
	h = 0.0785 C + 0.0050	234 - 238	0.990	0.82
	h = 0.0760 C + 0.0125	238 - 242	0.990	1.29
Piperonyl Butoxide	h = 0.0853 C + 0.1953	206 - 214	0.994	2.11
Fenitrothion	h = 0.0257 C - 0.0143	200 - 210	0.998	2.50

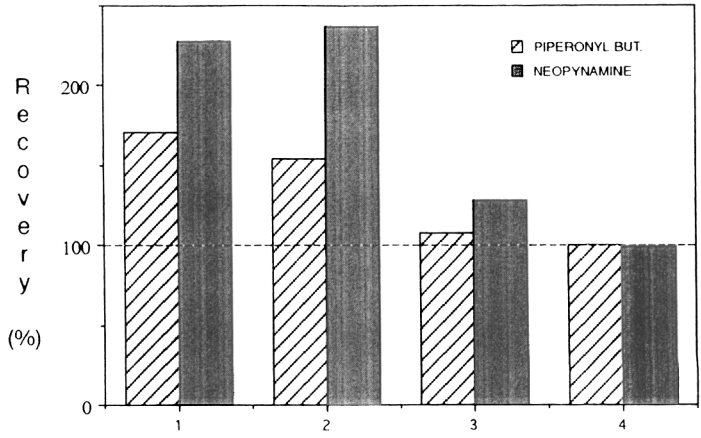

Figure 2. Recovery values obtained for the determination of neopynamine and piperonyl butoxide in commercial formulation.
METHODS: 1, conventional absorption spectrophotometry; 2, liquid chromatography; 3, liquid chromatography-zero order spectrophotometry; 4, liquid chromatography-second derivative spectrophotometry. Results are the average of six determinations.

Table 2. Analysis of commercial formulations by liquid chromatography-second derivative spectrophotometry.[a]

Formulation No	Piperonyl butoxide		Neopynamine		Fenitrothion	
	Stated concentration (10^{-5} M)	Found (10^{-5} M)	Stated concentration (10^{-5} M)	Found (10^{-5} M)	Stated concentration (10^{-5} M)	Found (10^{-5} M)
1	20.3	20.7	4.0	3.9	-	-
2	20.0	20.8	-	-	10.0	10.1
3	-	-	5.0	5.4	8.0	7.9

[a] The values are based on repetitive measurements on seven samples.

liquid chromatography (2), and liquid chromatography using the conventional spectra of the peak (3) and second-order derivative spectra (4). Results obtained with the binary mixture neopynamine-piperonyl butoxide are represented (Fig. 2). Procedure 4 (proposed in this work) offers the more precise recovery. Similar results are obtained with the other binary mixtures.

The procedure devised was applied to the resolution of three binary mixtures of the insecticides assayed. These mixtures were prepared artificially with a composition similar to commercially available formulations. Results are summarized in Table 2.

This method make possible the analysis of compound showing appretiable overlapping in their chromatographic peak without modification of the experimental variables used. Analysis is possible if the compounds showed different derivative spectra.

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