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Note

Diode array detector in liquid chromatography

II. Enhanced sensitivity via first derivative ($dA/d\lambda$) chromatograms

M. J. MILANO and ELI GRUSHKA
Department of Chemistry, State University of New York at Buffalo, Buffalo, N.Y. 14214 (U.S.A.)
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The use of a rapid scanning diode array spectrometer as a detector for liquid chromatography has been recently reported by this laboratory[1] and by others[2]. In this work[1] a novel approach to the deconvolution of overlapping chromatographic peaks using first derivative spectra was presented. A solute with an absorbance maximum in the UV can be effectively eliminated or deconvoluted from the chromatogram by plotting $dA/d\lambda$ at its corresponding first derivative zero crossing point. The application of this approach to a mixture of phenylalanine and tryptophan showed that it could be used to resolve qualitatively grossly overlapping peaks without prior knowledge of the individual spectra[1]. In designing a detector the problem of sensitivity is of utmost importance. For that purpose the present detector has a low-volume (8-μl) flow cell. It was expected that the use of first derivative chromatograms would cause the measurement to be less sensitive than in the case of straight absorbance chromatograms, since the generation of first derivative spectra is usually accompanied by a decrease in signal-to-noise ratio (S/N). A closer examination of these data, however, has indicated that the sensitivity of the instrument can be improved by plotting $dA/d\lambda$ chromatograms at the solute first derivative peaks.

EXPERIMENTAL

The details of the diode array spectrometer, data acquisition system, and chromatographic equipment have been presented[1]. The data from each experiment are stored on magnetic tapes as a series of 332 spectra, each with 128 resolution elements over the range of 220–330 nm. A 9-point quadratic/cubic least squares function was used to calculate $dA/d\lambda$ values.

RESULTS AND DISCUSSION

The sensitivity of the absorbance and first derivative measures were compared using data previously collected for mixtures of benzene, benzyl chloride, and anisole. S/N (peak height/$\frac{1}{2}$ peak-to-peak noise) was calculated for each solute from absorbance chromatograms at 254 nm, absorbance chromatograms at the solute's peak wavelength, and $dA/d\lambda$ chromatograms at the solute's first derivative peak wavelength.

TABLE I

COMPARISON OF SIGNAL-TO-NOISE RATIOS FOR ABSORBANCE AND FIRST DERIVATIVE METHODS

Compound measured	*Concentration (ng/μl)*	*Units*	*Wavelength (nm)*	*S/N*
Benzene	29	A	254	<1
		$dA/d\lambda$	263	3.0
Benzyl chloride	18	A	254	3.3
		A	260	4.8
		$dA/d\lambda$	271	4.6
Anisole	17	A	254	7
		A	271	20
		$dA/d\lambda$	281	48
Benzene	176	A	254	5
		$dA/d\lambda$	263	11
Benzyl chloride	110	A	254	9
		A	260	12
		$dA/d\lambda$	271	23
Anisole	100	A	254	20
		A	271	77
		$dA/d\lambda$	281	210
Tryptophan	2100	A	254	133
		A	278	150
		$dA/d\lambda$	290	240

The results are summarized in Table I. In each case the S/N from the derivative chromatogram is higher than from the absorbance chromatogram at 254 nm. In addition, the first derivative measures are better than the optimum absorbance measures for all except the diluted sample of benzyl chloride. The improvement in sensitivity obtained through the use of a first derivative measure can be seen clearly in the case of the diluted mixture benzene, benzyl chloride, and anisole. In the absorbance chromatogram at 254 (Fig. 1) the benzene peak is lost within the noise. The first derivative chromatogram at 263 nm (Fig. 2) shows the benzene peak at 3.4 min, which is the retention time of this solute as verified by injection of standard solutions.

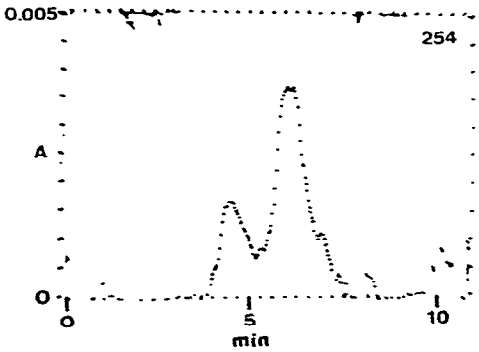

Fig. 1. Absorbance chromatogram at 254 nm.

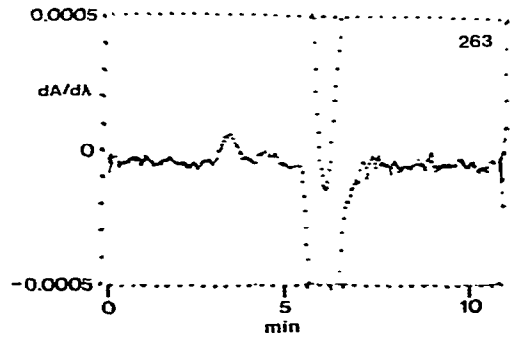

Fig. 2. Derivative chromatogram at 263 nm.

The first derivative measure also gave improved tryptophan S/N for the unresolved mixture of tryptophan and phenylalanine in a separate experiment. Due to the incomplete separation a similar S/N comparison is not possible for phenylalanine.

Although the data are admittedly small, the results do indicate a general trend toward improved S/N with the first derivative measure. O'Haver and Green have observed that the effect of the derivative measure is to trade off systematic error for random error[3]. The single beam detector used in this work would be expected to lead to a relatively large error due to lamp fluctuations and drift. Since the array detector effectively samples all wavelengths simultaneously, these errors become systematic in absorption spectra and can be reduced via the first derivative. Random high-frequency noise should, on the other hand, be relatively small due to the signal averaging and least squares smoothing techniques used.

In conclusion, our results indicate that the use of first derivative detection in liquid chromatography not only allow deconvolution of overlapping peaks, but also reduces the noise due to lamp fluctuations. It should be recognized, however, that both advantages are also a result of the array spectrometer used in this work. Although the basis of the deconvolution technique is general in nature, it requires the highest possible wavelength reproducibility. It is, therefore, doubtful if the technique could be used with a derivative spectrometer employing mechanical wavelength control. In addition, the use of a dual beam system would even be more effective in reducing noise due to lamp fluctuations. However, dual beam rapid scanning detectors using either array[2] or conventional spectrometers[4] are considerably more complex than the system used here and can introduce additional sources of error.

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