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RAPID-SCANNING, MULTI-CHANNEL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETECTION OF ZIMELDINE AND METABOLITES WITH THREE-DIMENSIONAL GRAPHICS AND CONTOUR PLOTTING

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SUMMARY

The linear photodiode array detector in high-performance liquid chromatography generates a three-dimensional data matrix, which is conventionally presented as a pseudo-isometric plot. A new graphical technique in this context is to present the data as a two-dimensional contour diagram, where isoabsorptive contours are plotted as a function of wavelength and time. The relative merits and demerits of these complementary approaches are discussed with respect to a study on the anti-depressant drug zimeldine and its principal metabolites. Several digital methods developed to access the three-dimensional data set are examined, particularly with regard to tests for peak homogeneity. Although spectral slices at wavelengths indicated in the contour plot, and the absorbance-ratio method, are limited in their sensitivity to non-homogeneity, spectral suppression and the second-derivative transformation of the elution profile are shown to be generally applicable to this problem. The total absorbance chromatogram is advocated as a new technique for presenting a rapid, general survey of spectral information within a specified range (*e.g.*, 200–400 nm) as a function of elution time, analogous with total ion current chromatograms in gas chromatography–mass spectrometry. The relative sensitivity of the linear photodiode array detector is shown to be critically dependent on the bandwidth employed for detection. Comparison with a regular single-channel detector indicates that the multi-channel detector is at least six times more sensitive for zimeldine and two metabolites, norzimeldine and zimeldine N-oxide, under identical chromatographic conditions.

INTRODUCTION

Rapid-scanning spectrophotometers have been advocated for several years as suitable monitors for flexible wavelength detection in high-performance liquid chromatography (HPLC)[1–9]. Developments have centred on electromechanical devices[1–3], or on multi-channel detectors based on either the TV vidicon tube[4,5] or the linear

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photodiode array[5–9]. The advent of commercially available diode array detectors has opened up several new strategies for detection in HPLC, particularly when combined with bench-top microcomputers for data capture, processing and evaluation[8–12].

The three-dimensional data matrix (A,λ,t), representing spectra (A,λ) acquired at sequential time intervals (t), can be readily accessed by computer-aided methods to generate the elution profile at any wavelength (λ) and the spectrum at any time (t)[7,8]. Moreover, the total data matrix can be presented as a three-dimensional projection in isometric or pseudo-isometric format for visual inspection[9–12]. Although "hidden lines" can be readily removed in the three-dimensional projection, the complete picture is seen only if the viewpoint is rotated around the data set by interactive graphics or by repeated forward and reversed plotting of the data matrix[9,11].

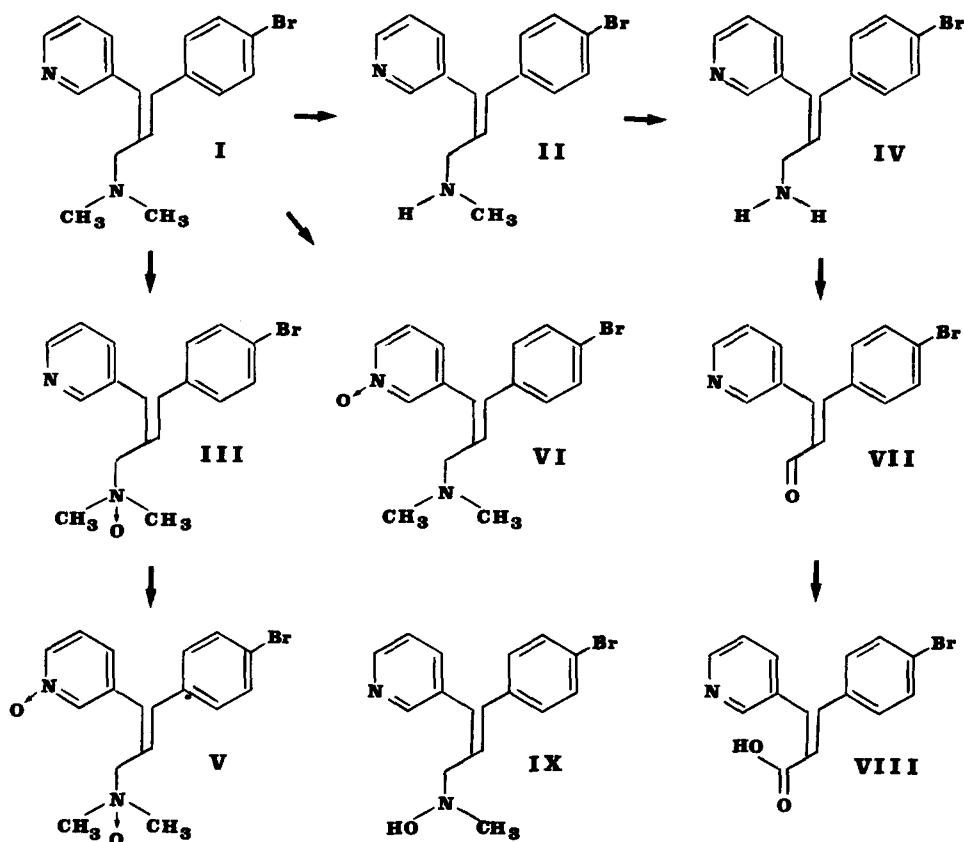
An attractive alternative approach, recently developed in this laboratory, is to transfer the three-dimensional data matrix effectively to two dimensions, using the cartographic technique of contour plotting. In this method, isoabsorptive contours plotted in the (λ,t) plane map the (A,λ,t) surface at stepped intervals. In essence, the data set is viewed from a point vertically above the (λ,t) plane, the absorbance levels being represented by contours in this plane.

A number of digital algorithms for manipulating spectrochromatographic data have recently been reported[7,8,11–13]. In particular, the question of chromatographic peak homogeneity has been addressed by exploiting additional information generated by rapid-scanning detectors in the wavelength domain[12,13]. For example, the absorbance ratio at two characteristic wavelengths can be plotted as a function of time. This can give an indication of homogeneity throughout the peak profile, provided that the absorption spectra of the co-eluting components are sufficiently different. A similar proviso applies to the method of spectral suppression[12,13], where the spectrum of a known component is mathematically deconvoluted by monitoring a suitable function of the difference in absorbance at two optimized wavelengths[12]. The presence of co-eluting impurities is observed as a positive or negative excursion in the difference-absorbance chromatogram, even if the retention positions of the components are identical[12,13].

Differentiation of the elution profile to give the second- or fourth-derivative chromatogram has recently been shown to serve as a useful diagnostic test for peak purity[12,14]. At a suitable detection wavelength, minor co-eluting components undetectable by the unaided eye can be resolved as sharper features adjacent to the principal peak, provided that the peaks do not elute at the same retention time. In another method, all the spectral data acquired at sequential time intervals are employed in a least-squares deconvolution routine. This permits co-eluting peaks of defined composition to be readily resolved and presented as a "deconvolution chromatogram"[12]. The constraint that accurate spectral standards are required for each component can in practice be relaxed, if the spectrum of an unknown co-eluting impurity can be captured on the leading or trailing edge of the composite elution profile[12].

Some of these digital techniques have been examined in this study on the antidepressant drug zimeldine and its known active and inactive metabolites. Zimeldine (I) is a new bicyclic antidepressant drug, which specifically inhibits the uptake of serotonin. It therefore differs from the tricyclic antidepressants, such as imipramine, which can block both noradrenaline and serotonin uptake[15].

Zimeldine is extensively metabolized in man to give N-dealkylation, deami-



Zimeldine is extensively metabolized in man to give N-dealkylation, deamination and oxidation products. Most of these metabolites are considered to be psychopharmacologically inactive, except for the major metabolite norzimeldine (II). Zimeldine N-oxide (III) and a minor metabolite (IV) have also been reported[16]. Metabolites II and IV are produced by successive demethylation at the exocyclic N-alkyl side-chain. Oxidation proceeds readily to the N-oxides III and V. The putative metabolite VI formed by oxidation at the pyridyl ring nitrogen has also been proposed[18]. Metabolites VII and VIII, formed by oxidative deamination, and the geometric isomer of VIII, have also been reported in man; however, the urinary conjugates anticipated for these metabolites have not been isolated[16]. Additional metabolites have been described by Lundström *et al.*[16], and the hydroxylamine IX has also been proposed as a putative metabolite.

Norzimeldine has been shown to exert greater pharmacological activity than zimeldine[17] and possesses about the same specificity for inhibiting the uptake of serotonin[17]. It has therefore been suggested that, in view of the ease of demethylation of the parent drug, zimeldine may act as a pro-drug for norzimeldine[15].

This paper reports the application of a number of new computer-aided techniques, based on photodiode array spectrometry, for the analysis and characterization of zimeldine and its primary metabolites. The relative sensitivity of diode array

spectrometry and conventional single-channel UV detection have also been compared with respect to the requirements of therapeutic drug monitoring and metabolic profiling.

EXPERIMENTAL

Reagents and materials

A 0.05 *M* solution of sodium hydrogen sulphate (AnalaR grade, BDH, Poole, U.K.) was prepared with glass-distilled water and filtered through a Millipore 0.45-μm MA filter using an all-glass apparatus. The eluent was degassed under reduced pressure in an ultrasonic bath for 10 min. Acetonitrile (HPLC "S" grade, Rathburn Chemicals, Walkerburn, U.K.) was used as received.

Zimeldine*, (*Z*)-3-(4-bromophenyl)-N,N-dimethyl-3-(3-pyridyl)allylamine dihydrochloride (I), norzimeldine (II), zimeldine N-oxide (III), 3-(4-bromophenyl)-3-(3-pyridyl)allylamine (IV), zimeldine N,N-dioxide (V), 3-(4-bromophenyl)-N,N-dimethyl-3-(3-pyridyl)-2-propenal (VII), zimeldine acrylic acid (VIII) and 3-(4-bromophenyl)-N-hydroxy-N-methyl-3-(3-pyridyl)allylamine (IX) were prepared as standard solutions in the HPLC mobile phase (10–100 μg/ml). These materials, together with N,N-dimethyl-N-octylamine (DMOA; ICN Pharmaceuticals, New York, NY, U.S.A.) and the *E*-isomer of II, used as an internal standard (X), were provided by Astra Läkemedel (Södertälje, Sweden).

Equipment and methods

The liquid chromatographic system consisted of a solvent-metering pump (Altex Model 110A; Altex Scientific, CA, U.S.A.), a Rheodyne injection valve (Model 7125; Rheodyne, Berkeley, CA, U.S.A.) provided with a 20-, 50- or 100-μl loop. The 100 × 2.9 mm I.D. stainless-steel column was slurry-packed with 5 μm C_{18}-Nucleosil (Macherey, Nagel & Co., Düren, F.R.G.). The rapid scanning spectrophotometer employed was a Hewlett-Packard (Waldbronn, F.R.G.) Model 1040A, supplied with a 4.5-μl flow cell. The spectral resolution was 2 nm per diode (with an interpolated increment of 1 nm) in the range 200–600 nm. The HP 1040A system consists of a detector mainframe connected by a HP-IB interface bus (IEEE 488) to an HP-85 personal computer and HP 82901M dual 5.25-in. flexible disc drives.

Conventional single-channel detection was carried out with a Pye Unicam LC–UV detector (Pye Unicam, Cambridge, U.K.) and a 10-mV potentiometric recorder (Model 56; Perkin-Elmer, Beaconsfield, U.K.). The flow-cell volume of this detector was 8 μl.

Zimeldine and its metabolites were weighed on a Cahn Model C29 electromicrobalance (Cahn, CA, U.S.A.).

HPLC procedure

The chromatographic support was 5-μm C_{18}-Nucleosil, the mobile phase being acetonitrile–0.05 *M* sodium hydrogen sulphate with 6×10^{-4} *M*, N,N-dimethyl-N-octylamine (DMOA; pH 1.9–2.0) (10:90, v/v). The addition of the ion-pairing agent DMOA has been shown to give improved peak shape and chromatographic

* Zimeldine (I) and metabolites II-IX have the *Z*-configuration[19].

efficiency[20], although a non-optimal eluent composition was used to create peak overlap conditions for the purposes of this work. A flow-rate of 0.5–1.0 ml/min was used, the injection volumes being 20–100 μl. The wavelength for single-channel detection was 250 or 270 nm.

Pseudo-isometric graphics routines

Pseudo-isometric three-dimensional plots of spectrochromatograms were obtained using a hidden-line removal algorithm as described previously[11]. The file sequence can be readily reversed in order to present the data on an inverted time axis. Sections of the spectrochromatogram can be selectively plotted for more detailed examination[11].

Contour plot presentation

A contour presentation of a three-dimensional data matrix such as the (A,λ,t) spectrochromatogram is more convenient, although perhaps less familiar, than isometric or pseudo-isometric plots. In a contour plot all the information is displayed in a single plane, so that small peaks are not "hidden" by larger foreground peaks, as in the isometric presentation. However, peaks less intense than the lowest contour value will not be represented in the contour plot.

The contour diagram is produced by connecting points of equal absorbance by contour lines. The equiabsorptive points are calculated by linear interpolation between neighbouring points in either the wavelength or the time domain, to find the (λ,t) pair corresponding to the absorbance of the contour. This requires at least two adjacent spectra to be held in the computer simultaneously. Particular care is required in drawing contours around saddle points.

One feasible way of producing a contour plot is to track each contour around the (λ,t) plane, and to plot the diagram contour by contour. However, this method is not suitable with a large data set, such as a spectrochromatogram, as the full matrix cannot be held in the microcomputer memory. This method requires the sequential transfer of each spectrum from disk once for each contour plotted. A preferable solution, adopted in the present work, is to calculate the appropriate portions of all equiabsorptive contours, while two sequential spectra are in computer memory. This implementation, where the array need only be read in and translated once, is a much more efficient approach.

From a contour diagram the wavelength maxima and elution times of peaks are readily obtained. The peak shape may be deduced from the spacing between contours; if the features of a peak are not perpendicular to the wavelength axis, this can give an indication of peak non-homogeneity.

With experience, the evaluation of contour diagrams is not significantly more difficult than the evaluation of pseudo-isometric plots. However, it is perhaps more necessary with contour plots to slice through the data matrix to obtain the corresponding spectra and chromatograms, in order to make the data readily accessible to the unaided eye.

RESULTS AND DISCUSSION

Detection by diode array spectrometry in HPLC clearly generates a consider-

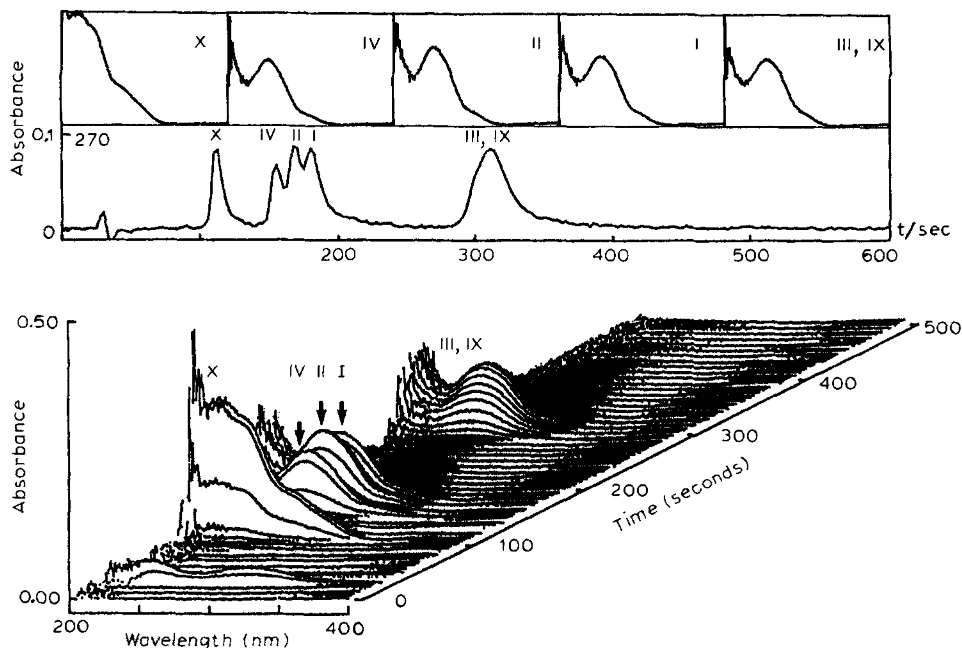


Fig. 1. Pseudo-isometric (A,λ,t) presentation of zimeldine and metabolites captured at 2.5-sec intervals during elution, together with the conventional chromatogram at 270 nm and UV spectra (210–400 nm) of zimeldine (I), its metabolites (II, III, IV, IX) and the internal standard (X) at 10–100 μg/ml in the HPLC eluent. For chromatographic conditions, see text; injection volume, 50 μl; reference wavelength, 550 nm; bandwidth, 100 nm; analytical detection wavelength, 270 nm; bandwidth, 5 nm.

able amount of data, for which several new strategies are available for post-run graphical and numerical evaluation. The increased information content is evident in the pseudo-isometric projection of the spectrochromatogram, where spectral data have been acquired at 2.5-sec intervals during the separation of the zimeldine system (Fig. 1). The conventional chromatogram obtained by slicing through the three-dimensional data set at 270 nm reveals a degree of peak overlap induced by the non-optimal chromatographic conditions selected. Supplementary information can also be obtained by retrieving the spectrum at the apex of each chromatographic peak. The marked similarity of the metabolite structures I–IV and IX is confirmed by comparison of the relatively featureless spectra, whose λ_{max} values fall between 250 and 256 nm. In contrast, the *E*-configuration analogue (X) of norzimeldine (II) is seen to posess a distinctly different chromphore system and is characterized by different retention properties in this HPLC separation.

An alternative approach to represent the data is to generate a contour plot (Fig. 2), which has the advantage of clearly illustrating the maximum absorbance wavelength for detection of each of the components separated. The contour plot may also indicate the most suitable detection wavelengths to use in order to show features not readily observed in the normal single-wavelength elution profile. This is illustrated in the chromatogram sliced at 230 nm (Fig. 3), where an impurity on the trailing edge of the zimeldine peak is evident.

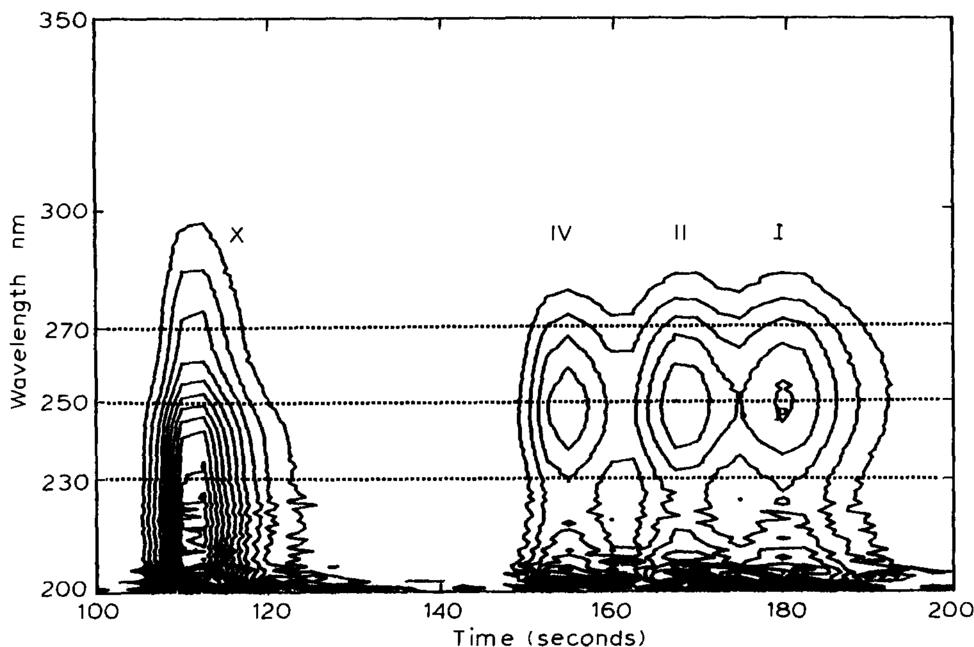


Fig. 2. Contour plot of zimeldine (I), norzimeldine (II), the primary amine (IV) and the internal standard (X), illustrating the optimum wavelength for detection; contour interval, 0.02 a.u.

Such information may also be observed in the composite elution profile produced as the total absorbance chromatogram from 200 to 400 nm (Fig. 4). In this instance a second impurity on the zimeldine peak is detected. It seems reasonable to suggest that this latter mode of presentation, which is analogous to total ion current monitoring in gas chromatography-mass spectrometry (GC-MS), should find use for conducting a rapid preliminary survey of complex separations in metabolic profiling and analytical toxicology.

A further technique recently proposed for resolution enhancement purposes is the derivative chromatogram, more especially the second derivative (d^2A/dt^2), for use in pharmaceutical[14,21] and forensic analysis[2]. In the second derivative chromatogram of the zimeldine system (Fig. 5), peaks I, II and IV are clearly resolved, whereas peaks III and IX are only partially resolved by this technique. Clearly, the usefulness of this method is determined both by the wavelength selected and by the degree of peak overlap observed. With two co-eluting components, the derivative method would not permit the components to be discriminated. It is possible, however, that a three-dimensional presentation of the sharpened second-derivative peaks as (d^2A/dt^2, λ, t) would permit the presence of closely eluting peaks to be more readily detected at the different wavelengths. As the second derivative is bipolar, the graphical presentation could be simplified by truncating the positive satellites and reversing the polarity, so that the second derivative peaks projected above the (λ,t) plane.

The technique of plotting the absorbance ratio at two different wavelengths, calculated continuously over the elution profile, can be helpful in detecting overlapping or unresolved peaks (Fig. 6). This method however, is, of use only when the

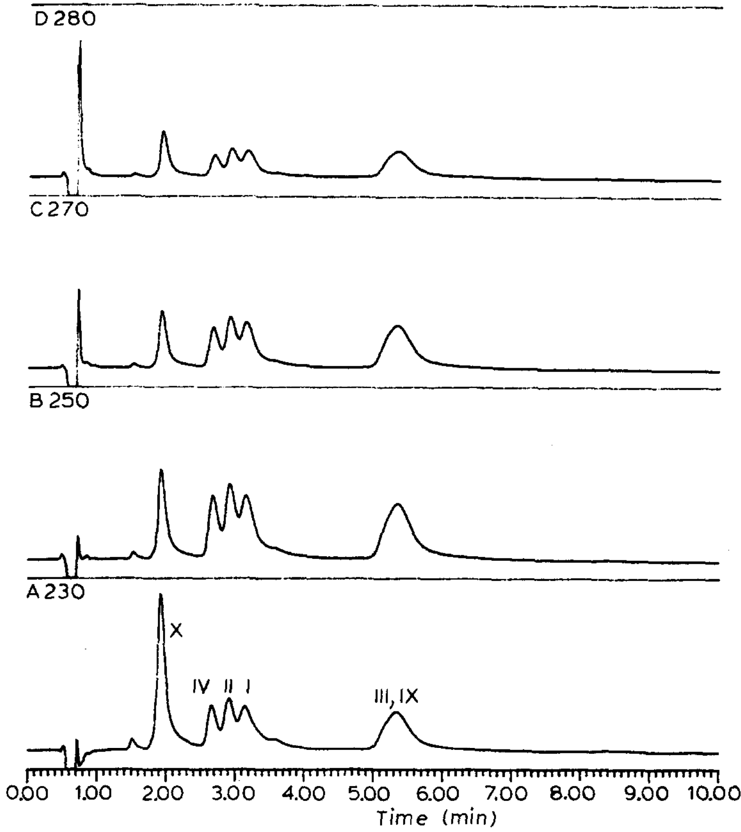


Fig. 3. Chromatograms obtained by slicing the contour plot (Fig. 2) at 230–280 nm for zimeldine (I), metabolites II, III, IV and IX and the internal standard (X). Reference wavelength, 550 nm; bandwidth, 100 nm; bandwidth 4 nm at the analytical wavelengths. For chromatographic conditions, see text.

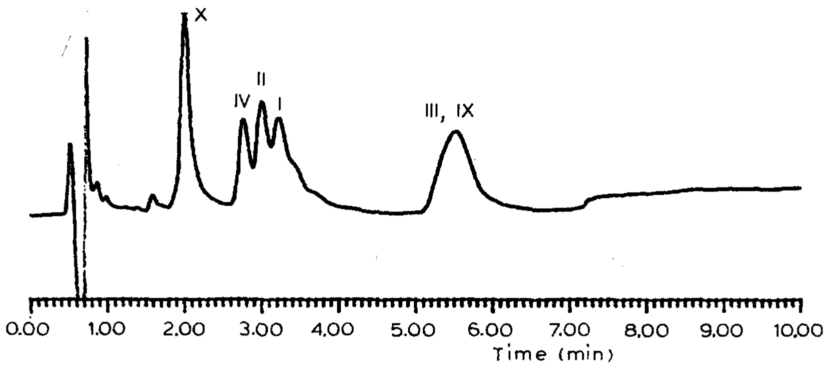


Fig. 4. Total absorbance chromatogram from 200 to 400 nm for zimeldine (I), metabolites II, III, IV and IX and the internal standard (X).

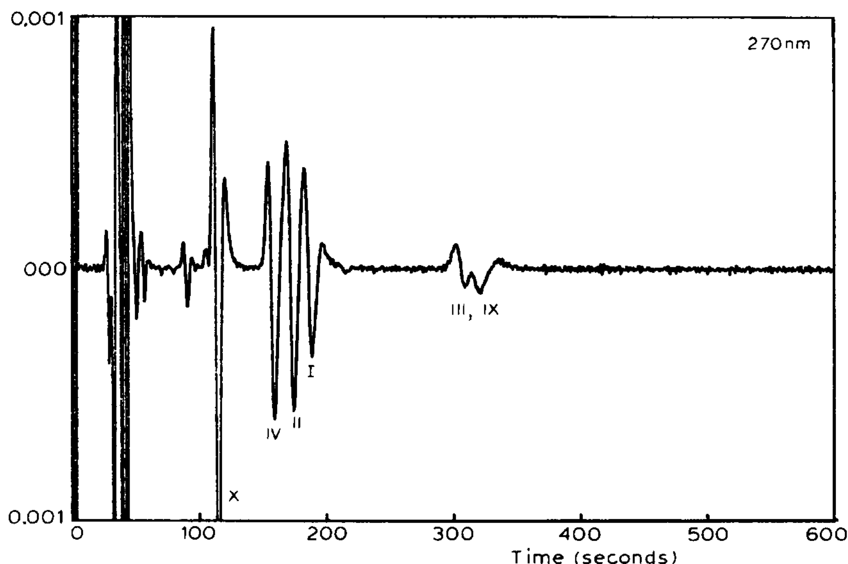


Fig. 5. Second-derivative chromatogram in the time domain (d^2A/dt^2) of zimeldine (I), metabolites II, III, IV and IX and internal standard (X). Detector wavelength, 270 nm; bandwidth, 4 nm; reference wavelength 550 nm; bandwidth 100 nm. For chromatographic conditions, see text.

individual spectra differ significantly; its application to this particular mixture of metabolites is therefore limited. Clearly, the close spectral similarity of III and IX does not permit discrimination by this method.

The technique of spectral suppression, however, has found use in pharmaceutical and forensic analysis for the detection of co-eluting impurities[12,13]. The construction consists of finding the absorbance ratio at two carefully selected wavelengths, λ_1 and λ_2, for the compounds of interest. Given this ratio K^x for compound X, then a plot of $\Delta A = A_{\lambda 1} - K^x A_{\lambda 2}$ as a function of time should be zero for compound X. Any co-eluting compound will yield a positive or negative response, which is directly related to concentration. When applied to the pair of overlapping peaks III and IX, using the two wavelengths 244 and 288 nm (based on the ratio of the two spectra[12]), the absorbance ratios 2.762 and 0.279 were obtained to give the two spectral suppression equations:

$$\Delta A_1 = A_{244} - 2.762\, A_{288} \quad (1)$$

$$\Delta A_2 = A_{288} - 0.279\, A_{244} \quad (2)$$

Eqn. 1 suppresses the response to compound IX, so that only peak III is seen. The second equation suppresses III to detect IX. The consequent deconvolution of these two compounds (Fig. 7) illustrates the general point that spectral suppression can be used only when the spectrum of one component is well defined. Even in cases where spectra are closely similar, satisfactory discrimination can be obtained. These equations do not suppress the other peaks in the chromatogram; they do, however, alter the selectivity of the system, as can be seen from the improved resolution of the

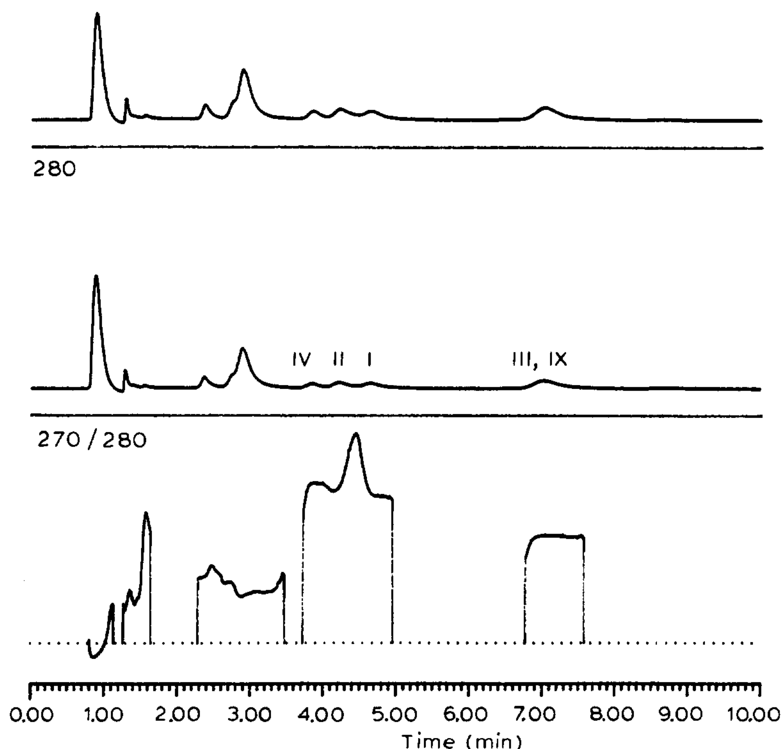


Fig. 6. Conventional and absorbance-ratio chromatograms of zimeldine (I) and metabolites II, III, IV and IX at 270 and 280 nm. Bandwidth, 4 nm. For chromatographic conditions, see text.

impurity on the trailing edge of compound I. By altering the value of the constant K^x, the zimeldine peak could be readily suppressed for better detection of this impurity. Although spectral suppression yields an increase in selectivity, this must be traded off against the inherent decrease in sensitivity due to the subtraction of absorbances. The method does, however, have an advantage over the absorbance-ratio technique, in that the response can be related to concentration. Spectral suppression should find wide use in the determination of chromatographic peak purity in a variety of biomedical application areas[12].

Direct comparison of the linear photodiode array (LDA) sensitivity with that of a typical single-channel detector (Pye Unicam LC–UV), was performed using the detectors in series. Results were also acquired by reversing the sequence of the detectors. The average values of five replicate 100-μl injections for each of three single components are given in Table I, where a six-fold increase in the limit of detection is observed for the LDA detector relative to the single-channel detector employed. Using optimal chromatographic conditions, lower detection limits have been reported

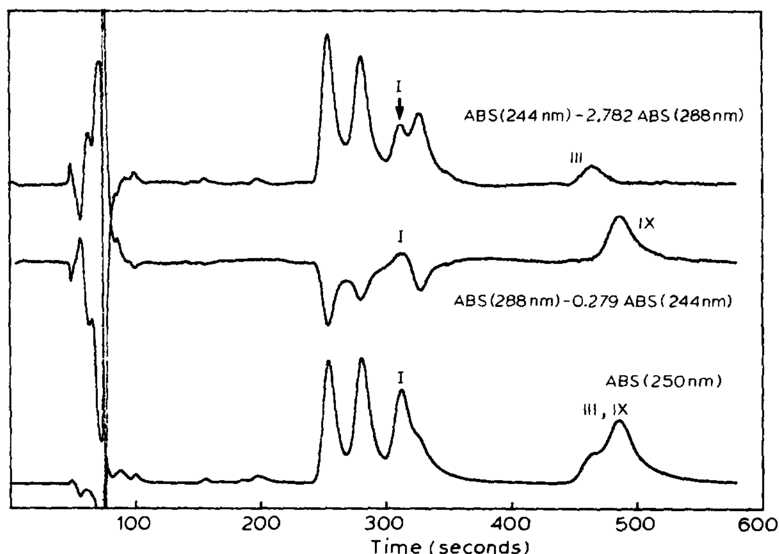


Fig. 7. Conventional and spectral suppression chromatograms showing resolution of peaks III and IX. The analytical wavelengths (bandwidth 4 nm) and equations are as shown; reference wavelength, 550 nm; bandwidth, 100 nm. For chromatographic conditions, see text.

for zimeldine[20]. It is possible to improve the detection limits further when using LDA detection, by increasing the bandwidth of the analytical wavelength, as illustrated in Fig. 8. The optimum bandwidth for norzimeldine at 250 nm was found to be 24 nm (*i.e.*, 238–262 nm), using a reference bandwidth of 100 nm at 550 nm, to give a two-fold improvement in signal-to-noise ratio. Further work in these laboratories has shown that this approach yields adequate sensitivity in studies on zimeldine and its metabolites in biological samples, either using extraction procedures[20,23] or by direct injection of urine samples[24]. The digital techniques proposed here and their

TABLE I

COMPARATIVE DETECTION LIMITS FOR RAPID-SCANNING LDA AND SINGLE-CHANNEL UV DETECTORS

Zimeldine or its metabolites were dissolved in eluent; five replicate 100-μl injections of each single-component solution were made with the detectors in series, followed by five injections with the detector sequence reversed. Nominal wavelength for each detector, 250 nm; analytical bandwidth for rapid-scanning LDA detector, 4 nm; reference bandwidth for LDA detector, 100 nm at 550 nm; absorbance range for LDA detector, 0.5 ma.u.f.s.; absorbance range for single-channel detector, 5 ma.u.f.s. On-column detection limits calculated in nanograms on the basis of a signal-to-noise ratio equivalent to 2. For chromatographic conditions, see text.

No.	*Compound*	*Detection limit (ng)*	
		LDA	*Single-channel*
I	Zimeldine	0.48	3.3
II	Norzimeldine	0.52	4.4
III	Zimeldine N-oxide	1.9	10.7

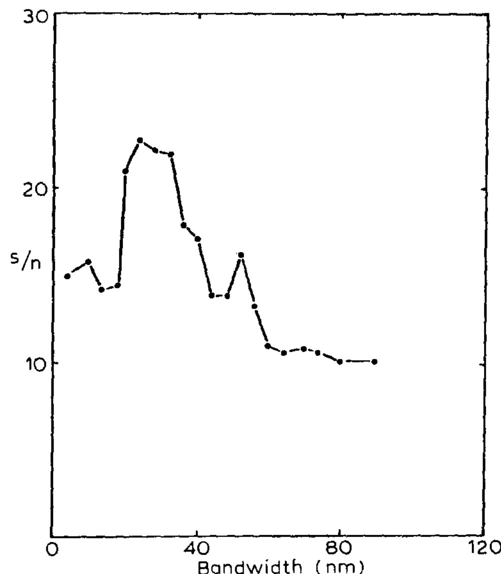


Fig. 8. Mean signal-to-noise ratio (s/n) for detection of 100-μl samples of norzimeldine (II) at 0.1123 μg/ml as a function of detector bandwidth at 250 nm. The bandwidth expresses the total wavelength window located symmetrically about the nominal 250-nm wavelength. The bandwidth at the reference wavelength (550 nm) was a constant 100 nm. Sampling rate, 160 msec. For chromatographic conditions, see text.

further refinement form the basis of continuing work in the context of therapeutic drug monitoring and metabolic profiling.

CONCLUSIONS

The rapid-scanning photodiode array detector generates a considerable amount of data in HPLC as the three-dimensional (A,λ,t) matrix. This matrix can be presented in one of two complementary formats: the three-dimensional pseudo-isometric plôt in the form of a "mountain range", or the cartographically equivalent contour plot in two dimensions. Each of these presentations has its merits, but in essence the visual accessibility of the pseudo-isometric plot or, indeed, of the truly isometric plot rotated in the (λ,t) plane by user-interactive graphics control, is probably on balance less versatile than the contour diagram. Just as some experience is required in interpreting geographical two-dimensional maps, so does the contour plot require the chromatographer to extend his experience in translating the encoded three-dimensional information to the context of his application. In this respect, the recent advent of colour graphics in LDA detection should facilitate the interpretation of contour diagrams in HPLC, bearing in mind the wide application of colour graphics in other areas of analysis, such as secondary ion mass spectrometry[25].

Of the digital techniques developed for accessing the (A,λ,t) matrix, those which address the question of peak homogeneity should find wide application in studies on complex mixtures, such as drug metabolites. The use of spectral information for the deconvolution of overlapping peaks and for advanced chemometric

techniques based on principal component analysis and pattern recognition should further extend the applications potential of rapid-scanning photodiode array HPLC detectors in the biomedical sciences.

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REFERENCES

1 A. Bylina, D. Sybilska, Z. Grabowski and J. Kosewski, *J. Chromatogr.*, 83 (1973) 357–362.
2 R. E. Dessy, W. G. Nunn, J. Titus and W. R. Reynolds, *J. Chromatogr. Sci.*, 14 (1976) 195–200.
3 G. I. Baram, M. A. Grachev, N. I. Komarova, M. P. Perelroyzen, Yu. A. Bolvanov, S. V. Kuzmin, V. V. Kargaltsev and E. A. Kuper, *J. Chromatogr.*, 264 (1983) 69–90.
4 A. E. McDowell and H. L. Pardue, *Anal. Chem.*, 49 (1977) 1171–1176.
5 L. N. Klatt, *J. Chromatogr. Sci.*, 17 (1979) 225–235.
6 M. J. Milano, S. Lam, M. Savonis, D. B. Pautler, J. W. Pav and E. Grushka, *J. Chromatogr.*, 149 (1978) 599–614.
7 G. E. James, *Can. Res.*, 13(8) (1980) 39–43.
8 A. F. Fell, H. P. Scott, R. Gill and A. C. Moffat, *Chromatographia*, 16 (1982) 69–78.
9 R. T. Ghijsen, B. F. H. Drenth, F. Overzet and R. A. de Zeeuw, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 192–198.
10 S. A. George and A. Maute, *Chromatographia*, 15 (1982) 419–425.
11 A. F. Fell, H. P. Scott, R. Gill and A. C. Moffat, *J. Chromatogr.*, 273 (1982) 3–17.
12 A. F. Fell, H. P. Scott, R. Gill and A. C. Moffat, *J. Chromatogr.*, 282 (1983) 123–140.
13 G. T. Carter, R. E. Schiesswohl, H. Burke and R. Yang, *J. Pharm. Sci.*, 71 (1982) 317–321.
14 J. C. Berridge, *Chromatographia*, 16 (1982) 172–174.
15 D. Brown, D. H. T. Scott, D. B. Scott, M. Meyer, D. Westerlund and J. Lundström, *Eur. J. Clin. Pharmacol.*, 17 (1980) 111–116.
16 J. Lundström, T. Hogberg, T. Gosztonyi and T. de Paulis, *Arzneim.-Forsch.*, 31 (I) (1981) 486–494.
17 L. Bertilsson, J. R. Tuck and B. Siwers, *Eur. J. Clin. Pharmacol.*, 18 (1980) 483–487.
18 T. Hogberg and J. Lundström, *Acta Chem. Scand. B*, 36 (1982) 85–89.
19 T. Hogberg, *Acta Chem. Scand. B*, 34 (1980) 629.
20 D. Westerlund and E. Erixson, *J. Chromatogr.*, 185 (1979) 593–603.
21 A. F. Fell, *Proc. Anal. Div. Chem. Soc.*, 15 (1978) 260–267.
22 R. Gill, T. S. Bal and A. C. Moffat, *J. Forensic Sci. Soc.*, 22 (1982) 165–171.
23 D. Westerlund, L. B. Nilsson and Y. Jaksch, *J. Liq. Chromatogr.*, 2 (1979) 373–405.
24 F. Neilson-Kudsk and A. K. Pederson, *Acta Pharmacol. Toxicol.*, 42 (1978) 298.
25 B. K. Furman and G. H. Morrison, *Anal. Chem.*, 52 (1980) 2305–2310.