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APPLICATIONS OF RAPID-SCANNING MULTICHANNEL DETECTORS IN CHROMATOGRAPHY

PLENARY LECTURE

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SUMMARY

A review of progress in the field of multichannel detection in column and thin-layer chromatography is presented, together with some novel applications of a computer-based, linear photodiode array UV—visible spectrophotometer for detection in high-performance liquid chromatography (HPLC). Computer-aided methods for simultaneous monitoring of the elution profile at three wavelengths with automatic peak detection and capture of UV spectra are described. The continuous calculation of absorbance ratios during elution is discussed as an index of peak homogeneity. A novel technique for the enhancement of qualitative identification in HPLC, based on transformation of captured spectra to the second derivative or to the decadic logarithm, is proposed. These developments are exemplified by a model system of diacetylmorphine and its principal metabolites and degradation products, morphine and 6-acetylmorphine. The potential utility of three-dimensional projections of (A,λ,t) data is discussed in the context of pharmaceutical, bioanalytical and forensic applications.

INTRODUCTION

Of the numerous physical methods advocated as detectors in column liquid chromatography (LC) and in thin-layer chromatography (TLC), those based on the measurement of transmittance, absorbance or, in the case of TLC, reflectance of radiation in the UV—visible range have found most application

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[1]. In the absence of a truly universal detector, the UV—visible spectrometer, based on the combination of a monochromator and photomultiplier tube (PMT), has proved to have a wide dynamic range, high signal-to-noise ratio (SNR) and long-term reliability for the detection of those compounds of pharmaceutical and bioanalytical interest with appreciable absorption in the UV—visible spectrum. However, many analytes display only moderate absorptivity and a relative lack of selectivity, which leads to problems of sensitivity and specificity, especially in bioanalytical studies. These problems may sometimes be overcome by exploiting natural or selectively-induced fluorescence for fluorimetric detection, which in favourable cases may be two or more orders of magnitude more sensitive as a chromatographic detector [2].

A major limitation of conventional UV—visible and fluorimetric detectors in chromatography is that only one wavelength resolution element is registered at a time. Although this may have the advantage of simplicity, the disadvantage implied by lack of detection flexibility and the consequent use of non-optimal wavelengths for the detection of multiple components in a chromatogram, imposes a significant limitation on the information-gathering power of the chromatographic method. To a certain degree this disadvantage can be overcome by the use of a scanning spectrophotometer in the stop—flow mode for LC [3] or at specified positions on the chromatoplate in TLC [4].

More convenient, however, are the rapid-scanning spectrophotometric detectors based on electronic, electromechanical or multichannel devices. One of the earliest approaches to rapid-scanning UV detection in column LC was the device of Bylina et al. [5], based on the ingenious combination of a conventional monochromator and PMT with a cathode-ray tube. The high-intensity quartz phosphor screen functioned as a radiation source. Electronic scanning of the screen in the axis of the monochromator slit caused the point source to generate a spectrum in the range 230—750 nm, permitting the transmission spectrum of the eluate to be recorded in 10 msec and the absorbance ratio at any wavelength pair to be calculated. An electromechanical analogue of this device is the oscillating mirror detector developed by Denton et al. [6] for high-performance liquid chromatography (HPLC). This was successfully used to demonstrate three-dimensional presentation of spectral data (A,λ,t) acquired as "time-slices" in the elution profile of uracil, cytosine and adenine. The normal elution chromatogram could be presented at stationary wavelength, optimised for each component. However, the low source intensity of the Bylina device and electromechanical problems of wavelength registration in Denton's detector have led to their being superceded by optical multichannel methods of detection in chromatography.

The advent of optical multichannel detectors based on the linear photodiode array (LDA), the silicon intensified target (SIT) vidicon or the charge-coupled device (CCD) has transformed the technology of detection systems in spectroscopy [7—10] and consequently in column LC [11—20], TLC [21—23] and in gel electrophoresis [23, 24]. These devices are, of course, limited to the detection of analytes with appreciable absorption or fluorescence in the UV—visible spectrum. They do, however, offer particular advantages for detection in chromatography. The rapid acquisition of spectral data should lead to a time-domain multiplex advantage, since simultaneous detection of N spectral

resolution elements reduces the observation time by a factor of N to yield an improvement in SNR by $\sqrt{N}$. An intrinsic advantage of the optical multichannel detector is that spectral data in digital form can be readily stored, manipulated and presented in any desired format. The wide variety of digital algorithms available for transformation of spectral data has led to the proposal of several new strategies for detection in LC [20].

The SIT vidicon, first proposed as a detector for HPLC by Rogers [25], was evaluated by McDowell and Pardue [14], who used a grating polychromator and reversed optics to disperse the eluate transmission spectrum across the front surface of the detector. In the plane of dispersion, each of the several hundred photosensitive diodes acts as a spatially separated resolution element, corresponding to a specific nominal wavelength and monitored by a scanning electron beam [7, 8]. The entire spectrum is registered in computer memory and can be manipulated by a number of digital techniques: ensemble averaging (to improve SNR); presentation of chromatograms at a number of observation wavelengths; and spectral deconvolution of overlapping chromatographic peaks. These concepts were extended by Klatt [15], who used the SIT vidicon to present spectral data during elution, followed by post-run processing by laboratory computer to generate three-dimensional (A,λ,t) surface projections of oil sample eluates, to characterise different degrees of hydrogenation. Moreover, the dispersed spectrum can be optically segmented across the vidicon tube surface to give sub-nanometer resolution if required [16].

The SIT vidicon has also shown promise as a fluorescence detector in HPLC. This approach has been developed by Christian and co-workers [9, 10, 17] to generate the three-dimensional emission—excitation matrix $(I_f, \lambda_f, \lambda_{ex})$, using a computer-aided videofluorimeter based on the SIT vidicon and twin polychromators. The emission—excitation matrix, captured at any time t during the elution of petroleum hydrocarbon fractions, has been shown to yield highly characteristic qualitative data of $(I_f, \lambda_f, \lambda_{ex}, t)$ in four-dimensional space [17]. More recently, Warner and co-workers [18, 19] have applied similar techniques for detection in HPLC and have made an extensive analysis of the data reduction algorithms required to apply the videofluorimetric detector efficiently to petrochemical analysis.

Christian and co-workers [21] have further examined the potential of the SIT vidicon for detection in two spatial dimensions in TLC. By illuminating the HPTLC chromatoplate with uniform excitation radiation, the fluorescence spectrum can be produced by dispersion at sequential points along the elution axis d_1 of a single track. This method yields a three-dimensional record (either as a contour plot or as an isometric projection) of (I_f, λ_f, d_1) without mechanical scanning, and has been successfully applied to resolve the overlapping spots of two porphine derivatives [21]. By sacrificing the emission wavelength variable and using a fixed-emission wavelength filter, the system can be modified to record the fluorescence intensity over the entire surface of the HPTLC chromatoplate to give a three-dimensional record of $(I_f^{\lambda(f)}, d_1, d_2)$, where the spatial dimension d_2 is normal to the elution track d_1 [21]. Thus three-dimensional isometric plots can be presented for multiple elution tracks on a single chromatoplate, or for separations achieved by the technique of two-dimensional TLC (reserving the term "dimension" for its correct mathematical

connotation [26]). The SIT vidicon has also been employed for rapid non-mechanical scanning of the densitometric profile of separations by TLC [22, 23] or by gel electrophoresis [23, 24].

Early work using the LDA detector either in single-beam [11] or in dual-beam configuration [12] demonstrated its potential usefulness as an LC detector much less expensive than the SIT vidicon. The 256-element Reticon LDA required Peltier cooling (−30°C) to sufficiently reduce the background noise level. Digital methods for ensemble averaging (or integrating) spectra at the rate of 20 or more per second were used to improve the SNR. Milano et al. [11] demonstrated the practicality of presenting chromatograms at any wavelength in the 256-nm range captured. They were the earliest to use the first derivative spectrum to eliminate a component from a chromatogram. In principle, the observation wavelength is selected to coincide with the λ_{max} of the component to be eliminated, where $dA/d\lambda = 0$ [11, 13]. Unfortunately, the method yields bipolar chromatograms and suffers from lack of sensitivity, except for sharp peaks whose spectral inflection points are close to the observation wavelength.

A more general method recently proposed [20] for the improved resolution of overlapping bands in HPLC, involves transformation of the elution profile to its second or higher even derivative in the time domain (d^2A/dt^2, d^4A/dt^4). As discussed later in this paper, the even derivative of a Gaussian band is sharper than the original band. This effect can therefore be exploited to enhance the resolution of peaks in HPLC [20, 27] and in TLC [28], albeit at the expense of a deterioration in the SNR. Another technique for the analysis of overlapping chromatographic peaks is the method of spectral deconvolution [13, 15], which requires among other things that the spectra to be deconvoluted are well defined and sufficiently dissimilar [20].

Several digital techniques for extending detection capability in LC have been given renewed impetus by the recent commercial development of microcomputer-controlled LDA spectrophotometers. These techniques include:

calculation of absorbance ratios at a pair of wavelengths specified for each peak as an index of chromatographic homogeneity [5];

generation of second and higher even derivative spectra in the wavelength domain for enhanced qualitative discrimination [20];

transformation of the elution profile to its corresponding second and higher even derivative in the time domain for the improved detection and resolution of overlapping peaks [20, 27, 28];

deconvolution in the wavelength domain of peaks which overlap in the elution profile, using zero order, first or higher derivative transformation of the absorption spectra [13, 15, 20];

conversion of spectral data to logarithmic format for direct comparison in computer-aided library searches [29, 30].

Moreover, the availability of data in digital form, stored and manipulated by an on-board microcomputer, permits the facile execution of conventional operations:

scaling to improve detection of both minor and major components in one chromatogram;

ensemble averaging for improved SNR throughout the spectrum;

"clustering" of adjacent diodes for improved SNR at a given nominal wavelength;

subtraction of spectra for compensation;

or division of spectra for qualitative comparison purposes.

The addition of a bench-top microcomputer to the LDA spectrophotometric detector enables multiwavelength plotting of chromatograms and the presentation of pseudoisometric or isometric three-dimensional displays of (A,λ,t) data [31].

The present paper reports the development of computer-aided algorithms, based on a commercial LDA spectrophotometer, and their application in HPLC for the detection and characterisation of a model system of diacetylmorphine (heroin) and its potential metabolites and degradation products. This drug system was selected as being illustrative of problems encountered in pharmaceutical manufacture, in bioanalytical investigations and in forensic toxicology.

EXPERIMENTAL

Reagents and materials

HPLC grade acetonitrile (Rathburn Chemicals, Walkerburn, Great Britain) was used as received. Tetrabutylammonium phosphate (Pic A reagent; Waters Assoc., Milford, MA, U.S.A.) was diluted to 0.005 *M* with glass-distilled water and then filtered through a Millipore® 0.45-μm MA filter using an all-glass apparatus. Eluent was degassed under reduced pressure in an ultrasonic bath for 10 min. Diacetylmorphine (DAM), 6-acetylmorphine (6AM) and morphine (MOR) were kindly provided by the Home Office Central Research Establishment, Aldermaston, Great Britain and were shown to be chromatographically homogeneous. The internal standard *p*-hydroxybenzoic acid (PHBA; Koch-Light, Colnbrook, Great Britain) and sulphuric acid (BDH, Poole, Great Britain) were of reagent grade. Standard solutions of DAM, 6AM and MOR were prepared in eluent as used for HPLC (vide infra) or in acetonitrile—0.02 *M* sulphuric acid (4:1). The internal standard PHBA was incorporated at 50 μg/ml (for pharmaceutical dosage forms) or at 0.20—0.40 μg/ml for dilute solutions at biological concentrations, to give peak height comparable with the anticipated peak heights of interest.

Equipment

The modular liquid chromatograph assembled in the laboratory comprised a constant-flow LC pump with integral pulse damping (Gilson Model 302; Villiers-Le-Bel, France), a Rheodyne injection valve (Model 7125; Berkeley, CA, U.S.A.), furnished with either 20-μl or 50-μl loops. The 100 x 5 mm I.D. stainless-steel column (Shandon Southern Instruments, Runcorn, Great Britain) was slurry-packed with a microparticulate aminopropyl bonded silica material (5-μm APS-Hypersil; Shandon Southern Instruments) by the upward displacement technique recommended by the manufacturers.

The optical multichannel detector system employed was the Hewlett-Packard (Palo Alto, CA, U.S.A.) Model HP 8450A UV—visible spectrophotometer, equipped with an 8-μl quartz flow-cell (Model 178-32 QS; Hellma, Mühlheim-Baden, G.F.R.). Spectral resolution was 1 nm per diode in the UV (200—400 nm) and 2 nm per diode in the visible range (400—800 nm). The

spectrophotometer was configured via the RS232C port (9600 Baud) with a Model HP 85 laboratory microcomputer, equipped with 32 kbyte total RAM storage, input-output ROM, print-plot ROM, matrix ROM, and RS232C and HP-IB IEEE-488 interface ports. Two configurations were employed for the present work. In configuration A (Fig. 1A), the HP 85 microcomputer was connected via the HP-IB port to a graphics plotter (Model HP 7225B) and a dual 8-inch floppy-disk mass storage unit (HP 9895A). The alternative configuration (Fig. 1B) employed the on-board tape unit of the microcomputer as a program source. The mass storage unit was connected via HP-IB directly to the spectrophotometer for rapid transfer and storage of "time-slice" spectral data during elution, the fastest sampling rate being 2.3 sec per UV spectrum. Configuration B has the merit of being identical both for data acquisition and data processing, and permits the storage of 1200 UV spectra. However, other configurations proposed by the manufacturers [31] require total instrument shut-down and the disconnection of equipment, to allow the mass storage unit to be reconfigured with the microcomputer for subsequent data processing. In both configuration A and B, the HP 85 microcomputer has been designated as controller, over-riding the central control of the spectrophotometer itself, an arrangement which has proved convenient in practice [32].

A software package ("KEYBOARD") establishes direct control of the LDA spectrophotometer by the HP 85 microcomputer. This allows instructions for

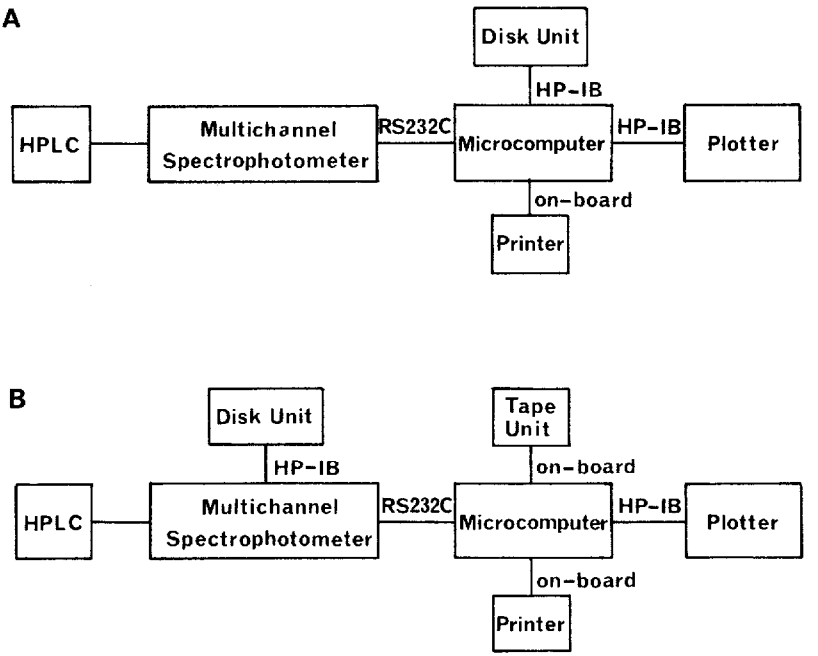

Fig. 1. Configurations of HP 8450A linear photodiode array spectrophotometer and peripherals used for multichannel detection in HPLC. Inter-connection (HP-IB, RS232C) is as indicated, the printer and tape drive units being integral with the HP 85 microcomputer. (A) System for multiwavelength monitoring under HP 85 control, captured spectra being stored by the spectrophotometer. (B) System for continuous spectral capture at 2.5-sec intervals under HP 85 control, captured spectra being stored by the twin disk-drive unit, followed by post-run processing to generate three-dimensional (A,λ,t) chromatograms.

any valid operation by the spectrophotometer to be entered via the alphanumeric keyboard of the HP 85 and translated into a local system code. A suite of programs has been developed to enable the spectrophotometer to function as a detector for HPLC [32]. Using configuration A, absorbance values at up to three wavelengths can be sent to the HP 85 every second and plotted directly on its monitor screen. A peak detection algorithm, based on the first derivative of the elution profile above a selected minimum threshold level, can be used to instruct the spectrophotometer to measure and store a UV spectrum automatically. The spectrophotometer can store up to 92 UV spectra, which can also be captured by manual interrupt. At the end of the chromatographic run, the spectra can be transferred from the spectrophotometer to disk for permanent storage and post-run processing. Firmware routines available on the LDA spectrophotometer include smoothing, transformation to the first or second derivative, spectral deconvolution, logarithmic spectra and spectral subtraction. Peak heights in the chromatogram can be measured automatically at each of the observation wavelengths and corrected for baseline drift. The alternative configuration (B), which allows "time-slice" spectra to be acquired for storage as the (A,λ,t) matrix, permits digital averaging of spectra within the sampling interval. This technique enables the SNR of spectra to be improved, a feature which is of importance for measurement at low absorbance.

Graphics routines have been developed for each of the instrument configurations. The chromatographic data acquired by system A can be presented at each of the selected observation wavelengths, with the absorbance ratio at two wavelengths superimposed. Captured spectra can be plotted above the chromatogram and compared with plots of normalised standard spectra retrieved from data archive. The captured spectra and their derivative or logarithmic transformations can also be compared with the corresponding standard spectra. "Time-slice" spectra acquired using configuration B can be presented as a three-dimensional plot of (A,λ,t). This routine requires typically 30—45 min to generate a pseudo-isometric projection of ca. 240 files at a selected angle (25° in the present report). A hidden-line removal algorithm eliminates any spectral data which would normally be obscured by a peak in the foreground at the observation angle selected. Simple manipulation of the spectral data files enables the projection to be reversed, so that bands hidden by foreground peaks can be more easily observed. Moreover, any particular region in the (A,λ,t) chromatogram can be isolated and presented separately for closer examination if required.

HPLC method and procedure

The published method of Baker and Gough [33] was adapted, using a shorter (100-mm) column packed with 5-μm aminopropyl bonded silica from an alternative source (APS-Hypersil) and an optimised eluent flow-rate of 1.2 ml/min to give more rapid separation. The eluent composition was acetonitrile—0.005 *M* tetrabutylammonium phosphate (85:15, v/v). A test mixture of DAM (retention time 4.0 min) was used to routinely check the column performance, the average number of theoretical plates being 35,000—40,000 plates/m. The retention times for the internal standard (PHBA) and

for morphine were 2.9 and 9.2 min, respectively. The HPLC flow-cell was referenced against air, the detector baseline being balanced for 10 sec prior to chromatography.

After introducing a sample via the loop valve injector, the spectral data were acquired every 1 or 2 sec using configuration A, and at 2.5-sec intervals using configuration B. The observation wavelengths were 254 nm for PHBA and 280 nm for DAM, 6AM and MOR. Data at 300 nm were used to check the baseline. UV spectra were acquired on-line for peaks detected by the gradient-sensitive algorithm described above and stored by the spectrophotometer. At the end of the chromatogram the corrected absorbance of each peak above a pre-set threshold was automatically measured and listed on the HP 85 printer.

Analytical curves of peak height (and of peak height ratio with respect to the internal standard) against analyte concentration were rectilinear and passed through or close to the origin at both low (0—1 mg/ml for DAM) and high sensitivities (0—4 μg/ml for DAM, 6AM and MOR). For replicate 50-μl injections (n = 8) of each component the relative standard deviations (R.S.D.) of corrected peak heights at 280 nm (and of the corresponding peak height ratios to internal standard at 254 nm, in brackets) were: DAM 200 ng, 2.7% (1.3%): 6AM 80 ng, 7.3% (6.6%); MOR 160 ng, 10.5% (10.1%); and for PHBA (10 ng) at 254 nm, 2.3%. At higher concentrations, replicate 10-μl injections (n = 8) of each component gave the following R.S.D. data for peak heights at 280 nm (with peak height ratio to internal standard at 254 nm, in brackets): DAM 5 μg, 2.7% (1.2%); 6AM 2 μg, 2.3% (1.2%); MOR 4 μg, 2.9% (3.5%); and for PHBA (0.5 μg) at 254 nm, 1.9%. The R.S.D. of peak retention times varied from 0.96—1.05% (n = 8). After the chromatographic run, the zero order spectrum captured for each peak was plotted and coded in elution sequence directly above the chromatogram. Standard spectra were superimposed for direct comparison. Spectral data were then further manipulated by smoothing or derivative routines, the results of which could be plotted separately and compared directly with standard spectra similarly treated. When configuration B was employed, the UV spectra (200—400 nm) acquired on disk at 2.5-sec intervals during chromatography were processed by a graphics routine to plot three-dimensional (A,λ,t) chromatograms at 25° in forward or reverse projection, as described above. Superfluous baseline scans between the peaks were suppressed for greater clarity of presentation.

RESULTS AND DISCUSSION

The detection of DAM at high sensitivity, well-resolved from its principal metabolites, is illustrated in Fig. 2. UV spectra could be captured for all components at these low concentrations, although the relatively high noise level necessitated the application of 7-point Savitzky—Golay smoothing for satisfactory comparison of spectra with standards. However, the residual noise level led to unsatisfactory first and second derivatives of the captured spectra. The on-column detection limits at 280 nm, defined in terms of twice peak-to-peak noise level, were: DAM, 15 ng; 6AM, 25 ng; MOR, 65 ng. These limits can be reduced by an order of magnitude by measurement between 210 and 220 nm, where the absorptivities of DAM and its metabolites are greater. The

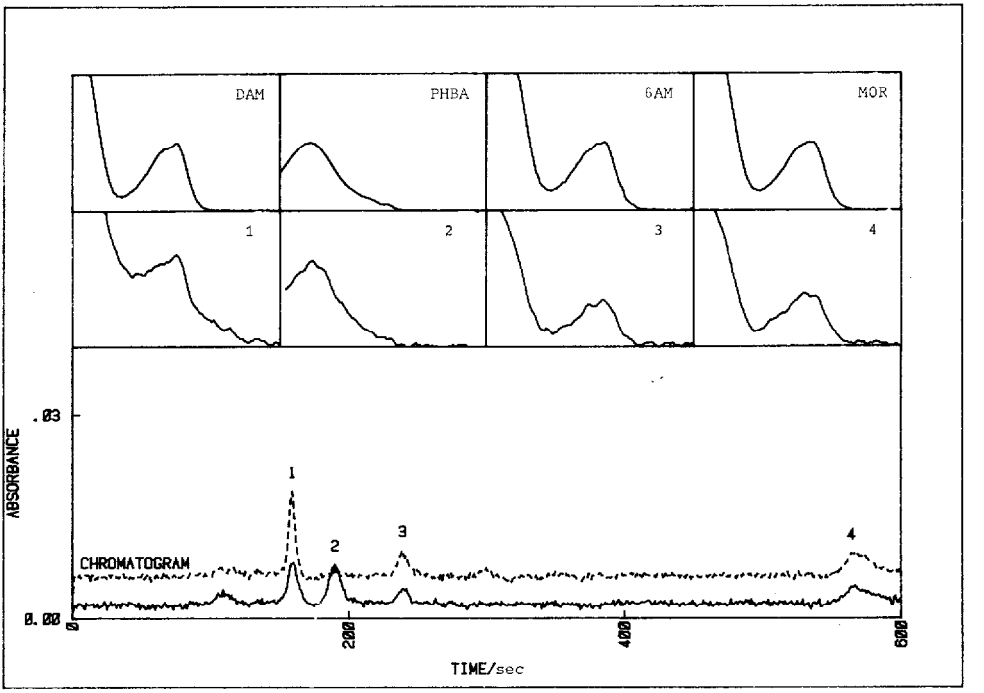

Fig. 2. Chromatograms at 254 nm (——) and 280 nm (— — —) of: (1) diacetylmorphine (DAM) (100 ng); (2) *p*-hydroxybenzoic acid (PHBA) (internal standard, 10 ng); (3) 6-acetylmorphine (6AM) (103 ng); and (4) morphine (MOR) (232 ng). Chromatographic conditions, see text; injection volume, 50 μl. Smoothed UV spectra (230—330 nm) captured during elution (lower row), compared with normalised reference spectra (upper row), correspond to each peak as coded.

quantitative performance at 280 nm is improved somewhat by use of an internal reference standard, PHBA, optimally detected at 254 nm. This flexibility in wavelength selection exemplifies a very useful feature of multichannel detection in HPLC.

At concentrations of DAM and its degradation products, comparable with those found in pharmaceutical dosage forms or forensic samples, low-noise UV spectra can be readily acquired during elution for confirmation of identity (Fig. 3). The absorbance ratio at two pre-determined wavelengths (A_{254}:A_{280}), calculated continuously and superimposed above the chromatogram, yields a sensitive measure of peak homogeneity during elution [34]. In principle, the absorbance ratio profile for a pure component peak should resemble a square-wave function, as is seen for DAM in Fig. 3. Any co-eluting impurity changes the absorbance ratio to higher or lower values, depending on the absorptivities at the pair of wavelengths selected, the concentration and the exact retention position of impurity peak relative to the analyte peak of interest. The numerical value of the absorbance ratio is a qualitative index of identity and gives an estimate of peak purity [34]. A constant absorbance ratio throughout the peak profile gives a good indication of peak homogeneity. The absorbance ratio concept has been usefully extended by Carter et al. [35], who recently used the multichannel detector to eliminate a major component mathematically to reveal various underlying impurities, as a technique for method validation

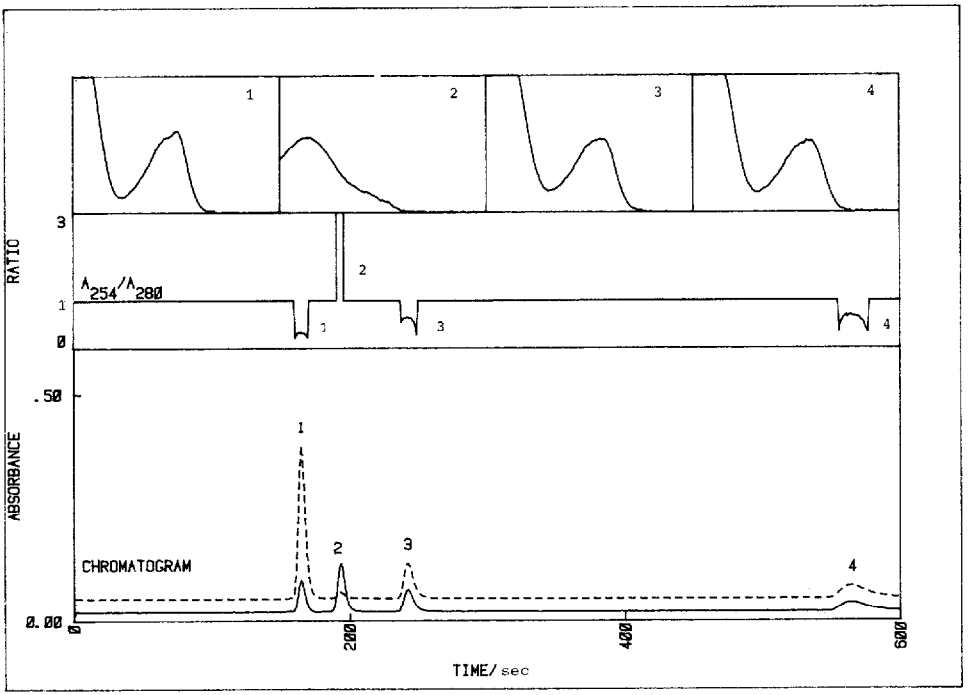

Fig. 3. Chromatograms at 254 nm (——) and 280 nm (— — —) of: (1) diacetylmorphine (4.8 μg); (2) *p*-hydroxybenzoic acid (internal standard, 0.5 μg); (3) 6-acetylmorphine (2 μg); and (4) morphine (2 μg). Chromatographic conditions, see text; injection volume, 10 μl. The continuous absorbance ratio (A_{254}:A_{280}) superimposed above the chromatogram and the normalised UV spectra (230—330 nm) captured during elution (upper row) correspond to each peak as shown.

in pharmaceutical analysis.

A number of functions have been explored for enhanced qualitative evaluation of spectral data captured during elution. The second or higher even derivative spectrum has recently been proposed for identification purposes in pharmaceutical [36], biochemical [37, 38], environmental [39] and forensic analysis [40]. This proposal is based on the fact that, in general, the fine structure and inflection points observed in a zero order UV spectrum are transformed to more finely structured, bipolar peaks in the first or higher derivative ($dA/d\lambda$, $d^2A/d\lambda^2$, . . .). The second derivative is seen as a sharpened, inverted profile, with a minimum corresponding to the maximum in the original peak. Insofar as second and higher even derivative spectra bear a similarity to the zero order spectrum, they are more easily interpreted than the odd derivative functions and have found wider popularity.

In Fig. 4 the zero order spectra of 6AM and MOR, captured during elution (Fig. 3), are compared with the unsmoothed second derivative spectra. The zero order, first derivative, second derivative, and $\log_{10}$ (A) spectra of DAM are compared in Fig. 5. It can be readily seen that, whereas the zero order spectral profiles are relatively smooth and featureless, the second derivative functions present a number of sharpened features for comparison. The second derivative spectra of 6AM and MOR are very similar, an observation

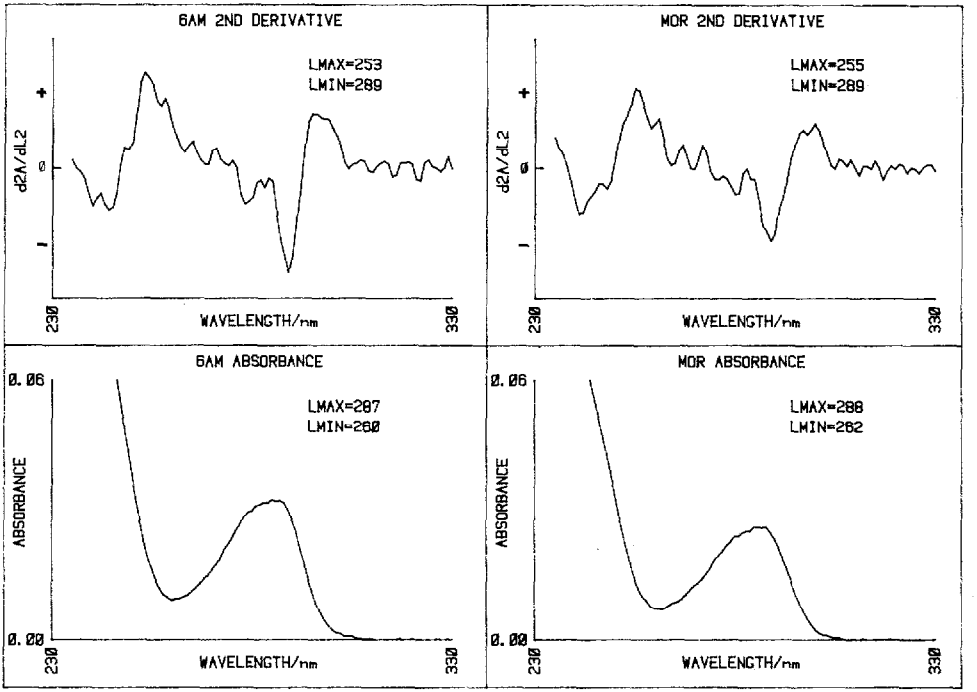

Fig. 4. Zero order and second derivative UV spectra of 6-acetylmorphine (6AM) and morphine (MOR) peaks (cf. Fig. 3).

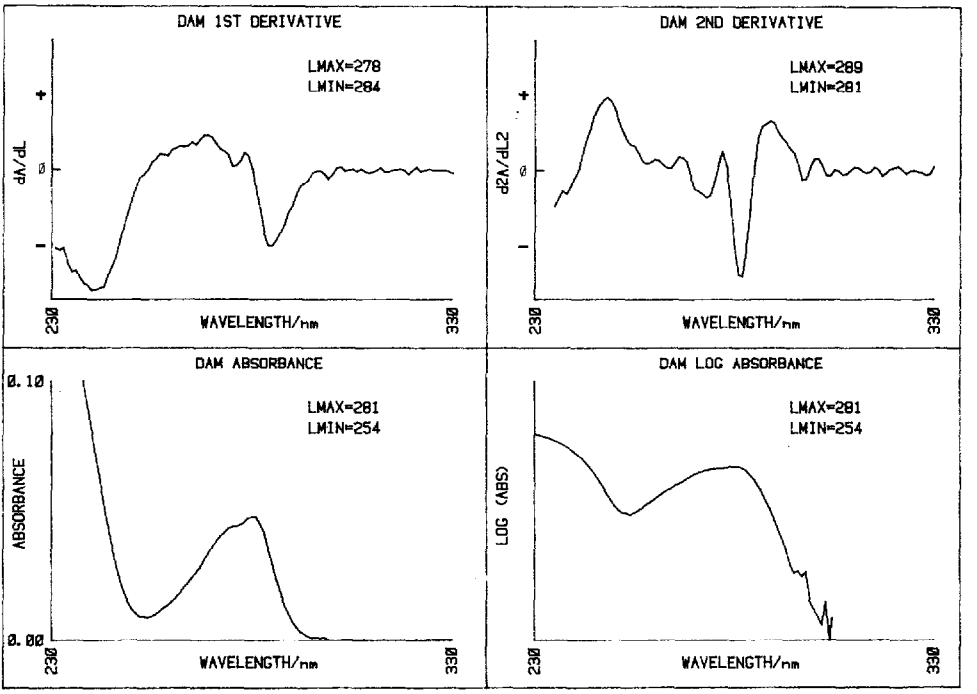

Fig. 5. Zero order, first derivative, second derivative and $\log_{10}$ (A) UV spectra of diacetylmorphine (DAM) peak (cf. Fig. 3).

attributable to the shared feature of a 3-hydroxyl group in the aromatic ring (Fig. 4). However, both 6AM and MOR are readily distinguished from DAM in the second derivative spectrum (Fig. 5). Here the inflection near 275 nm in the zero order spectrum of DAM is seen as a sharpened feature in the second derivative, which although inverted, can be readily related to the original spectrum. The first derivative spectrum of DAM is, however, a relatively featureless disperse function and is more difficult to relate to the zero order profile. The $\log_{10}$ (A) presentation of DAM is relatively uninformative. However, it does offer an alternative method for normalisation, since the concentration term in the Beer—Lambert expression is thereby reduced to a constant displacement of the spectrum on the $\log_{10}$ ordinate, the shape of the profile remaining constant. The noisy baseline at high wavelength reflects the logarithm of values close to zero. Theoretical considerations suggest that the second derivative of $\log_{10}$ (A) spectra should offer a characteristic, concentration-independent profile for spectral comparison purposes [29]. The combination of retention data with zero order and second derivative UV spectra offers a highly selective means for characterising components separated by HPLC.

The presentation of a three-dimensional chromatogram as a pseudo-isometric projection of (A,λ,t) data, using configuration B, is illustrated in Fig. 6, where DAM can be clearly distinguished from 6AM and MOR although the angle of projection partially obscures the internal standard PHBA. By digital manipulation of the data files the viewpoint can be reversed, so that the internal standard and 6AM peaks are more readily visible (Fig. 7). Alternatively, the data files for 6AM could be suppressed for better observation of hidden peaks, as discussed above. Similar three-dimensional projections of the sample used in

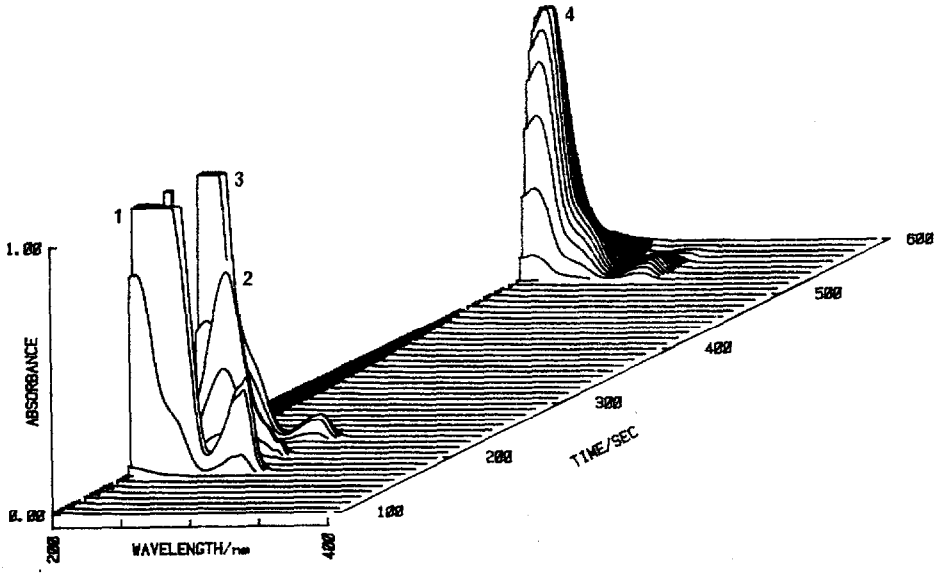

Fig. 6. Pseudo-isometric (A,λ,t) presentation of sequential spectra captured at 2.5-sec intervals during elution. Hidden lines and superfluous baseline spectra have been omitted for clarity. Chromatographic conditions, see text; injection volume, 10 μl. (1) Diacetylmorphine (5.0 μg); (2) *p*-hydroxybenzoic acid (internal standard, 0.50 μg); (3) 6-acetylmorphine (2.0 μg); and (4) morphine (4.0 μg).

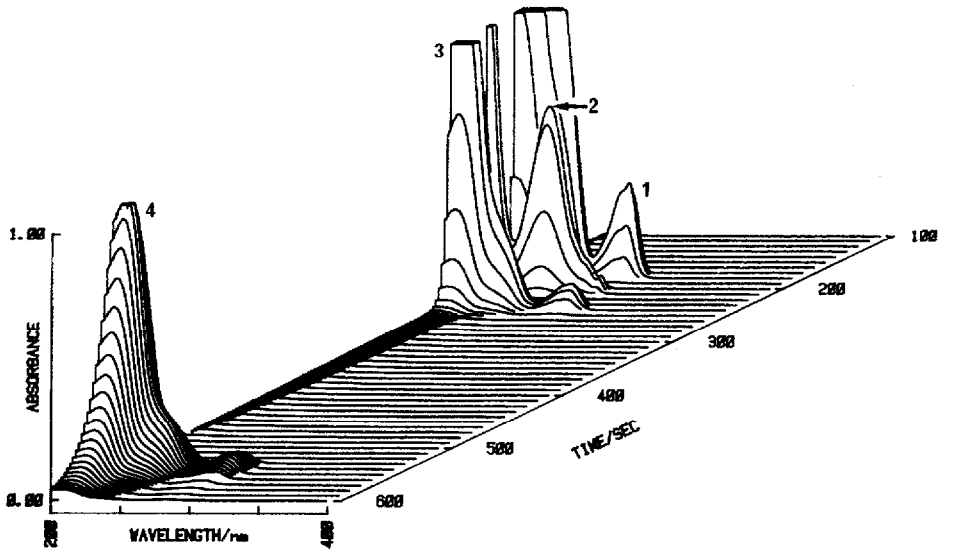

Fig. 7. Reversed presentation of pseudo-isometric (A,λ,t) data in Fig. 6, in order to facilitate observation of hidden features.

Figs. 6 and 7 have been successfully obtained after 100-fold dilution, using the technique of digital integration during the 2.5-sec sampling period [32]. The present configuration for generating (A,λ,t) chromatograms (Fig. 1B) enables immediate post-run data processing to be performed by the HP 85 microcomputer and has been found to be fast, reliable and flexible in use.

It should perhaps be emphasised that the presentation of a three-dimensional projection is only one of many options open to the chromatographer for processing multichannel data. It can be argued that the availability of the (A,λ,t) data as a three-dimensional matrix in digital memory is in itself of greater potential usefulness than the pseudo-isometric projection. The data matrix can be sectioned at any time to give the absorption spectrum for qualitative characterisation or at any wavelength to generate the conventional elution profile. If required, the wavelength of the section can be varied as a function of time to present each peak at its optimum wavelength in the chromatogram. Moreover, the absorbance ratio can be calculated at any pair of wavelengths for each peak. The three-dimensional chromatogram is, however, an attractive and effective means of presenting complex qualitative relationships in a chromatogram. As such it will find use in the biomedical sciences for the characterisation of drugs and their metabolites. This application of three-dimensional chromatography has recently been elegantly confirmed in the screening of metabolites in body fluids [41, 42].

Spectral deconvolution of peaks overlapping in the time domain offers an additional technique for the characterisation of complex chromatograms, provided that the components are defined and available in spectral archive. Work continuing in our laboratory indicates that there is considerable potential in pharmaceutical analysis for deconvolution using zero order and higher derivative spectra [43]. Further benefits may be anticipated from the use of principal component analysis and pattern recognition techniques applied to

three-dimensional data generated in HPLC and TLC [44]. The combination of these computer-aided concepts with rapid-scanning multichannel detectors presages several new experimental strategies for chromatographic detection in the life sciences.

CONCLUSIONS

The optical multichannel detector offers a rapid and versatile approach to detection in HPLC and TLC. By combining retention time data with rapidly-scanned spectra captured during elution, drugs, their degradation products and metabolites can be characterised with greater specificity. Peak homogeneity can be examined by use of absorbance ratios and qualitative features can be enhanced by generating the second derivative of the captured UV spectrum. By monitoring the chromatogram at wavelengths selected according to known spectral characteristics, the quantitative performance of the detector can be optimised.

The acquisition of rapidly scanned spectral data during elution permits several new strategies to be developed for more flexible detection in HPLC. These include the presentation of three-dimensional projections of (A,λ,t) chromatograms for qualitatitive purposes and the optimised selection of detection wavelength after chromatographic separation.

Further developments in optical multichannel detectors based on 16-bit and 32-bit dedicated microcomputers will give faster data handling and greater sensitivity for bioanalytical applications. Such devices will enable digital data-bases of spectral and chromatographic data to be established and rapidly accessed for the application of pattern recognition algorithms and related techniques in the biomedical sciences.

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