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THE DEVELOPMENT OF A MULTIWAVELENGTH DETECTOR FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A relatively low cost multiwavelength ultra-violet absorbtion detector for high-performance liquid chromatography has been developed which allows up to four wavelengths to be monitored during an analysis. It is based on a rotating disc which supports four narrow bandpass interference filters. Some examples of the multiwavelength results indicate the potential of such an approach. The typical noise level (10^{-4} absorbance units) and detection limit ($\lesssim$ 1 ng) of the instrument are comparable to those of the more conventional single wavelength detectors.

INTRODUCTION

Traditionally ultra-violet (UV) absorption high-performance liquid chromatographic (HPLC) detectors have been based on a single wavelength design. Even with a manually tunable monochromator, only one wavelength can be monitored conveniently during a chromatogram. The results from such systems are totally dependent on retention time for identification, as is the validity of any subsequent quantitation. The obvious attractions of multiwavelength detection have been underlined by the reports of several workers using two single wavelength detectors in series[1–3]. The limitation of this approach is that to extend the scheme to three or more wavelengths becomes inefficient in terms of cost, space and chromatographic performance–which will degrade as a result of the band spreading introduced by each detector in turn.

By developing a single detector which could monitor up to four wavelengths it was felt that the two main advantages of multiwavelength detection would be achieved:

(i) More definitive identification of compounds based on retention times plus up to three absorbance ratios.

(ii) Absorbance ratios across a peak could be used to check peak homogeneity, *i.e.*, to confirm that what appears as a single peak on the chromatogram is not a composite of two or more unresolved compounds.

At least two commercial attempts have been made to produce multiwavelength

detectors[4], the final systems costing more than five times that of their single wavelength counterparts. The aim of this work was to produce a multiwavelength detector which would be much closer in price to the original single wavelength systems.

THE MULTIWAVELENGTH DETECTOR

The detector assembly

A schematic diagram of the detector is shown in fig. 1—further details of the individual components are given in Table I. The basic optical arrangement is similar to that of a single beam fixed wavelength detector. Light from a deuterium lamp is focused onto a narrow band pass interference filter; the transmitted radiation then passes through a HPLC flow cell behind which is positioned a photomultiplier. In this detector the normal static UV filter has been replaced by a disc which holds four interference filters (one in each quadrant). The disc is fixed to a stepping motor which rotates the filter assembly at 2 cycles per second. Thus with each completed revolution of the disc the photomultiplier sees a signal associated with each of the four filters in turn.

Signal monitoring

As each of the four filters comes into position in front of the flow cell one of the four aluminium flags attached to the edge of the disc triggers an optical light sensor (see fig. 2). There is an additional flag attached to the disc with its own independent optical sensor, and this is used as a reference point for the start of each rotation, thereby ensuring that the order in which the filters are monitored always follows the same sequence.

Separation and storage of the signals for each filter is achieved using a micro-

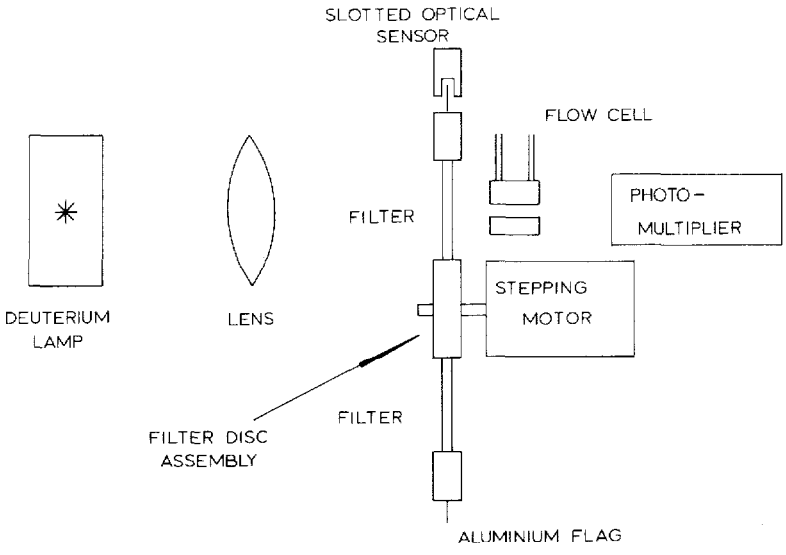

Fig. 1. A schematic diagram of the detector.

TABLE I

TECHNICAL DETAILS FOR THE DETECTOR

Light source:	Deuterium lamp	
Narrow bandpass interference filters:	λ_{max} (nm)	Half bandwidth (nm)
	217	20
	254	12
	268	15
	279	12
Diameter of filters:	32 mm	
Diameter of the rotating disc:	125 mm	
Photomultiplier tube:	R372	
Photomultiplier supply:	Brandenburg	Model 475R
Pre-amplifier:	National semiconductor	LM11
Logarithmic amplifier:	Burr Brown	4127
Optical sensors:	Radio Spares	304 506
Microcomputer:	Zilog 1/20A with a dual 8-in. floppy disc and printer 1 analogue/digital converter 12 bit 2 digital/analogue converter 12 bit	
Chart recorders:	Servoscribe Model RE 541.20	
Stepping motor and drive card:	PKS-Digiplan 1218-Stepping motor assembly	

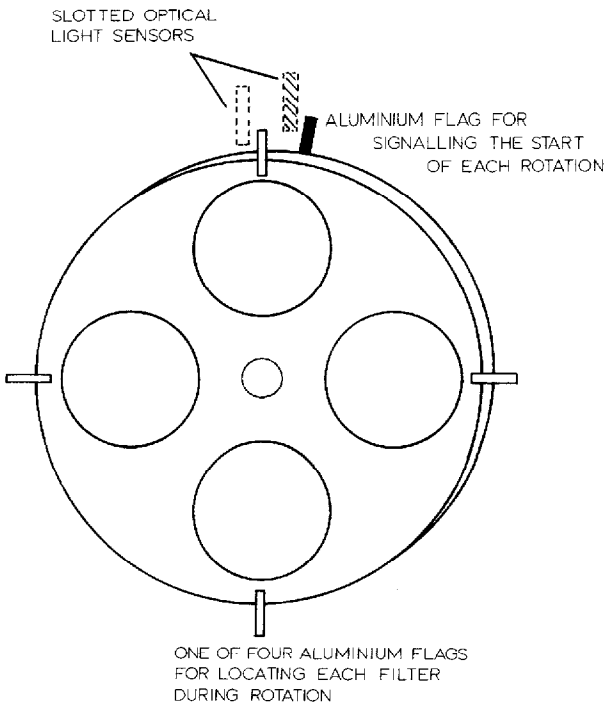

Fig. 2. Schematic diagram of the rotating disc holding four narrow bandpass filters.

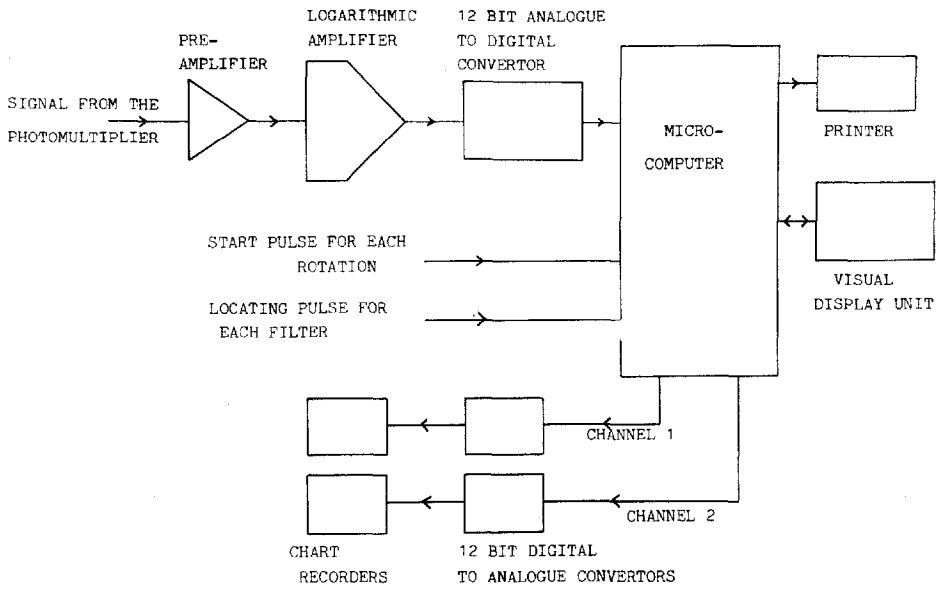

Fig. 3. The signal processing system.

computer. A diagram of the signal processing arrangement is shown in fig. 3. The initial signal from the photomultiplier is connected to a pre-amplifier stage before being linked to a logarithmic amplifier which gives an output proportional to absorbance. This final voltage is connected to an analogue to digital convertor before being linked to the microcomputer. The specific digital values which represent an absorbance reading for each filter are only accepted and stored by the microcomputer when it is triggered by a pulse from the appropriate optical sensor. In addition the "START" flag is used to ensure that the sequence in which the filter readings are sampled remains synchronised.

During analysis the microcomputer also allows signals to be re-monitored via two 12-bit digital to analogue convertors. Thus the digital values which represent the absorbance readings for any two of the four wavelengths can be selected and monitored on a pair of chart recorders to produce chromatograms in the normal manner. An additional option is for one these analogue lines to be used to plot a ratio of the absorbances for two selected filters—this again can be achieved during the analysis.

Processing the results

At the end of the analysis the absorbance values for peaks above a given pre-set threshold are calculated for all four wavelengths, together with the retention times. The absorbance measurements are based on a reduction of the digital data to a peak height measurement. The results for absorbance, retention time and ratioed absorbance are then presented in a tabulated form on the printer.

RESULTS

The results reported below have been divided into three sections. (The chromatographic conditions used in each of the three sections are given in Table II.

TABLE II

CHROMATOGRAPHIC CONDITIONS

Systems: I = MDA–nortriptylene; II = barbiturates; III = alprenolol–timolol ("beta blockers"). Amount injected: 20 μl using a Rheodyne valve.

	System I	*System II*	*System III*
Column:	15 cm × 1/4 in. O.D. Syloid 74 (non-baked)	10 cm × 1/4 in. O.D. Hypersil (5 μm) ODS	12.5 cm × 1/4 in. O.D. Spherisorb silica
Eluent:	0.01 *M* NH_4ClO_4–methanol (+ 1 ml 0.1 *M* NaOH in 1 l methanol), pH = 6.6–6.7	Methanol–water (40:60) (aqueous phase = 1.56 g $NaH_2PO_4 \cdot 2H_2O$ + 12–78 g Na_2HPO_4, pH = 8.5 in 1 l water)	As for I
Flow-rates	1 ml/min	2 ml/min	0.5 ml/min

Firstly a mixture of methylenedioxyamphetamine (MDA) and nortriptylene has been used to demonstrate the type of information that is made available with this multiwavelength detector. The detection limits of the detector have also been assessed under these same conditions.

Secondly the potential of the detector for identifying barbiturates (a group of drugs commonly encountered in the forensic laboratory) was tested. The basis for such identification relies heavily on the reproductibility of the absorbance ratios measured from the multiwavelength detector's signals.

Finally the detector was applied to two compounds which virtually co-elute under the prevailing chromatographic conditions—alprenolol and timolol, both of which are used as "beta blockers".

It was hoped that by monitoring absorbance ratios across the observed peaks it would be possible to differentiate between single and co-eluting compounds.

General performance of the multiwavelength detector

The chromatograms in Fig. 4 are for the analysis of a mixture of MDA and nortriptylene monitored at four separate wavelengths, corresponding to each of the four filters mounted on the rotating disc. The dramatic changes in relative peak heights that are observed indicate how much additional information can be made available by multiwavelength detection.

It was convenient to also assess the detection limits of the detector using the same chromatographic conditions. A comparison is presented of the results from the 254-nm filter with the disc stationary and with the disc rotating thereby assessing any degradation in performance in operating in the multiwavelength mode. The chromatograms in Fig. 5 show the performance for 10 ng and 1 ng injected of the MDA–nortriptylene mixture: (a) and (b) were recorded with the disc stationary; while (c) and (d) nominally represent the same chromatograms but with the disc rotating. The noise levels observed are similar with only a slight increase apparent in the multiwavelength mode.

This performance is equivalent to an approximate noise level of 10^{-4} absorbance units (a.u.), with a corresponding detection limit of about 1 ng. These values are similar to those quoted for single wavelength detectors.

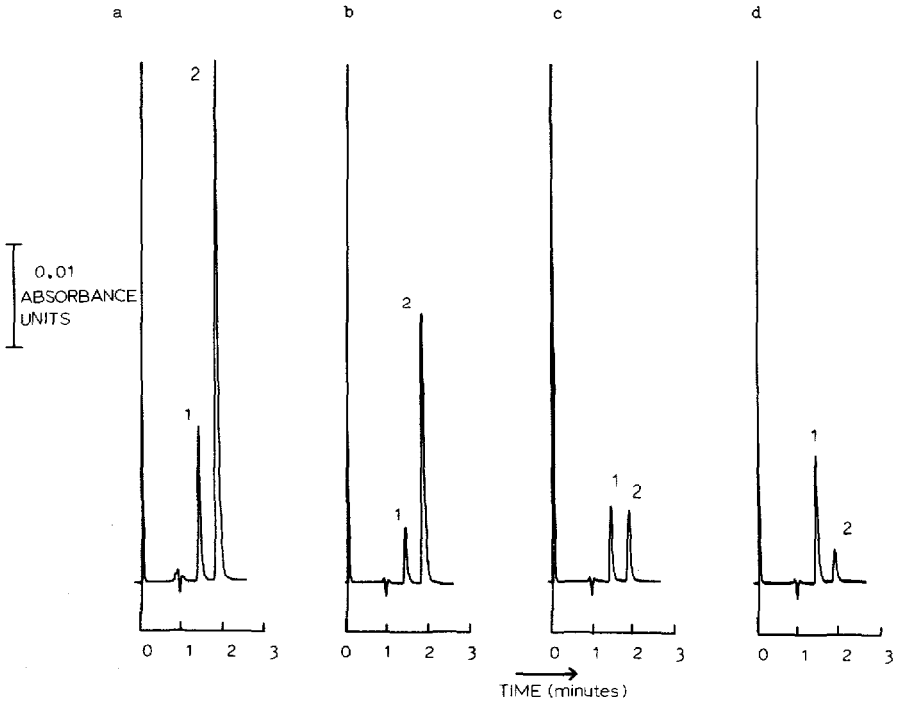

Fig. 4. Chromatograms of MDA (1) and nortriptylene (2) (100 ng each) monitored at four different wavelengths: 217 (a), 254 (b), 268 (c) and 279 nm (d)

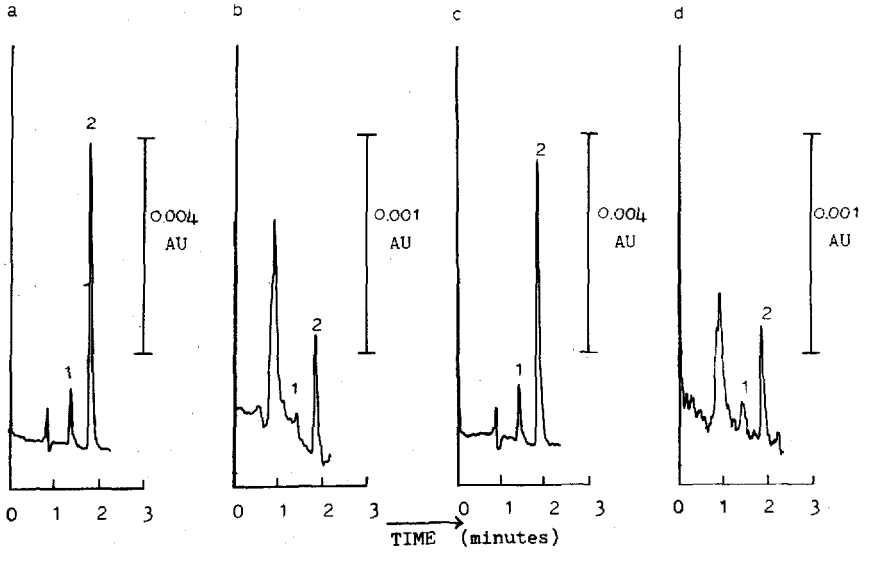

Fig. 5. Chromatograms showing the detection limit for the MDA (1)–nortriptylene (2) 254-nm filter Stationary disc: a, amount injected 10 ng; b, 1 ng. Rotating disc: c. amount injected 10 ng; d, 1 ng.

TABLE III

PRECISION OF THE ABSORBANCE RATIO MEASUREMENTS

Conditions	*No. of analyses*	*Relative standard deviations (%) for each ratio*		
		254:217	*268:217*	*279:217*
(a) Over 1 day	10	0.94	4.17	10.1
(b) Over 7 days	16	0.99	4.78	9.80
(c) Encompassing a 20-fold concentration range	6	2.26	3.57	8.76

Reproducibility of absorbance ratios

By analysing a mixture of three barbiturates (cyclobarbitone, butobarbitone and quinalbarbitone) the reproducibility of the absorbance ratios was measured. It is anticipated that the identification of any group of compounds using a multiwavelength HPLC detector will rely heavily on the differences between such ratios, hence the emphasis on reproducibility.

Table III summarises the standard deviations for the three ratios 254:217 (nm), 268:217 (nm) and 279:217 (nm)—for the three barbiturates, while retention times and absorbance ratios are given in Table IV.

The limiting factor on the short term precision for these ratios equates closely to the noise level of the detector (*i.e.*, 10^{-4} a.u.).

Comparisons of the relative standard deviations achieved over a 1-day and 7-day period, together with those obtained on standards encompassing a twenty fold difference in concentration suggest that a reproducibility of less than 5% (R.S.D.) should be routinely reached on peaks of > 0.002 a.u.

Profiling unresolved peaks

The absorbance ratios referred to in the previous section were all measured at the peak maximum. It possible with this detector to monitor a chosen ratio at frequent intervals as the peak elutes (although as the "wings" of the peak are reached the errors rapidly increase). By monitoring the ratio across the peak the analyst should be able to check that the peak does in fact represent a single compound.

An example of this application is shown in fig. 6. Chromatograms (a) and (b)

TABLE IV

RETENTION TIMES AND ABSORBANCE RATIOS

Measured under the conditions quoted in Table II, System II.

Compound	t_R *(min)*	*Absorbance ratio*		
		254:217	*268:217*	*279:217*
Cyclobarbitone	2.8	0.498	0.242	0.108
Butobarbitone	3.4	0.402	0.140	0.053
Quinalbarbitone	9.6	0.373	0.145	0.056

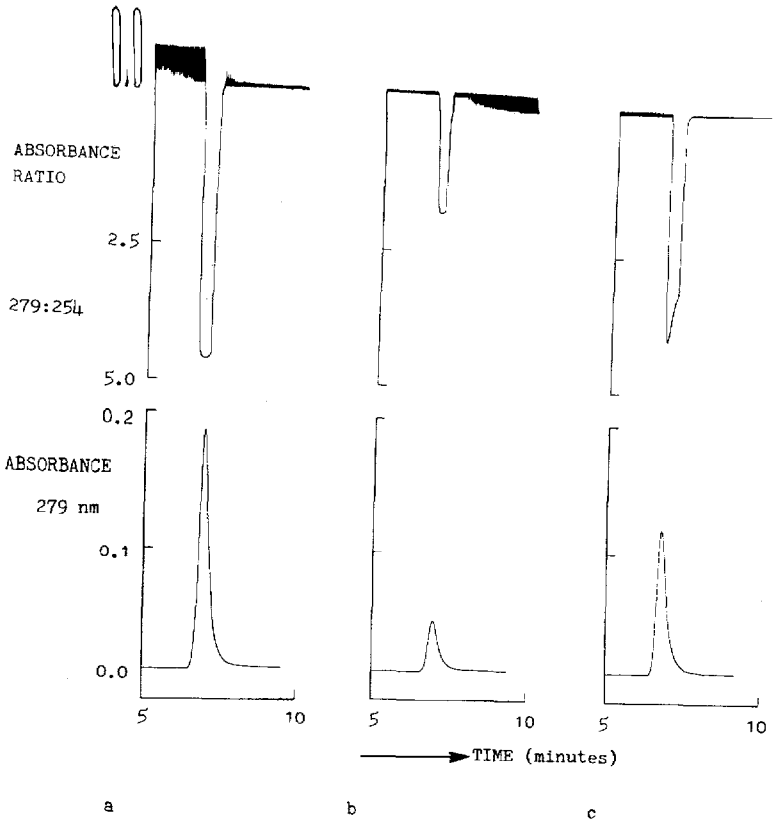

Fig. 6. An example of peak absorbance ratios monitored during the analysis of 2 μg timolol (a) 2 μg alprenolol (b) and 1 μg timolol + 1 μg alprenolol (c).

show the peaks for timolol and alprenolol monitored at 279 nm. Presented with these chromatograms is the plot of the 279:254 absorbance ratio. Within the limitations of the detector these ratio plots approximate to the expected square wave as the single compounds elute. The equivalent result for a mixture of the two compounds is shown in fig. 6c. Although the chromatogram monitored at 279 nm appears to show a single peak, the plot of the 279:254 absorbance ratio indicates a continuous change in value as the peak elutes, with no plateau region as was observed for the single component peaks. This result confirms that with the detector operating in a ratio mode it can provide a very quick and easy test for peak homogeneity.

CONCLUSIONS

The results presented here show that the multiwavelength detector, based on a rotating disc supporting four narrow bandpass filters, performs well in terms of both sensitivity and reproducibility when compared to the more standard single wavelength detectors. The additional information obtained by such a system will greatly enhance the identification of components which would otherwise be dependent on retention time data alone.

An alternative method of achieving a multiwavelength system could have been based on a photodiode array. However, such an approach would have increased the cost of the detector box by approximately a factor of five. A modification in which

the Zilog is replaced by the much less expensive PET (Commodore) microcomputer is nearing a successful completion. This will ensure that the final cost of this form of multiwavelength HPLC detection will be much closer in price to the original single wavelength design than any present alternative.

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