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Note

Evaluation of a rotating disc multiwavelength ultraviolet high-performance liquid chromatographic detector for the identification of barbiturates and other drugs

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In forensic science laboratories it is important to be able to identify drugs of abuse, and in a previous paper[1] it was shown that UV absorbance ratios and relative retention time data obtained from high-performance liquid chromatograms are excellent parameters for identification purposes, even for a large group of compounds such as the barbiturates, which have very similar UV profiles. Absorbance ratios at two different wavelengths can be measured by coupling two detectors in series as previously described[1], by simultaneously monitoring with a detector incorporating a linear diode array[2], or by carrying out rapid sequential monitoring with a rotating disc detector[3,4]. The latter form of detection was developed in this laboratory and this paper evaluates its performance in identifying barbiturates and other drugs.

EXPERIMENTAL

Solutions of barbiturates, a variety of other drugs found in association with them in commercial formulations, and a series of blind trial samples were examined under the following conditions.

Chromatographic conditions

An ACS pump (Model 400; Applied Chromatography Systems, Luton, U.K.) was used to deliver the eluent, methanol–phosphate buffer (40:60), at 2 ml min^{-1}. The phosphate buffer was prepared by weighing accurately sodium dihydrogen phosphate (1.56 g) and disodium hydrogen phosphate (12.78 g) into a volumetric flask (1 l) and making up to the mark with distilled water. The pH of the eluent as measured with a pH meter (Jenway, Essex, U.K.) was 8.4.

The analytical column was a 10 cm × 4.6 mm I.D. stainless-steel tube, slurry packed with 5-μm Hypersil ODS (Shandon Southern Products, Runcorn, U.K.), and thermostated at 30°C. Injections onto the column were via a Rheodyne valve (Rheodyne, CA, U.S.A.) fitted with a 20-μl loop. To prevent dissolution of silica in this column, a guard column (10 cm × 4.6 mm I.D. packed with silica) was placed between the pump and the injection valve.

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TABLE I

TECHNICAL DETAILS FOR THE DETECTOR

Narrow bandpass interference filters:	max. (nm)	Half bandwidth (nm)
	217	20
	240	12
	254	13
	265	12
Diameter of filters:	25 mm	
Diameter of rotating disc:	125 mm	
Microcomputer:	PET (Commodore) model 4032 with dual 5¼ disc drive Model 8050 Tracor printer Model 4022	
Programming language:	Basic-Pseudo compiled using PETSPEED (Oxford Computer Systems)	
Chart recorder:	Dual-pen Kipp & Zonen Model BD9	
Output channels to recorder:	Normally 217-nm and 240-nm signals	
Rotation speed of filter disc:	2 cps	

Detector conditions

The rotating disc detector is essentially as described in previous reports[3,4]. Details of the narrow bandpass interference filters and other technical information are given in Table I.

For each rotation of the disc the few values representing the absorbances at each filter were extracted by the machine code programmed interface and stored in the PET computer. Details of this interface system have been reported in a separate publication[4].

During the chromatographic run signals from two wavelengths (usually 217 and 240 nm) were continuously plotted on a chart recorder to yield normal chromatograms. On completion of a run the microcomputer examined the stored data from all four wavelengths, determined where peak maxima occurred, measured the absorbance at the maxima and ratioed these values. Absorbances, absorbance ratios, and retention times of each chromatographic peak were then listed with the aid of a printer. The data from a 20-min chromatographic run took less than 2 min to be processed.

RESULTS AND DISCUUSSIOON

Choice of test compounds and chromatographic conditions

Barbiturates were selected for an in-depth study as they represent a group of compounds which are subject to abuse and have closely similar UV profiles. They were also studied earlier by using two detectors mounted in series[1] and hence data from both experiments are comparable. Since carrying out the earlier investigation we have changed chromatographic conditions to use a commercially available packing material and eluent conditions similar to those used by Gill *et al.*[5] in their barbiturate study.

Importance of eluent pH control

In carrying out this work it was noticed initially that when different batches

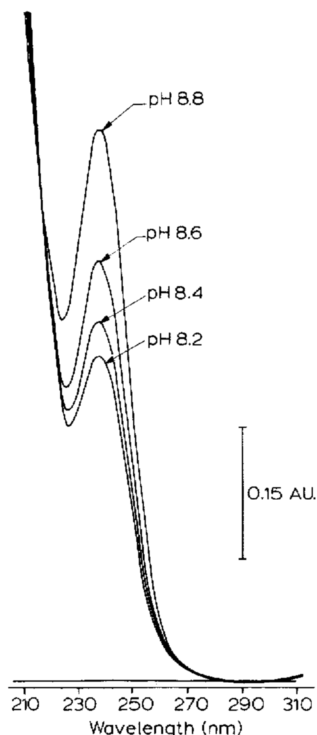

Fig. 1. Effect of eluent pH on the UV absorbance profile of butobarbitone.

of eluent were prepared with the same nominal pH, as measured by a pH meter, some variation in the absorbance ratio of a given barbiturate would occur. This was particularly noticeable with the A_{217}/A_{254} ratio. Fig. 1 shows the way in which the absorbance spectrum of a typical barbiturate (butobarbitone) varies as a function of pH in the range pH 8.2–8.8. Absorbance values abstracted from Fig. 1 were used to derive the ratios shown in Table II and these confirm that the A_{217}/A_{254} ratio is very sensitive to pH.

In order to achieve acceptable pH control batches it was found necessary to accurately weigh out the components of the buffer solution as described in the experimental section.

TABLE II

EFFECT OF ELUENT pH ON ABSORBANCE RATIOS OF BUTOBARBITONE

Eluent pH	*Absorbance ratios*	
	A_{217}/A_{254}	A_{240}/A_{254}
8.2	4.24	3.15
8.4	4.10	3.38
8.6	3.41	3.28
8.8	2.36	3.29

TABLE III

PRECISION OF RELATIVE RETENTION TIME AND ABSORBANCE RATIOS

Precision determined from fourteen analyses over a period of four weeks.

	Relative retention time	*Absorbance ratio*		
		A_{217}/A_{254}	A_{240}/A_{254}	A_{265}/A_{254}
Average relative standard deviation (%)	0.63	1.55	1.08	7.98

Long-term precision of absence ratios, retention times, detector linearity and barbiturate discrimination

To utilise the chromatographic parameters determined by the rotating disc detector for qualitative identification some knowledge of their variability is necessary. Table III summarises results obtained and by comparison with the values obtained in the earlier study[1] it will be noted that improvements have been made.

Of the three absorbance ratios used A_{265}/A_{254} gave the lowest precision; this can be attributed to the fact that the test compounds displayed very low absorbances at 265 nm with most values recorded being just greater than the noise level of the detector, *i.e. ca.* $1 \cdot 10^{-4}$ AU. The linearity of the detector at all four wavelengths was very good with the response of the test compounds being linear over a range of 0–5 μg which encompassed an absorbance range of 0–0.5 AU. Because of the improved

TABLE IV

RELATIVE RETENTION TIME (*RRT*) DATA AND ABSORBANCE RATIOS FOR OTHER DRUGS INCLUDED IN BARBITURATE FORMULATIONS

RRT data measured with respect to heptabarbitone (4.8 min).

Drug	*RRT*	*Absorbance ratio*		
		A_{217}/A_{254}	A_{240}/A_{254}	A_{265}/A_{254}
Paracetamol	0.24	0.81	1.00	0.40
Theophylline	0.25	1.58	0.82	1.83
Theobromine	0.25	1.43	0.83	1.69
Orciprenaline	0.26	> 10.0	1.39	2.49
Caffeine	0.32	1.68	0.86	1.80
Phenacetin	0.65	0.74	0.98	0.39
Bromvaletone	0.67	6.58	1.27	0.28
Chlormezanone	0.68	> 10.0	4.47	0.54
Ephedrine	0.77	> 10.0	0.72	0.47
Hyoscine	0.81	> 10.0	1.28	0.46
Mephensin	0.83	7.45	0.78	2.26
Phenytoin	1.29	7.20	1.64	0.21
Carbromal	1.31	5.60	1.24	0.33
Mepenzolate bromide	3.83	Very broad peak		
Ergotamine	Not detected			
Thenyldiamine	Not detected			
Reserpine	Not detected			

precision of the rotating disc detector both in the long and short term it was found possible to characterise all of the barbiturates examined. However, if a mixture of co-eluting compounds with very similar ratios was examined, *e.g.* allobarbitone and phenobarbitone, it was impossible to verify that two components were present. This situation would occur with any multiwavelength detection system, and only a change of chromatographic conditions would solve this problem.

Chromatographic characteristics of other drugs, and blind trials

Barbiturates often occur in admixture with other drugs and Table IV presents the chromatographic features of a number of such compounds. Although several of these co-elute with the barbiturates the very considerable differences in absorbance ratios permitted discrimination. These results confirmed the potential value of absorbance rationing for qualitative analysis.

Blind trials with several current barbiturate formulations led to the correct identification of component barbiturates without having to perform any extraction prior to their analysis. The average time taken to analyse any one of these samples was about 35 min. An example of the results obtained by the analysis of Tuinal, *i.e.*, a commercial formulation, is illustrated in Fig. 2.

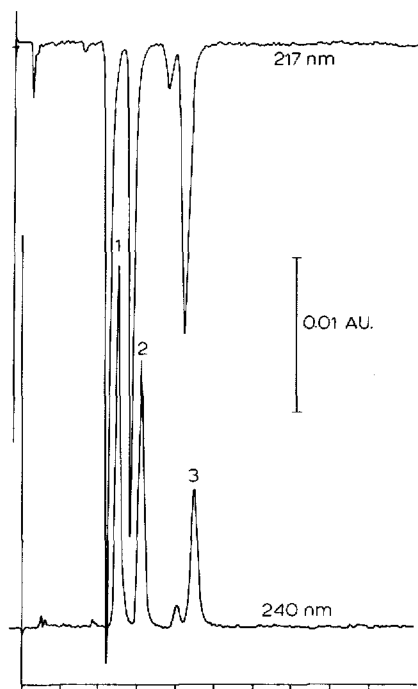

Fig. 2. Analysis of a barbiturate formulation (Tuinal). Peaks: 1, heptabarbitone (standard added for calculation of relative retention times); 2, amylobarbitone; 3, quinalbarbitone. Time interval 2 min, and all other conditions as stated in text.

CONCLUSIONS

From this trial it has been established that some of the disadvantages experienced with obtaining absorbance ratios from two UV detectors coupled together in series can be minimised with this rotating disc multiwavelength detector. Apart from reduced costs and analysis time, and improvements in chromatographic performances, this multiwavelength detector offers excellent reproducibility. In analyses where UV profiles of compounds vary with pH (*e.g.* barbiturates) enhanced long-term reproducibility can be achieved by controlling accurately the pH of the eluent.

The 28 barbiturates studied could all be positively identified using absorbance ratios and relative retention time data generated with this detector. Furthermore, barbiturates could be correctly identified in formulations without involving any extraction procedure. Other drugs if present in these formulations could be identified thus reflecting that the four filter wavelengths chosen are sufficient for discriminating between a large range of drugs.

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