Journal of Chromatography, 200 (1980) 271–276
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,143

Note

Use of dual-wavelength UV detection in high-performance liquid chromatography for the identification of barbiturates

P. C. WHITE

Metropolitan Police Forensic Science Laboratory, 109 Lambeth Road, London SE1 7LP (Great Britain)

(Received July 14th, 1980)

Barbiturates are a class of drug that are widely abused, and the need to identify them in forensic science laboratories is becoming increasely important. Current methods of analysis by gas–liquid chromatography[1,2] and high-performance liquid chromatography (HPLC)[3,4] although being capable of appreciable sub-classification of this group of drugs do not provide unequivocal characterisation of all of them. Mass spectrometry[5–7] and nuclear magnetic resonance spectroscopy[8–10] have been used but involve expensive instrumentation, and are only effective when used in conjunction with separative techniques.

For routine screening purposes, therefore, it would be very useful if the discriminating power of the chromatographic techniques could be improved. Absorbance ratios using two detectors in series have been used in this laboratory to improve identification in HPLC separations[11]. In a recent paper[12] this technique was employed to study a large number of drugs including seventeen barbiturates, by using dual-wavelength detection at 254 and 280 nm. However the wavelengths chosen were not the most appropriate for barbiturate analysis, and the long term reproducibility of the results was poor. The method reported here shows that the combined use of absorbance ratios A_{220}/A_{254} and A_{240}/A_{254} give greater discrimination, and that together with retention data it is possible to positively identify 27 out of 29 barbiturates.

EXPERIMENTAL

An Altex pump (Altex Scientific, CA, U.S.A.) was used to deliver solvent, methanol–0.1% aqueous ammonium carbonate (40:60), at 1.5 ml min^{-1}. The analytical column was a 12.5 cm × 4.6 mm I.D. stainless-steel tube, slurry-packed with octadecyltrichlorosilylated silica (16% loading; particle size 3–7 μm; as prepared by a method previously described[13]). Injections were made onto an 8 μm stainless-steel gauze at the top of the column, via a "T"-piece stop-flow system as described by Wheals[14]. To prevent dissolution[15,16] of the material in this column and thereby enable reproducible retention data to be obtained over a long period of time, a guard column (20 cm × 4.6 mm I.D., packed with silica) was placed between the pump and the injection port. The eluent was monitored at 220 or 240 nm by a variable-wavelength UV detector (CE 2012, Cecil, Cambridge, Great Britain, or a Spectro-Monitor III, LDC, Riviera Beach, FL, U.S.A.), and at 254 nm with a fixed-wavelength

0021-9673/80/0000–0000/$02.25

UV detector (UV Monitor III, LDC). The detectors were coupled together by a short length of PTFE micr›bore tubing, and the chromatograms were recorded simultaneously.

Barbiturate standards were prepared by dissolving approximately 1 mg of the drug in 1 ml of methanol–2 *N* ammonium hydroxide–1 *N* ammonium nitrate (27:2:1).

RESULTS AND DISCUSSION

The precision of relative retention time (RRT) data and of absorbance ratios was calculated for a mixture containing six barbiturates, over both short and long term periods of analysis. The average relative standard deviations are summarised in Table I.

TABLE I

PRECISION OF LONG AND SHORT RETENTION TIME AND ABSORBANCE MEASUREMENTS

Precision of retention time data determined from each set of twenty measurements; precision of absorbance ratio data determined from each set of ten measurements.

Period of analysis	*Average relative standard deviation (%)*			
	Retention time		*Absorbance ratio*	
	Absolute	*Relative*	A_{240}/A_{254}	A_{220}/A_{254}
One day	0.58	0.59	0.92	1.62
Six weeks	2.82	1.63	2.06	3.97

Equivalent results were obtained when either of the variable wavelength detectors were employed, or when other columns of the same packed material were used.

The structures together with the names of all the barbiturates that were analysed are listed in Table II.

The relative retention times (RRT) and absorbance ratio measurements that were obtained from the analysis of these barbiturates are given in Table III.

The reproducibility results showed that for any particular set of determinations there were only very small variations in the standard deviation (σ), and therefore a value of $\pm 4\sigma$ was imposed on all the results to determine if the barbiturates were separated. From the data given in Table III and by applying the standard deviations for the short and long term reproducibilities, barbiturates were identified by comparing either the relative retention times or a combination of these and the absorbance ratios. When based upon this method of identification certain barbiturates could not be positively identified and these are listed in Table IV.

However the results clearly show even when the largest standard deviation is applied, that the use of the RRT data and two absorbance ratios enabled all the barbiturates to be identified positively, with the exception of butalbital and butobarbitone.

The A_{240}/A_{220} ratio calculated from the A_{220}/A_{254} and A_{240}/A_{254} ratios (Table III) was found to give additional information regarding the structures of some of the

TABLE II

NAMES AND STRUCTURES OF BARBITURATES ANALYSED

Y=O unless otherwise stated; R=H unless otherwise stated.

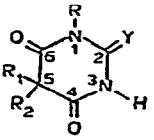
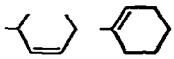
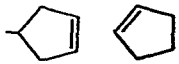
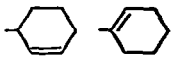
Barbiturate 	R_1	R_2
Allobarbitone	$CH_2 \cdot CH=CH_2$	$CH_2 \cdot CH=CH_2$
Amylobarbitone	$CH_2CH_2CH(CH_3)_2$	CH_2CH_3
Aprobarbitone	$CH(CH_3)_2$	$CH_2 \cdot CH=CH_2$
Barbitone	CH_2CH_3	CH_2CH_3
Butalbital	$CH_2CH(CH_3)_2$	$CH_2 \cdot CH=CH_2$
Butobarbitone	$CH_2CH_2CH_2CH_3$	CH_2CH_3
Cyclobarbitone		CH_2CH_3
Cyclopentobarbitone		$CH_2 \cdot CH=CH_2$
Enallylpropymal (R=CH_3)	$CH(CH_3)_2$	$CH_2 \cdot CH=CH_2$
Heptabarbitone		CH_2CH_3
Hexethal	$CH_2CH_2CH_2CH_2CH_2CH_3$	CH_2CH_3
Hexobarbitone (R=CH_3)		CH_3
Ibomal	$CH_2 \cdot C(Br)=CH_2$	$CH(CH_3)_2$
Idobutal	$CH_2CH_2CH_2CH_3$	$CH_2 \cdot CH=CH_2$
Metharbitone (R=CH_3)	CH_2CH_3	CH_2CH_3
Methohexitone (R=CH_3)	$CH(CH_3)C \equiv CCH_2CH_3$	$CH_2 \cdot CH=CH_2$
Methylphenobarbitone (R=CH_3)	C_6H_5	CH_2CH_3
Nealbarbitone	$CH_2 \cdot C(CH_3)_3$	$CH_2 \cdot CH=CH_2$
Pentobarbitone	$CH(CH_3)CH_2CH_2CH_3$	CH_2CH_3
Phenobarbitone	C_6H_5	CH_2CH_3
Phenylmethylbarbitone	C_6H_5	CH_3
Probarbitone	$CH(CH_3)_2$	CH_2CH_3
Quinalbarbitone	$CH(CH_3)CH_2CH_2CH_3$	$CH_2 \cdot CH=CH_2$
sec.-Butobarbitone	$CH(CH_3)CH_2CH_3$	CH_2CH_3
Sigmodal	$CH_2 \cdot C(Br)=CH_2$	$CH(CH_3)CH_2CH_2CH_3$
Talbutal	$CH(CH_3)CH_2CH_3$	$CH_2 \cdot CH=CH_2$
Thialbarbitone (Y=S)		$CH_2 \cdot CH=CH_2$
Thiopentone (Y=S)	$CH(CH_3)CH_2CH_2CH_3$	CH_2CH_3
Vinbarbitone	$C(CH_3)=CHCH_2CH_3$	CH_2CH_3

TABLE III

RELATIVE RETENTION DATA AND ABSORBANCE RATIOS

RRT data were measured with respect to heptabarbitone.

Barbiturate	*RRT*	*Absorbance ratio*		
		A_{240}/A_{254}	A_{220}/A_{254}	A_{240}/A_{220}
Barbitone	0.44	6.25	5.68	1.10
Phenylmethylbarbitone	0.47	4.46	5.21	0.86
Phenobarbitone	0.49	4.46	4.17	1.07
Allobarbitone	0.53	4.81	4.17	1.15
Probarbitone	0.62	5.21	6.25	0.82
Vinbarbitone	0.67	4.17	3.47	1.20
Aprobarbitone	0.71	4.17	4.81	0.87
Cyclobarbitone	0.71	3.68	3.29	1.12
Metharbarbitone	0.73	3.13	6.94	0.45
Ibomal	0.74	3.47	3.91	0.89
Butalbital	0.87	4.46	4.17	1.07
Butobarbitone	0.87	4.46	4.17	1.07
sec.-Butobarbitone	0.93	4.17	4.81	0.87
Methylphenylbarbitone	0.94	2.08	3.29	0.63
Cyclopentylallobarbitone	0.95	3.13	3.47	0.90
Heptabarbitone (4.30 min)	1.00	3.47	3.47	1.00
Idobutal	1.00	4.17	3.91	1.07
Talbutal	1.12	3.68	3.68	1.00
Thialbarbitone	1.14	0.91	0.66	1.36
Nealbarbitone	1.25	3.29	3.29	1.00
Hexobarbitone	1.35	2.40	4.81	0.50
Amylobarbitone	1.35	3.91	3.47	1.12
Thiopentone	1.43	1.01	0.58	1.73
Enallylpropymal	1.49	2.23	5.21	0.43
Pentobarbitone	1.58	3.29	3.91	0.84
Quinalbarbitone	1.95	2.98	3.29	0.91
Sigmodal	2.00	2.31	3.13	0.74
Hexethal	3.22	3.47	3.47	1.00
Methohexitone	3.31	1.95	4.81	0.41

TABLE IV

BARBITURATE IDENTIFICATION

Method of identification	*Standard deviation applied*	*Barbiturates not positively identified*
RRT	Short term	Phenylmethylbarbitone, phenobarbitone, aprobarbitone, cyclobarbitone, metharbarbitone, ibomal, butalbital, butobarbitone, *sec.*-butobarbitone, methylphenylbarbitone, cyclopentylallobarbitone, heptabarbitone, idobutal, talbutal, thialbarbitone, hexobarbitone, amylobarbitone
	Long term	As for short term plus barbitone, allobarbitone and vinbarbitone
RRT + A_{220}/A_{254} ratio	Short term	Butalbital, butobarbitone, methylphenylbarbitone, cyclopentylallobarbitone
	Long term	Barbitone, phenylmethylbarbitone, allobarbitone, butalbital, butobarbitone, methylphenylbarbitone, cyclopentylallobarbitone
RRT + A_{220}/A_{254} and A_{240}/A_{254} ratios	Short term	Butalbital, butobarbitone
	Long term	Butalbital, butobarbitone

barbiturates. The two compounds with very high ratios were those that contained sulphur, namely, thialbarbitone (ratio 1.36) and thiopentone (1.73). The 1-methyl substituted barbiturates gave low ratios *i.e.*, methohexitone (0.41), enallylpropymal (0.43), metharbarbitone (0.45), hexobarbitone (0.50) and methylphenylbarbitone (0.63). Chromatograms illustrating the variations in absorbance of the 1-methyl substituted and thiobarbiturates are shown in Figs. 1 and 2, respectively, and to show how significantly different these barbiturates were from those not belonging to either of these groups heptabarbitone and butobarbitone were included in the mixtures.

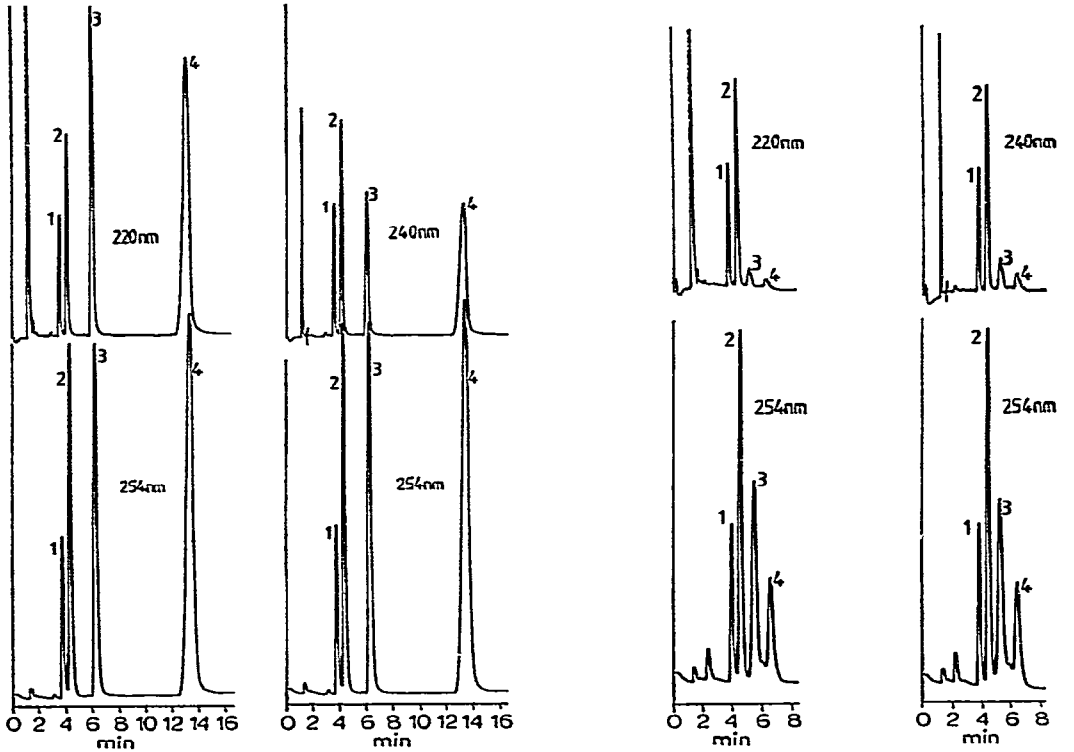


Fig. 1. Chromatograms illustrating the variations in absorbance of some 1-methyl substituted barbiturates at various wavelengths. Peaks: 1 = butobarbitone, 2 = heptabarbitone, 3 = enallylpropymal and 4 = methohexitone. Sensitivity at 254 nm, 0.032 a.u.f.s. and at 220 and 240 nm, 0.2 a.u.f.s. Other conditions as stated in text.

Fig. 2. Chromatograms illustrating the variations in absorbance of some thiobarbiturates at various wavelengths. Peaks: 1 = butobarbitone, 2 = heptabarbitone, 3 = thialbarbitone and 4 = thiopentone. Sensitivity at 254 nm, 0.032 a.u.f.s. and at 220 and 240 nm, 0.2 a.u.f.s. Other conditions as stated in text.

CONCLUSIONS

The use of dual wavelength UV detection in conjunction with HPLC affords an excellent method for the discrimination of barbiturates. Provided that a guard

column is incorporated between the pump and the injection system, reproducible results can be obtained. By combining the retention data and the A_{220}/A_{254} and A_{240}/A_{254} absorbance ratios, 27 barbiturates could be positively identified, even over extended periods of analysis. Butalbital and butobarbitone could not be positively identified. The ratio of the A_{220}/A_{254} and A_{240}/A_{254} ratios give useful structural information which enabled the thiobarbiturates and the 1-methyl substituted barbiturates to be identified.

ACKNOWLEDGEMENT

I would like to thank I. Jane for all his helpful advice.

REFERENCES

1 E. G. C. Clarke, *Isolation and Identification of Drugs*, The Pharmaceutical Press, London, 1969.
2 D. J. Berry, *J. Chromatogr.*, 86 (1973) 89.
3 U. R. Tjaden, J. C. Kraak and J. F. K. Huber, *J. Chromatogr.*, 143 (1977) 183.
4 R. Gill and A. C. Moffat, *Report No. 324*, HOCRE, Aldermaston, 1979.
5 W. Arnold and H. F. Grutzmacher, *Tetrahedron Lett.*, (1966) 1365.
6 G. Rücker and G. Boln, *Arch. Toxicol.*, 25 (1969).
7 L. V. Jones and M. J. Whitehouse, in preparation.
8 G. A. Neville and H. W. Avdovich, *Can. J. Pharm. Sci.*, 4 (1969) 51.
9 G. Rücker, *Arch. Pharm.*, 299 (1966) 688.
10 G. Döring and H. Lackner, *Arch. Toxicol.*, 26 (1970) 220.
11 R. N. Smith and M. Zetlein, *J. Chromatogr.*, 130 (1977) 314.
12 J. K. Baker, R. E. Skelton and Cheng-Yu Ma, *J. Chromatogr.*, 168 (1979) 417.
13 B. B. Wheals, *J. Liquid Chromatogr.*, 2 (1979) 91.
14 B. B. Wheals, *J. Chromatogr.*, 122 (1976) 85.
15 A. Wehrli, J. C. Hildenbrand, H. P. Keller, R. Stampfli and R. W. Frei, *J. Chromatogr.*, 149 (1978) 199.
16 J. G. Atwood, G. J. Schmidt and W. Slavin, *J. Chromatogr.*, 171 (1979) 109.