Journal of Chromatography, 301 (1984) 165–172
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,960

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY RETENTION DATA FOR 84 BASIC DRUGS OF FORENSIC INTEREST ON A SILICA COLUMN USING AN AQUEOUS METHANOL ELUENT

B. LAW, R. GILL* and A. C. MOFFAT
Central Research Establishment, Home Office Forensic Science Service, Aldermaston, Reading, Berkshire RG7 4PN (U.K.)
(Received June 12th, 1984)

SUMMARY

High-performance liquid chromatography retention characteristics have been measured for 84 basic drugs of forensic interest using a silica column with a methanol–aqueous ammonium nitrate eluent. The drugs are from two classes of major interest, namely, the narcotic analgesics (including antagonists, metabolites and analogues) and drugs structurally and pharmacologically related to amphetamine.

INTRODUCTION

The application of chromatographic methods to the identification of drugs can only be effected if suitable reference data are available. In the areas of thin-layer chromatography (TLC) and gas–liquid chromatography (GLC) where standardised systems are in common use, large collections of TLC[1] and GLC[2] retention data for compounds encountered in forensic analysis have been generated. The production of comparable data for high-performance liquid chromatography (HPLC) has been hampered by a number of factors *viz.* the wide range of HPLC systems in current use, the lack of a universal method for retention measurement and the wide variation in the properties of HPLC packing materials of the same type from different manufacturers.

The use of silica HPLC columns with polar eluents (aqueous methanol containing ammonia and ammonium salts in particular) has become widespread in forensic laboratories for the screening and quantitative analysis of basic drugs. These HPLC systems were first introduced by Jane[3] and although the mechanisms of separation are poorly understood they have found wide applicability for the analysis of drugs in biological fluids and the examination of illicit drug preparations. Applications include tricyclic antidepressants[4], morphine[5,6], LSD[7], amiodarone[8] and its metabolite[9], quinidine[10], nitrazepam[11] and a wide range of other basic drugs[12–16]. Unfortunately, the retention data reported in the literature for these systems have been obtained using a variety of silica packing materials, including some which were home-made. The U.K. Forensic Science Laboratories have recently adopted Spher-

isorb S5W as the standard silica packing and this development has prompted us to obtain retention data on this material using the most common eluent, methanol–aqueous ammonium nitrate buffer pH 10.1, (9:1, v/v). Retention characteristics have been measured for 84 drug compounds of forensic interest falling into two broad classes. The first group is formed by the narcotic analgesics and includes antagonists, metabolites and related compounds while the second consists of the structural and pharmacological analogues of amphetamine which includes stimulants, hallucinogens, sympathomimetics and putrefactive amines.

EXPERIMENTAL

Chromatography was carried out using a Waters 6000A HPLC pump which was used to deliver eluent to a 250 × 5 mm I.D. stainless-steel column packed with Spherisorb S5W (Phase Separations, Queensferry, U.K.). The column was packed using a slurry procedure with methanol as the dispersing and pressurising solvent. Because of the alkaline nature of the eluent, a short column, dry packed with silica (40 μm), was included between the pump and injector to minimise dissolution of the analytical packing material. Injections were made with a Rheodyne 7125 injection valve fitted with a 20-μl sample loop. Detection was carried out at 254 nm using a Perkin-Elmer LC75 spectrophotometric detector.

The eluent consisted of methanol–aqueous ammonium nitrate buffer (9:1, v/v). The buffer was prepared by adding 94 ml ammonia (35%, w/w) (AnalaR grade, BDH, Poole, U.K.) and 21.5 ml nitric acid (70%, w/w) (AristaR grade, BDH) to 884 ml water and then adjusting the pH to 10.1 with ammonia. The flow-rate was 2 ml/min throughout. This eluent is as used by the Metropolitan Police Forensic Science Laboratory[17].

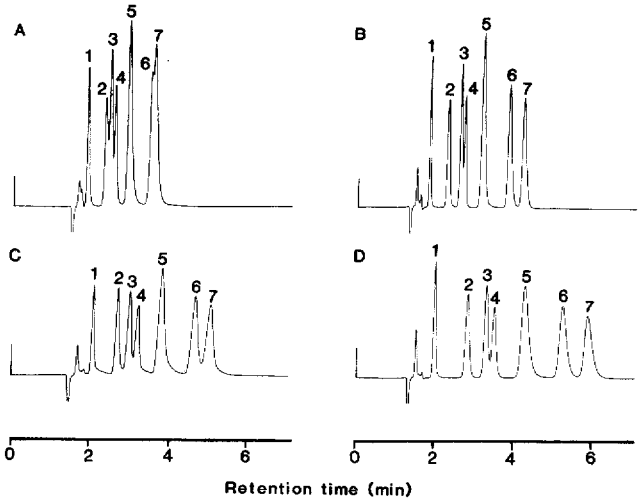


Fig. 1. Chromatograms showing the separation of seven basic drugs on four different commercial brands of HPLC silica (A, Hypersil; B, Spherisorb S5W; C, Nucleosil 50-5; D, Zorbax BP-SIL). All columns 250 × 5 mm I.D. Eluent: methanol–ammonium nitrate buffer pH 10.1 (9:1, v/v). Flow-rate: 2 ml/min. Detection: 254 nm (0.16 a.u.f.s.). Peaks: 1 = phendimetrazine; 2 = phenylpropanolamine; 3 = phentermine; 4 = amphetamine; 5 = morphine; 6 = ephedrine; 7 = methylamphetamine.

Drugs were obtained from the collection at the Central Research Establishment, Home Office Forensic Science Service and were dissolved in methanol at *ca.* 1 mg/ml with a trace of dilute acid where necessary to aid dissolution. Approximately

TABLE I

HPLC RETENTION DATA FOR NARCOTIC ANALGESICS AND RELATED COMPOUNDS (ARRANGED IN ALPHABETICAL ORDER)

Compound	*Retention time (min)*	*Capacity factor (k')*
Acetylcodeine	2.35	0.78
Benzylmorphine	2.69	1.03
Buprenorphine	1.39	0.05
Codeine	2.92	1.21
Codeine-N-oxide	2.98	1.26
Dextromoramide	1.44	0.09
Dextropropoxyphene	1.57	0.19
Diamorphine	2.19	0.66
Dihydrocodeine	4.62	2.50
Dihydromorphine	4.95	2.75
Dipipanone	2.12	1.61
Ethoheptazine	3.36	1.55
Ethylmorphine	2.74	1.06
Etorphine	1.46	1.11
Fentanyl	1.46	1.11
Hydrocodone	4.18	2.17
Hydroxypethidine	2.00	0.51
Levallorphan	1.93	1.46
Levorphanol	5.55	3.20
Methadone	2.68	1.03
6-Monoacetylmorphine	2.37	0.80
Morphine	3.03	1.30
Morphine-3-glucuronide	3.38	1.56
Morphine-N-oxide	2.88	1.18
Nalorphine	1.70	0.29
Naloxone	1.55	0.17
Norcodeine	5.95	3.51
Norlevorphanol	2.68	1.03
Normethadone	2.02	0.53
Normorphine	6.50	3.92
Norpethidine	4.01	2.04
Norpipanone	1.78	0.35
Noscapine	1.52	0.15
Oxycodone	2.44*	0.85*
Papaverine	1.53	0.16
Pentazocine	2.21	0.67
Pethidine	2.05	0.55
Phenazocine	1.72	0.30
Phenoperidine	1.45	0.10
Pholcodine	3.47	1.63
Piritramide	1.51	0.14
Thebacon	2.44	0.85
Thebaine	2.56	0.94

* Tailing peak.

0.5–5-μl samples were injected. The void volume was determined by injecting 10 μl of aqueous ethanol (50:50, v/v) and this value was used to calculate the capacity factors (k') of the compounds injected.

TABLE II

HPLC RETENTION DATA FOR NARCOTIC ANALGESICS AND RELATED COMPOUNDS (ARRANGED IN ORDER OF INCREASING RETENTION TIME)

Compound	*Retention time (min)*	*Capacity factor (k')*
Buprenorphine	1.39	0.05
Dextromoramide	1.44	0.09
Phenoperidine	1.45	0.10
Fentanyl	1.46	0.11
Etorphine	1.46	0.11
Piritramide	1.51	0.14
Noscapine	1.52	0.15
Papaverine	1.53	0.16
Naloxone	1.55	0.17
Dextropropoxyphene	1.57	0.19
Nalorphine	1.70	0.29
Phenazocine	1.72	0.30
Norpipanone	1.78	0.35
Levallorphan	1.93	0.46
Hydroxypethidine	2.00	0.51
Normethadone	2.02	0.53
Pethidine	2.05	0.55
Dipipanone	2.12	0.61
Diamorphine	2.19	0.66
Pentazocine	2.21	0.67
Acetylcodeine	2.35	0.78
6-Monoacetylmorphine	2.37	0.80
Thebacon	2.44	0.85
Oxycodone	2.44*	0.85*
Thebaine	2.56	0.94
Norlevorphanol	2.68	1.03
Methadone	2.68	1.03
Benzylmorphine	2.69	1.03
Ethylmorphine	2.74	1.06
Morphine-N-oxide	2.88	1.18
Codeine	2.92	1.21
Codeine-N-oxide	2.98	1.26
Morphine	3.03	1.30
Ethoheptazine	3.36	1.55
Morphine-3-glucuronide	3.38	1.56
Pholcodine	3.47	1.63
Norpethidine	4.01	2.04
Hydrocodone	4.18	2.17
Dihydrocodeine	4.62	2.50
Dihydromorphine	4.95	2.75
Levorphanol	5.55	3.20
Norcodeine	5.95	3.51
Normorphine	6.50	3.92

* Tailing peak.

RESULTS AND DISCUSSION

It is well known that different commercial brands of HPLC silica can give rise to widely differing separations. This is well illustrated in Fig. 1 which shows the

TABLE III

HPLC RETENTION DATA FOR AMPHETAMINE AND ITS STRUCTURAL AND PHARMACOLOGICAL ANALOGUES (ARRANGED IN ALPHABETICAL ORDER)

NE = Not eluted.

Compound	*Retention time (min)*	*Capacity factor (k')*
Adrenaline	2.15*	0.63*
Amphetamine	2.62	0.98
Bromo-STP	2.81	1.13
Benzphetamine	1.52	0.15
Caffeine	1.66	0.26
Chlorphentermine	2.40	0.82
Dopamine	NE	NE
Diethylpropion	1.53	0.16
Dimethylamphetamine	3.82	1.89
Ephedrine	3.68	1.79
Fencamfamin	2.27	0.72
Fenethyline	1.68	0.27
Fenfluramine	2.48	0.88
4-Hydroxyamphetamine	2.79	1.11
Levodopa	NE	NE
Mazindol	1.58	0.20
Mephentermine	4.59	2.48
Mescaline	4.18	2.17
Methylamphetamine	4.05	2.07
Methyldopa	NE	NE
Methyldopate	NE	NE
Methylenedioxy amphetamine	2.61	0.98
Methylephedrine	3.73	1.83
Methylphenidate	1.80	0.36
Noradrenaline	NE	NE
Normetanephrine	2.74	1.08
Norpseudoephedrine	2.42*	0.83*
Pemoline	1.50	0.14
Phendimetrazine	1.74	0.32
Phenelzine	1.81	0.37
2-Phenethylamine	3.05	1.31
Phentermine	2.45	0.86
Phenylephrine	3.49	1.64
Phenylpropanolamine	2.25	0.70
Pipradrol	2.23	0.69
Prolintane	2.98	1.26
Pseudoephedrine	3.65	1.77
STP	2.81	1.13
Tranylcypromine	1.66	0.26
Trimethoxyamphetamine	3.28	1.48
Tyramine	3.26	1.47

* Tailing peak.

separation of seven basic drugs on four different brands of HPLC silica using the methanol–ammonium nitrate eluent described in this paper. All columns were 250 × 5 mm I.D., packed in the same way and eluted with the same batch of eluent; all

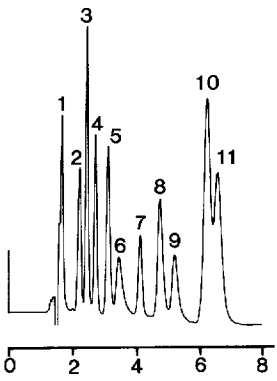
TABLE IV

HPLC RETENTION DATA FOR AMPHETAMINE AND ITS STRUCTURAL AND PHARMACOLOGICAL ANALOGUES (ARRANGED IN ORDER OF INCREASING RETENTION TIME)

NE = Not eluted.

Compound	*Retention time (min)*	*Capacity factor (k')*
Pemoline	1.50	0.14
Benzphetamine	1.52	0.15
Diethylpropion	1.53	0.16
Mazindol	1.58	0.20
Tranylcypromine	1.66	0.26
Caffeine	1.66	0.26
Fenethyline	1.68	0.27
Phendimetrazine	1.74	0.32
Methylphenidate	1.80	0.36
Phenelzine	1.81	0.37
Adrenaline	2.15*	0.63*
Pipradrol	2.23	0.69
Phenylpropanolamine	2.25	0.70
Fencamfamin	2.27	0.72
Chlorphentermine	2.40	0.82
Norpseudoephedrine	2.42*	0.83*
Phentermine	2.45	0.86
Fenfluramine	2.48	0.88
Methylenedioxy amphetamine	2.61	0.98
Amphetamine	2.62	0.98
Normetanephrine	2.74	1.08
4-Hydroxyamphetamine	2.79	1.11
Bromo-STP	2.81	1.13
STP	2.81	1.13
Prolintane	2.98	1.26
2-Phenethylamine	3.05	1.31
Tyramine	3.26	1.47
Trimethoxyamphetamine	3.28	1.48
Phenylephrine	3.49	1.64
Pseudoephedrine	3.65	1.77
Ephedrine	3.68	1.79
Methylephedrine	3.73	1.83
Dimethylamphetamine	3.82	1.89
Methylamphetamine	4.05	2.07
Mescaline	4.18	2.17
Mephentermine	4.59	2.48
Dopamine	NE	NE
Levodopa	NE	NE
Methyldopa	NE	NE
Methyldopate	NE	NE
Noradrenaline	NE	NE

* Tailing peaks.



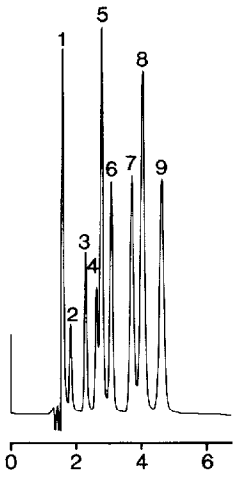


Fig. 2. Separation of some narcotic analgesic drugs and related compounds on a silica column (Spherisorb S5W 250 × 5 mm I.D.). Eluent: methanol–aqueous ammonium nitrate buffer pH 10.1 (9:1, v/v). Flow-rate: 2 ml/min. Detection: 254 nm. Peaks: 1 = dextropropoxyphene; 2 = dipipanone; 3 = 6-monoacetylmorphine; 4 = methadone; 5 = morphine; 6 = morphine-3-glucuronide; 7 = norpethidine; 8 = dihydrocodeine; 9 = dihydromorphine; 10 = norcodeine; 11 = normorphine.

Fig. 3. Separation of amphetamine analogues on a silica column (Spherisorb S5W 250 × 5 mm I.D.). Eluent: methanol–aqueous ammonium nitrate buffer pH 10.1 (9:1, v/v). Flow-rate: 2 ml/min. Detection: 254 nm. Peaks: 1 = diethylpropion; 2 = methylphenidate; 3 = phenylpropanolamine; 4 = amphetamine; 5 = 4-hydroxyamphetamine; 6 = 2-phenethylamine; 7 = ephedrine; 8 = methylamphetamine; 9 = mephentermine.

other experimental conditions were also maintained constant. It can be seen that although the elution order remains the same on the four columns, the capacity factors for the drugs show enormous differences (>200%). To facilitate the rapid transfer of HPLC methods from one laboratory to another it is essential that these laboratories all use the same packing material. Although satisfactory separations can be achieved with any of the four materials used in Fig. 1, and probably with most commercial silicas, we have standardised on Spherisorb S5W on which the present data were generated.

The retention times and k' values for the 43 narcotic analgesic drugs, listed alphabetically and in order of increasing retention, are given in Tables I and II respectively. Comparable data for the 41 amphetamine type compounds are given in Tables III and IV. With the exception of dopamine, levodopa, methyldopa, methyldopate and noradrenaline, all drugs injected were eluted from the column. Furthermore, peak shapes were generally very good except for adrenaline, norpseudoephedrine and oxycodone. The chromatographic system showed excellent stability with little drift in retention times over the course of a working day. To prolong the life of the column, the entire system was flushed with methanol–water (9:1, v/v) at the end of every day. Figs. 2 and 3 show typical separations of drugs from the narcotic analgesic and amphetamine groups respectively.

It can be seen that the HPLC system is particularly suitable for drug screening

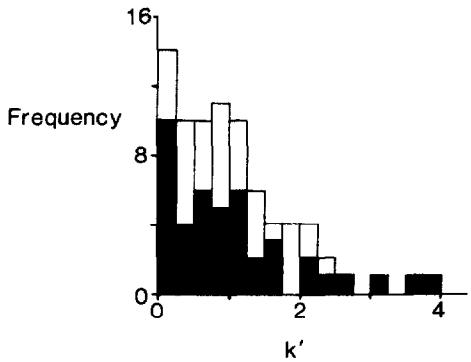


Fig. 4. Frequency distribution of k' values for 79 drug compounds. Narcotic analgesics (black) and amphetamines (white).

purposes, *e.g.* examination of illicit drug preparations. It is capable of eluting a wide range of compounds within a relatively small retention range ($k' = 0.05$–3.92 for the narcotic analgesics and $k' = 0.14$–2.48 for the amphetamines) allowing rapid analysis times of approximately 7 min per sample. Furthermore the good peak shapes give excellent detection sensitivity. However, the limited discriminating power of this system, arising from the large number of compounds eluting within a small retention range, means that any identification can only be tentative and must be confirmed by other techniques, *e.g.* GLC[18]. Fig. 4 shows the frequency distribution of the k' values for the 79 compounds which could be eluted and reference to this histogram can give a useful indication of the reliability of any identification based on HPLC. For example, it is clear that compounds having k' values greater than 2 are the ones most reliably discriminated using this system.

REFERENCES

1 A. H. Stead, R. Gill, T. Wright, J. P. Gibbs and A. C. Moffat, *Analyst*, 107 (1982) 1106.
2 R. E. Ardrey and A. C. Moffat, *J. Chromatogr.*, 220 (1981) 195.
3 I. Jane, *J. Chromatogr.*, 111 (1975) 227.
4 I. D. Watson and M. J. Stewart, *J. Chromatogr.*, 110 (1975) 389.
5 I. Jane and J. F. Taylor, *J. Chromatogr.*, 109 (1975) 37.
6 M. W. White, *J. Chromatogr.*, 178 (1979) 229.
7 J. Christie, M. W. White and J. M. Wiles, *J. Chromatogr.*, 120 (1976) 496.
8 R. J. Flanagan, G. C. A. Storey and D. W. Holt, *J. Chromatogr.*, 187 (1980) 391.
9 R. J. Flanagan, G. C. A. Storey, W. D. Holt and P. B. Farmer, *J. Pharm. Pharmacol.*, 34 (1982) 638.
10 R. G. Achari, J. L. Baldridge, T. R. Koziol and L. Yu, *J. Chromatogr. Sci.*, 16 (1978) 271.
11 H. Kelly, A. Huggett and S. Dawling, *Clin. Chem.*, 28 (1982) 1478.
12 R. G. Achari and E. E. Theimer, *J. Chromatogr. Sci.*, 15 (1977) 320.
13 K. Sugden, G. B. Cox and C. R. Loscombe, *J. Chromatogr.*, 149 (1978) 377.
14 B. B. Wheals, *J. Chromatogr.*, 187 (1980) 65.
15 B. A. Bidlingmeyer, J. K. Del Rios and J. Korpi, *Anal. Chem.*, 54 (1982) 442.
16 R. J. Flanagan, G. C. A. Storey, R. K. Bhamra and I. Jane, *J. Chromatogr.*, 247 (1982) 15.
17 I. Jane, (1983) personal communication.
18 A. C. Moffat, A. H. Stead and K. W. Smalldon, *J. Chromatogr.*, 90 (1974) 19.