Journal of Chromatography, 631 (1993) 207–213
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2595

Porous graphitized carbon and octadecylsilica columns in the separation of some monoamine oxidase inhibitory drugs

Esther Forgács, Klára Valkó and Tibor Cserháti

Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest (Hungary)

Kálmán Magyar

Department of Pharmacology, Semmelweis University of Medicine, Budapest (Hungary)

ABSTRACT

The retentions of seventeen monoamine oxidase inhibitory drugs (propargylamine derivatives) were determined on a porous graphitized carbon (PGC) column using unbuffered methanol–water and acetonitrile–water eluents and on an octadecylsilica column with methanol–water eluents using constant and varying buffer concentrations. Principal component analysis showed that the retention characteristics of the PGC and octadecylsilica columns differ considerably. A marked deviation between the selectivities of methanol and acetonitrile organic modifiers was observed on PGC. The buffer concentration has a significant impact on the retention of drugs on the octadecylsilica column. The type of substitution (heterocyclic, condensed ring, substituted benzene) accounts for the retention differences irrespective of the type of column, eluent and buffer concentration.

INTRODUCTION

Porous graphitic carbon (PGC) columns have been developed in the last decade [1,2]. The development of this highly pH-stable type of column was motivated by the fact that the application of silica or silica-based supports in high-performance liquid chromatography (HPLC) is limited by the low stability of silica at high pH values [3] and by the undesirable electrostatic interactions between the polar substructures of solutes and the free silanol groups not covered by the hydrophobic ligand [4]. PGC columns have been used for the separation of diastereoisomers [5], geometric isomers [6] and various alkaline compounds [7] such as tioconazole derivatives. The effect of various physico-chemical parameters of solutes on their retention behaviour on PGC columns has been studied in detail and the importance of electronic interactions between solutes and the stationary phase has been emphasized [8].

Reversed-phase HPLC (RP-HPLC) has been extensively used to determine the hydrophobicity (lipophilicity) of various compounds [9,10]. To increase the accuracy of the lipophilicity determination, linear correlations were calculated between the log k' value and the concentration of organic modifier in the eluent. The intercept of the correlation was considered as a good estimate of lipophilicity [11] and the slope was considered to be related to the specific hydrophobic surface area [12]. The good correlation between the intercept and slope values indicates the structural homogeneity of solutes [13]. When the compound contains one or more dissociable polar substituents, the pH values of the

Correspondence to: Esther Forgács, Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest, Hungary.

0021-9673/93/$06.00

eluent [14,15] and the ionic strength [16] modify the retention. The buffers used to control the pH value of the eluent can modify the chromatrographic system in two different manners: they change the pH value and at the same time they increase the ionic strength of the eluent. As each buffer contains more or less dissociable salts the two effects cannot be separated experimentally in common chromatographic practice. To our knowledge, their relative impact on the retention behaviour of polar solutes has never been studied in detail.

Various substituted propargylamine derivatives are promising therapeutic compounds as monoamine oxidase inhibitory drugs [17,18], but their exact mode of action has not been elucidated in detail [19].

Principal component analysis (PCA) [20] have frequently been used to extract maximum information from retention data matrices of considerable dimensions [21,22]. The advantages of the application of PCA in chromatography is that it allows a reduction in the number of variables whilst maintaining most of information content. PCA is suitable not only for calculations of two–two variables relationships, but also for the simultaneous study of all-variables relationships.

The objectives of our investigation were to determine the retentions of seventeen monoamine oxidase inhibitory drugs on PGC and on octadecylsilica columns with various eluent systems, to evaluate the retention data with multivariate mathematical statistical methods and to find the relationship between the retention characteristics of the columns.

EXPERIMENTAL

The PGC column (Shandon Hypercarb, 100 × 4.7 mm I.D., particle diameter 7 μm) was purchased from Shandon Scientific (Runcorn, UK). The HPLC system consisted of a Liquopump Model 312 pump (LaborMIM, Budapest, Hungary), a Cecil CE-212 variable-wavelength UV detector (Cecil Instruments, Cambridge, UK), a Valco (Houston, TX, USA) injector with a 20-μl sample loop and a Waters Model 740 integrator (Waters–Millipore, Milford, MA, USA). The flow-rate was 1ml/min and the detection wavelength was 254 nm. Methanol–water and acetonitrile–water mixtures were used as eluents with methanol and acetonitrile concentrations ranging from 95 to 77 and from 97 to 64% (v/v), respectively. Buffers were not used as it was previously established that the inert surface of PGC makes the application of buffers unnecessary. The measurements were also carried out on an octadecylsilica column (LiChrospher C_{18}, 150 × 4 mm I.D.; ChromLab, Budapest, Hungary), using methanol–10 m*M* K_2HPO_4 mixtures containing 60–90% (v/v) of methanol.

To study the effect of buffer concentrations, the same eluents were applied, always containing a 10 m*M* K_2HPO_4 end concentration. The application of constant and changing buffer concentrations was motivated by the supposition that the buffer concentration may also influence the retention of the polar drugs. The other chromatographic conditions were as before.

The structures of the monoamine oxidase inhibitory drugs are shown in Table I. The drugs were dissolved in methanol or acetonitrile at a concentration of 0.05 mg/ml. The retention time of each compound was determined in each eluent with three consecutive determinations. As the correlation between the log k' value and the organic phase concentration is generally linear in HPLC, we also applied linear equations to describe the dependence of log k' on the organic mobile phase concentration:

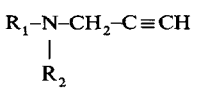
$$\log k' = \log k'_0 + bC \quad (1)$$

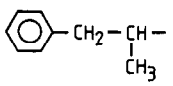
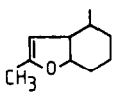
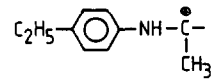
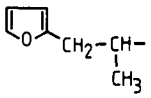
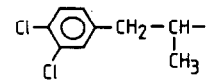
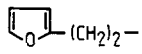
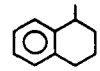
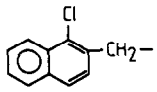
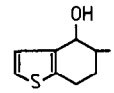
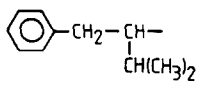
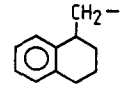
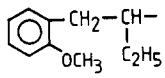
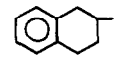
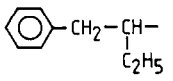
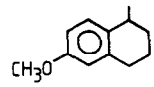
where k' = capacity factor, k'_0 = capacity factor extrapolated to zero organic modifier content in the mobile phase (intercept, related to molecular lipophilicity), b change in log k' value caused by unit change (1% v/v) of organic mobile phase concentration (slope, related to the specific hydrophobic surface areas) and C = organic mobile phase concentration (%, v/v).

To find the similarities and dissimilarities between the retention behaviour of columns and drugs, PCA was applied. The parameters of eqn. 1 were the eight variables (slope and intercept values for PGC column with methanol–water and acetonitrile–water eluents, slope and intercept values for an octadecylsilica column with constant and changing buffer concentration) and the seventeen drugs were the observations. For the easier visualization of the results the two dimensional non-linear map [23] of PC loadings and PC variables was also calculated.

TABLE I

CHEMICAL STRUCTURE OF MONOAMINE OXIDASE INHIBITORS



No. of compound	R_1	R_2	No. of compound	R_1	R_2
1 (+) 2 (−)		CH_3 CH_3	10		CH_3
3		CH_3	11		H
4		H	12		CH_3
5		CH_3	13		CH_3
6		CH_3	14		CH_3
7		CH_3	15		CH_3
8		CH_3	16		CH_3
9		C_4H_7	17		CH_3

RESULTS AND DISCUSSION

Each drug showed narrow and symmetrical peaks in each eluent system (Figs. 1 and 2), that is, the carbon column can be successfully used for the separation of this class of monoamine oxidase inhibitory drugs. The parameters of eqn. 1 are compiled in Tables II–V. The relationship between the logarithm of the capacity factor and the concentration of organic phase in the eluent was significantly

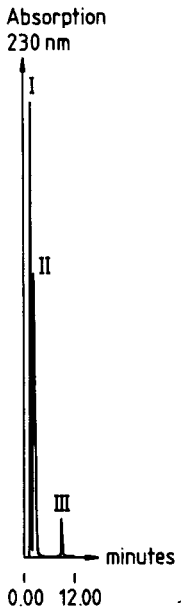


Fig. 1. Separation of manoamine oxidase drugs on the porous graphitized carbon column. Eluent, methanol–water (82:18, v/v); flow-rate, 1 ml/min; detection, 230 nm. I = dead volume; II and III refer to drugs **5** and **17** in Table I, respectively.

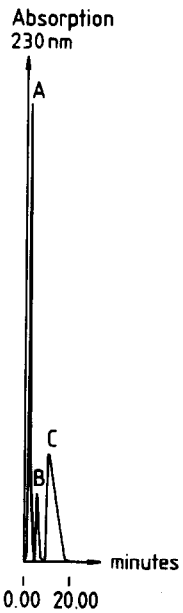


Fig. 2. Separation of monoamine oxidase drugs on the porous graphitized carbon column. Eluent, acetonitrile–water (92:8, v/v); flow-rate, 1 ml/min; detection, 230 nm. A = dead volume; B and C refer to drugs **8** and **12** in Table I, respectively.

linear in each instance that is, the retention of propargylamine derivatives decreases linearly with increasing concentration of the organic component in the eluent. This finding further indicates that the propargylamine derivatives follow the general rule also on the PGC column (Tables II and III); no anomalous retention behaviour was observed. The slope and intercept values differ from each other, which means that these drugs can be easily separated on the PGC column using either acetonitrile–water or methanol–water eluents. The result that the slope and intercept values of a solute are different in eluents containing methanol or acetonitrile proves the different selectivity of the organic modifiers.

The parameters compiled in Tables II–V make possible the calculation of retention time differences for each pair of propargylamine derivatives at each eluent composition:

$$t_1 - t_2 = t_0(10^{a_1+b_1C} - 10^{a_2+b_2C}) \quad (2)$$

where a and b are the intercept and slope values for compounds **1** and **2** at an organic phase concentration C.

TABLE II

PARAMETERS OF THE LINEAR CORRELATIONS BETWEEN THE LOG k' VALUE OF MONOAMINE OXIDASE INHIBITORY DRUGS AND THE METHANOL CONCENTRATION (C%, v/v) IN THE ELUENT

Porous graphitized carbon column. Numbers refer to drugs in Table I. Log $k' = k'_0 + bC$

No. of drug	Log k'_0	$-b \times 10^{-2}$	$s_b \times 10^{-3}$	r
1	1.01	4.47	4.13	0.9721
2	1.01	4.47	4.13	0.9721
3	1.06	6.69	3.89	0.9902
4	1.87	52.20	9.81	0.9817
5	1.04	5.99	3.47	0.9875
6	0.94	1.73	3.94	0.9425
7	0.99	2.56	4.56	0.9986
8	1.06	5.40	3.92	0.9848
9	0.97	2.03	2.08	0.9621
10	1.00	4.03	5.40	0.9351
11	1.39	26.96	37.31	0.9311
12	0.97	1.48	1.26	0.9773
13	0.97	1.74	1.69	0.9768
14	1.04	2.44	1.19	0.9909
15	1.00	2.59	1.50	0.9899
16	1.10	1.07	16.35	0.9181
17	1.14	3.73	7.27	0.9329

TABLE III

PARAMETERS OF THE LINEAR CORRELATIONS BETWEEN THE LOG k' VALUE OF MONOAMINE OXIDASE INHIBITORY DRUGS AND THE ACETONITRILE CONCENTRATION (C%, v/v) IN THE ELUENT

Porous graphitized carbon column. Number refer to drugs in Table I. Log $k' = \log k'_0 + bC$

No. of drug	Log k'_0	$-b \times 10^{-2}$	$s_b \times 10^{-3}$	r
1	0.94	4.28	9.90	0.9371
2	0.94	4.28	9.90	0.9371
3	1.14	13.19	15.73	0.9361
4	1.10	10.51	3.24	0.9955
5	1.27	17.43	15.32	0.9569
6	0.97	2.83	2.57	0.9707
7	1.07	6.98	3.15	0.9922
8	1.06	5.65	4.06	0.9823
9	1.03	4.73	3.39	0.9849
10	0.98	5.71	8.07	0.9138
11	0.99	2.24	1.46	0.9807
12	1.03	3.45	3.12	0.9651
13	1.00	4.07	5.21	0.9548
14	1.02	4.50	1.63	0.9927
15	1.01	3.84	4.89	0.9265
16	1.06	4.86	5.24	0.9671
17	1.11	6.26	3.47	0.9913

TABLE IV

PARAMETERS OF THE LINEAR CORRELATIONS BETWEEN THE LOG k' VALUE OF MONOAMINE OXIDASE INHIBITORY DRUGS AND THE METHANOL CONCENTRATION (C%, v/v) IN THE ELUENT

Changing buffer concentration. Octadecylsilica column. Numbers refer to drugs in Table I. Log $k' = \log k'_0 + bC$

No. of drug	Log k'_0	$-b \times 10^{-2}$	$s_b \times 10^{-3}$	r
1	3.13	3.89	1.34	0.9981
2	3.13	3.89	0.96	0.9991
3	3.32	4.04	0.65	0.9996
4	2.18	3.28	1.72	0.9958
5	2.68	3.61	2.53	0.9926
6	3.91	4.33	1.72	0.9975
7	3.88	4.28	1.19	0.9988
8	3.76	4.39	1.72	0.9984
9	5.31	5.50	1.12	0.9939
10	3.00	3.76	1.18	0.9984
11	2.99	3.85	0.81	0.9929
12	4.34	5.05	1.02	0.9959
13	3.43	3.86	1.49	0.9977
14	2.17	3.13	1.71	0.9974
15	3.62	4.06	0.46	0.9998
16	2.91	3.53	1.59	0.9969
17	3.23	3.67	0.99	0.9989

TABLE V

PARAMETERS OF THE LINEAR CORRELATIONS BETWEEN THE LOG k' VALUE OF MONOAMINE OXIDASE INHIBITORY DRUGS AND THE METHANOL CONCENTRATION (C%, v/v) IN THE ELUENT

Constant (10 mM) buffer concentration. Octadecylsilica column. Numbers refer to drugs in Table I. Log $k' = \log k'_0 + bC$

No. of drug	Log k'_0	$-b \times 10^{-2}$	$s_b \times 10^{-3}$	r
1	−1.86	2.95	2.31	0.9939
2	−1.86	2.95	2.31	0.9939
3	3.27	3.94	1.98	0.9962
4	1.99	2.95	2.41	0.9901
5	2.59	3.45	1.10	0.9984
6	3.90	4.36	5.12	0.9800
7	4.23	4.83	3.64	0.9915
8	3.64	4.18	1.83	0.9971
9	6.54	7.03	4.68	0.9977
10	2.82	3.46	1.31	0.9978
11	2.94	3.71	2.01	0.9955
12	4.23	4.89	3.12	0.9938
13	4.12	4.76	4.88	0.9895
14	1.88	2.70	1.83	0.9931
15	4.18	4.79	6.68	0.9810
16	3.75	4.60	6.92	0.9781
17	3.96	4.60	6.63	0.9798

The slope and intercept values differ considerably in eluents with constant and changing buffer concentration (Tables IV and V). This indicates that with polar compounds the lipophilicity value determined in a buffered eluent may depend on the concentration of the buffer, and therefore its application in quantitative structure–activity relationship studies is questionable. We strongly advocate the application of various buffer and organic modifier concentrations that are not intercorrelated and the extrapolation of the log k'_0 values to zero organic modifier and zero buffer concentrations.

The parameters of the PCA describing the relationship between the retention characteristics of drugs on various columns are compiled in Table VI. Three principal components explained most (95.09%) of the total variance. This means that the eight variables can be substituted by three background (abstract) variables without a substantial loss of information. Unfortunately, PCA does not define these three background variables, it only indicates their mathematical possibility.

Each chromatographic parameter determined on

TABLE VI

RELATIONSHIP BETWEEN THE RETENTION PARAMETERS OF SOME MONOAMINE OXIDASE INHIBITORY DRUGS

Results of principal component analysis.

No. of PC component	Eigenvalues	Variance explained (%)	Total variance explained (%)
1	4.57	57.07	57.05
2	1.72	21.45	78.52
3	1.33	16.57	95.09
4	0.31	3.85	98.94

Parameter[a]	Principal component loadings		
	1	2	3
A, intercept (MET)	−0.70	0.00	0.71
A, slope (MET)	−0.66	−0.01	0.74
A, intercept (ACN)	−0.34	0.92	−0.08
A, slope (ACN)	−0.45	0.85	−0.13
B, intercept (VAR)	0.95	0.16	0.18
B, slope (VAR)	0.88	0.16	0.28
B, intercept (CONST)	0.92	0.20	0.25
B, slope (CONST)	0.88	0.25	0.29

[a] A = Porous graphitized carbon column; MET = methanol–water eluents; ACN = acetonitrile–water eluents; B = octadecylsilica column; VAR = varying buffer concentration; CONST = constant buffer concentration.

the octadecylsilica column has a high loading in the first principal component, that is, the first PC can be related to the reversed-phase separation mechanism irrespective of constant or varying buffer concentrations. The retention parameters of the PGC column are distributed according to the type of organic modifier; the acetonitrile–water and methanol–water eluents have high loadings in the second and third PCs, respectively. This indicates that methanol and acetonitrile have different selectivities and the type of organic modifier has a considerable effect on the separation of the monoamine oxidase inhibitory drugs.

The conclusions drawn from the two dimensional non-linear map of PC loadings (Fig. 3) entirely support our previous conclusions. The retention characteristics of PGC (cluster A) and octadecylsilica (cluster B) columns form two widely separated clusters, which means that the retention characteristics of the columns are markedly different. The four

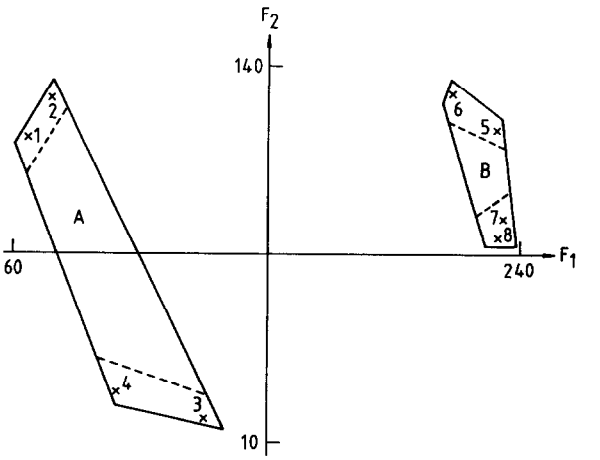


Fig. 3. Two dimensional non-linear map of principal component loadings. No. of iterations, 36; maximum error, $4.58 \cdot 10^{-3}$; 1 and 2 = intercept and slope values, PGC column, methanol organic modifier; 3 and 4 = intercept and slope values, PGC column, acetonitrile organic modifier; 5 and 6 = intercept and slope values, octadecylsilica column, varying buffer concentration; 7 and 8 = intercept and slope values, octadecylsilica column, constant buffer concentration.

eluent systems are also separated, but, the differences between the organic modifiers are higher than the differences between the effect of constant or varying buffer concentrations.

The monoamine oxidase inhibitory drugs form distinct clusters according to the type of substituents (Fig. 4). This result suggests that heterocyclic, condensed ring or substituted benzene substructures account for the retention behaviour irre-

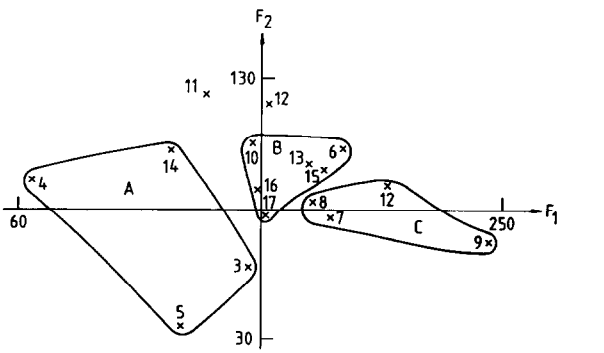


Fig. 4. Two-dimensional non-linear map of principal component variables. No. of iterations, 130; maximum error, $1.55 \cdot 10^{-2}$; A = heterocyclic substituents; B = substituents with condensed ring; C = substituents with benzene derivatives.

spective of the type of column and eluent composition. The position of compounds 11 and 12 indicates that the increased polarity and the number of chlorine atoms also influence the retention.

ACKNOWLEDGEMENT

This work was supported by grant OTKA 2670 of the Hungarian Academy of Sciences.

REFERENCES

1 M. T. Gilbert, J. H. Knox and B. Kaur, *Chromatographia*, 16 (1982) 138.
2 J. H. Knox, B. Kaur and G. R. Millward, *J. Chromatogr.*, 352 (1986) 3.
3 A. Berthod, *J. Chromatogr.*, 549 (1991) 1.
4 H. Tayar, H. Waterbend and B. Testa, *J. Chromatogr.*, 305 (1985) 320.
5 B. Kaur, *LC · GC*, 3 (1990) 41.
6 D. Berek and J. Novak, *Chromatographia*, 30 (1990) 582.
7 J. C. Berridge, *J. Chromatogr.*, 449 (1988) 317.
8 R. Kaliszan and K. Osmialowski, *J. Chromatogr.*, 333 (1990) 499.
9 W. J. Haggerty and E. A. Murrill, *Res. Dev.*, 25 (1974) 30.
10 J. M. McCall, *J. Med. Chem.*, 18 (1975) 549.
11 K. Valkó, S. Olajos and T. Cserháti, *J. Chromatogr.*, 499 (1990) 361.
12 C. Horvath, W. Melander and I. Molnar, *J. Chromatogr.*, 125 (1976) 129.
13 K. Valkó, *J. Liq. Chromatogr.*, 7 (1984) 1405.
14 B. Rittich, M. Polster and O. Kralik, *J. Chromatogr.*, 197 (1980) 43.
15 G. Vigh, J. Varga-Puhony, J. Hlavay and E. Pap, *J. Chromatogr.*, 236 (1982) 51.
16 E. Pap and G. Vigh, *J. Chromatogr.*, 259 (183) 49.
17 J. Knoll, E. S. Vizi and G. Somogyi, *Arzneim.-Forsch.*, 18 (1968) 109.
18 J. Knoll and K. Magyar, *Adv. Biochem. Psychopharmacol.*, 5 (1972) 393.
19 J. Knoll, Z. Ecsery, K. Magyar and É. Sátory, *Biochem. Pharmacol.*, 27 (1978) 1739.
20 K. V. Mardia, J. T. Kent, J. M. Bibby, *Multivariate Analysis*, Academic Press, London and New York, 1979.
21 T. Cserháti and Z. Illés, *Chromatographia*, 31 (1991) 152.
22 T. Cserháti, *J. Chromatogr.*, 543 (1991) 425.
23 J. W. Sammon, Jr., *IEEE Trans. Comput.*, C18 (1969) 401.