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Chemometric comparison of recent chromatographic and electrophoretic methods in a quantitative structure–retention and retention–activity relationship context

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Abstract

The retention characteristics of 21 basic pharmaceutical substances with a considerable difference in hydrophobicity (octanol–water partition coefficients, log *P*, between −0.026 and 6.45) are considered on an immobilized artificial membrane column, with a micellar liquid chromatography and a micellar electrokinetic capillary chromatography method. Utilising principal component analysis (PCA), it is seen that although the main retention principle is the same, the above methods as well as more classical RP-HPLC methods vary in secondary retention mechanisms. Combining the results of different methods a differentiation of the substances into their pharmacological families can be seen with PCA. The high correlations of the retention characteristics with log *P* and a biological parameter seem little affected by the method used.
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Keywords: Principal component analysis; Quantitative structure–retention relationships; Quantitative retention–activity relationships; Immobilized artificial membranes

1. Introduction

In the pharmaceutical industry a focal point of research for drug candidate molecules is the early and rapid estimation of their partitioning over a biological membrane and thus their potential bioactivity. Although the partitioning of drugs is determined by several variables, it is often mainly attributed to their hydrophobic character [1,2]. Thus, in view of a fast screening of new drug candidates, frequently the logarithms of the retention factors (log *k*) from classical RP-HPLC are correlated with hydrophobicity represented by the partition coefficient, log *P* [1–3]. This results in so-called quantitative structure retention relationships (QSRR). However, according to Dorsey et al. [4] biological partitioning is generally entropy driven as opposed to the bulk-phase hydrocarbon–water partitioning (e.g. occurring in classical RP-HPLC), which is overall enthalpy driven. Consequently new techniques are proposed combining the advantages of chromatographic or electrophoretic methods [5] with a partitioning expected to mimick the biological one. This usually implies inserting in existing techniques amphiphilic structures (e.g. micelle and/or liposome

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forming agents) because of their membrane forming characteristics.

Stationary phase models of membranes such as immobilised artificial membrane (IAM) columns [6,7] and methods using micelles in their mobile phase, like micellar liquid chromatography (MLC) [8,9] and micellar electrokinetic capillary chromatography (MECC) [10–12], are already described as possible alternatives for classical RP-HPLC. The studies reveal QSRR as well as quantitative retention activity relationships (QRAR) using biological parameters. However, little comparison between these methods is made concerning their retention characteristics nor are they situated opposed to other methods.

Chemometric techniques have been successfully applied on retention results from several chromatographic systems, revealing information about the behavior of both substances and systems [13–15]. Since the retention results for the different systems are usually inter-correlated, mostly principal component analysis (PCA) is performed. This technique allows an easy visual assessment of the comparability of several systems, which is a major advantage.

In this work the elution behaviour of a set of pharmaceutical substances was determined with MLC (using micelles) and MECC (using mixed micelles) methods, and compared with published results on an IAM column. The extent to which these methods differ both amongst themselves as well as compared to other ''classic'' chromatographic systems in information rendered about the characteristics of the substances, is examined with PCA. Moreover, it is investigated whether the correlation of the retention factors with log *P* is affected by the method used, allowing to make suggestions for their use in QSRR. Finally, a limited correlation study with Caco-2 cell permeability coefficients (P_c), which represent in-vitro membrane partitioning, should indicate the potential prediction properties for membrane partitioning of the different methods.

2. Experimental

2.1. Materials

Alprenolol·HCl, atenolol, carbamazepine, chlorpheniramine maleate, clonidine·HCl, desipramine·HCl, diphenhydramine·HCl, imipramine·HCl, nadolol, pindolol, promazine·HCl, propranolol·HCl, ranitidine·HCl, trifluoperazine·HCl, sodium dodecyl sulphate (SDS, 99% purity), taurodesoxycholate and phosphatidylcholine were from Sigma (St. Louis, MO, USA or Steinheim, Germany). Bisoprolol fumarate, sotalol, thioridazine, timolol maleate, potassium iodide, sodium dihydrogenphosphate, *n*-propanol and NaOH were from Merck (Darmstadt, Germany). Acebutolol was a gift from Rhône-Poulenc Rorer (Vitry sur Seine, France). Chlorpromazine·HCl came from Fluka Chemie (Buchs, Switzerland). Cimetidine was a gift from Smith-Kline Beecham (Herts, UK) and esmolol·HCl from Du Pont–De Nemours (Le Grand Saconnex, Switzerland).

2.2. Methods

The MLC measurements were based on a protocol described earlier [16,17]. The chromatograph was composed of an isocratic L-6000 pump, an L-7400 spectrophotometric detector and a D-7500 integrator, all Merck-Hitachi (Tokyo, Japan). A Kromasil C_{18} analytical column (5 μm, 120×4.6 mm I.D.) from Scharlau (Barcelona, Spain) was used. The flow-rate was 1 ml/min, the injection volume 20 μl and the detection wavelength 225 nm. Stock solutions containing 100 μg/ml of the drugs were prepared in an ethanol–0.05 *M* sodium dodecyl sulphate (SDS) aqueous solution (10–90%, v/v). The mobile phase (MP) consisted of SDS (0.15 *M*)–*n*-propanol (15%, v/v) in a 0.01 *M* sodium dihydrogenphosphate buffer (pH 7.4). Before the addition of *n*-propanol, the pH was adjusted to 7.4 with NaOH. The MP was filtered through Nylon membranes of 0.45 μm pore size and 47 mm diameter from Schleicher & Schuell (Dassel, Germany). The MLC retention (or capacity) factor *k* was calculated with the dead time measured as the time at which a potassium iodide (4.2 μg/ml aqueous solution) peak appears.

For MECC the protocol described by Hanna et al. [10] was followed. A Spectroforesis Ultra CE system with a Fast Scanning UV 3000 detector from Thermo Separation Products (Riviera Beach, FL, USA) was used. The fused-silica capillary with an internal diameter of 50 μm was 57 cm long (50 cm effective

length). The experiments took place at 25 °C and UV-absorbance at 214 nm was measured. A 40 m*M* solution of taurodesoxycholate in a 50 m*M* borate buffer (pH 8.0) (=TDC buffer) was used as solvent for sample and MP preparation. The MP consisted of TDC buffer to which 25 m*M* of phosphatidylcholine was added. This solution was stirred during 3 to 6 h until a clear solution of mixed micelles (taurodesoxycholate–phosphatidylcholine) was obtained. The MP had to be prepared daily and in between measurements a wash protocol (Table 1) should be applied. The drug solutions at 1000 μg/ml were prepared in the TDC buffer. They were filtered over a Chromafil type A filter with pore size 0.2 μm from Machery-Nagel (Düren, Germany). In this anionic MECC system at pH 8 the investigated basic substances appeared under neutral as well as cationic form. The MECC retention (or migration) factor k for a substance, be it uncharged or cationic, with a migration time t_r could then be determined by [10,18]:

$$k = \frac{t_r - t_0}{t_0(1 - t_r/t_m)} \quad (1)$$

As a marker of the migration time of the bulk solution (t_0) a 5% v/v aqueous solution of methanol was added to the sample solution prior to injection. The volume ratio marker/sample was 1:2. The migration time of the micelles (t_m) was marked by the baseline perturbation caused by the buffer. Although Eq. (1) is generally described for the calculation of the k of neutral substances, according to Strasters et al. [18] usually strong ion pairing is expected between cationic substances and anionic monomers and thus the equation can also be applied to cationic substances.

The IAM retention results k were taken from Nasal et al. [13]. Their MP consisted of an acetonitrile–phosphate buffer (pH 7.0) (20–80%, v/v).

Table 1
The wash protocol in between measurements for MECC

Wash liquid	Time (min)	Temperature (°C)
1.0 *M* NaOH	2	200
0.1 *M* NaOH	2	500
0.1 *M* NaOH	2	200
H_2O	2	200

The log P values were calculated by applying the freely available LOGKOW program of the Environmental Science Center of Syracuse Research Corporation (Syracuse, NY, USA) (http://esc_plaza.syrres-.com/interkow/kowdemo.htm). The acid–base dissociation constants, pK_a, were obtained from the ACD/pK_a database 4.06 (1999) of the Advanced Chemistry Development Corporation (Toronto, Canada). The chemometric calculations were executed with the Matlab 4.2c.1 program from the MathWorks (Natick, MA, USA). For the PCA an in-house Matlab toolbox for multivariate calibration was utilised.

3. Results and discussion

3.1. Retention measurements

The retention factors (k) of 21 basic (pK_a between 7.82 and 10.4) pharmaceutical substances belonging to five different pharmacological families (Table 2), were determined with MLC, MECC and IAM as defined in the experimental part. From Table 3, it can be seen that the retention order of the three methods largely follows the log P of the substances. However, between the methods the order differs somewhat for substances with similar log P values, which marks a variation between them.

For each of the three methods only one mobile phase (MP) was required to elute, within a reasonable time-span, the substances covering a very large hydrophobicity range. This is a definite advantage of these techniques opposed to classical RP-HPLC [8], which demands several MPs and an extrapolation procedure to acquire comparable retention results [19].

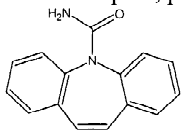
3.2. Principal component analysis

The retention results of Table 3 can be considered an $n \times m$ matrix, where n represents the objects (the substances) and m the variables (k from different methods). With PCA the number of original variables is reduced to a few latent ones called principal components (PCs), that still contain the main information from the original data set. The first new variable (PC1) is chosen in the direction of the

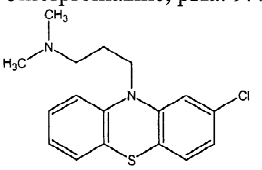
Table 2
The pharmacological class, the structures and pK_a [a] of the 21 investigated substances

Family A: Psychotropics

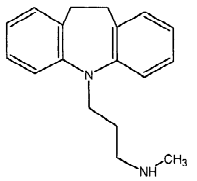
Carbamazepine, pKa: 9.17



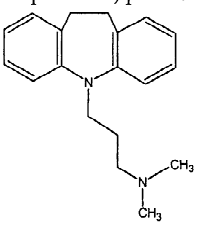
Chlorpromazine, pKa: 9.43



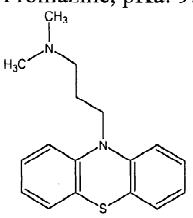
Desipramine, pKa: 10.40



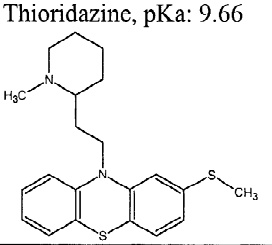
Imipramine, pKa: 9.49



Promazine, pKa: 9.43

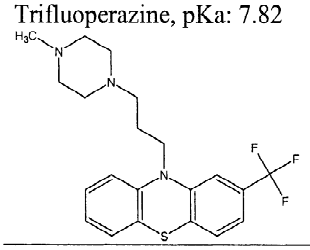


Thioridazine, pKa: 9.66

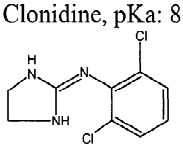


Family B: α-Adrenoreceptor agonists

Trifluoperazine, pKa: 7.82

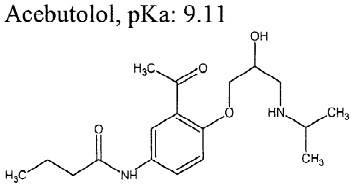


Clonidine, pKa: 8.01

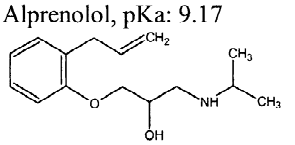


Family C: β-Adrenolytics

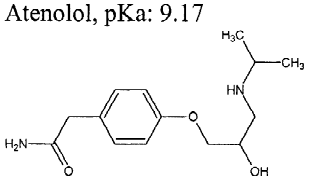
Acebutolol, pKa: 9.11



Alprenolol, pKa: 9.17



Atenolol, pKa: 9.17



Metoprolol, pKa: 9.18

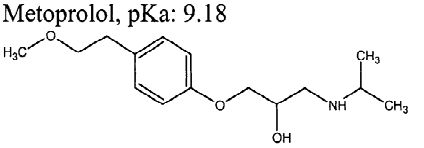
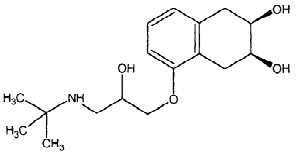
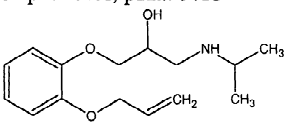


Table 2. Continued

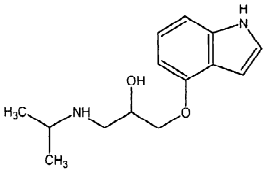
Nadolol, pKa: 9.17



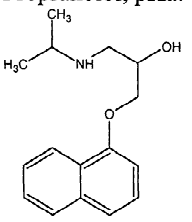
Oxprenolol, pKa: 9.13



Pindolol, pKa: 9.21

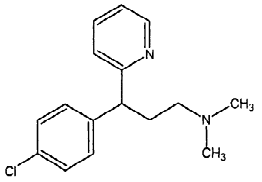


Propranolol, pKa: 9.15

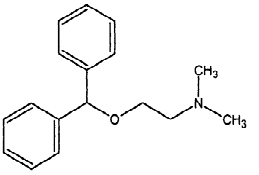


Family D: Histamine H1-receptor antagonists

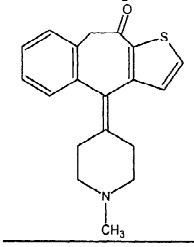
Chlorpheniramine, pKa: 9.33



Diphenhydramine, pKa: 8.76

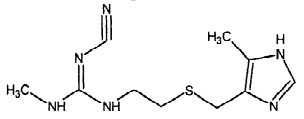


Ketotifen, pKa: 8.75

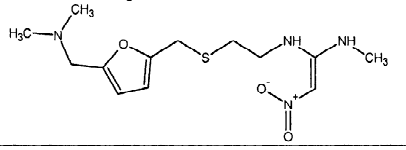


Family E: Histamine H2-receptor antagonists

Cimetidine, pKa: 6.72



Ranitidine, pKa: 8.40



[a]Only the pK_as relevant for the experimental range (pH 7–8) are mentioned.

largest variance in the data. The second PC is defined in such a manner that it is orthogonal to the first one and it represents a maximum of variance that was not explained by PC1, etc. Mathematically each PC can be described as a linear combination of the original variables where the importance of each original variable is given by its so-called loading. This linear combination yields for each object on each PC, values, the so-called scores. From PCA two main types of plots are obtained, namely the score plots which give information about the objects, here the substances, and the loading plots representing the importance and correlation of the variables, in this case the methods. The easy visualisation of complex

Table 3
The retention results of the 21 substances with MLC, MECC and IAM

Pharmaceutical substances [a]—Class [b]	Retention factors k and retention order ()			Log P
	MLC	MECC	IAM	
1. Atenolol—C	1.91 (2)	0.54 (2)	0.71 (2)	−0.026
2. Ranitidine—E	1.96 (3)	0.57 (3)	0.96 (3)	0.294
3. Cimetidine—E	1.105 (1)	0.42 (1)	0.54 (1)	0.574
4. Nadolol—C	3.46 (5)	1.69 (4)	1.86 (4)	1.169
5. Acebutolol—C	4.38 (7)	2.75 (7)	4.00 (10)	1.191
6. Pindolol—C	3.77 (6)	3.25 (9)	3.85 (8)	1.483
7. Metoprolol—C	7.40 (9)	1.94 (6)	2.72 (7)	1.694
8. Oxprenolol—C	11.60 (12)	4.75 (10)	3.85 (8)	1.831
9. Clonidine—B	6.11 (8)	1.69 (4)	2.57 (6)	1.888
10. Carbamazepine—A	2.40 (4)	2.95 (8)	2.47 (5)	2.248
11. Propranolol—C	15.45 (13)	40.76 (15)	21.88 (18)	2.597
12. Alprenolol—C	18.73 (15)	14.13 (12)	8.28 (11)	2.814
13. Difenhydramine—D	18.08 (14)	12.12 (11)	10.14 (12)	3.106
14. Ketotifen—D	9.85 (10)	25.59 (14)	14.72 (17)	3.640
15. Chlorpheniramine—D	11.18 (11)	22.25 (13)	11.35 (14)	3.819
16. Promazine—A	20.50 (16)	105.30 (19)	14.62 (16)	4.560
17. Desipramine—A	28.71 (20)	65.10 (17)	10.74 (13)	4.798
18. Imipramine—A	24.16 (17)	54.41 (16)	12.50 (15)	5.009
19. Trifluoperazine—A	31.79 (21)	92.30 (18)	66.07 (21)	5.108
20. Chlorpromazine—A	28.11 (18)	165.24 (21)	27.23 (19)	5.205
21. Thioridazine—A	28.25 (19)	125.46 (20)	56.49 (20)	6.449

[a] The substances are sorted according to ascending hydrophobicity (log P).
[b] A, B, C, D, E represent the different pharmacological families as mentioned in Table 2.

information in this way can be regarded as a definite advantage of the technique.

Usually some type of simple transformation (or scaling) is applied to the original data before it is analyzed with PCA. Here autoscaling [20] is applied on the logarithm of the retention results, giving rise to variables that are independent of the measurement units, that have equal range and therefore equal importance.

3.2.1. Comparing the three methods: PCA on their autoscaled log k

In order to evaluate the retention mechanisms of the three methods against each other PCA was performed on their autoscaled log k. On the PC1-PC2 loading plot along PC1, which represents 75.82% of the variance, the loadings are very similar and positive (Fig. 1a). This indicates that the main retention characteristic of the three methods might be the same. Along PC2 the difference between MLC on the one hand and MECC and IAM on the other is expressed. On Fig. 1b the PC3-loadings essentially represent the contrast between MECC and IAM. Thus the retention principles of IAM and MECC appear to resemble each other most.

On the PC1–PC2 score plot (Fig. 1c) the substances are numbered according to increasing log P (see Table 3). From this plot it appears the partition of the substances along PC1 is mainly based on their hydrophobicity. This is confirmed by the high correlation between the scores and log P ($r = 0.94$) as seen in Fig. 2. As a consequence, hydrophobicity is the main retention characteristic of the methods.

Since molecules covering a wide range of log P values can belong to the same pharmacological family (e.g. family C, the β-adrenolytics), along PC1 no complete separation of the substances into their families is expected. However, since in our test set the log P values of the substances belonging to the different families show little overlap, some separation can be seen anyway (Fig. 1c). The variation in the substances behavior introduced by the differences between MLC and MECC, IAM as indicated on the PC2–PC3 score plot do not give a good differentia-

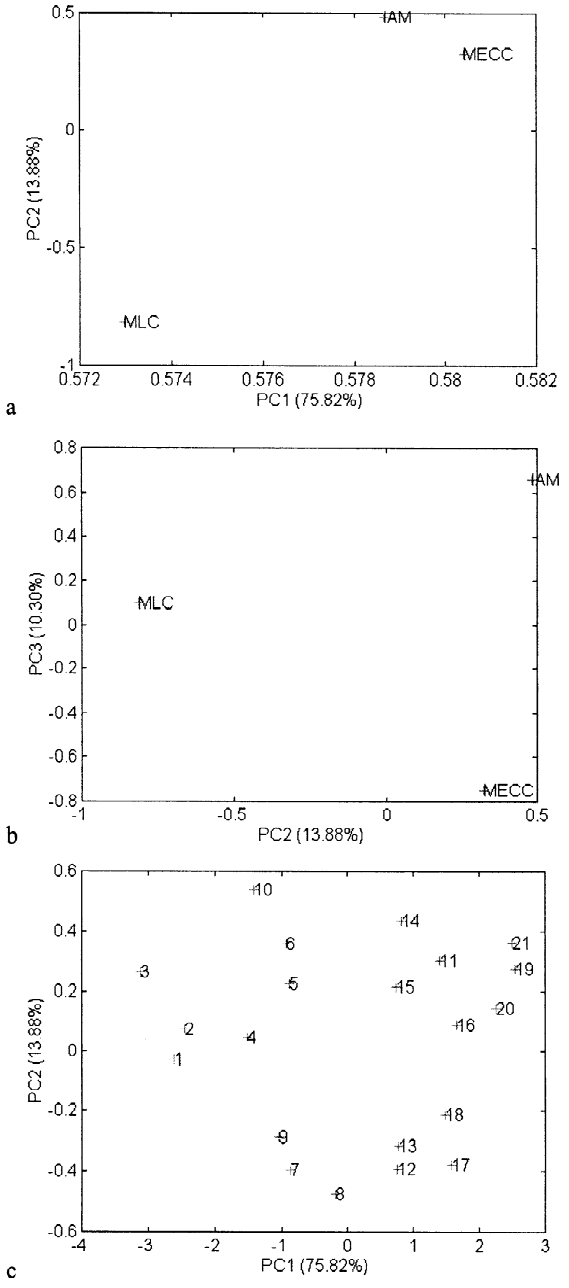


Fig. 1. PCA plots for the three methods: (a) PC1–PC2 loading plot, (b) PC2–PC3 loading plot, (c) PC1–PC2 score plot. No. 1–21 represent the substances as in Table 3.

tion (score plot not shown). The retention characteristics of the three methods might be too similar. Substances belonging to a same family, i.e. containing a common structural feature can have very diverse, spacious side chains. A masking effect of these chains might make it impossible for a chromatographic system or resembling chromatographic systems to detect the underlying basic structure.

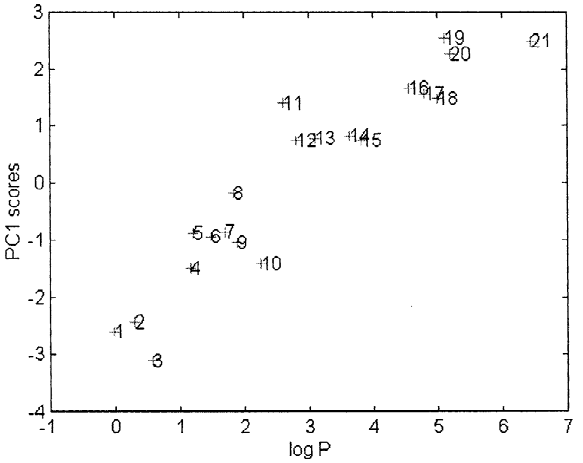


Fig. 2. PC1 scores of the autoscaled data for the three methods versus the log *P*. No. 1–21 represent the substances as in Table 3.

3.2.2. Situating the three methods: PCA on the autoscaled log k of ten chromatographic systems

Since the biological activity is determined by several variables, it appears logical that diverse chromatographic systems might be necessary when attempting to relate chromatographic and biological data. The original data matrix is extended with the retention factors of the substances on seven RP-HPLC systems taken from Ref. [13]. They show a large variety in stationary phase packing materials and in the pH of the mobile phases. Thus the three methods are situated opposed to a diverse set of ''classic'' systems (CS), in which no amphiphilic structures are inserted. These chromatographic systems included a chiral α_1-acid glycoprotein (AGP) column at pH 6.5 (CS1), a Suplex pKb-100 column at pH 2.5 (CS2), a Suplex pKb-100 column at pH 7.4 (CS3), a RP-Spheri column at pH 2.5 (CS4), a RP-Spheri column at pH 7.0 (CS5), an Aluspher RP-select B column at pH 7.3 (CS6) and a Unisphere PBD column at pH 11.7 (CS7). Performing PCA on the ten systems allows to situate the three methods considered relative to the other chromatographic systems in an easy way.

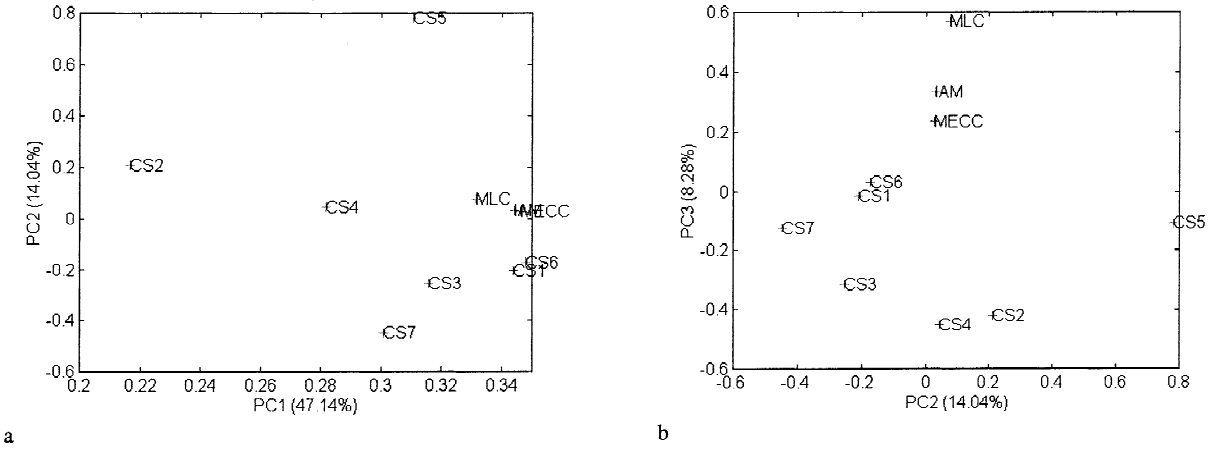


Fig. 3. PCA loading plots for the 10 methods: (a) PC1–PC2 plot, (b) PC2–PC3 plot.

In Fig. 3a, the PC1-loadings of all methods are relatively similar and positive. Thus the main retention principle appears analogue for all ten systems and in analogy with the three methods discussed above, PC1 possibly represents a hydrophobicity axis. As shown further, this is indeed the case. The systems with the lowest loadings on PC1 are those with a low pH of 2.5. At this pH the dissociation of the basic substances will be somewhat different from the other systems which all have a pH of at least 6.5. Along PC2 the three new methods carry no weight, a variance between the CS is expressed here, which seems to be mainly due to the pH differences in the basic part of the pH-scale. The PC3-loadings essentially represent the contrast (8.28%) between the three studied methods (especially MLC) and the other seven RP-HPLC methods (Fig. 3b). At this level the retention characteristics of the three methods (mainly MLC) seem to differ from the seven others. Possibly the different thermodynamic signature of micelle–water partitioning is expressed here [12].

On the PC1–PC2 score plot (Fig. 4a) the substances are numbered according to increasing log *P*. Their separation along PC1 again seems mainly due to hydrophobicity (Fig. 5, $r = 0.96$) and thus the main retention mechanism on all ten systems appears to be based on this.

Again some but no complete separation of the different families can be seen on the PC1–PC2 score

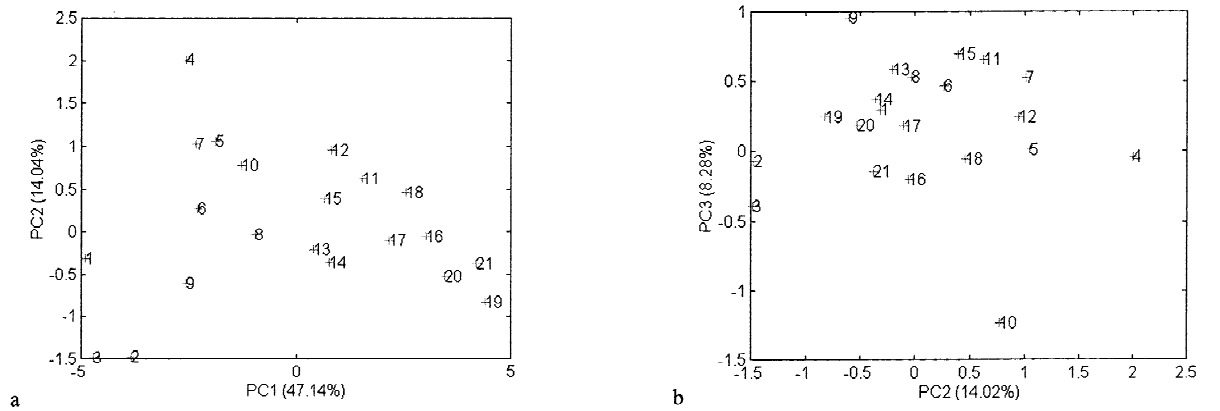


Fig. 4. PCA score plots for the 10 methods. No. 1–21 represent the substances as in Table 3. (a) PC1–PC2 plot, (b) PC2–PC3 plot.

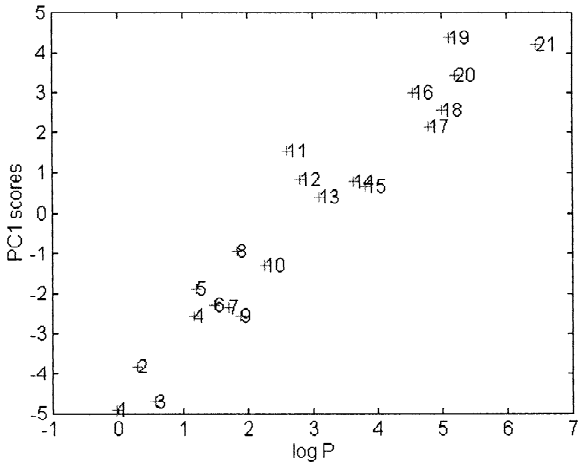


Fig. 5. PC1 scores of the autoscaled data for the 10 methods versus the log *P*. No. 1–21 represent the substances as in Table 3.

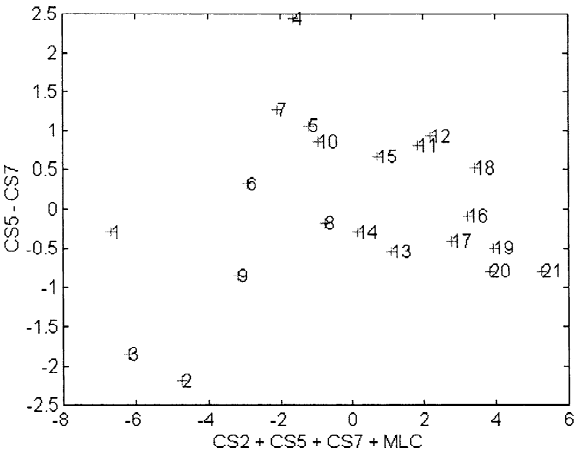


Fig. 6. The summed autoscaled log *k* of CS2, CS5, CS7 and MLC versus the autoscaled log *k* of CS5 minus CS7. No. 1–21 represent the substances as in Table 3.

plot. On the PC2–PC3 score plot however a separation of the different families can be detected (Fig. 4b). This is opposed to previous findings [13] where after performing PCA on the retention results from the seven ''classic'' systems no separation is achieved (plots not shown). One can therefore conclude that both the differences in retention characteristics between the ''classic'' (PC2) and the new methods (PC3) are necessary to detect a separation in the pharmacological families. Thus the battery of CSs seem to yield essentially a plot of hydrophobicity (PC1) against differences in ''classic'' and new methods (PC2–PC3).

The main information in the data can be reproduced by only four systems (CS7, CS5, CS2 and MLC) i.e. the most extreme systems in Fig. 3. The sum of their retention characteristics represents hydrophobicity (see PC1) while the subtraction of the values of CS7 from CS5 shows the difference between the classic systems (see PC2) (Fig. 6). From the difference between CS2 and MLC (Fig. 7) the contrast of the ''classic'' versus the old methods is seen (see PC3). These findings are confirmed by the high correlations that are found between the PC scores and (i) the sum of the autoscaled log *k* of the four systems (r with PC1 = 0.98), (ii) the autoscaled log *k* of CS7 minus CS5 (r with PC2 = 0.98) and (iii) the autoscaled log *k* of MLC minus CS2 (r with PC3 = 0.77).

As a consequence the separation of the different families as seen on Fig. 6 is very comparable to that found on the PC1–PC2 score plot (Fig. 4a). The separation on Fig. 7 can be compared to the one found on the PC2–PC3 score (Fig. 4b) although differences are seen due to the lower correlation between the autoscaled log *k* of MLC minus CS2 and the scores on PC3.

Since on most loading plots (Figs. 1a and 3) MECC and IAM are found in each other's vicinity, they should give very correlated information. Indeed,

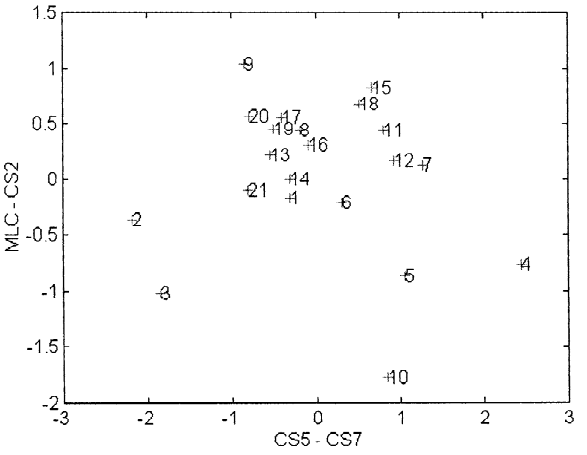


Fig. 7. The autoscaled log *k* of CS5 minus CS7 versus the autoscaled log *k* of MLC minus CS2. No. 1–21 represent the substances as in Table 3.

Table 4
Correlation matrix between the log *k*s obtained with the different methods

	MLC	MECC	IAM
MLC	1	0.9263	0.9112
MECC	–	1	0.9537
IAM	–	–	1
Seven others[a]	0.74–0.87	0.78–0.96	0.77–0.92

[a] log *k*s taken from Nasal et al. [5].

from Table 4, MECC and IAM seem most correlated. Moreover they also tend to show a slightly higher correlation with the seven other methods than MLC. Compared to the CS the three methods are highly correlated and give very similar information, as can be observed both from Fig. 3b and Table 4.

Compared to the CS the IAM, MLC and MECC methods use amphiphilic structures in either mobile and/or stationary phase. For MLC it is shown the solute retention is influenced by the net surface charge of the stationary phase as well as by the unusual nature of the micelle–solute interaction [4]. Likewise for MECC [21] and IAM [6] it is claimed the separation principle is based both on charge and partition differences. As a consequence the extra interaction introduced by the amphiphilic structures may cause the retention difference seen on PC3 (Fig. 3b) and the lower correlations of the new versus the more ''classic'' methods (Table 4). The occurrence of a more extreme electrostatic interaction of an amphiphilic structure might explain why both IAM and MECC give such similar results and are somewhat apart from MLC. The MLC system uses the negatively charged SDS as micelle-forming agent and their attraction to the positively charged substances might be rather important. The IAM and the MECC techniques on the other hand use phospholipid structures (lecitine) which are neutral or slightly negative (mixed micelles of MECC) at the applied pHs. Thus here electrostatic attraction might not be involved as much in the retention as with MLC. It should also be remarked that the used amphiphilic structure appears to play a more important role than the difference in technique (LC versus EC, use of adsorbed versus permanently bound amphiphilic structures).

3.3. QSRR with log P, log D

Originally the log *P* of a substance is defined for its unionised form. As the partition characteristics change for the ionised form a derived parameter, log *D*, is calculated. Each substance's log *P* is corrected proportional to the amount of ionised form present during the experiment [22]. For basic molecules when pH is near pK_a:

$$\log D = \log P - \log\left(1 + 10^{pK_a - pH}\right) \quad (2)$$

Contrary to what is expected the correlations of the log *k* for the different systems with log *D* are less good as with log *P* (Table 5). A possible explanation is that the pH of a given system might be to far from the pK_a to obtain proper estimates from Eq. (2). This is definitely the case for CS2 and CS4. Thus to compare the different methods for their predictability of hydrophobicity from retention results, the correlations between log *k* and log *P*, rather then between log *k* and log *D* should be considered, especially when pH is far from most pK_a values.

Several statistical parameters were calculated to evaluate the linear relation between a retention parameter and log *P* (Table 6). One can observe that in general the log *k*s are best correlated with log *P*. Although the correlation coefficient (*r*) between log *k* from MECC and log *P* is the highest, the model with log *k* from MLC and log *P* fits somewhat better (lowest quality coefficient (QC)). The best RMSPE is given by IAM. However, the differences between the methods are small for all parameters and thus the

Table 5
The correlation coefficient *r* between the log *k* and log *D* or log *P*

System	log *D*	log *P*
MLC (pH 7.3)	0.7974	0.8831
MECC (pH 8.0)	0.8908	0.9500
IAM (pH 7.0)	0.8537	0.9080
CS1 (pH 6.5)	0.8687	0.9283
CS2 (pH 2.5)	0.6344	0.7816
CS3 (pH 7.4)	0.9307	0.9361
CS4 (pH 2.5)	0.7382	0.8993
CS5 (pH 7.0)	0.5500	0.7180
CS6 (pH 7.3)	0.8479	0.9148
CS7 (pH 11.7)	0.8927	0.8926

Table 6
Statistical parameters to evaluate the linear relation between a retention parameter and log *P*, with *r* the correlation coefficient between (log) *k* and log *P*, QC the quality coefficient and RMSPE the root mean squared prediction error of the models: (log) $k = a \log P + b$

	r	QC	RMSPE
k MLC	0.9118	32.05	4.48
log *k* MLC	0.8831	22.61	0.29
k MECC	0.8400	74.13	32.66
log *k* MECC	0.9500	27.09	0.30
k IAM	0.7454	88.67	13.48
log *k* IAM	0.9080	30.72	0.25
log *k* seven others	0.94–0.72	18.50–40.97	0.25–0.88

use of any of the three methods for correlation studies with log *P* will depend on practical considerations. The other, more classic, methods do not give rise to better correlations nor predictions.

3.4. QRAR with Caco-2 results

A limited correlation study of five β-blocking agents (alprenolol, atenolol, metoprolol, oxprenolol and pindolol) with the cellular permeability coefficients (P_c) of Caco-2 monolayers, taken from Ref. [23], was made. From Table 7 one can conclude the *k*s are somewhat better correlated with the permeability coefficients for Caco-2 than the log *k*s. The retention on MLC seems to represent this permeability best. Since, according to Woodrow and Dorsey [12], the thermodynamic signature of micelle–water partitioning is similar to biological partitioning, as opposed to octanol–water, better correlations with in vitro biological parameters can be expected. However, for this very limited set there is only little difference with the other methods.

Table 7
Correlation coefficients *r* between the Caco-2 permeability P_c and the retention factors

	P_c Caco-2[a]
k MLC	0.9918
log *k* MLC	0.9526
k MECC	0.9396
log *k* MECC	0.9212
k IAM	0.9382
log *k* IAM	0.8612
k seven others	0.8869–0.9660
log *k* 7 others	0.8614–0.9759

[a] P_c values taken from Palm et al. [23].

4. Conclusions

From the chemometric analysis it appears that the main retention mechanism of the new as well as of the more classic methods is based on hydrophobicity. Nevertheless it can be seen that MLC, MECC and IAM have other characteristics compared to more conventional methods. These differences appear useful to differentiate substances into their pharmacological classes on PCA score plots. Inserting these methods into a battery of chromatographic systems covering a whole range of retention mechanisms could be useful to model QRAR.

MECC and IAM, although highly correlated to MLC, show a higher correlation amongst each other. It is suspected the used surfactant plays a major role in that. The difference in technique used (CE or LC) is of less importance.

Good relationships between log *P* and the log *k* of the three methods were found. Even better relationships seem to exist with P_c. No better correlations with ''classic'' methods were found. Thus depending on the availability of material and knowledge the use of one of the three new methods for log *P* studies could be preferred since they only require one mobile phase to determine a retention parameter that can be correlated with log *P*.

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