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# Application of chemometrically processed chromatographic data for pharmacologically relevant classification of antihistamine drugs

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ABSTRACT

For a set of 22 drugs known to modify physiological effects of endogenous histamine, high-performance liquid chromatographic (HPLC) retention data were determined employing two reversed-phase columns, seven compositions of methanol–buffer eluent and three pH values (acidic, neutral and alkaline) of the buffer. Logarithms of capacity factors, normalized to 100% buffer eluent (log $k'_w$), were determined by extrapolation of the respective data in five HPLC systems studied. Chemometric analysis of the 5 × 22 matrix of log $k'_w$ data obtained in five HPLC systems for 22 drug solutes allowed the extraction of two main principal components which accounted for 96% of total data variance. The distribution of individual drugs on the plane determined by the two first principal component axes forms the patterns, which are in excellent agreement with the established pharmacological classification of the structurally diverse compounds studied. It was demonstrated that systematic information extracted by chemometric analysis of behaviour of solutes in diverse HPLC systems has direct relevance to the pharmacological properties of the solutes. The approach developed here should facilitate the preselection of drug candidates, at the same time reducing the costs and the use of laboratory animals.

## INTRODUCTION

Dynamic, non-destructive processes involved in chromatographic separations resemble the essential processes at the basis of drug action (except for metabolism). The analogy is especially relevant with reversed-phase high-performance liquid chromatography (RP-HPLC), as hydrophobic–hydrophilic equilibria determine both chromatographic distribution and the penetration of a drug within a living system, in addition to its affinity to a receptor.

Until recently, the applications of RP-HPLC in medicinal chemistry and molecular pharmacology concentrated on the determination of convenient scales of drug hydrophobicity [1]. Much effort has been devoted to producing an RP-HPLC system mimicking the common reference hydrophobicity scale which is provided by the octanol–water partition system [2]. However, it must be realised that what is measured as the hydrophobicity of solutes depends on the partition system employed [3]. Different hydrophobicity scales reflect some "phobia" of solutes towards the aqueous phase but this "phobia" depends considerably on the environment. There is no single, unique, universal, continuous, unequivocally defined and pharmacologically distinguished hydrophobicity scale. Consequently, there is no justification to prefer information on properties of drug solutes provided by an individual RP-HPLC system over information gained from measurements performed in another chromatographic system [4].

We assume that systematic information extracted from diverse RP-HPLC retention data can be more appropriate for the prediction of the net effects of complex pharmacokinetic and pharmacodynamic processes than information based on an individual

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one-dimensional hydrophobcity scale. To extract systematic information from diverse (yet usually highly intercorrelated) sets of data, the multivariate chemometric methods of data analysis must be applied.

Factorial methods of analysis of chromatographic data have been successfully employed for the classification of solutes and/or chromatographic systems according to their physico-chemical properties (*e.g.*, refs. 5 and 6). Combining the normal-phase thin-layer chromatographic and other physico-chemical data for amino acids, Wold *et al.* [7] applied the approach to determine the pharmacological activity of a series of peptides. Recently, we subjected to principal component analysis a large set of RP-HPLC data previously determined for a series of eighteen imidazoline derivative drugs of various pharmacological activity [8]. The grouping of these agents according to retention behaviour correlated surprisingly well with their established pharmacological classification. As the approach offers the potential of facilitating the search for new drugs, at the same time reducing the costs and the use of laboratory animals, we further tested its validity. Here, the results are reported of principal component analysis of several RP-HPLC hydrophobicity parameters determined for a series of drugs known to affect the biological activity of histamine.

## EXPERIMENTAL

### *Materials*

The following drugs were chromatographed after dissolution in the mobile phase: anatazoline hydrochloride (a gift from Polfa, Warsaw, Poland), burimamide (Smith Kline & French, Welwyn Garden City, UK), chloropyramine hydrochloride (a gift from Polfa, Kraków, Poland), cimetidine (a gift from Polfa, Rzeszów, Poland), cinnarizine (a gift from Polfa, Warsaw, Poland), dimethindene maleate (a gift from Zyma, Munich, Germany), diphenhydramine hydrochloride (a gift from Polfa, Kraków, Poland), disodium cromoglycate (a gift from Lek, Ljubljana, Slovenia), famotidine (a gift from Polfa, Starogard, Poland), isothipendyl hydrochloride (a gift from ASTA Pharma, Frankfurt, Germany), ketotifen fumarate (a gift from Polfa, Warsaw, Poland), khellin (Fluka, Buchs, Switzerland), mepyramine maleate (a gift from Rhône-Poulenc, Dagenham, UK), metiamide (Smith Kline & French), nizatidine (a gift from Lilly France, Saint-Cloud, France), pheniramine hydromaleate (a gift from Hoechst, Frankfurt, Germany), pizotifen maleate (a gift from Sandoz, Nürnberg, Germany), promethazine hydrochloride (a gift from Polfa, Jelenia Góra, Poland), ranitidine hydrochloride (a gift from Polfa, Starogard, Poland), roxatidine acetate hydrochloride (a gift from Albert-Roussel Pharma, Wiesbaden, Germany), tripelenamine hydrochloride (a gift from Teva Pharmaceutical Industries, Petah Tiqva, Israel) and tymazoline hydrochloride (a gift from Polfa, Warsaw, Poland).

A Suplex pKb-100 deactivated hydrocarbon-bonded silica column (15 cm × 4.6 mm (I.D.) (particle size 5 $\mu$m) was purchased from Supelco (Bellefonte, PA, USA) and a Unisphere-PBD polybutadiene-encapsulated alumina column (10 cm × 4.6 mm (I.D.) (partice size 8 $\mu$m) from Biotage, (Charlottesville, VA, USA).

Deuteromethanol ($CH_3O^2H$) was purchased from IBJ (Swierk, Poland).

### *Apparatus*

The chromatographic system consisted of a Model L-6200A pump, a Model L-4250 UV–VIS detector and a Model D-2500 chromato-integrator (all from Merck–Hitachi, Vienna, Austria). The experiments were carried out using a flow-rate of 1 ml/min and the columns were thermostated at 22°C.

### *Chromatographic conditions*

Chromatography was carried out polycratically using eluents with following proportions (v/v) of methanol to buffer: 80:20, 70:30, 60:40, 50:50, 40:60, 30:70 and 20:80. Buffers of pH 2.20, 7.40 and 11.40 were prepared by adding 0.2 *M* NaOH to a solution of 0.04 *M* $CH_3COOH$, 0.04 *M* $H_3PO_4$ and 0.04 *M* $H_3BO_3$.

Capacity factors were calculated assuming a constant dead volume of the column. The dead volumes were determined by measuring signals of deuteromethanol ($CH_3O^2H$) chromatographed with neat methanol ($CH_3OH$) [9].

Logarithms of capacity factors (log $k'$) for individual solutes chromatographed in a given chromatographic system were regressed against the volume fraction of methanol in the eluent. The linear

part of the relationship was extrapolated to a hypothetical capacity factor corresponding to 0% of methanol (100% buffer) in the mobile phase. The resulting retention parameters, normalized to pure buffer, log $k'_w$, were subjected to further analysis.

*Chemometric analysis*

A 5 × 22 matrix of log $k'_w$ parameters determined in five HPLC systems for 22 solutes was subjected to statistical analysis by the principal component method [10]. A standard, commercially available statistical package was employed, run on a personal computer.

## RESULTS AND DISCUSSION

Structural formulae of the compounds studied are given in Fig. 1. There are two main pharmacological groups represented by the drugs analysed [11]. One group are classified as classical antihistaminics (antiallergics) which are antagonists of the type $H_1$ of histamine receptor. The second large group are antiulcerative drugs which block the type $H_2$ of histamine receptor. Ketotifen, sodium cromoglycate and khellin are used for the prophylaxis of bronchial asthma. Pizotifen is an antimigraine drug and cinnarizine is recommended for improving the brain blood circulation.

All the drugs are assumed to modify somehow the effects of endogenous histamine. The hypothesis on the structural similarity of the drugs to one of the two energetically favoured conformations of histamine [12] and the rational design of $H_2$-antagonists [13] imply differences in the physico-chemical properties of the compounds. If these differences could be measured by convenient HPLC methods then the procedure of preselection of potential drug candidates could be greatly facilitated.

Measurements of chromatographic retention were performed at neutral and acidic pH when employing the Suplex pKb-100 hydrocarbon-bound silica column. The chemical stability of the Unisphere-PBD polybutadiene-encapsulated alumina column allowed retention measurements at acidic, neutral and alkaline pH. As the compounds studied are mostly organic bases their retention at pH 2.20 on the silica-based column was low and several solutes (**2**, **4**, **9**, **14**, **15** and **19** in Fig. 1) were excluded from the stationary zone in the whole range of eluent composition studied. However, these compounds were retained using the alumina-based column also operated at pH 2.20. On the other hand, exclusion of some solutes **4**, **8** and **15**) was observed with the alumina based column at pH 11.40. Well measurable retention data were obtained on both columns at pH 7.40. With the alumina-based column only acidic cromoglycate was excluded.

Very good linearity of the relationship of log $k'$ *versus* methanol concentration in the eluent was observed for the most of the solutes when chromatographed at pH 7.40 and 11.40 in a wide eluent composition range (see Fig. 2 for illustration). The linearity range was limited to the higher buffer concentrations for most of the compounds chromatographed at acidic pH. The number of the highest consecutive concentrations of the buffer used to extrapolate linearly log $k'$ data to log $k'_w$ is given in Table I together with the respective log $k'_w$ values. For excluded solutes a value of $-1$ was assumed for chemometric analysis.

The log $k'_w$ data in Table I indicate that typical $H_2$-antagonists are generally less retained than $H_1$-antagonists. However, relative ordering of the agents differs in all five RP-HPLC systems studied. Hence, specific properties of individual chromatographic systems affect the specific retention of a given solute.

Differences in the properties of solutes which manifest themselves in a systematic manner may be of relevance for their diverse pharmacological behaviour. To check this hypothesis, the data matrix in Table I was subjected to principal component analysis (PCA). PCA is a statistical procedure aimed at the reduction of the dimensionality of data space but providing concentration of systematic information previously dispersed over many variables in a few common abstract factors [10].

The PCA of the log $k'_w$ data in Table I yielded two main factors accounting together for about 96% of the variance. The first principal component accounted for 90.3% of the total variance and the second for 5.7% of the variance. The loadings (weights) of the two main principal components by the five variables of the chromatographic analysis (Fig. 3) indicate that the first factor (*W*1) is loaded mostly by retention data determined at pH 7.40 on Suplex pKb-100 and at pH 11.40 on Unisphere-PBD. In both instances one can presume an effec-

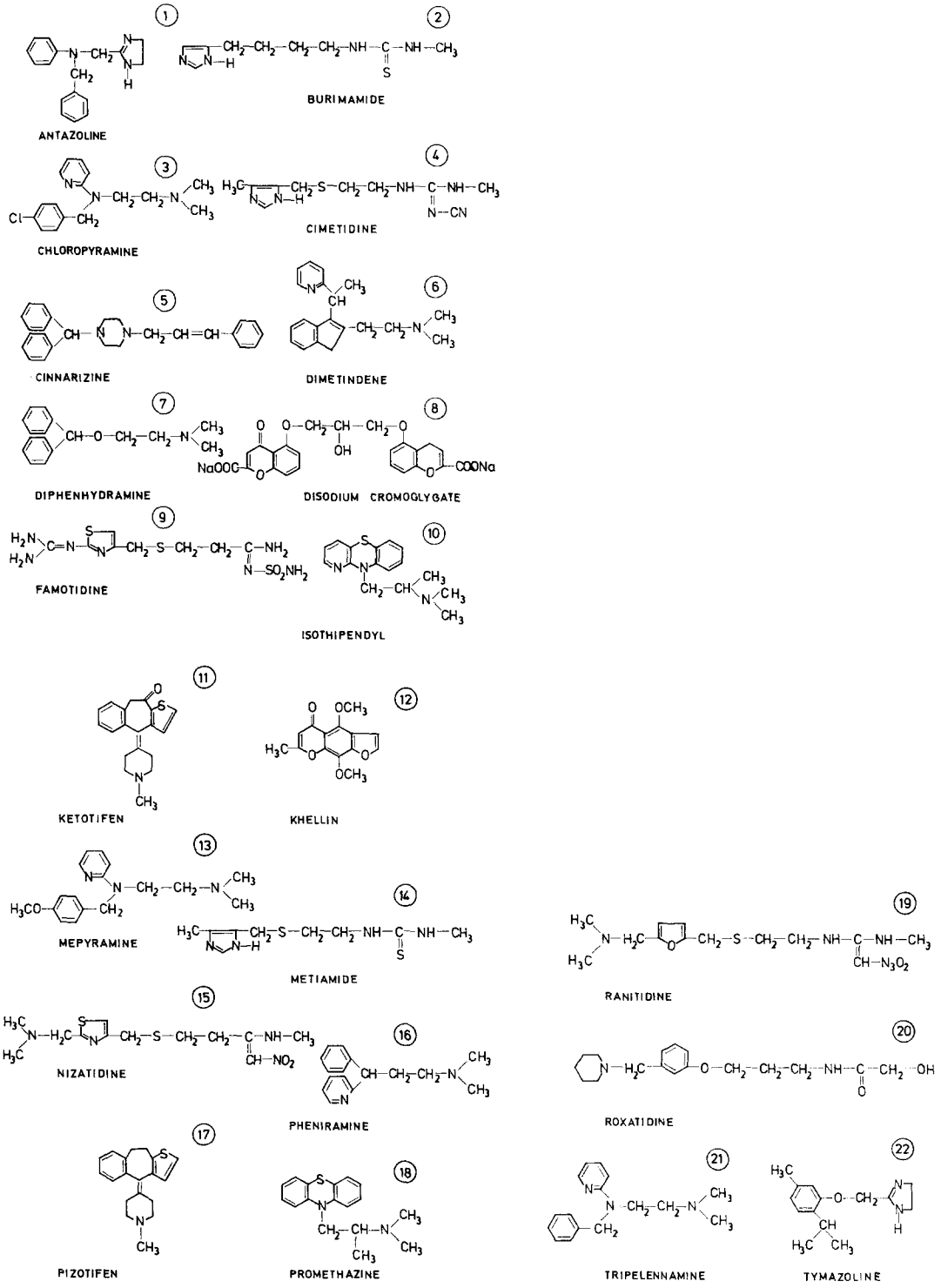


Fig. 1. Structural formulae of drug solutes studied.

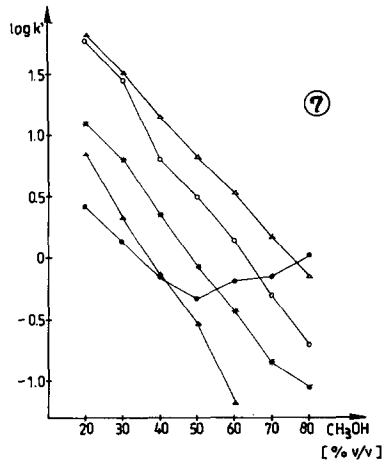


Fig. 2. Typical relationships between logarithms of capacity factors (log $k'$) and percentage (v/v) of methanol in eluent for diphenhydramine chromatographed in five HPLC systems: △ = Suplex pKb-100, pH 7.40; ○ = Unisphere-PBD, pH 7.40; * = Unisphere-PBD, pH 11.40; ▲ = Suplex pKb-100, pH 2.20; ● = Unisphere-PBD, pH 2.20.

tive suppression of specific, polar solute–stationary phase interactions due to the suppression of the ionization of the solutes and deactivation of the stationary phase support material. Thus, $W1$ is postulated to extract information on abilities of the solutes to take part in the molecular-size-related, nonspecific, dispersive intermolecular interactions. The second principal component ($W2$) is loaded mostly by retention data determined on alumina-based Unisphere-PBD column at acidic and neutral pH. $W2$ can be assumed to reflect the abilities of the fully or partially ionized basic solutes to participate in structurally specific, polar (ionic, dipole–dipole, dipole–induced dipole, charge-transfer) intermolecular interactions.

Principal component object (solute) scores were calculated for individual compounds. The positions of the drugs on the plane determined by the two first principal component axes (PC1 and PC2) are displayed in Fig. 4. There are two clear clusters of sol-

TABLE I

LOGARITHMS OF CAPACITY FACTORS OF DRUG SOLUTES EXTRAPOLATED TO PURE BUFFER AS THE MOBILE PHASE (LOG $k'_w$) FROM FIVE HPLC SYSTEMS

Number of mobile phase concentrations used for linear extrapolation is denoted by $n$. For excluded solutes log $k'_w = -1$ was assumed.

| No.[a] | Suplex pKb-100 | | | | Unisphere-PBD | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | pH 2.20 | | pH 7.40 | | pH 2.20 | | pH 7.40 | | pH 11.40 | |
| | log $k'_w$ | $n$ | log $k'_w$ | $n$ | log $k'_w$ | $n$ | log $k'_w$ | $n$ | log $k'_w$ | $n$ |
| **1** | 1.8063 | 4 | 2.4829 | 6 | 0.8102 | 4 | 1.8797 | 7 | 1.7027 | 6 |
| **2** | −1 | | 1.0777 | 6 | −0.6040 | 3 | 0.4621 | 5 | −1.0041 | 3 |
| **3** | 1.2718 | 5 | 3.0857 | 5 | 0.9504 | 4 | 2.9227 | 6 | 2.4413 | 4 |
| **4** | −1 | | 1.4185 | 4 | 0.5141 | 3 | 0.100 | 2 | −1 | |
| **5** | 3.6665 | 5 | 4.5896 | 4 | 2.7722 | 4 | 4.8184 | 5 | 4.5332 | 4 |
| **6** | 1.4333 | 6 | 2.8009 | 6 | 0.7147 | 4 | 2.2400 | 7 | 1.9607 | 6 |
| **7** | 1.8521 | 5 | 2.4835 | 6 | 0.8999 | 4 | 2.6170 | 6 | 1.9383 | 6 |
| **8** | −1.0800 | 2 | 2.2643 | 6 | 1.3939 | 5 | −1 | | −1 | |
| **9** | −1 | | 0.8113 | 7 | 0.3027 | 3 | 0.4189 | 6 | −0.7400 | 2 |
| **10** | 2.0595 | 5 | 2.9256 | 6 | 1.3740 | 4 | 2.4681 | 7 | 2.5455 | 6 |
| **11** | 1.6011 | 6 | 2.8090 | 5 | 1.2823 | 4 | 2.4950 | 7 | 1.8976 | 5 |
| **12** | 2.4974 | 6 | 2.3777 | 7 | 0.6595 | 6 | 0.6738 | 5 | 0.5587 | 6 |
| **13** | 0.2599 | 3 | 2.3865 | 6 | 0.5465 | 4 | 2.0513 | 7 | 0.4622 | 4 |
| **14** | −1 | | 1.1579 | 7 | 0.3997 | 3 | 0.3706 | 4 | −0.3768 | 4 |
| **15** | −1 | | 1.1229 | 7 | 0.3294 | 4 | 0.5500 | 2 | −1 | |
| **16** | 0.4443 | 5 | 1.8109 | 7 | 0.1825 | 4 | 1.1495 | 6 | 1.1568 | 5 |
| **17** | 2.4328 | 6 | 3.7361 | 4 | 1.9797 | 4 | 3.5771 | 5 | 3.1903 | 5 |
| **18** | 2.2227 | 6 | 3.7144 | 4 | 1.7941 | 3 | 3.4184 | 6 | 3.0831 | 6 |
| **19** | −1 | | 1.2764 | 6 | 0.3019 | 4 | 0.3320 | 5 | −1.1445 | 4 |
| **20** | −1.2500 | 2 | 1.3134 | 7 | −0.5500 | 2 | 0.5392 | 7 | 0.8782 | 4 |
| **21** | 0.7102 | 5 | 2.3295 | 6 | 0.4458 | 4 | 1.9938 | 7 | 1.6395 | 6 |
| **22** | 1.7398 | 6 | 2.2431 | 6 | 0.8952 | 4 | 2.1513 | 6 | 1.6954 | 6 |

[a] Solutes are numbered as in Fig. 1.

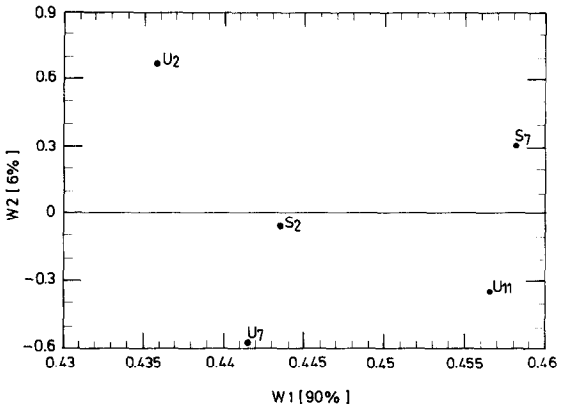


Fig. 3. Two-dimensional scatter plot of two first principal component weights, *W*1 and *W*2, due to the individual HPLC system employed: $S_7$ = Suplex pKb-100, pH 7.40; $U_{11}$ = Unisphere-PBD, pH 11.40; $S_2$ = Suplex pKb-100, pH 2.20; $U_7$ = Unisphere-PBD, pH 7.40; $U_2$ = Unisphere-PBD, pH 2.20.

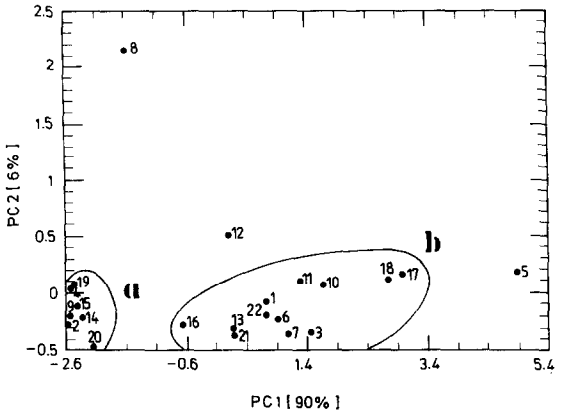


Fig. 4. Two-dimensional scatter plot of scores by individual drug solutes in two first principal components, PC1 and PC2.

utes in Fig. 4 and three solutes show distinctive chromatographic behaviour. Undoubtedly, compounds **2**, **4**, **9**, **14**, **15**, **19** and **20** form a compact cluster **a** of drugs unequivocally classified as antagonists of the $H_2$ type of histamine receptor [11,14–16]. This is in spite of evident diversity of chemical structures within the group of $H_2$-antagonists studied.

Among twelve compounds forming cluster **b** there are ethylendiamine derivatives (**3**, **13** and **21**), imidazoline derivatives (**1** and **22**), phenothiazine derivatives (**10** and **18**), an oxyethylamine derivative (**7**), an arylalkylamine derivative (**16**) and an indene derivative (**6**). All these are classified and employed clinically as typical antagonists of $H_1$ receptor [11, 17, 18]. To the cluster **b** belong also ketotifen (**11**) and pizotifen (**17**). Although the clinical indication for ketotifen is prophylaxis of bronchial asthma and for pizotifen it is migraine, both ketotifen [19] and pizotifen [20] are reported to possess antagonistic activity towards $H_1$-type histamine receptors.

Compounds **8** and **12** clearly do not belong to cluster **a** nor **b**. Khellin (**12**) is a miolytic agent [21] and its derivative, disodium cromoglycate (**8**), decreases the liberation of histamine which accompanies the antigen-antibody reaction [22]. None of the drugs is known to interfere with the $H_1$ or $H_2$ receptors.

Cinnarizine (**5**) could perhaps be included in cluster **b**. This drug has diverse sites of actions, including some antihistaminic properties [23]. Central effects ascribed to the drug are certainly connected with its high hydrophobicity (and thus brain barrier permeation), as is reflected by a high PC1 score.

The data discussed above demonstrate the applicability of the chemometrically processed retention data, generated in diverse HPLC systems, for predicting the pharmacological classification of drug solutes. As representative sets of reliable HPLC data can readily be obtained for large series of compounds, the approach developed here appears suitable for the preselection of drugs and other substances with a given, required property.

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