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ORIGINAL PAPER

Evaluation of molecular descriptors and HPLC retention data of analgesic and anti-inflammatory drugs by factor analysis in relation to their pharmacological activity

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Abstract Factor analysis (FA) was performed for some analgesic, anti-inflammatory and antipyretic drugs to model relationships between molecular descriptors and HPLC retention parameters. FA performed using 26 sets of structural parameters, 26 sets of HPLC retention data, and considering all parameters together (52 parameters) led to the extraction of two main factors. The first principal component (factor 1) accounted for 65–73% of the variance in the data. The second principal component (factor 2) explained 27–35% of data variance. Moreover, of the 52 parameters tested, the highest influence on factor value was found with chromatographic parameters and selected structural parameters (i.e., energy quantum-chemical parameters and electron affinity specifying parameters). Additionally, the pattern of distribution of individual drugs within the plane determined by the two principal components (factors 1 and 2) was in good agreement with their pharmacological (analgesic, anti-inflammatory and antipyretic) properties. The findings are discussed from the point of view of structure–activity relationships.

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Introduction

Analgesics (also known as painkillers) are members of a diverse group of drugs used to relieve pain. The word *analgesic* is derived from the Greek *an-* ("without") and *algos-* ("pain"). Analgesic drugs act in various ways on the peripheral and central nervous systems and include non-steroidal anti-inflammatory drugs (NSAIDs) such as acetaminophen [1, 2], aminophenazone [2], acetylsalicylic acid (ASA) [2, 3], diclofenac [4, 5], etodolac [4–7], ketorolac [6, 8], nimesulide [9, 10], noramidopyrine [11, 12], piroxicam [5], salicylamide [11, 12], and sulindac [13], as well as synthetic drugs with narcotic properties such as tramadol [14], and many others. Analgesics are usually drugs with antipyretic (lowering elevated body temperature and relieving pain without impairing consciousness) and, in higher doses, anti-inflammatory effects. NSAIDs are usually indicated for the treatment of acute or chronic conditions where pain and inflammation are present. Research continues into their potential for prevention of colorectal cancer, and treatment of other disorders such as cancer and cardiovascular disease. Analgesics are generally indicated for the symptomatic relief of the following disorders: rheumatoid arthritis, osteoarthritis, inflammatory arthropathies (e.g., ankylosing spondylitis, psoriatic arthritis, and Reiter's syndrome), acute gout, dysmenorrhoea (menstrual pain), metastatic bone pain, headache and migraine or postoperative pain. They have also been found to be invaluable in palliative care to alleviate the severe, chronic, disabling pain of terminal conditions such as cancer.

Factor analysis (FA)—a chemometric technique based on principle component analysis (PCA)—belongs to a set of data-processing methods aimed at extracting and visualizing systematic patterns or trends in large data sets. By FA, the number of variables in a data set can be reduced by finding linear combinations of those variables that explain most of the variability. Unfortunately, the independent variables applied are often mutually inter-correlated. For this reason, inter-correlated chemical, spectroscopic, chromatographic and other data that are often unsuitable for direct multiple regression analysis can be subjected to multivariate analysis such as FA. In other words, using FA, all those original parameters that are interrelated by simple or multiple correlations are combined linearly to two orthogonal principal components (factors). To date, FA (or PCA) has been applied to the classification of a number of compounds (drugs) using HPLC retention [15–19], TLC [20–23] or other [24] data.

The aim of the present study was to determine the relationships between HPLC retention parameters of a series of drugs differing in chemical structure and characterized by similar pharmacological (analgesic, anti-inflammatory and antipyretic) activity and their structural parameters obtained by molecular modeling calculations applying the FA method. The following 12 compounds were selected: acetaminophen, aminophenazone, acetylsalicylic acid, diclofenac, etodolac, ketorolac, nimesulide, noramidopyrine, piroxicam, salicylamide, sulindac and tramadol. These drugs include weak carboxylic acids (ASA, diclofenac, etodolac, ketorolac, sulindac), free bases (aminophenazone and tramadol), sulfonic acids (noramidopyrine), sulfonamides (nimesulide), amides (salicylamide), phenols (acetaminophen) and enoloacids (piroxicam). The aim of the work was to evaluate the relationships between structural molecular descriptors along with chromatographic data obtained for the studied analgesics in terms of their pharmacological activity by means of FA.

Materials and methods

Drugs

In all experiments the following drugs were investigated: (1) acetylsalicylic acid (ASA), (2) salicylamide, (3) diclofenac (as sodium salt), (4) noramidopyrine (as sodium salt), all from Polpharma, Starogard Gdański, Poland; (5) acetaminophen from Rhône-Poulenc, Köln, Germany; (6) aminophenazone from Polfa, Pabianice, Poland; (7) etodolac from Teva Pharmaceutical Industries, Petah Tikva, Israel; (8) ketorolac from Ranbaxy, New Delhi, India; (9) nimesulide from Chemex, Vienna, Austria; (10) piroxicam from Jelfa, Jelenia Góra, Poland; (11) sulindac from Dipharma, Basiliano, Italy and (12) tramadol (as hydrochloride) from Grünenthal, Aachen, Stolenberg, Germany.

Structural parameters

The structures of the tested compounds were investigated by molecular modeling with the use of HyperChem 7.5 software (HyperCube, Gainesville, FL). First, the structures of the compounds were pre-optimized geometrically with the molecular mechanics force field procedure (with MM+ method). This allowed preparation of structures for further optimization steps. The resulting structures were then optimized by means of the quantum-based, semi-empirical AM1 method, and by applying the Polak-Ribiere algorithm with a gradient limit of 0.01 kcal $Å^{-1}$.

The following molecular descriptors were considered: total energy (TE), binding energy (BE), atom interaction energy (IAE), electronic energy (EE), heat of formation (HF), highest occupied molecular orbital energy (EHOMO), lowest unoccupied molecular orbital energy (ELUMO), ionization energy (potential) (IE_IP) and electron affinity (EA). EA was calculated as the difference between the heat of molecular positive ion formation and neutral molecules (ionization potential) or between molecular negative ion and neutral molecules (electron affinity), expressed in electronvolts. Electronegativity (EN) was calculated as an arithmetic mean of ionization potential and electron affinity according to Mulliken [25, 26]. The "hardness" of molecules (HARD) was calculated according to Parr and Pearson [27] as well as Robles and Bartolotti [28] and presented as half of the difference between the ionization potential and the electron affinity. Additionally, the following values were used: the highest (ED_MAX) and the lowest (ED_MIN) free electron density, electron orbital density HOMO (ED_HOMO) and LUMO (ED_LUMO), the values of the highest positive (MAX_POS) and negative (MAX_NEG) charge of atoms that constitute a molecule, the difference between the highest positive and negative charges of atoms constituting a molecule (DELTA_Q), total dipole moment (TDM), energy equivalent to the length of the longest electron transition for which the energy value of an oscillator was different than zero (E_L), and the value of the most intensive electron transition for which the energy value of an oscillator took the maximum value of electron transition (E_MAX; the value of wave numbers calculated into eV), as well as the maximum energy value of the oscillator (OS_EMAX).

Moreover, the following additional structural parameters were considered: partial values of molar volume in water (V_MOL) and the interaction energy with water (E_INT) calculated by the ChromSword 1.0 program (Merck, Darmstadt, Germany). The logarithms of the *n*-octanol-

water partition coefficient (LG_P), which reflect the hydrophobicity of the drugs studied, were calculated according to Nys and Rekker [29]. Molecular refractivity (MR) was calculated as the sum of the bond refractivities for all pairs of connected atoms.

Chromatographic analysis

Chromatographic analysis was performed with a Waters SM 2690 Alliance HPLC system equipped with a PDA 996 diode detector (Waters, Milford, MA) and a Compaq Deskpro computer (Compaq, Houston, TX) with the Millennium 3.2 program for data collection and process control. The following HPLC columns were employed: Nucleosil C18 AB column, 50×3.0 mm i.d. (Macherey-Nagel, Düren, Germany), packed with octadecylsilica with particle size 5 μm; Nucleogel 100-5 RP column, 150×4.6 mm i.d. (Macherey-Nagel,), packed with polystyrene copolymer cross-linked by divinylbenzene with particle size 5 μm; Aluspher RP select B column, 125×4.0 mm i.d. (Merck), packed with aluminum oxide with chemically bounded polybutadiene, with particle size 5 μm.

The compounds studied were chromatographed by applying isocratic conditions to the columns described above at ambient temperature. The mobile phases were acetonitrile:0.01 M phosphate buffer at pH 2.5, 7.0 or 11.0; methanol:0.01 M phosphate buffer at pH 2.5, 7.0 or 11.0; tetrahydrofuran:0.01 M phosphate buffer at pH 2.5, 7.0 or 11.0 with the following proportions 70:30, 60:40, 50:50, 40:60 and 30:70 (% v/v). However, in the case of the Nucleosil C18 AB column, experiments were performed

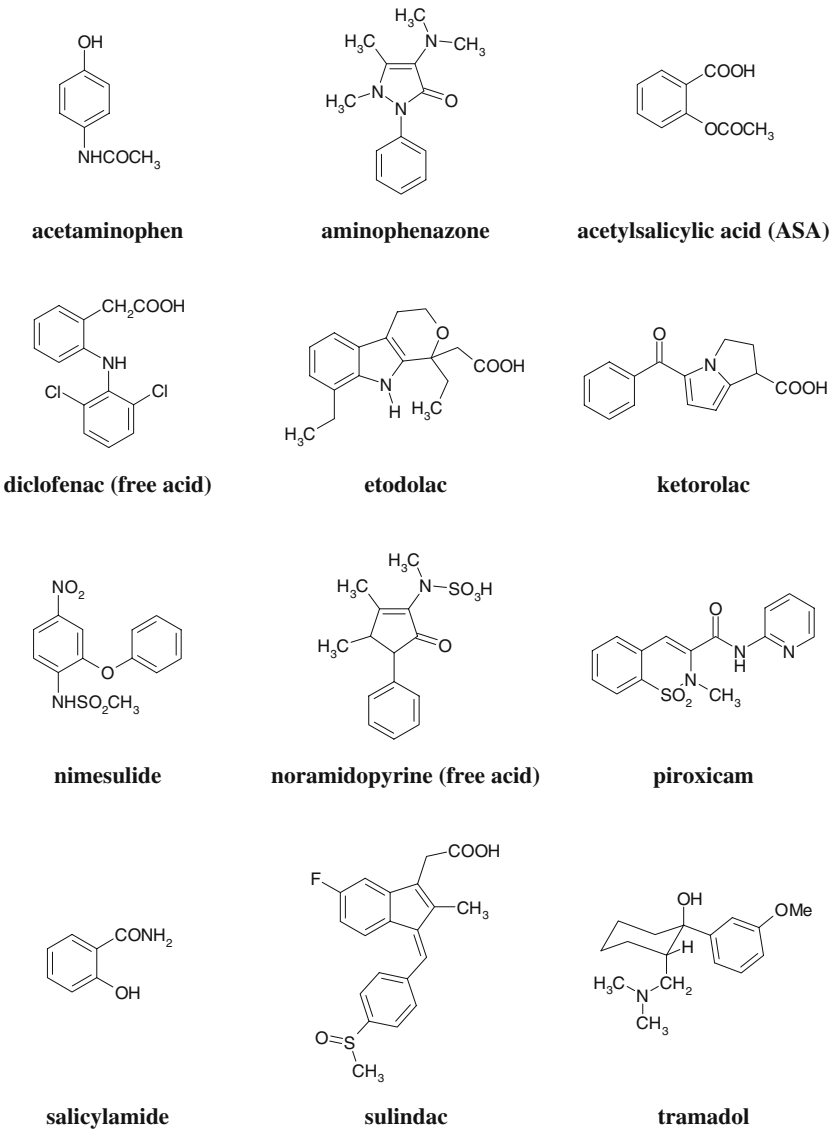

Fig. 1 Chemical structures of the studied compounds

Table 1 Values of HPLC retention data and molecular descriptors used in factor analysis (FA). *ASA* Acetylsalicylic acid. See Materials and methods for definitions of molecular parameters

Compound		HPLC retention data											
No.	Name	LGK W2NAL	LGK W2NAS	LGK W7NAL	LGK W7NAS	LGK W2NML	LGK W2NMS	LGK W7NML	LGK W7NMS	LGK W2NTL	LGK W2NTS	LGK W7NTL	LGK W7NTS
1	Acetaminophen	0.02	0.05	0.06	0.11	0.08	0.12	0.07	0.12	−0.01	0.17	−0.02	0.17
2	Aminophenazone	−0.19	0.37	0.44	1.0	−0.01	0.09	1.4	2.5	−0.11	0.06	0.14	0.48
3	ASA	0.71	1.1	−0.07	0.09	1.5	2.5	0.06	0.09	0.71	1.1	−0.12	0.12
4	Diclofenac	3.0	5.0	1.1	2.7	4.8	6.0	3.5	5.1	2.7	5.0	0.82	2.4
5	Etodolac	2.7	4.5	0.93	2.5	4.7	6.8	3.5	4.5	2.6	4.7	0.88	2.4
6	Ketorolac	1.5	2.9	0.10	−0.22	3.0	4.9	1.8	3.3	1.4	2.6	−0.01	0.08
7	Nimesulide	2.6	4.1	1.7	3.2	3.3	4.8	2.5	4.5	2.4	4.2	1.4	2.9
8	Noramidopyrine	−0.05	0.15	0.01	0.01	0.10	0.07	0.10	0.11	−0.11	0.01	−0.04	−0.22
9	Piroxicam	1.3	2.1	0.09	−0.01	2.4	3.5	1.7	3.6	1.0	1.8	0.04	0.08
10	Salicylamide	0.50	0.86	0.46	0.78	1.1	1.8	0.8	1.6	0.74	1.3	0.67	1.3
11	Sulindac	1.6	3.5	0.02	0.18	4.0	6.5	27	5.1	0.78	1.7	0.01	0.14
12	Tramadol	0.08	0.22	0.42	0.78	0.70	2.1	1.4	1.9	−0.07	0.08	0.29	0.33

Compound		HPLC retention data											
No.	Name	LGK W2GL	LGK W2GSQ	LGK W7GL	LGK W7GSQ	LGK W7GHL	LGK W7GHS	LGK W11GL	LGK W11GS	LGK W2AL	LGK W2ASQ	LGK W7AL	LGK W7ASQ	LGK W11AL
1	Acetaminophen	−0.16	0.31	−0.04	0.74	0.01	0.52	0.06	0.35	−0.28	−0.23	−0.30	−0.03	−0.56
2	Aminophenazone	−0.23	0.99	0.70	2.7	1.1	1.8	1.4	2.2	−0.72	−1.4	−0.63	0.89	0.10
3	ASA	0.82	1.5	0.13	2.1	−0.04	0.20	0.10	0.25	0.40	0.31	−1.5	−1.2	−0.84
4	Diclofenac	2.7	4.4	1.2	2.7	1.8	3.2	2.3	3.9	2.5	3.6	−0.12	0.67	−0.04
5	Etodolac	2.2	3.7	1.1	2.8	1.7	3.3	2.0	3.5	1.9	2.8	0.63	2.0	−0.11
6	Ketorolac	1.8	3.1	1.3	2.4	0.60	1.7	0.87	1.8	1.4	1.6	−0.66	−0.02	−0.69
7	Nimesulide	2.7	4.1	2.6	4.3	2.1	3.2	1.4	3.1	1.6	2.6	0.30	0.52	−0.33
8	Noramidopyrine	−0.43	0.58	−0.06	0.71	−0.22	0.62	0.23	0.44	0.59	0.71	−1.7	−0.12	−1.4
9	Piroxicam	1.9	2.9	0.73	2.3	0.88	1.9	1.1	2.1	0.85	0.99	−0.61	0.44	−0.57
10	Salicylamide	0.68	1.3	0.89	2.3	0.78	1.3	0.06	0.16	0.26	0.85	0.30	0.84	−0.71
11	Sulindac	1.9	3.7	0.37	2.7	0.35	2.1	1.2	2.9	1.5	3.0	−0.34	0.71	−0.72
12	Tramadol	−0.02	0.92	1.4	2.9	1.3	2.1	1.8	2.5	−1.1	1.3	1.7	2.9	1.1

Compound		HPLC retention data				Molecular descriptors									
No.	Name	LGK W11AS	V_MOL	E_I NT	LG_P	MR	TE	BE	IAE	EE	HF	IE_IP	EA	EN	HARD
1	Acetaminophen	−0.33	126	−111	−0.56	41	−46026	−2125	−43901	−211886	−57	8.0	−0.41	3.8	4.2
2	Aminophenazone	1.2	201	−102	−0.76	68	−65415	−3435	−61980	−427308	71	7.0	−0.99	3.0	4.0
3	ASA	−0.16	136	−123	0.29	43	−58671	−2335	−56336	−285517	−142	8.9	−1.2	3.9	5.1
4	Diclofenac	1.1	223	−124	3.7	75	−81620	−3310	−78310	−487947	−54	7.8	−0.80	3.5	4.3
5	Etodolac	0.90	239	−149	2.5	80	−83818	−4399	−79420	−612908	−108	7.5	−0.52	3.5	4.0

6	Ketorolac	0.45	184	−157	1.4	69	−75323	−3578	−71745	−462515	−45	8.2	−1.1	3.6	4.6
7	Nimesulide	−0.10	220	−146	−0.99	81	−93050	−3472	−89578	−594540	−35	8.8	−2.2	3.3	5.5
8	Noramidopyrine	0.08	227	−161	−5.3	83	−92060	−3790	−88271	−630482	−39	7.2	−1.9	2.6	4.6
9	Piroxicam	−0.60	231	−227	−4.5	89	−96718	−3927	−92792	−683306	−42	7.8	−1.9	2.9	4.8
10	Salicylamide	0.23	117	−97	0.10	36	−42435	−1845	−40590	−185297	−52	8.9	−0.83	4.0	4.9
11	Sulindac	0.24	289	−168	3.7	99	−102256	−4653	−97603	−698329	−86	8.3	−2.1	3.1	5.2
12	Tramadol	0.61	245	−147	2.8	77	−74742	−4347	−70395	−551781	−78	7.9	−0.22	3.8	4.1

Compound		Molecular descriptors												
No.	Name	E HOMO	E LUMO	ED_MAX	ED_MIN	ED_HOMO	ED_LUMO	MAX_POS	MAX_NEG	DELTA_Q	TDM	E_L	E_MAX	OS_MAX
1	Acetaminophen	−8.6	0.04	1.9	0.76	1.5	1.0	0.31	−0.36	0.67	3.2	3.6	5.6	0.89
2	Aminophenazone	−8.5	−0.10	1.9	0.76	1.2	0.94	0.31	−0.32	0.62	3.4	3.9	5.9	0.81
3	ASA	−9.8	−0.56	1.9	0.72	1.9	1.12	0.36	−0.37	0.73	2.2	4.0	5.8	1.1
4	Diclofenac	−8.6	−0.22	2.0	0.75	1.2	1.0	0.31	−0.38	0.69	0.94	3.9	5.3	0.48
5	Etodolac	−8.2	0.14	1.9	0.72	0.93	1.9	0.32	−0.39	0.71	0.97	3.7	5.2	1.1
6	Ketorolac	−9.0	−0.44	1.9	0.72	1.1	1.5	0.34	−0.35	0.69	1.6	4.3	4.3	0.57
7	Nimesulide	−9.8	−1.3	1.9	0.71	1.0	0.92	2.8	−0.95	3.8	4.0	4.3	4.3	0.44
8	Noramidopyrine	−8.7	−0.95	1.9	0.72	1.2	0.97	2.8	−0.93	3.7	3.2	3.7	5.2	0.52
9	Piroxicam	−8.8	−0.93	1.9	0.70	0.78	0.81	2.9	−0.93	3.8	3.6	3.2	4.8	0.62
10	Salicylamide	−9.5	−0.32	1.9	0.75	1.3	1.1	0.35	−0.44	0.79	2.4	3.8	6.3	0.97
11	Sulindac	−9.0	−1.2	2.0	0.76	0.95	1.2	1.4	−0.78	2.2	6.6	3.3	4.8	0.71
12	Tramadol	−8.9	0.41	1.9	0.80	1.2	0.98	0.15	−0.33	0.48	1.2	3.9	6.0	0.97

only at pH 2.5 and 7.0, because stationary phases based on silica gel are sensitive to media with pH values >8 and hydrolysis of the chemically bound phase with silica and silica dissolution were observed. The detection wavelength was 254 nm. Additionally, all the mobile phases used in HPLC were filtered through a GF/F glass microfiber filter (Whatman, Maidstone, UK) and degassed by ultrasonication immediately before use. The compounds studied were dissolved in methanol.

The logarithms of the HPLC retention factors (log k) for particular chromatographed compounds in the given chromatographic system were regressed against the volume fraction of organic modifier in the eluent. The linear part of the relationship was extrapolated to a hypothetical retention factor corresponding to 0% of organic modifier in the mobile phase. The resulting retention parameters were normalized to pure buffer using linear or quadratic extrapolation and defined as log $k_{w(L)}$ or log $k_{w(sq)}$, respectively. Those HPLC retention parameters were further subjected to FA. The naming convention used was derived from buffer pH, column name, type of organic modifier in the mobile phase used in chromatographic system, and type of data extrapolation, as follows:

LGKW2NAL—Nucleosil C18 AB, acetonitrile:buffer pH 2.5, linear extrapolation
LGKW2NAS—Nucleosil C18 AB, acetonitrile:buffer pH 2.5, quadratic extrapolation
LGKW7NAL—Nucleosil C18 AB, acetonitrile:buffer pH 7.0, linear extrapolation
LGKW7NAS—Nucleosil C18 AB, acetonitrile:buffer pH 7.0, quadratic extrapolation
LGKW2NML—Nucleosil C18 AB, methanol:buffer pH 2.5, linear extrapolation
LGKW2NMS—Nucleosil C18 AB, methanol:buffer pH 2.5, quadratic extrapolation
LGKW7NML—Nucleosil C18 AB, methanol:buffer pH 7.0, linear extrapolation
LGKW7NMS—Nucleosil C18 AB, methanol:buffer pH 7.0, quadratic extrapolation
LGKW2NTL—Nucleosil C18 AB, tetrahydrofuran: buffer pH 2.5, linear extrapolation
LGKW2NTS—Nucleosil C18 AB, tetrahydrofuran: buffer pH 2.5, quadratic extrapolation
LGKW7NTL—Nucleosil C18 AB, tetrahydrofuran: buffer pH 7.0, linear extrapolation
LGKW7NTS—Nucleosil C18 AB, tetrahydrofuran: buffer pH 7.0, quadratic extrapolation
LGKW2GL—Nucleogel 100-5 RP, acetonitrile:buffer pH 2.5, linear extrapolation
LGKW2GSQ—Nucleogel 100-5 RP, acetonitrile:buffer pH 2.5, quadratic extrapolation
LGKW7GL—Nucleogel 100-5 RP, acetonitrile:buffer pH 7.0, linear extrapolation
LGKW7GSQ—Nucleogel 100-5 RP, acetonitrile:buffer pH 7.0, quadratic extrapolation
LGKW7GHL—Nucleogel 100-5 RP, acetonitrile:buffer pH 7.0, linear extrapolation
LGKW7GHS—Nucleogel 100-5 RP, acetonitrile:buffer pH 7.0, quadratic extrapolation
LGKW11GL—Nucleogel 100-5 RP, acetonitrile:buffer pH 11.0, linear extrapolation
LGKW11GS—Nucleogel 100-5 RP, acetonitrile:buffer pH 11.0, quadratic extrapolation
LGKW2AL—Aluspher RP select B, acetonitrile:buffer pH 2.5, linear extrapolation
LGKW2ASQ—Aluspher RP select B, acetonitrile: buffer pH 2.5, quadratic extrapolation
LGKW7AL—Aluspher RP select B, acetonitrile:buffer pH 7.0, linear extrapolation
LGKW7ASQ—Aluspher RP select B, acetonitrile: buffer pH 7.0, quadratic extrapolation

Table 2 FA loadings using structural parameters

Structural parameters	Factor 1	Factor 2
V_MOL	−0.9212[a]	−0.0890
E_INT	0.6809	0.4346
LG_P	0.0131	0.5190
MR	−0.9382[a]	−0.2515
TE	0.8582[a]	0.4384
BE	0.9304[a]	−0.0397
IAE	0.8481[a]	0.4581
EE	0.9264[a]	0.2866
HF	−0.1721	−0.0100
IE_IP	0.6564	−0.4188
EA	0.3089	0.9237[a]
EN	0.7273[a]	0.4415
HARD	0.1953	−0.8996[a]
EHOMO	−0.5885	0.6132
ELUMO	0.1710	0.9564[a]
ED_MAX	−0.2401	−0.4751
ED_MIN	0.1370	0.6487
ED_HOMO	0.8145[a]	0.1777
ED_LUMO	−0.1232	0.4303
MAX_POS	−0.4095	−0.8434[a]
MAX_NEG	0.4151	0.8597[a]
DELTA_Q	−0.4118	−0.8489[a]
TDM	−0.1793	−0.6236
E_L	0.3857	0.0562
E_MAX	0.5037	0.5456
OS_EMAX	0.3726	0.6040

[a] Factor loadings among the variables with values higher than 0.7

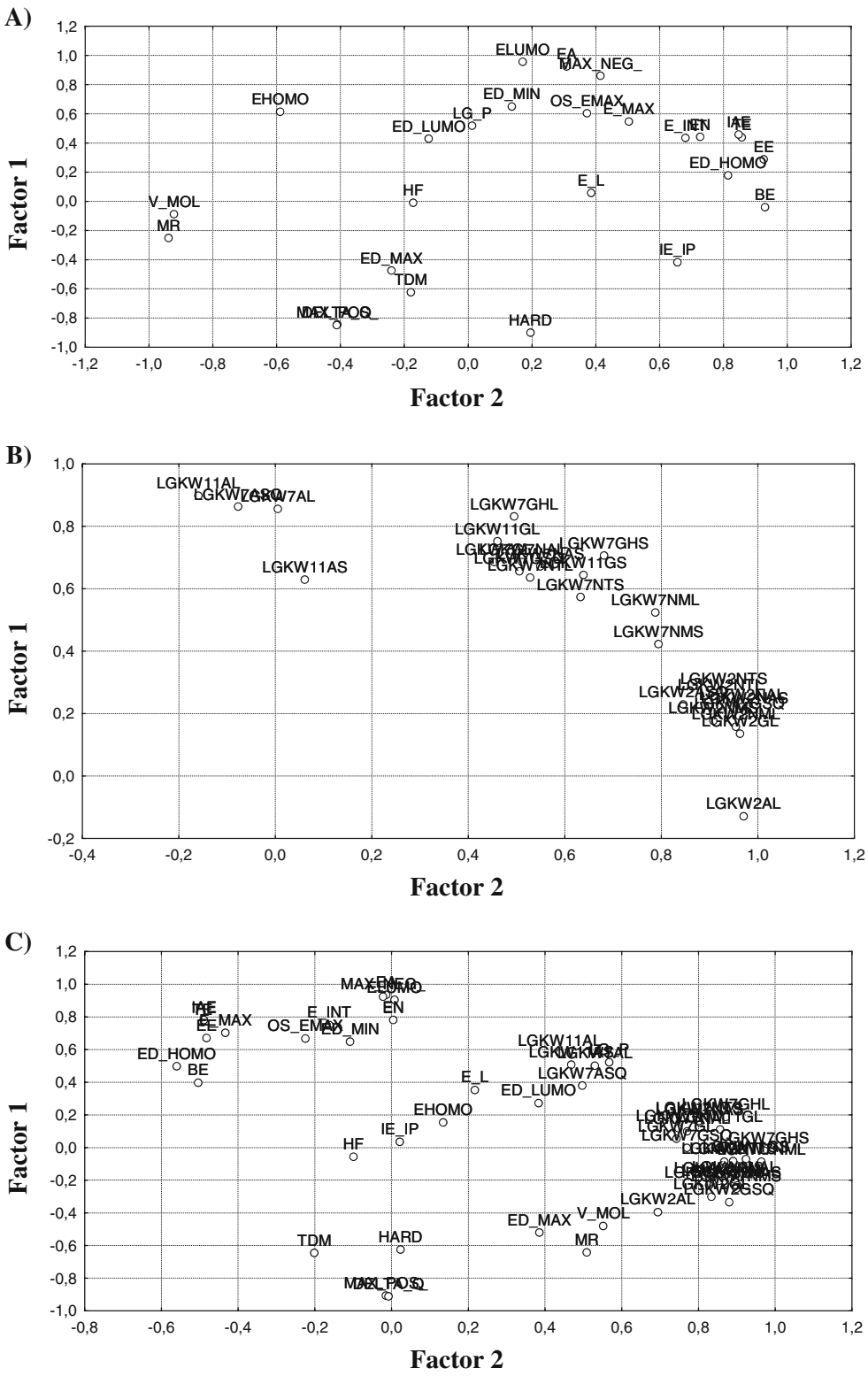

Fig. 2 Two-dimensional scatter plots of the loadings of the first two factors using **a** structural parameters, **b** HPLC retention data, or **c** structural parameters and HPLC retention data

LGKW11AL—Aluspher RP select B, acetonitrile: buffer pH 11.0, linear extrapolation
LGKW11AS—Aluspher RP select B, acetonitrile:buffer pH 11.0, quadratic extrapolation.

Statistical analysis

The chemometric analysis allowing discussion of quantitative structure–activity relationships (QSAR) was performed

Table 3 FA loadings using HPLC retention data

HPLC retention data	Factor 1	Factor 2
LGKW2NAL	0.9675[a]	0.2206
LGKW2NAS	0.9728[a]	0.2083
LGKW7NAL	0.5120	0.6852
LGKW7NAS	0.5497	0.6715
LGKW2NML	0.9548[a]	0.1577
LGKW2NMS	0.9079[a]	0.1773
LGKW7NML	0.7875[a]	0.5240
LGKW7NMS	0.7942[a]	0.4226
LGKW2NTL	0.9232[a]	0.2533
LGKW2NTS	0.9301[a]	0.2724
LGKW7NTL	0.5285	0.6354
LGKW7NTS	0.6328	0.5739
LGKW2GL	0.9635[a]	0.1358
LGKW2GSQ	0.9622[a]	0.1918
LGKW7GL	0.4540	0.6861
LGKW7GSQ	0.5061	0.6558
LGKW7GHL	0.4951	0.8312[a]
LGKW7GHS	0.6813	0.7067[a]
LGKW11GL	0.4604	0.7520[a]
LGKW11GS	0.6386	0.6445
LGKW2AL	0.9707[a]	−0.1297
LGKW2ASQ	0.8457[a]	0.2297
LGKW7AL	0.0053	0.8557[a]
LGKW7ASQ	−0.0770	0.8629[a]
LGKW11AL	−0.1594	0.8978[a]
LGKW11AS	0.0614	0.6292

[a] Factor loadings among the variables with values higher than 0.7

with using Statistica 5.1 software (StatSoft, Tulsa, OK) with the application of FA with optimized Varimax method for factors rotation.

Results and discussion

The chemical structures of the considered compounds are presented in Fig. 1. The values of all 52 structural parameters used for the 12 selected compounds are presented in Table 1. The results of FA representing the first two loadings (factor 1 and 2) of each variable and the two-dimensional scatter plots obtained with the use of various sets of parameters as structural parameters, HPLC retention data, all parameters (comprising all 52 parameters from the structural parameters and HPLC retention data) are collected in Table 2 and Fig. 2a, Table 3 and Fig. 2b, and Table 4 and Fig. 2c, respectively. Factor loadings among the variables with values higher than 0.7 are indicated in the tables. FA led to the extraction of two main factors from all

Table 4 FA loadings using all data

All data	Factor 1	Factor 2
LGKW2NAL	0.8953[a]	−0.1981
LGKW2NAS	0.9017[a]	−0.2324
LGKW7NAL	0.7705[a]	0.0983
LGKW7NAS	0.8032[a]	0.1532
LGKW2NML	0.8704[a]	−0.2177
LGKW2NMS	0.8538[a]	−0.2048
LGKW7NML	0.9641[a]	−0.0874
LGKW7NMS	0.8987[a]	−0.2519
LGKW2NTL	0.8676[a]	−0.0860
LGKW2NTS	0.8906[a]	−0.0835
LGKW7NTL	0.7484[a]	0.1188
LGKW7NTS	0.8020[a]	0.1625
LGKW2GL	0.8343[a]	−0.3023
LGKW2GSQ	0.8804[a]	−0.3345
LGKW7GL	0.7427[a]	0.0533
LGKW7GSQ	0.7700[a]	−0.0021
LGKW7GHL	0.8717[a]	0.1874
LGKW7GHS	0.9719[a]	−0.0193
LGKW11GL	0.8564[a]	0.1108
LGKW11GS	0.9240[a]	−0.0713
LGKW2AL	0.7042[a]	−0.3951
LGKW2ASQ	0.8278[a]	−0.2304
LGKW7AL	0.5301	0.5006
LGKW7ASQ	0.4976	0.3790
LGKW11AL	0.4383	0.5782
LGKW11AS	0.4689	0.5064
V_MOL	0.5522	−0.4813
E_INT	−0.1621	0.7490[a]
LG_P	0.5678	0.5210
MR	0.5091	−0.6421
TE	−0.4931	0.7681[a]
BE	−0.5039	0.3968
IAE	−0.4888	0.7802[a]
EE	−0.4819	0.6702
HF	−0.0989	−0.0566
IE_IP	0.0219	0.0343
EA	−0.0138	0.9352[a]
EN	0.0048	0.7805[a]
HARD	0.0232	−0.6253
EHOMO	0.1347	0.1525
ELUMO	0.0075	0.9049[a]
ED_MAX	0.3851	−0.5215
ED_MIN	−0.1077	0.6473
ED_HOMO	−0.5602	0.4967
ED_LUMO	0.3834	0.2722
MAX_POS	−0.0148	−0.9064[a]
MAX_NEG	−0.0220	0.9230[a]
DELTA_Q	−0.0078	−0.9121[a]
TDM	−0.2011	−0.6460

Table 4 (continued)

All data	Factor 1	Factor 2
E_L	0.2171	0.3522
E_MAX	−0.4328	0.7029[a]
OS_EMAX	−0.2246	0.6675

[a] Factor loadings among the variables with values higher than 0.7

analyzed groups of parameters. In the set of structural parameters (Fig. 2a) the first factor accounted for 67% of the data variance and the second for 33%. On the other hand, in the HPLC retention data set (Fig. 2b), and when considering all 52 parameters (Fig. 2c), the first factor accounted for 73.2% and 64.6% of the data variance, respectively, and second for 26.8% and 35.4%, respectively. The data obtained indicate that the majority of the information contained in the original data matrix can be explained by two principal components. It can be interpreted that two principal components contain almost the whole information held previously in original variables. Moreover, in the set of structural parameters (Fig. 2a), factor 1 depended mostly on molar volume (V_MOL), molar refractivity (MR), total energy (TE), binding energy (BE), atom interaction energy (IAE), electron energy (EE), electronegativity (EN) and electron orbital density HOMO (ED_HOMO), whereas factor 2 depended mostly on electron affinity (EA), hardness (HARD), LUMO energy (ELUMO), the values of the highest positive (MAX_POS) and negative (MAX_NEG) charge of atoms that constitutes a molecule and the difference between the highest positive and negative charges of atoms constituting a molecule (DELTA_Q). The results were in accordance with previous observations [30] for similar considerations on structural parameters. Namely, factor 1 presented mainly properties connected with molecular bulkiness (e.g., V_MOL, MR or TE), whereas factor 2 presented properties related to electronic parameters (e.g., ELUMO, MAX_POS, MAX_NEG or DELTA_Q).

In the case of HPLC retention data (Fig. 2b), factor 1 depended mostly on chromatographic-data-linked hydrophobicity parameters (log k_w) achieved mainly at pH 2.5 on all tested columns, buffers, type of organic modifier in mobile phase and type of data extrapolation, as well as log k_w values obtained only on Nucleosil C18 AB column at pH 7.0. On the other hand, factor 2 depended mainly on log k_w parameters obtained at pH 7.0 but only on columns packed with stationary phases other than octadecylsilica, i.e., Nucleogel 100-5 RP and Aluspher RP select B columns packed with polystyrene copolymer cross-linked by divinylbenzene and aluminium oxide with chemically bounded polybutadiene, respectively. These observations indicated that, in the case of retention data obtained on stationary phases based on polystyrene copolymer cross-linked by divinylbenzene or aluminium oxide with chemically bounded polybutadiene, there was a greater influence from the polar properties of the molecules of the studied compounds, rather than their bulkiness or mass.

In the dataset considering all parameters (Fig. 2c), factor 1 depended only on the majority of chromatographic

Table 5 FA scores of the studied compounds

Compound		Structural parameters[a]		HPLC retention data[b]		All data[c]	
No.	Name	Factor 1	Factor 2	Factor 1	Factor 2	Factor 1	Factor 2
1	Acetaminophen	1.0456	0.6015	−1.0472	−0.6845	−1.2696	0.7849
2	Aminophenazone	−0.1986	0.8386	−1.2331	0.8583	−0.4690	0.6542
3	ASA	1.6881	−0.3670	−0.2405	−1.3482	−1.0567	0.3373
4	Diclofenac	−0.2681	0.5157	1.4641	0.5574	1.4967	0.4407
5	Etodolac	−1.0309	1.3545	1.0776	0.8893	1.5020	0.6681
6	Ketorolac	0.0284	0.1713	0.5466	−0.7141	0.1345	−0.0117
7	Nimesulide	0.1347	−1.8468	1.0970	0.9176	1.1997	−0.9607
8	Noramidopyrine	−0.7647	−0.8255	−0.7357	−1.2018	−1.2640	−1.3614
9	Piroxicam	−1.0063	−1.0683	0.2569	−0.5618	−0.1940	−1.5448
10	Salicylamide	1.7548	−0.0226	−0.5205	0.0276	−0.5578	0.9627
11	Sulindac	−1.0315	−0.7307	0.7671	−0.5882	0.3754	−1.2319
12	Tramadol	−0.3513	1.3793	−1.4323	1.8484	0.1027	1.2626

[a] FA performed only for structural parameters

[b] FA performed only for HPLC retention data

[c] FA performed for structural parameters together with HPLC retention data

parameters (except for data obtained on Aluspher RP select B column at pH 7.0 and 11.0). However, factor 2 depended mainly on each compound's interaction energy with water (E_INT), total energy (TE), electron affinity (EA) and electronegativity (EN), LUMO energy (ELUMO) and the values of the highest positive (MAX_POS) and negative (MAX_NEG) charge of atoms that constitute a molecule, the difference between the highest positive negative charges

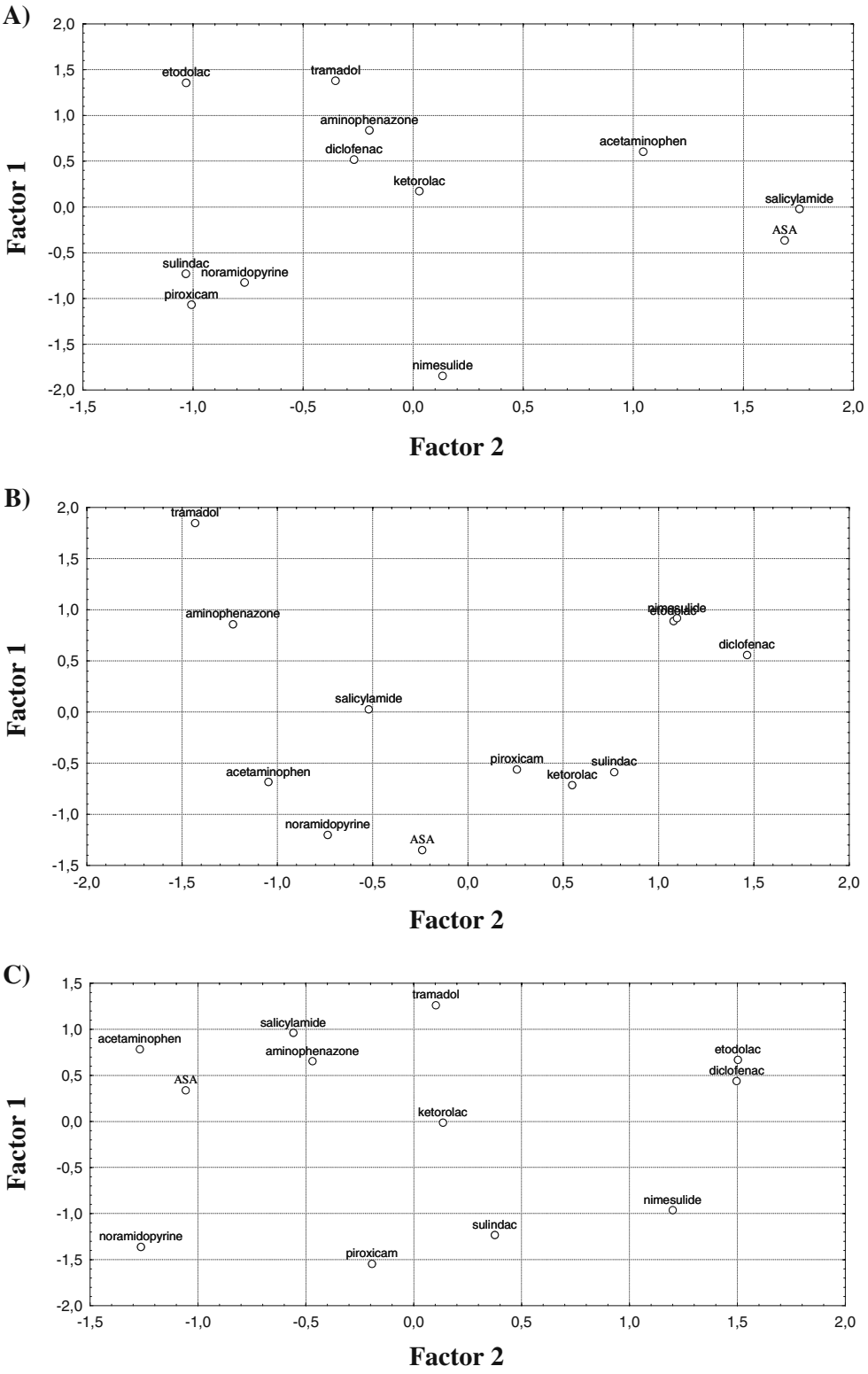

Fig. 3 Two-dimensional scatter plots of the scores of individual drugs in the first two factors extracted from **a** structural parameters, **b** HPLC retention data, or **c** structural parameters together with HPLC retention data

of atoms constituting a molecule (DELTA_Q) and the value of the most intensive electron transition for which the energy value of an oscillator took the maximum value (E_MAX).

As noted above, almost all the information (total data variance) can be explained by the first two principal components. Therefore, specific compounds can be compared on the basis of two principal component scores (objects) plots. Principal component scores calculated for all studied compounds and their individual positions on the plane determined by the two factor axes and performed only for structural parameters, only for HPLC retention data, and for all parameters considered above are presented in Table 5 and Fig. 3a–c. Comparing activities of the selected compounds was quite difficult because of the need to compare the results of pharmacological research performed under the same conditions. Moreover, most of the studied compounds possess various pharmacological properties (analgesic, anti-inflammatory, antipyretic and also anti-rheumatic), and their activities in all these aspects should be estimated. The classification of anti-inflammatory drugs according to their analgesic, anti-inflammatory and antipyretic activity based on literature data is presented in Table 6. Moreover, it is important to note that in some previous works [15–17] it was established that compounds characterized by their identical mechanism of action in FA charts form clusters, e.g., classifications of compounds of α- and β-adrenergic action, antagonists of histamine receptors H_1 and H_2 and psychotropic drugs.

The positions of particular compounds on the plane determined by factors 1 and 2 obtained for structural parameters is characterized by an arrangement of three clusters (Fig. 3a). The first contains noramidopyrine, piroxicam and sulindac, with nimesulide further away. All these compounds are characterized by strong (piroxicam and sulindac) or mild (noramidopyrine and nimesulide) analgesic and diverse (low to strong) anti-inflammatory activity, with additional mild antipyretic activity of noramidopyrine and sulindac (Table 6) [31–36]. Moreover, all presented compounds have a sulfur atom as part of their structure. Another cluster on the scatter diagram in Fig. 3a comprises ASA, salicylamide (a derivative of salicylic acid), with acetaminophen (a derivative of *p*-aminophenole) some way off. Acetaminophen, as well as derivatives of salicylic acid, are characterized by strong antipyretic and analgesic activity with mild anti-inflammatory properties (Table 6). The remaining drugs, i.e., tramadol, aminophenazone, diclofenac and ketorolac, which are all compounds with unsubstituted or chlorine or methoxy-substituted phenyl groups with some linked aromatic systems (e.g., pyrazole, *o*-aminophenylacetic acid residues, pyrrolepyrrolidine or cycloheksanol), form the last cluster, which is characterized by variable analgesic, anti-inflammatory and antipyretic activity (Table 6). The analgesic and anti-inflammatory activity of aminophenazone can be lower or higher, respectively, compared to the analgesic and anti-inflammatory activity of salicylic acid derivatives, whereas diclofenac is characterized by similar or lower anti-inflammatory and antipyretic activity compared to salicylates. On the other hand, ketorolac is characterized by mild, and tramadol by strong, analgesic activity combined with low anti-inflammatory and no antipyretic properties [37–39].

The positions of particular compounds on the plane determined by factors 1 and 2 obtained by HPLC retention data is presented in Fig. 3b. A small cluster containing diclofenac, etodolac and nimesulide can be observed in this scatter diagram, which is somewhat related to their strong anti-inflammatory and anti-rheumatic activity. Moreover, a further two clusters can be identified, comprising (1) piroxicam, ketorolac and sulindac; and (2) acetaminophen,

Table 6 Classification of anti-inflammatory drugs according to their analgesic, anti-inflammatory and antipyretic activity[a]

No.	Compound	Analgesic activity	Anti-inflammatory activity	Antipyretic activity
1	Acetaminophen	(+++) [37]; (++) [2]	(++) [37]	(+++) [37]
2	Aminophenazone	(++) [2]	(++++) [2]	(−)
3	ASA	(+++) [37]; (+) [2]	(++) [37]	(+++) [37]
4	Diclofenac	(+) [37]; (+++) [38]	(+++) [37] (++) [38]	(+) [37]; (+)[38]
5	Etodolac	(+) [31]	(+++) [31]	(−)
6	Ketorolac	(++) [37]; (+) [38]	(+) [37]	(+) [37]
7	Nimesulide	(++) [32]; (++) [33]	(++) [32]; (++) [33]	(−)
8	Noramidopyrine	(++) [34]	(+) [34]	(++) [34]
9	Piroxicam	(+++) [31]; (+++) [35]	(+++) [31]; (+++) [35]	(−)
10	Salicylamide	(+++) [37]	(++) [37]	(+++) [37]
11	Sulindac	(+++) [36]	(++) [35]	(++) [35]
12	Tramadol	(+++) [39]	(−)	(−)

[a] Analgesic, anti-inflammatory and antipyretic activity: ++++ very strong, +++ strong ++ mild, + low, − no activity data

noramidopyrine and ASA, which can be correlated with mild or strong analgesic activity in the case of compounds from cluster (1), and mild or strong antipyretic activity in the case of compounds from cluster (2) presented above (Table 6).

Figure 3c presents the positions of particular compounds on the plane determined by factors 1 and 2 obtained for all 52 parameters. In this case, particular compounds were generally more scattered compared to the plots in Fig. 3a,b. This time, three clusters comprising only (1) etodolac and diclofenac, (2) salicylamide and aminophenazone, and (3) acetaminophen and ASA can be distinguished, which can be correlated with strong anti-inflammatory activity in the case of compounds from cluster (1), moderate and very strong analgesic and anti-inflammatory activity, respectively, for aminophenazone, compared to ASA in the case of compounds from cluster (2), and the same antipyretic, anti-inflammatory and analgesic activity in the case of compounds from cluster (3).

Conclusions

To summarize the observations presented above, the pattern of distribution of individual drugs on the plane determined by two principal components (factors 1 and 2) obtained on the basis of structural parameters and log k_w values was in good agreement with both their physicochemical characteristics and their pharmacological features.

Based on the results discussed above, the following more detailed conclusions may also be proposed.

FA extracted two factors from among the whole group of 52 parameters; however, depending on the character and number of the parameters used, the first principle component (factor 1) accounted for 65–73% of variance in the data, and second principal component (factor 2) explained 27–35% of data variance.

Of the 52 parameters used, those with most influence on factor value were chromatographic parameters and selected structural parameters (relevant to energy quantum-chemical parameters and electron affinity specifying parameters).

The approach proposed after optimization of datasets could be used in the preliminary classification of analgesic and anti-inflammatory drugs, and could also be incorporated into QSAR analysis as part of a strategy for the design of new drugs.

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