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Application of perfluorinated acids as ion-pairing reagents for reversed-phase chromatography and retention-hydrophobicity relationships studies of selected β-blockers

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ABSTRACT

The addition of the homologous series of perfluorinated acids-trifluoroacetic acid (TFAA), pentafluoropropionic acid (PFPA), heptafluorobutyric acid (HFBA) to mobile phases for reversed-phase high-performance liquid chromatography (RP-HPLC) of β-blockers was tested. Acidic modifiers were responsible for acidification of mobile phase (pH 3) ensuring the protonation of the β-blockers and further ion pairs creation. The effect of the type and concentration of mobile phase additives on retention parameters, the efficiency of the peaks, their symmetry and separation selectivity of the β-blockers mixture were all studied. It appeared that at increasing acid concentration, the retention factor, for all compounds investigated, increased to varying degrees. It should be stressed that the presence of acids more significantly affected the retention of the most hydrophobic β-blockers. Differences in hydrophobicity of drugs can be maximized through variation of the hydrophobicity of additives. Thus, the relative increase in the retention depends on either concentration and hydrophobicity of the anionic mobile phase additive or hydrophobicity of analytes. According to QSRR (quantitative structure retention relationship) methodology, chromatographic lipophilicity parameters: isocratic $\log k$ and $\log k_w$ values (extrapolated retention to pure water) were correlated with the molecular ($\log P_{o/w}$) and apparent ($\log P_{app}$) octanol–water partition coefficients obtained experimentally by countercurrent chromatography (CCC) or predicted by Pallas software. The obtained, satisfactory retention-hydrophobicity correlations indicate that, in the case of the basic drugs examined in RP-HPLC systems modified with perfluorinated acids, the retention is mainly governed by their hydrophobicity.

1. Introduction

β-Adrenoceptor blocking drugs are important substances in the pharmaceutical industry. They are widely used in the treatment of neurological, neuropsychiatric and cardiovascular disorders. So far, several high-performance liquid chromatography (HPLC) methods have been developed for their analysis [1–8]. Among chromatographic techniques reversed-phase systems have been most popular (RP-HPLC). Analysis of β-blockers, as basic compounds possessing secondary amino groups, requires special mobile phase modification in reversed-phase systems.

Basci et al. applied alkyl sulfonates (pentanesulfonate, hexanesulfonate, heptanesulfonate, etc.) and organic amines (diethylamine, tetraethylammonium, *N*,*N*-dimethyloctylamine, etc.) as mobile phase additives [4]. The elution behaviour of β-blockers was examined on a C_{18} column with a phosphate buffer–acetonitrile mobile phase at pH 3.0 in the presence of the above-mentioned additives. While alkyl-sulfonates acted as ion-pairing reagents, the organic amines were responsible for masking of residual silanols. Kazakevich described the application of chaotropic mobile phase additives in the mobile phase at low pH [5,6]. The increasing retention of basic compounds was in agreement with the theory of chaotropicity associated with Hofmeister series of salt. Berthod et al. compared effectiveness of additives in the form of the ionic liquids (IL): 1-butyl-3-methylimidazolium ($BMIMBF_4$), 1-octyl-3-methylimidazolium tetrafluoroborate (OMIM BF_4) and triethylamine (TEA) on peak shape, elution behavior and resolution of β-blockers utilizing a conventional reversed-phase Kromasil C_{18} column [7]. IL cation was shown to have been responsible for the silanol screening effect; nevertheless, one may say that the retention of the analytes depended mainly on the hydrophobic nature and chaotropic character of ILs anions.

Several reversed-phase chromatographic methods using micellar mobile phases have also been described for the analysis of β-blockers. Rapado-Martinez et al. applied sodium dodecyl sulfate (SDS) as mobile phase modifier at acidic conditions, too (pH 3) [8].

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Table 1
Regression parameters obtained for investigated drugs from the relationships log k versus volume fraction of methanol in the mobile phase containing 30 mM of different acidic additives.

Investigated compounds	30 mM HFBA		30 mM PFPA		30 mM TFAA		30 mM AA	
	$\log k_w$	R^2	$\log k_w$	R^2	$\log k_w$	R^2	$\log k_w$	R^2
Acebutolol	4.3785	0.9960	2.7662	0.9962	2.2110	0.9993	2.3913	0.9959
Alprenolol	4.7249[a]	0.9818[a]	3.7593	0.9994	3.2957	0.9962	2.5129	0.9998
Atenolol	2.3896	0.9968	1.1695	0.9974	0.6680	0.9972	0.9444	0.9925
Labetalol	4.4620[a]	0.9783[a]	3.4098	0.9997	3.2112	0.9975	2.2672	0.9989
Metoprolol	4.4943	0.9960	2.8124	0.9971	2.2032	0.9996	2.0239	0.9983
Nadolol	3.7176	0.9963	2.2159	0.9968	1.6217	0.9986	1.6292	0.9942
Pindolol	3.3280	0.9947	1.9270	0.9982	1.3987	0.9997	1.3175	0.9933
Propranolol	4.7065[a]	0.9783[a]	3.5536	0.9987	3.3503	0.9996	2.4598	0.9997
Sotalol	2.3106	0.9968	1.1067	0.9973	0.5329	0.9963	0.9444	0.9926

[a] The regression coefficients were calculated for linear part of log k versus φ (MeOH) relationships in the range of 0.45–0.60 volume fraction of methanol.

In the present study, the homologous series of volatile perfluorinated acids (trifluoroacetic acid, TFAA; pentafluoropropionic acid, PFPA; heptafluorobutyric acid, HFBA) were applied as hydrophobic anionic ion-pairing reagents for reversed-phase high-performance liquid chromatography (RP-HPLC) of basic drugs. In this paper, they were applied in the analysis of small basic ionizable compounds, belonging to β-blockers, differing in their hydrophobicity. So far, these acids especially trifluoroacetic acid have been used as a mobile phase additive in RP-HPLC of peptides [9–15] and other pharmaceuticals [16]. Usefulness of this additive for improvement of analysis efficiency was confirmed in ultra-performance liquid chromatography (UPLC) systems capable of generating pressures up to 1000 bar [16]. Influence of anionic ion-pairing reagents of varying hydrophobicity ($CH_3COO^- < TFA^- < PFPA^- < HFBA^-$) and concentration (5–40 mM) on retention parameters, efficiency and separation selectivity of β-blockers was investigated.

In order to investigate the optimal conditions for obtaining log P values from RP-HPLC measurements the relationships between RPLC retention data sets with the molecular ($\log P_{o/w}$) and apparent ($\log P_{app}$) octanol–water partition coefficients were analyzed.

2. Experimental

2.1. Reagents

Investigated compounds (listed in Tables 1 and 2) were obtained from Sigma (St. Louis, MO, USA). Pentafluoropropionic and heptafluorobutyric acids were obtained from Sigma–Aldrich (St. Louis, Missouri). Trifluoroacetic acid (99%) was purchased from Merck (Darmstadt, Germany). Reagent-grade phosphoric acid and acetic acid (H_3PO_4) were obtained from POCH (Gliwice, Poland). HPLC grade methanol (MeOH) was purchased from Merck. The eluents were prepared by mixing the aqueous and HPLC grade organic solvent, then appropriate amounts of anionic ion-pairing reagent per 200 ml were added. The concentrations of the additives were in the range from 5 to 40 mmol l^{-1}, in the whole mobile phase. After mixing, the pH value was measured and adjusted to pH 3 by addition of saturated solution of NaOH. All mobile phases were filtered with Nylon 66 membrane filter (0.45 μm) Whatman (Maidstone, England) by the use of a filtration apparatus.

2.2. Apparatus

Experiments were performed using a LaChrom HPLC Merck Hitachi (E. Merck, Darmstadt, Germany) model equipped with diode array detector, column oven L-7350 and solvent degasser L-7612. The column (150 mm × 4.6 mm I.D.) was packed with 5-μm Zorbax Extend-C18 (pore size: 80 Å, surface area: 180 m^2/g) Agilent Technologies (Santa Clara, CA, USA); its void volume was determined to be 1.31 ml, by the injection of thiourea in acetonitrile–water (50:50) eluent system. Retention data were recorded at a flow-rate of 1 ml min^{-1}. The column was thermostated at 20 °C ± 0.1. Injected solutions were prepared at 0.1 mg ml^{-1} concentrations in aqueous methanol. The detection of the drugs was set at appropriate wavelength (λ_{max} = 220 nm) chosen according to the recorded spectra. Typical injection volumes were 3 μl. Duplicate injections were made.

3. Results and discussion

3.1. RP-HPLC system

Extend-C18 column applied in this study possesses unique method of protection of the silica from dissolution at a wide range of eluent pH. Bidentate bonding technology combined with a double-endcapping process provides high pH stability in the range of 2.0–11.5.

Table 2
Experimental β-blocker partition coefficients measured by countercurrent chromatography [7,20] and theoretical ones determined by Pallas software version 3.3.2.3.

Investigated compounds	pK_{a1}/pK_{a2}	$\log P_{o/w}$	$\log P_{app}$ at pH 3	$\log P_N$ (Pallas)	$\log P^+$ (Pallas)
Acebutolol	9.24	1.83	−2.08	2.03	−1.19
Alprenolol	9.60	3.15	−1.39	2.38	−0.91
Atenolol	8.07	0.25	Out of range	0.76	−2.18
Labetalol	7.4/8.7	1.00	−1.16	2.69	−0.67
Metoprolol	9.7	1.9	−2.08	1.85	−1.32
Nadolol	9.4	1.00	−2.6	1.61	−1.51
Pindolol	8.8/9.7	1.91	−2.2	1.43	−1.65
Propranolol	9.5	3.41	−1.09	2.66	−0.69
Sotalol	7.7/9.0	−0.77	−2.92	0.26	−2.56

$\log P_{o/w}$ Molecular log P values determined by the use of CCC.
$\log P_{app}$ at pH 3 Experimental log P values of cationic form measured at pH 3 by CCC.
$\log P_{N\ (Pallas)}$ log P of neutral form of drugs determined by the use of Pallas software.
$\log P_{+(Pallas)}$ log P of cationic form of β-blockers predicted by PrologD.

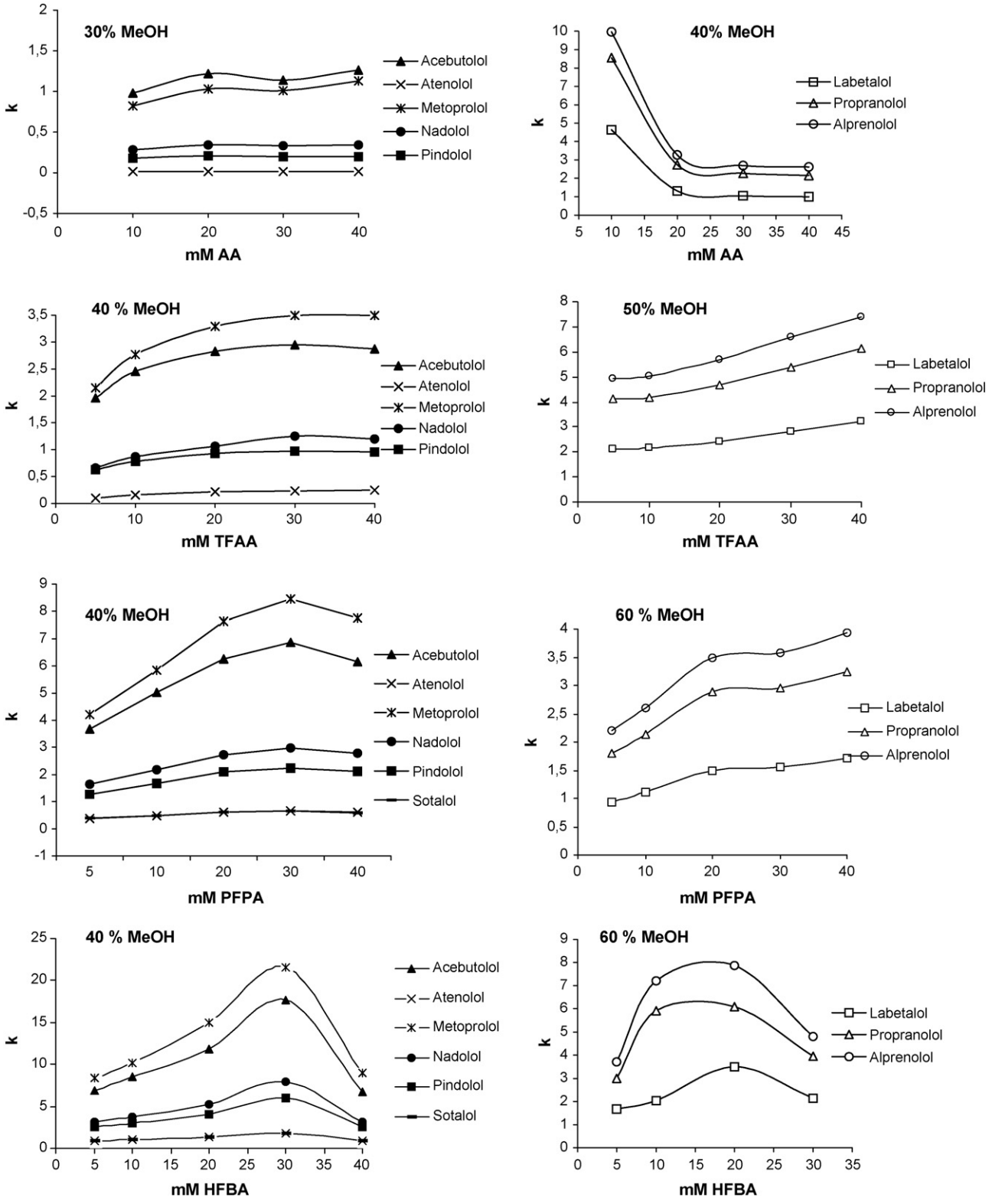

Fig. 1. Effect of ion-pairing reagent concentration in methanol/water mobile phase (acetic acid, AA; trifluoroacetic acid, TFAA; pentafluoropropionic acid, PFPA; heptafluorobutyric acid, HFBA) on retention coefficient of investigated β-blockers. For HPLC conditions see Section 2.

It should be noted that pH of organic–aqueous eluent used in the present study independently of the modifier type could be generally referred to as pH 3 or lower (2.8–2.9). Even underivatized silanol groups will remain neutral taking into account their pK_a which is about 4–5. In turn at pH 3 the investigated compounds: β-blockers are protonated.

3.2. Effect of different anionic ion-pairing reagents on retention behavior of β-blockers

Fig. 1 presents graphical representation of the effect of increasing different acids concentration on β-blockers retention factors. As seen in Fig. 1, increasing acetic acid (AA) concentration had

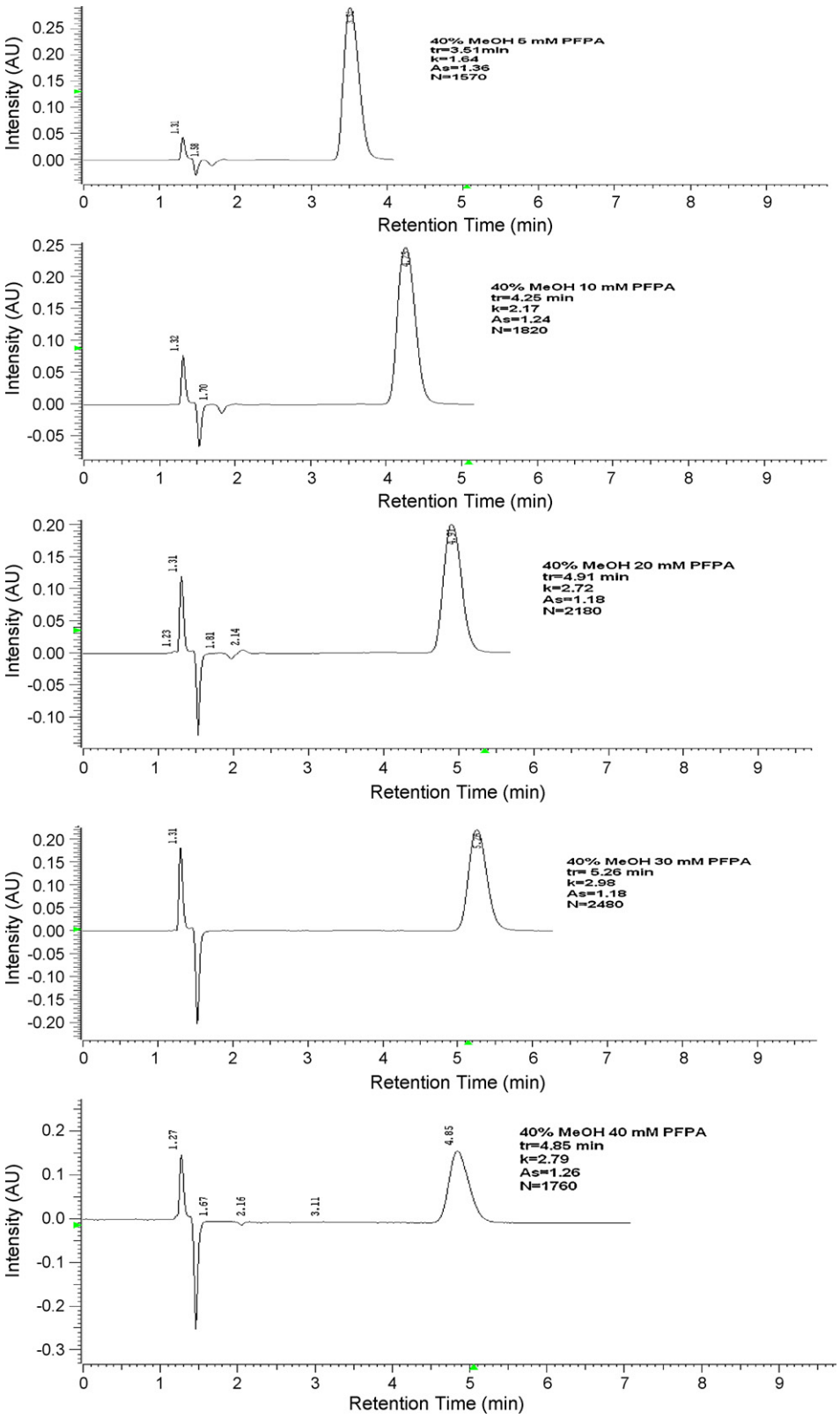

Fig. 2. The effect of different pentafluoropropionic acid (PFPA) concentration in the mobile phase on retention, peak symmetry and efficiency of nadolol.

no visible effect on retention of less hydrophobic drugs. The most flat profile was exhibited by the phosphoric acid (data not shown). Somewhat more marked effect was observed in the case of the most hydrophobic analytes (propranolol, alprenolol, labetalol). Their retention factors decreased with increasing acetic acid concentration due to increasing eluent ionic strength and low ion pairing properties of acetates. Protonated analytes were eluted faster and faster as wide asymmetric peaks.

In contrast to acetic and phosphoric acids, TFAA belonging to perfluorinated acidic modifiers exhibited quite different effect on

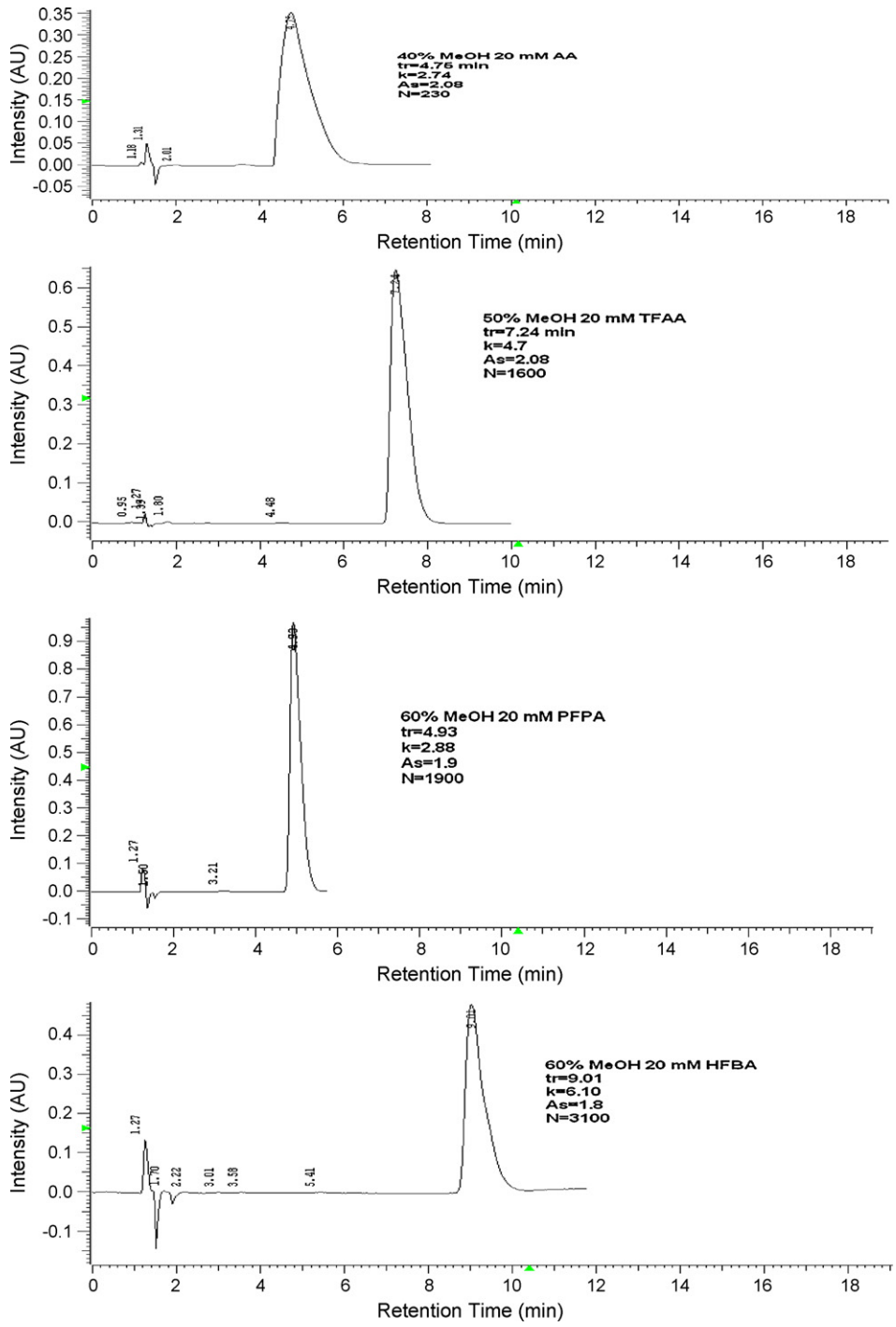

Fig. 3. The effect of different acidic additives on retention, peak symmetry and efficiency of propranolol.

basic compounds retention. It should be noted that this mobile phase additives can be referred to as liophilic compounds as they are the source of anions that have the ability for electrostatic interactions and low tendency to participate in dispersion interactions owing to weak polarizability of fluoroalkyl groups. Additionally, they are characterized by significant delocalization of their charge and at the same time they do not have deleterious properties of surfactant agents (surface modification of the bonded phase). In view of the investigation performed by Kazakevich and co-workers [17], the liophilic ions are not adsorbed on the stationary phase in methanol–water eluent systems, as methanol forms monomolecular adsorbed layer; which simply does not provide a suitable medium for retention. Thus, changes in retention of protonated basic analytes are driven first of all by the solvation–desolvation process occurring in the mobile phase. Solvated ionic analytes undergo low retention owing to the suppression of hydrophobic interaction. Increasing concentration of liophilic counterions leads to the disruption of the analyte solvation shell, increasing the analyte hydrophobicity. It also leads to increased interaction with the hydrophobic stationary phase and finally results in elevated retention. Results of the above-mentioned processes were observed experimentally for TFAA (and PFPA but only at higher methanol contents). As seen in Fig. 1, basic compounds exhibited increased retention with the increase in their concentration.

However, the relative rapidity of retention factor increase was the highest at lower concentration of acidic additives while at the high concentration a type of saturation effect was observed. In this region, further increase in the acidic additives concentration did not cause significant changes in retention. The influence of increasing concentration of acidic modifiers on retention, symmetry and efficiency of the peaks may be analyzed on the basis of the examples of chromatograms obtained for nadolol (Fig. 2).

The affinity of investigated compounds for the stationary phase also depends on hydrophobicity of the anion in the following order: $H_2PO_4^-$ < TFA^- < $PFPA^-$ < $HFBA^-$. Elongation of the carbon chain of a modifier with two carbon atoms, simultaneously keeping constant organic solvent content, makes it possible to achieve about a two-fold increase in the retention factor values. It should be stressed that increasing anion hydrophobicity results in not only increase in the retention factor but also in the improvement of overall peak shape and separation efficiency. This is noticeable with the increase in theoretical plate number and symmetry coefficient (Fig. 3).

On the other hand, the increase in retention factor depends on the hydrophobicity of the analyte. For more hydrophobic compounds higher retention increase is observed. Thus, for the most hydrophobic compounds such as labetalol, propranolol and alprenolol increase in retention according to increasing of perfluorinated acids concentration is out of the acceptable k range. Therefore the higher methanol content in the mobile phase is required.

An interesting observation may be made by analysis of Fig. 1 the decrease in retention underlined slightly at higher PFPA and more clearly at higher HFBA concentration. This phenomenon is connected with lowering of the theoretical plate numbers and poor peak symmetry. This probably resulted in the additional electrostatic interactions in the system and, resulted from the column modified by the adsorption of these anionic additives. It should be stressed however that this effect is observed only at higher concentration of these additives when excess adsorption takes place. Heptafluorobutyric acid possessing non-spherical shape exhibits some amphiphilic properties. In contrast to the liophilic ions showing weak interactions with reversed-phase surface, adsorption of amphiphilic ions on stationary phase occurs independently on the kind of organic solvent in the mobile phase [18]. At the applied conditions (suppression of silanophilic interactions by low pH of the mobile phase) the hydrophobic chain of anionic additives is probably inserted in the bonded organic layer with the polar functional group protruding toward the mobile phase. Adsorption of the excess amount of anionic additives (with amphiphilic properties) by the column gives rise to increase in the polarity of the stationary phase with reduction of hydrophobic effect dominating in retention mechanism. Thus, either electrostatic or hydrophobic interactions could influence the retention.

Absolute increase in the retention factors of the investigated drugs depends not only on acid type but also on the concentration of an organic modifier in eluent system. The highest values of Δk were calculated for mobile phase containing the lowest methanol concentration (Fig. 4). This may be attributed to the contribution of increasing concentration of organic solvent in the desolvation process instead of hydrophobic anions providing smaller increases in retention factors under the influence of increasing concentration of liophilic additives. Another possible explanation is connected with overall hydrophobic effect, or more generally solvophobic driving forces for the adsorbent associated with known tendency of solvent to reduce the space occupied by neutral ion-pair (BH^+A^-). This tendency increases with the increasing surface area of the solute molecules and the surface tension of the solvent finally intensifying the process of adsorption. Eluent systems with high content of organic solvent have relatively low surface tension, which is why

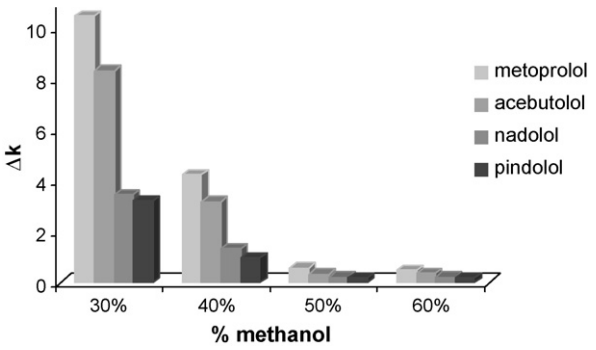

Fig. 4. Absolute increase in retention factors (Δk) of β-blockers obtained in different concentration of methanol in eluent systems modified with 5 and 30 mM PFPA.

suppression of hydrophobic interactions (lowering of retention) is observed.

3.3. Effect of different anionic ion-pairing reagents on separation selectivity of β-blockers mixture

Fig. 5 presents the effect of increasing counterion hydrophobicity on the elution behaviour of β-blockers at a constant concentration of each acid (20 mM). As it may be seen, the retention times of all five drugs increase with increasing hydrophobicity of acidic additive (AA < TFAA < PFPA < HFBA). It should be stressed that the elution order of all separated β-blockers still remains the same despite the additive kind of changes. The best separation was achieved with heptafluorobutyric acid as the mobile phase additive. To separate additionally the more hydrophobic analytes (labetalol, propranolol, alprenolol) by the use of this acidic additive, time consuming analysis giving k values bigger than 20 has to be conducted. Thus to avoid overall elution problem connected with elongated analysis time, gradient elution appears to be the method of choice. Fig. 6 illustrates separation of eight β-blockers obtained in one run owing to gradient elution.

3.4. Study of log k versus concentration of methanol in acidified mobile phase

Acidic reagents employed in this study in a concentration range of 5–30 mM (till saturation) enable either protonation of basic molecules or further ion-pairs formation owing to the presence of big hydrophobic anions. The analyzed drugs undergo retention as neutral complexes [BH^+A^-] formed mainly in the mobile phase. This statement could be applied, first of all, because methanol as a organic modifier forms a monomolecular adsorbed layer leading to, primarily, an adsorption type reversed-phase retention process, secondly because applied acidic additives (at appropriate concentration, lower than saturation level) in contrast to long chain ion-pairing reagents do not undergo hydrophobic adsorption on hydrophobic stationary phase protected from domination of ion-exchanging mechanism. That is why changes in retention observed under increasing methanol concentration in aqueous mobile phase enriched by acidic additives can be described by linear equation [19]:

$$\log k = -S\varphi + \log k_w$$

Graphs in Fig. 7 show an effect of methanol concentration in the mobile phases containing constant amount of acidic additives (30 mM) on $\log k$ for investigated β-blockers. For the most dependences, the correlation coefficient was approaching 0.9 (Table 1) in the whole range of the investigated concentrations. Correlations between $\log k$ and the concentration of methanol were not strictly linear only for mobile phases containing phosphoric acid.

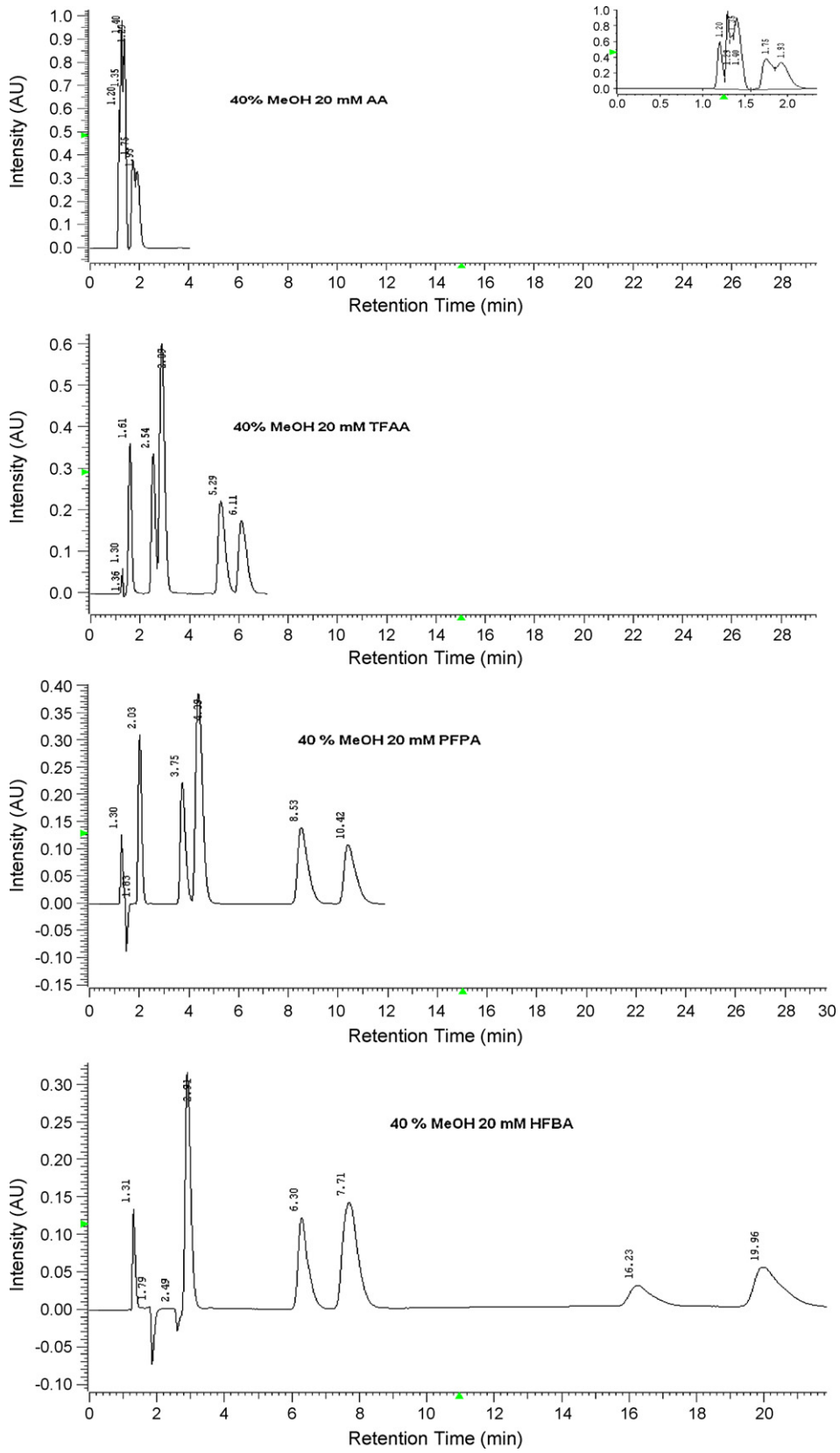

Fig. 5. Chromatograms of mixtures of β-blockers obtained by the use of different mobile phases. The peaks order: atenolol, pindolol, nadolol, metoprolol, acebutolol.

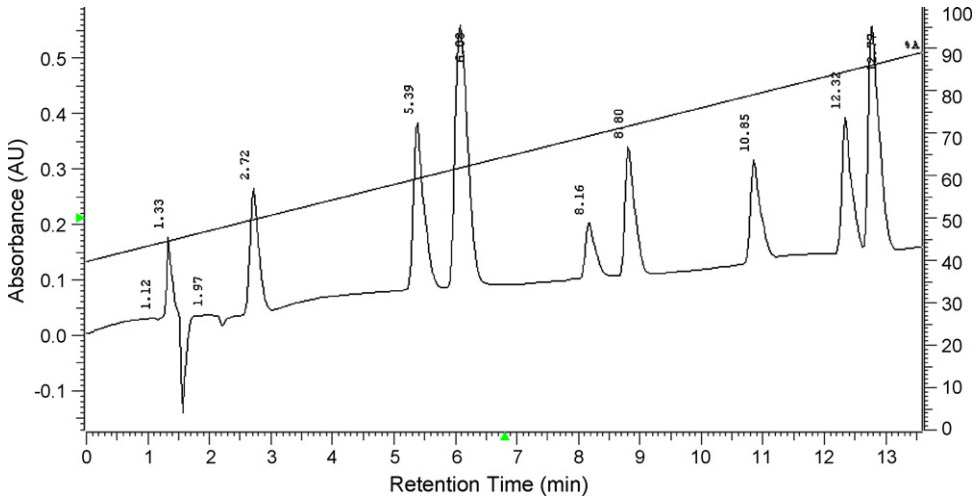

Fig. 6. Separation of β-blockers mixture. Gradient chromatographic conditions: 1 ml/min, gradient from 40 to 95% solvent A in 15 min, 20 °C, 220 nm. Solvent A was 20 mM PFPA in MeOH:water (9:1), solvent B was 20 mM PFPA in water. The peaks order: atenolol, pindolol, nadolol, metoprolol, acebutolol, labetaolol, propranolol, alprenolol.

On the basis of the experimentally obtained $\log k$ versus volume fraction (φ) of methanol relationships one can determine chromatographic lipophilicity parameters $\log k_w$, by extrapolation techniques.

3.5. Correlation between the retention and hydrophobicity of investigated β-blockers

As it can be seen in Fig. 8, representing relationships between $\log D$ (octanol–water distribution coefficient in its logarithmic form) values and pH, the investigated compounds exist in cationic forms in the examined conditions exhibiting simultaneously the lowest lipophilicity. The plots of $\log D$ against pH predicted by PrologD (a module of the Pallas system) enable to determine $\log P$ for neutral and cationic forms of drugs exactly at pH 2.9. Table 2 contains values predicted theoretically by Pallas software as well as determined experimentally by countercurrent chromatography (CCC) by Carda-Broch and Berthod [7,20] for molecular and cationic form of drugs at pH 3.

One of the oldest type of quantitative structure-retention relationship (QSRR) relates logarithms of retention factors ($\log k$) to logarithms of *n*-octanol–water partition coefficients ($\log P$). It should be stressed, however, that the success of this correlation is the most difficult to achieve for ionic compounds because originally, $\log P_{oct}$ is defined for neutral form. Presence of ionic groups causes deviation from linearity as a consequence of electrostatic interactions. Corrections to $\log P_{oct}$ taking into account ionization of the basic solutes at different pH could be calculated as follows [7,21,22]:

$$P_{app} = \frac{P_{o/w} + P^{+}_{o/w}(h/K_a)}{1 + (h/K_a)}$$

where $P_{o/w}$, $P^{-}_{o/w}$ and $P^{+}_{o/w}$ are the octanol–water partition coefficients of the molecular, anionic and cationic species, respectively. K_a is the acid–base dissociation constant and h the concentration of hydrogen ions.

Apart from these corrections, when analyzing ionizable compounds in reversed-phase mode, different modifiers must be added to the mobile phase to avoid decreases of system efficiency visible in the form of small theoretical plate numbers and worsened peak symmetry. In the present study, perfluorinated acids creating ion-associated complexes with higher retention and better efficiency were applied for this purpose.

To examine the quality of the correlation between retention and hydrophobicity, chromatographic parameters such as $\log k_w$ and isocratic $\log k$ values determined in an appropriate mobile phase were plotted against $\log P_{o/w}$ or $\log P_{app}$ determined experimentally by CCC or predicted by Pallas software. The correlation coefficients of these relationships are presented in Table 3.

Table 3
Correlation coefficients of $\log k$ versus $\log P$ for β-blockers in classical RPLC modified with acidic reagents.

Mobile phase modifier	Chromatographic lipophilicity parameters	Countercurrent chromatography parameters (CCC)		Pallas version 3.3.2.3 (PrologD module)	
		$\log P_N$ molecular	$\log P_+$ cationic	$\log P_N$ molecular	$\log P_+$ cationic
Acetic acid	$\log k_w$	0.5779	0.6904	0.8753	0.8752
	$\log k$ (30% MeOH)	0.6953	0.8487	0.9440	0.9455
Trifluoroacetic acid	$\log k_w$	0.6357	0.9207	0.9434	0.9446
	$\log k$ (40% MeOH)	0.6888	0.8979	0.9176	0.9193
	$\log k$ (45% MeOH)	0.7069	0.8895	0.9145	0.9163
	$\log k$ (50% MeOH)	0.7177	0.8764	0.9049	0.9069
Pentafluoropropionic acid	$\log k_w$	0.6729	0.8523	0.9304	0.9318
	$\log k$ (45% MeOH)	0.6955	0.8878	0.8917	0.8936
	$\log k$ (50% MeOH)	0.6981	0.8881	0.8807	0.8827
	$\log k$ (55% MeOH)	0.7115	0.8826	0.8793	0.8813
Heptafluorobutyric acid	$\log k_w$	0.6792	0.6738	0.8990	0.9003
	$\log k$ (45% MeOH)	0.6910	0.8916	0.8910	0.8929
	$\log k$ (50% MeOH)	0.7004	0.8806	0.8626	0.8646

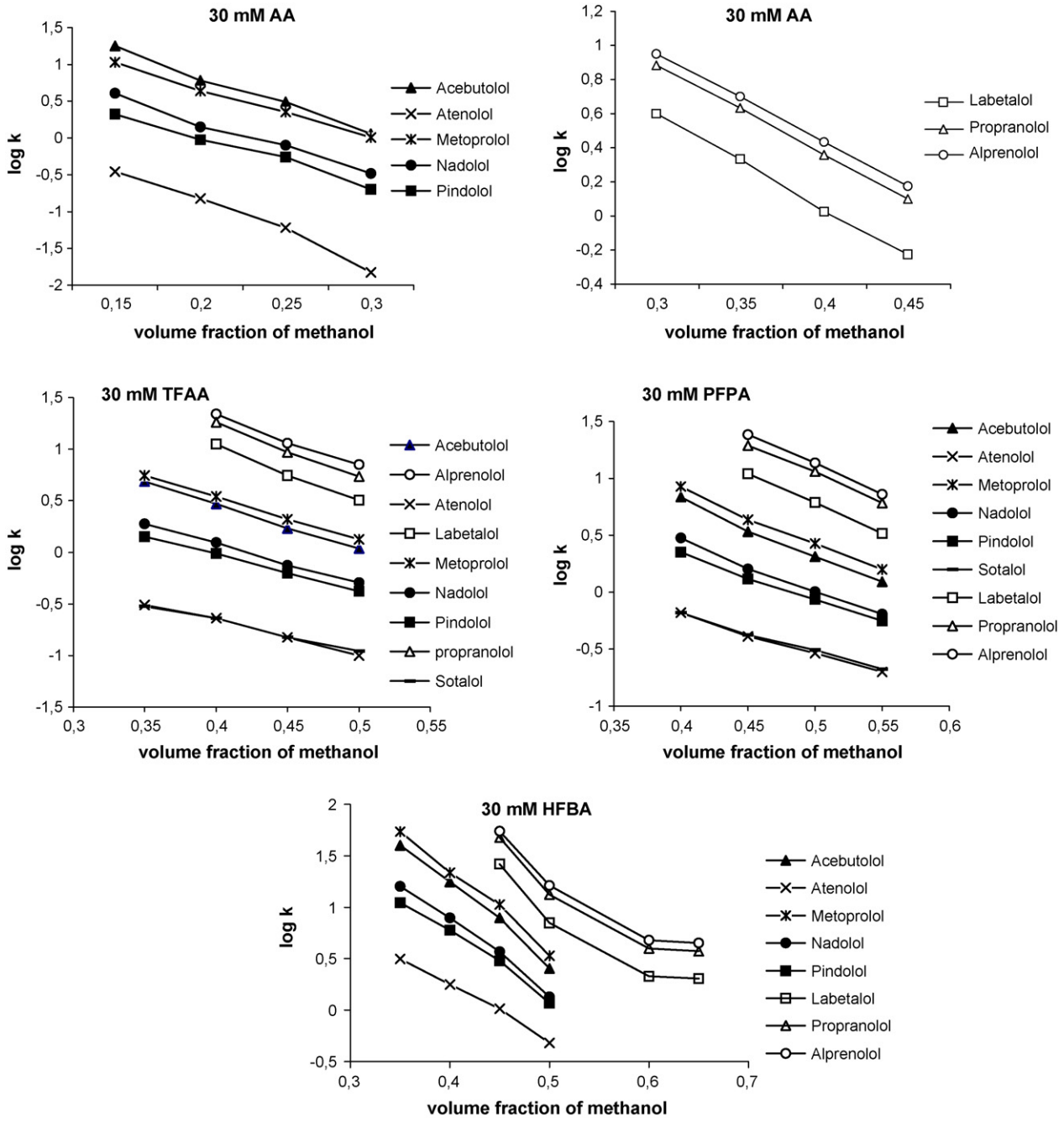

Fig. 7. Plots of log *k* against volume fraction of methanol in the mobile phase containing 30 mM of different acidic additives.

As it can be seen, the correlation coefficients obtained for experimentally determined lipophilicity parameters (CCC) were lower (0.8) than those predicted by Pallas software (0.9) independently of the modifier kind. It should be stressed, however, that regression coefficients improved slightly when log *P* values for cationic forms were used. This suggests that the differences in hydrophobicity of analytes decide about differences in retention, in spite of the electrostatic interaction between the protonated amine group of the drugs and the anionic additive. Considering correlation coefficients determined by computer software, it could also be noted that better correlations were obtained for less hydrophobic additives. Thus effectiveness of acidic modifier according to their usefulness for prediction of lipophilicity could be arranged as follows: TFAA > PFPA > HFBA. Another interesting observation is the fact that for pefluorinated acids log kw values give better correlations than

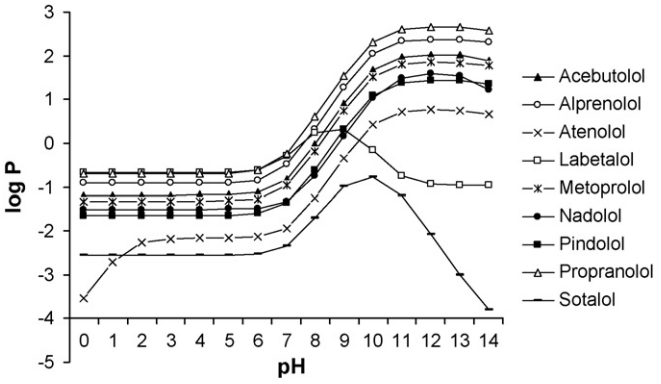

Fig. 8. The plots of log *D* against pH predicted by PrologD, which is a module of the Pallas system.

particular retention parameters ($\log k$) in contrast to data obtained for acetic acid. For sure it is directly connected with the linearity of $\log k$ versus φ relationships. To predict more precisely lipophilicity of analyte by RP chromatography, plots $\log k$ versus φ must be linear also outside the working organic solvent range. This was achieved in systems modified with short-chain perfluorinated acids.

4. Conclusions

In the present study, Zorbax Extend-C18 column together with methanol-aqueous mobile phase modified with perfluorinated acids in the range of 5–40 mM for separation and lipophilicity determination of chosen β-blockers was applied. It was determined that enhancement of retention, peak efficiency and peak symmetry could be obtained by the addition of these acids to the mobile phases and that the degree of enhancement depends on the type (TFAA < PFPA < HFBA) and concentration of counter-anion employed. The best separation selectivity was achieved by the use of mobile phase containing the most hydrophobic additive (HFBA).

The isocratic retention parameters ($\log k$) and $\log k_w$ for aqueous–organic phases modified with different acids were correlated with molecular and cationic octanol–water partition coefficients either experimentally measured by countercurrent chromatography or predicted by Pallas software. The quality of the correlations obtained for β-blockers appears to confirm the necessity of the ionization correction and usefulness of computer software for this purpose. The correlations were satisfactory for the $\log k_w$ versus $\log P$, as well as for $\log k$ (at different methanol concentration) versus $\log P$. It means that the extrapolation used to obtain the $\log k_w$ value does not introduce a cumulative error in the case of perfluorinated acids used as the mobile phase modifiers. Usefulness of acidic modifiers in QSRR studies could be arranged in the following order: TFAA > PFPA > HFBA.

Summarizing, it could be concluded that short chain perfluorinated acids may replace the need for addition of hydrophobic "ion-pairing" reagents, chaotropic salts or ionic liquids which have to be applied together with additional components of buffering systems in RP-HPLC of basic compounds. Although in the past, higher concentrations of such acidic modifiers were broadly avoided because silica based stationary phases could undergo degradation under highly acidic environment. Now, the approach to mobile phase modifiers can be revised owing to the advancement of silica based packings and availability of RP columns with excellent chemical stability. In the light of the conducted experiments, the use of perfluorinated acids for a chromatographic separation and lipophilicity determination of basic compounds may be now considered as one of methods for development strategy.

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