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ARTICLES

APPLICATION OF SULPHONATED STYRENE AND DIVINYLBENZENE COPOLYMERS WITH VARIOUS DEGREE OF CROSSLINKING FOR ION EXCLUSION CHROMATOGRAPHY

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Abstract. The chromatographic retention of neutral polar (alcohols, ketones, carboxylic acids, carbohydrates, and sweeteners) and compounds in ionized form (monobasic and dibasic organic acids) on cation exchange columns filled with sulfonated poly (styrene-divinylbenzene) with a crosslinking degree of 8% (Nautilus-IE) and 10% (Sevko AA). When using 5 mM sulfuric acid, the parameters of the retention of compounds were determined and new patterns were obtained that clarify the mechanism of retention of organic acids in the ionoexclusive chromatography variant. It was found that the retention of all studied compounds ($\log k'$) is directly proportional to the hydrophobicity values ($\log P_{exp}$). In this case, the electrostatic repulsion of organic acids from the sulfogroups of the cation exchanger shifts the $\log k'$–$\log P_{exp}$ dependences by a fixed amount proportional to the number of carboxyl groups. The possibility of using the Sevko AA column not only for amino acid analysis, but also for ionoexclusive chromatographic determination of organic acids and alcohols in samples of complex composition with simultaneous spectrophotometric and refractometric detection is shown.

Keywords: *ionoexclusive chromatography, sulfocation exchangers, alcohols, carboxylic acids, ketones, carbohydrates*

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Ionoexclusive chromatography (**IECH**) is a convenient method for the simultaneous determination and separation of polar water-soluble compounds such as low molecular weight organic acids, alcohols, ketones, aldehydes, carbohydrates, and some other classes of compounds in the analysis of food and beverages [1, 2]. As a rule, highly acidic cation exchangers are used for separation, such as sulfonated poly(styrene-divinylbenzene) (**PS-DVB**) with a low degree of crosslinking (4-8%), particle diameters from 5 to 10 microns and an ion exchange capacity of about 3-5 meq/g. Water or dilute solutions of strong acids (usually sulfuric acid) [3] or weak acids [4] with additives of organic solvents (acetonitrile, acetone, methanol, ethanol) are used as eluents in IECH [5]. It is also known to use carboxylated PS-DVB [6, 7], sulfonated super-crosslinked polystyrene [8, 9], modified silica gel [10], and some other sorbents in IECH.

High separation selectivity, low toxicity of eluents, and low cost of analysis are important advantages of IECH in comparison with hydrophilic interaction chromatography (**HIC**), which is also widely used for the separation and determination of polar compounds. It should be noted that in the case of HIC, eluents containing up to 85–95 vol. % of toxic solvents like acetonitrile and methanol are usually used [11, 12] or complex mixtures of organic solvents and water [13, 14].

On the other hand, the disadvantages of IECH include significant retention of long-chain and aromatic acids due to their interaction with the PS-DVB matrix of ion exchangers [12, 15]. Various detection methods are compatible with IEX: conductometric, spectrophotometric, refractometric, mass spectrometric and charged aerosol detector.

It should be noted that sulfonated PS-DVB with a slightly higher degree of crosslinking (10%) is widely used in classical amino acid analysis [10], which makes it possible to use it in IECH.

The purpose of this work is to study and compare the patterns of retention of various classes of polar compounds (alcohols, ketones, carboxylic acids and carbohydrates) by the IEX method on two sulfocation exchangers based on PS–DVB in H^+ form with the same ion exchange capacity, but with different degrees of crosslinking, and to evaluate the possibility of their use for the determination of carboxylic acids and alcohols in objects of complex composition, such as beer and apple cider. Special attention is paid to the study of

the mechanism of retention of neutral and ionic polar organic compounds under IECH conditions.

METHODS AND MATERIALS

Reagents. Polar compounds of several classes were used as models, including alcohols: *n*-propanol, *n*-butanol, *tret*-butanol, isobutanol, isopentanol, *n*-pentanol, propanediol-1,2, propanediol-1,3, butanediol-1,3, butanediol-1,4 chemical elements (Reakhim, Russia), methanol (Merck, Germany, ⩾ 99.97%), ethanol (Verein, Russia, ⩾ 95%), isopropanol (Merck, Germany, ⩾ 99.9%), ethylene glycol (Fluka, Switzerland, ⩾ 99%), Diethylene glycol (Reagent Component, Russia, 99.5%), Triethylene glycol (Reagent Component, Russia, 98.5%), tetraethylene glycol (TCI, Japan, ⩾95%); aldites: glycerin AR grade (Reachim, Russia), erythritol (DopDrops LLC, Russia); ketones: acetone, methyl ethyl ketone, methyl butyl ketone, methyl isobutyl ketone reagent grade (Reachim, Russia), diethyl ketone (Sigma-Aldrich, USA, ⩾99%), organic acids: oxalic, tartaric, citric, malic, fumaric, succinic, adipic, formic, acetic, butyric, valeric reagent grade (Reachim, Russia), ascorbic (TCI, Japan, ⩾99%), glyoxylic (Sigma-Aldrich, USA, ⩾99%), glycolic (Sigma-Aldrich, USA, ⩾99%), lactic (Soyuz Reagent, Russia, ⩾95%) and propionic (Chemical Line, Russia, ⩾99.5%); sugars: L-(+)-arabinose, D-(+)-fructose, D-(+)-glucose, D-(+)-maltose monohydrate reagent grade (Reachim, Russia); D-(+)-xylose (Megsk, Germany, ⩾ 99.97%), maltotriose AR grade (ICN Biomedicals, USA), inulin (Molecularmeal, China) and sweetener substances: sucralose (Spirulina Food LLC, Russia), rebaudioside A (Stevia Group LLC, Russia, 98%).

Deionized water (Portlab, Russia) and sulfuric acid (Uralkhiminvest, Russia) were used to prepare solutions of analytes and eluents.

Equipment. The chromatograph Chromatek-Kristall HPLC 2014 (Russia) was used, which includes a mobile phase degasser, a high-pressure pump, a column thermostat, spectrophotometric and refractometric detectors. The results were processed using the Chromatech Analyst 3.1 software.

Chromatographic columns filled with sulfonated PS-DVB in H^+ form were studied:

— Sevko AA (Sevko and Co., Russia), size 200 × 4.6 mm, particle diameter 7 μm, degree of crosslinking 10%, exchange capacity 4.6–5.0 meq/g;

— Nautilus-IE (Biochemistry ST, Russia), size 150 × 4.6 mm and 200 × 4.6 mm, particle diameter 10 microns, degree of crosslinking 8%, exchange capacity 4.6–5.0 meq/g.

All separations were performed using 5 mM sulfuric acid as the mobile phase at a volume rate of 0.45 ml/min. Before use, the mobile phase was filtered through a nylon membrane filter with a pore diameter of 0.45 microns. The sample volume was 20 μl. The separations were carried out at a chromatographic column temperature of 80 °C, and the refractometer cell was thermostated at 55 °C.

Preparation of samples for analysis. Liquid samples (kvass, beer, cider, brine) were pre-filtered through a membrane filter with a pore diameter of 0.45 microns, degassed until the foam disappeared and diluted 10 times in the eluent.

Examination of the sorbent surface. The surface morphology of the Nautilus-IE sorbent was studied using scanning electron microscopy on a Hitachi Tabletop Microscope TM3030Plus (Hitachi, Japan) with a voltage of 15 kV on an accelerating electrode.

DISCUSSION OF RESULTS

Mechanism of polar sorbate retention in ionoexclusive chromatography. The retention of polar sorbates on polymer sulfocation exchangers in protonated form is determined by a combination of several factors, including Donnan exclusion, hydrophobic interaction with the PS-DVB sorbent matrix, size exclusion, and electrostatic interaction with the sulfogroup [15-17]. The contribution of each of these processes to retention depends on the nature of the compounds being separated.

According to the theory, Donnan ion exclusion is the main mechanism of ion retention in the IECH. The hydrated cation exchange resin forms a hypothetical semi-permeable membrane (Donnan membrane) due to negatively charged functional groups, which conditionally divides the liquid phase into two parts. The first part is the liquid between the particles of the cation exchanger, and the second is the liquid inside the particles of the loosely crosslinked resin. This leads to the fact that neutral molecules penetrate through the membrane and are distributed between the two liquid phases and, therefore, are retained in the sorbent layer. At the same time, negatively charged ions are electrostatically repelled from the similarly charged groups of the cation exchanger and are weakly retained on the column. Partially ionized compounds are weakly repelled by the membrane and can be completely protonated in the acidic phase of the cation exchanger. Their retention times are intermediate between the retention times of fully ionized and neutral analytes. Thus, the retention of a compound depends on its effective charge, which is determined by the ratio of concentrations of its ionized and neutral forms. For example, in the case of organic acids, their retention decreases with decreasing pH, whereas fully dissociated inorganic acids are eluted undivided in the dead column volume [15-17].

According to the theory of IEX, the hydrophobic interaction of polar sorbates with the PS-DVB sorbent matrix prevails for molecules containing a long carbon

Table 1. Properties of organic acids, their retention and selectivity of separation on Sevko AA and Nautilus IE columns

Acid	$\log P_{exp}$ [a]	pK_{a1} [b] (25 °C, $I = 0$)	pK_{a2} [b] (25 °C, $I = 0$)	Sevko AA		Nautilus-IE	
				k'	α	k'	α
Monobasic acids							
Glyoxylic		3.46	-	0.43	-	0.61	-
Glycolic	-1.11	3.83	-	0.78	1.81	1.03	1.69
Lactic	-0.72	3.86	-	0.85	1.09	1.13	1.10
Formic	-0.54	3.75	-	1.05	1.24	1.29	1.14
Acetic	-0.17	4.76	-	1.21	1.15	1.50	1.16
Propionic	0.33	4.86	-	1.59	1.31	1.95	1.30
Butyric	0.79	4.83	-	2.18	1.37	2.62	1.34
Valeric	1.39	4.84	-	-	-	3.30	1.26
Polybasic acids							
Oxalic acid	-	1.25	4.27	0.07	-	0.11	-
Citric	-	3.13	4.76	0.26	3.71	0.37	3.36
Tartaric	-	3.04	4.37	0.30	1.15	0.44	1.19
Malic	-1.26	3.46	5.10	-	-	0.63	1.43
Succinic	-0.59	4.21	5.64	-	-	0.99	1.57
Fumaric	0.46	3.02	4.48	-	-	1.32	1.33
Adipic	0.08	4.42	5.42	-	-	1.63	1.23

Note: a logPexp values are taken from the EPA KOWWIN database [18]; b acid pKa values are taken from the NIST database [19].

chain or aromatic system. For such molecules, there is a stronger retention compared to the proposed mechanism of ion exclusion of ions. For example, long-chain (n_C > 3) aliphatic acids have similar dissociation constants (Table 1), but they differ significantly in retention in the IECH [17]. In this case, the contribution of electrostatic interactions remains constant, and retention (log$k$) is proportional to n_C or hydrophobicity ($\log_P$) (Tables 1 and 2).

The size exclusion is based on the fact that larger molecules are less likely to penetrate the resin, which is a porous three-dimensional network. Retention of the substance to be determined is determined by the Stokes radius and the permeability of the sorbent, which depends on the degree of crosslinking and swelling of the polymer matrix. The lower the degree of crosslinking, the greater the swelling and the higher the permeability of the ion exchanger. It is believed that the separation of sugars occurs according to size exclusion [5, 15].

Additional interaction of neutral compounds with the sulfone group is possible with the formation of hydrogen bonds or due to dipole-dipole interactions. It is also possible to implement a distribution mechanism between water bound to sulfonic groups inside the resin particles and the mobile phase outside the sorbent particles, which to a certain extent corresponds to the mechanism of hydrophilic chromatography. In this case, the analytes, in particular carbohydrates, are retained on sulfonated PS-DVB according to their hydrophilicity [17].

Characteristics of sorbents and columns. The patterns of retention of various polar compounds on Sevko AA and Nautilus IE columns filled with sulfocation exchangers based on PS-DVB having the same ion exchange capacity of 4.6–5.0 meq/g (in H^+ form), but different particle diameters (7 and 10 μm) and the degree of crosslinking (10 and 8%) were considered. The dead time was determined by the retention time of sulfuric acid, it was 2.46 and 2.31 minutes, respectively, for the Sevko AA and Nautilus IE columns with a length of 200 mm. According to the manufacturers' recommendations, the Nautilus-IE column is designed for the separation of polar compounds by the IEX method, and the Sevko AA column is designed for amino acid analysis. Microspherical cation exchangers with a uniform particle size distribution were used to fill the

Table 2. Retention and selectivity of separation of neutral polar organic compounds on Sevko AA and Nautilus IE columns

Сщьзщгте	$\log P_{exp}$ [a]	Sevko AA			Nautilus-IE		
		t_R, min	*k'*	α	t_R, min	*k'*	α
Monatomic alcohols							
Methanol	-0.77	6.58	1.67	-	7.05	2.06	-
Ethanol	-0.31	7.46	2.03	1.21	8.08	2.50	1.22
Isopropanol	0.05	8.20	2.33	1.15	8.99	2.91	1.16
tert-Butanol	0.35	8.77	2.56	1.10	9.75	3.24	1.11
n-Propanol	0.25	9.39	2.82	1.10	10.15	3.42	1.05
sec-Butanol	0.61	10.81	3.39	1.20	11.85	4.15	1.22
Isobutanol	0.76	11.28	3.58	1.06	12.27	4.36	1.05
n-Butanol	0.88	12.88	4.24	1.18	13.88	5.06	1.16
Isopentanol	1.16	16.01	5.51	1.30	17.31	6.57	1.30
n-Pentanol	1.51	19.76	7.03	1.28	21.07	8.23	1.25
Polyatomic alcohols							
Erythritol	-2.29	4.07	0.65	-	4.43	0.92	-
Glycerin	-1.76	4.59	0.87	1.33	5.02	1.17	1.28
Ethylene Glycol	-1.36	5.47	1.22	1.40	5.95	1.58	1.35
Diethylene glycol	-	5.48	1.23	1.01	6.14	1.66	1.05
Triethylene glycol	-1.75	5.51	1.24	1.01	6.30	1.73	1.04
Tetraethylene glycol	-	5.54	1.25	1.01	6.35	1.75	1.01
Propanediol-1,2	-0.92	5.74	1.33	1.06	6.38	1.77	1.01
Propanediol-1,3	-1.04	5.89	1.40	1.05	6.56	1.84	1.04
Butanediol-1,3	-	6.28	1.55	1.11	7.13	2.09	1.14
Butanediol-1,4	-	7.03	1.86	1.20	7.98	2.46	1.18
Ketones							
Acetone	-0.24	7.75	2.15		8.27	2.58	
Methylethylketone	0.29	9.82	2.99	1.39	10.43	3.52	1.36
Diethyl Ketone	0.99	12.74	4.18	1.4	13.44	4.82	1.37
Methyl Isobutyl Ketone	1.31	15.25	5.2	1.24	16.09	5.97	1.24
Methylbutyl Ketone	1.38	18.74	6.62	1.27	19.41	7.41	1.24
Sugars and sweeteners							
Inulin		2.48	0.01	-	2.40	0.04	-
Maltotriosis		2.73	0.11	11.52	2.67	0.16	3.90
Maltose		2.88	0.17	1.60	2.93	0.27	1.72
Glucose	-3.24	3.36	0.37	2.12	3.46	0.50	1.86
Xylose		3.54	0.44	1.20	3.70	0.60	1.21
Fructose		3.56	0.45	1.02	3.72	0.61	1.01
Arabinose		3.78	0.54	1.20	4.01	0.74	1.21
Sucralose	-0.51	4.17	0.69	1.29	4.68	1.03	1.40
Rebaudioside A		4.32	0.76	1.09	5.28	1.29	1.25

Note: a The logPexp values are taken from the EPA KOWWIN database [18] and work [20].

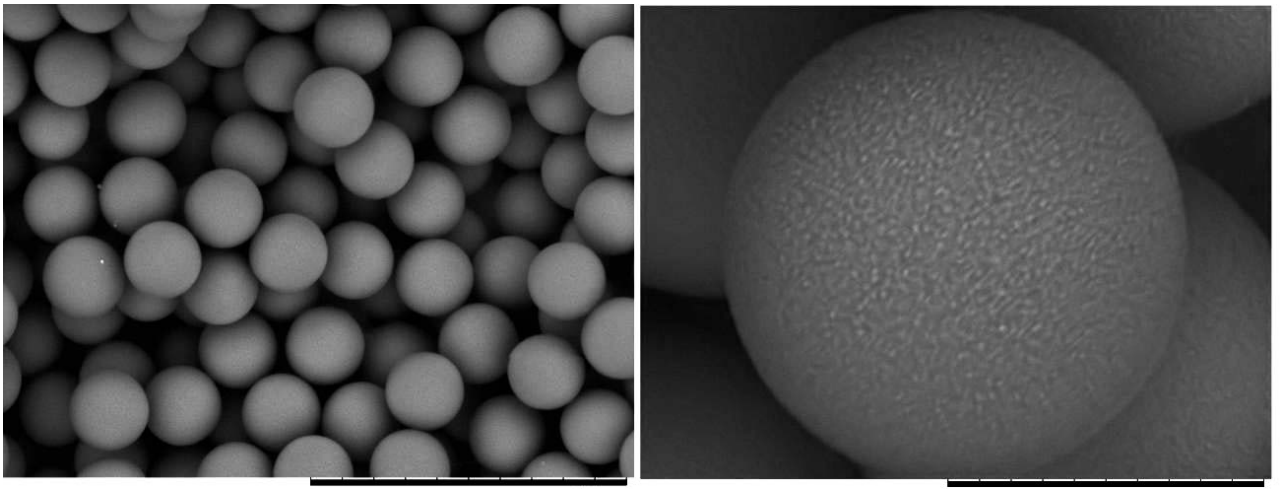

Fig. 1. Micrographs of Nautilus-IE cation exchanger particles.

chromatographic columns. Figure 1 shows a photograph of the Nautilus IE sorbent with a particle diameter of 10 microns, illustrating the uniformity of particles in size. The alcohol efficiency of the Sevko AA column reaches 28,000 tt/m, and in the case of Nautilus IE – 24,000 tt/m (Table 3). Accordingly, the reduced height equivalent to the theoretical plate (RHETT), which characterizes the quality of the column stuffing, is 5.1 and 4.1.

Chromatographic retention of six groups of polar water-soluble organic compounds was studied, including monatomic alcohols **(**methanol, ethanol, *n*-propanol, isopropanol, *tert*-butanol, btor-butanol, isobutanol, *n*-butanol, isopentanol, *n*-pentanol); polyatomic alcohols (ethylene glycol, diethylene glycol, triethylene glycol, tetraethylene glycol, propanediol-1,2, propanediol-1,3, butanediol-1,3, butanediol-1,4, glycerin, erythritol); ketones **(**acetone, methyl ethyl ketone, diethyl ketone, methyl isobutyl ketone, methyl butyl ketone); aliphatic carboxylic acids (oxalic, citric, tartaric, malic, fumaric, succinic, adipic, glyoxylic, glycolic, lactic, formic, acetic acid, propionic acid, fatty acid, valerian acid); carbohydrates **(**inulin, maltotriose, maltose, glucose, xylose, fructose, arabinose) and sweetener substances (sucralose, rebaudioside A).

For each compound, the retention time (t_R), retention factor (*k'*), separation selectivity relative to the previous peak (α), efficiency (*N*), resolution (*Rs*), and asymmetry of chromatographic peaks (*As*) were obtained, which are shown in Tables 1-3.

Sorbents obtained by sulfonation of microspherical PS-DVB particles have varying degrees of hydrophobicity. To assess hydrophobicity, it is convenient to use the values of $\alpha(CH_2)$, which are calculated from the retention difference of homologues with the number of carbon atoms $n_C > 3$ [21].

Homologue retention in chromatography is described by a linear relationship:

$$\log k' = \alpha(CH_2)n_C + \text{const}, \quad (1)$$

from the tangent of the slope angle, the contribution of the methylene group to the retention or methylene selectivity of $\alpha_(CH_2)$ can be estimated [18]. In this work, the retention of homologues of *n*-alkanols, α,ω-diols, and *n*-alkane acids was studied, for which $\log k'–n_C$ dependences were constructed (Fig. 2).

The values of $\alpha(CH_2)$ calculated from the difference in the log*k* values of *n*-butanol and *n*-propanol were 0.170 and 0.177 for the Nautilus IE and Sevko AA sorbents, respectively. Since $\alpha(CH_2)$ values characterize the hydrophobicity of sorbents, it can be concluded that the Sevko AA column is slightly more hydrophobic. The data obtained correspond to the values of $\alpha(CH_2)$ for ion exchangers based on a PS-DVB matrix [18]. The hydrophobicity of sorbents can also be estimated by the slope tangent of the linear dependence logk' on logP_{exp} of neutral polar organic compounds. The obtained values of the slope angle tangent were 0.26 ± 0.01 and 0.24 ± 0.01 (n = 21) for Sevko AA and Nautilus IE sorbents, respectively. These values confirm the higher hydrophobicity of the Sevko AA column compared to Nautilus IE.

Patterns of retention of neutral polar organic compounds. *Monatomic alcohols.* According to the data in Table 1, the retention of normal monatomic alcohols increases with increasing chain length. This is probably due to the increasing contribution of hydrophobic interactions between alkyl radicals, estimated by the logP_{exp} of alcohols, and the polymer matrix of sorbents (Fig. 3). In general, alcohols with a branched alkyl radical are retained less strongly than the corresponding isomers with a linear chain. This order of elution of compounds is associated with a relatively higher polarity of the hydroxyl group and alcohol in general and lower values (log*P*). It should be borne in mind that, due to

Table 3. Efficiency, resolution, and coefficients of assimilation of polar organic compounds on Sevko AA and Nautilus IE columns

Connection	Sevko AA			Nautilus IE		
	N, тт/m	R_s	As	*N*, тт/m	R_s	As
Monatomic alcohols						
Methanol	23600	-	0.66	18200	-	0.43
Ethanol	28300	2.27	0.59	20100	2.1	0.42
Isopropanol	24600	1.72	0.6	19200	1.68	0.49
tert-Butanol	20200	1.11	1.12	13000	1.13	0.5
n-Propanol	28700	1.19	0.92	-	0.6	-
sec-Butanol	22000	2.47	0.39	15600	2.38	-
Isobutanol	-	0.69	-	-	0.56	-
n-Butanol	25500	2.27	0.55	24500	2.23	0.38
Isopentanol	17200	3.45	0.94	17400	3.49	0.62
n-Pentanol	19700	3.19	0.74	23400	3.12	0.4
Polyatomic alcohols						
Glycerin	17400	-	0.67	17400	-	0.51
Ethanediol-1,2	19200	2.68	0.57	18200	2.53	0.57
Propanediol-1,3	20500	1.13	1.04	16800	1.37	0.76
Butanediol-1,3	22000	1.18	1.22	18800	1.32	0.9
Butanediol-1,4	21000	1.87	0.58	22000	1.74	0.47
Ketones						
Acetone	24900	-	0.74	21800	-	0.46
Methylethylketone	26600	4.23	0.48	23300	3.98	0.36
Diethyl Ketone	28100	4.8	0.48	24000	4.46	0.43
Methiisobutyl Ketone	24100	3.22	0.51	22800	3.18	0.44
Methylbutyl Ketone	24900	3.6	0.51	24300	3.35	0.46
Sugar						
Maltotriosis	7700	-	1.2	5800	-	1
Maltose	7800	0.52	0.9	5500	0.66	0.8
Glucose	10400	1.52	0.89	10000	1.68	0.6
Organic acids						
Oxalic	9700	-	1.5	9200	-	1.1
Citric	8400	1.57	-	9200	2.35	-
Tartaric	9200	0.47	-	10300	0.65	-
Glyoxylic	13700	1.12	0.87	15800	1.36	0.67
Glycolic	12000	2.75	0.22	16800	3.3	0.3

Table 3. End

Connection	Sevko AA			Nautilus IE		
	N, тт/m	R_s	As	*N*, тт/m	R_s	As
Lactic	-	0.53	-	-	0.7	-
Formic	22000	1.57	0.84	21300	1.12	0.72
Acetic	26000	1.41	0.6	25400	1.5	0.53
Propionic	29700	3.03	0.43	29700	3.07	0.36
Butyric	27000	4.02	0.91	25800	4.03	0.92

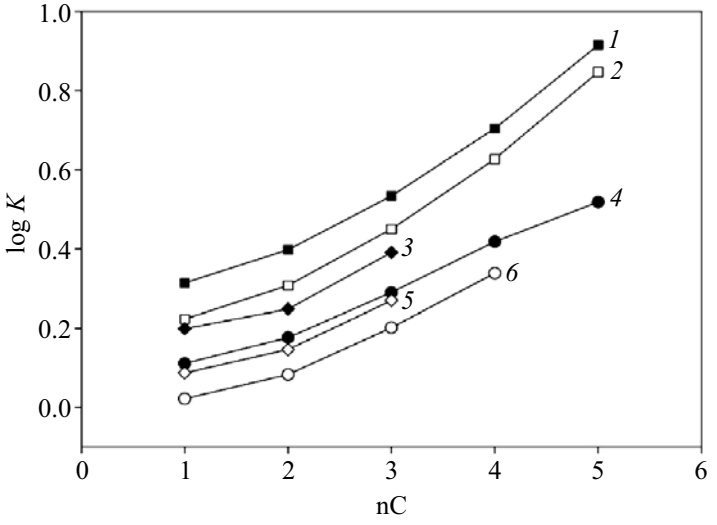

Fig. 2. Dependence of the retention of homologues of *n*-alkanols (*1*, *2*), diols (*3*, *5*) and *n*-alkane acids (*4*, *6*) on the number of carbon atoms on the Nautilus IE (*1*, *3*, *4*) and Sevko AA (*2*, *5*, *6*) columns.

screening with alkyl groups, the central carbon atom is less accessible for heterogeneous interaction with the surface of the sorbent's rigid polymer matrix compared to homogeneous interaction with octanol-2, used to determine $\log P_{exp}$ values. This effect determines the following order of elution of butanol isomers: *tert*-butanol *sec*-butanol < isobutanol < *n*-butanol.

The dependence of the elution order on hydrophobicity is linear for all studied neutral polar compounds (Fig. 3), including monatomic alcohols, with the exception of *tert*-butanol. The obtained alcohol retention patterns are valid for both studied chromatographic columns, while a linear correlation is observed between the retention factors of monatomic alcohols on the studied columns.:

$$k'_{\text{Nautilus-IE}} = 1.15 \times k'_{\text{Sevko AA}} + 0.21$$
$$(n = 10, r^2 = 0.999), \quad (2)$$

this confirms the same retention mechanism with a relatively weaker alcohol retention on the Sevko AA sorbent. This may be due to the fact that the Sevko AA cation exchanger is characterized by a higher degree of crosslinking and a smaller pore size. Because of this, it is more difficult for the sample molecules to penetrate the resin, which leads to a decrease in their retention. For this reason, cation exchangers with a low degree of crosslinking (usually 4%) are used to separate substances with a higher molecular weight, such as oligosaccharides, and sorbents with 6-8% crosslinking are usually used to separate mono- and disaccharides [17]. In addition, both columns are characterized by similar selectivity for most alcohols, except for the *tert*-butanol/*n*-propanol pair, where the highest selectivity coefficient is observed on the Sevko AA sorbent.

The possibility of simultaneous separation of a mixture of ten monatomic alcohols has been studied on Sevko AA and Nautilus-IE columns. The corresponding chromatograms are shown in Fig. 4. As can be seen (fig. 4, Table. 3), the Sevko AA column showed higher separation efficiency, resolution, and peak symmetry of alcohols. A noticeable improvement in resolution was observed for *tert*-butanol/n-propanol and *sec*-butanol/isobutanol pairs, whereas the peaks on Nautilus-IE are poorly resolved. The following correlation was found for

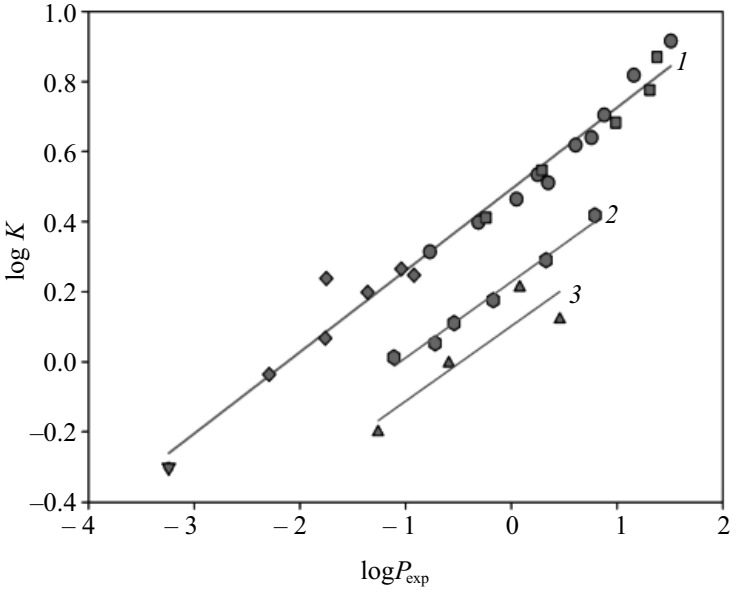

Fig. 3. The dependence of the retention (log*k*) of polar organic compounds on the Nautilus IE column on hydrophobicity (logP_{exp}). *1* – neutral compound ((●) – monohydric alcohols (■) – ketone (◆) – polyhydric alcohols, (▼) – glucose), *2* – monobasic acids (⬢) and *3* – polybasic acids (▲)

the retention of monatomic alcohols on the Nautilus IE column:

$$\log k' = 0.268 \cdot \log P_{exp} + 0.471 \quad (n = 10, r^2 = 0.967). \quad (3)$$

Polyatomic alcohols. An increase in retention time with an increase in the length of the carbon chain between hydroxyl groups was observed for polyatomic alcohols (Fig. 2, Table 2). Thus, retention on the Nautilus IE column increases from ethylene glycol (n_C =2, k' = 1.58) to propylene glycol (3, 1.77) and butylene glycol (4, 2.46). An increase in the number of hydroxyl groups in the molecules reduces hydrophobicity (logP_{exp}) and thus leads to a decrease in alcohol retention. This is manifested in the following order of elution of alcohols containing three carbon atoms, but a different number of hydroxyl groups: glycerol < propanediol isomers < propanol isomers. Accordingly, the retention of polyatomic alcohols is also proportional to their hydrophobicity, as shown in Fig. 3.

A comparison of the columns showed that stronger retention of both polyatomic and monatomic alcohols is observed on the Nautilus IE column compared to Sevko AA (Table 2). As for selectivity, it can be seen that the values of the selectivity coefficients differ for Sevko AA and Nautilus IE sorbents: Sevko AA sorbent demonstrated better selectivity for the separation of erythritol/glycerin, glycerin/ethylene glycol, tetraethylene glycol/propanediol-1,2 and butanediol-1,3/butanediol-1,4 pairs. At the same time, an increase in the separation selectivity of propanediol-1,3 and butanediol-1,3 was observed on Nautilus IE. The possibility of simultaneous separation of a mixture of five polyatomic alcohols on Sevko AA and Nautilus IE columns is demonstrated (Fig. 5).

According to the data obtained (Fig. 5, Table. 3) Sevko AA sorbent showed higher efficiency and peak symmetry. Despite this, a significantly better resolution for ethylene glycol/propanediol-1,3 and propanediol-1,3/butanediol-1,3 pairs was observed on the Nautilus-IE column compared to the Sevko AA sorbent. The opposite situation is observed for glycerol/ethylene glycol and butanediol-1,3/butanediol-1,4 pairs, where the Rs value is higher on the Sevko AA column.

Ketones. Patterns of retention of ketones and alcohols (Table 2, Fig. 3) are similar, while log*k'* increases linearly with increasing logP_{exp}:

$$\log k' = 0.255 \cdot \log P_{exp} + 0.467 \quad (n = 5, r^2 = 0.965). \quad (4)$$

It should be noted that the coefficients of the correlation equations (3) and (4) practically coincide, which indicates the determining contribution of hydrophobicity to the retention of these compounds. Figure 6 shows chromatograms of a model ketone mixture on Sevko AA and Nautilus-IE columns. From the data in Fig. 6 and Table. 3 It can be seen that both columns show high ketone efficiency and resolution, in addition, Sevko AA sorbent demonstrates higher separation efficiency and peak symmetry.

Carbohydrates and sweeteners. Retention of sugars and structurally similar sweeteners (Table 2) decreases with increasing degree of polymerization (molecular weight), therefore, it can be assumed that the order of sugar elution is due to size exclusion. Thus, trisaccharides

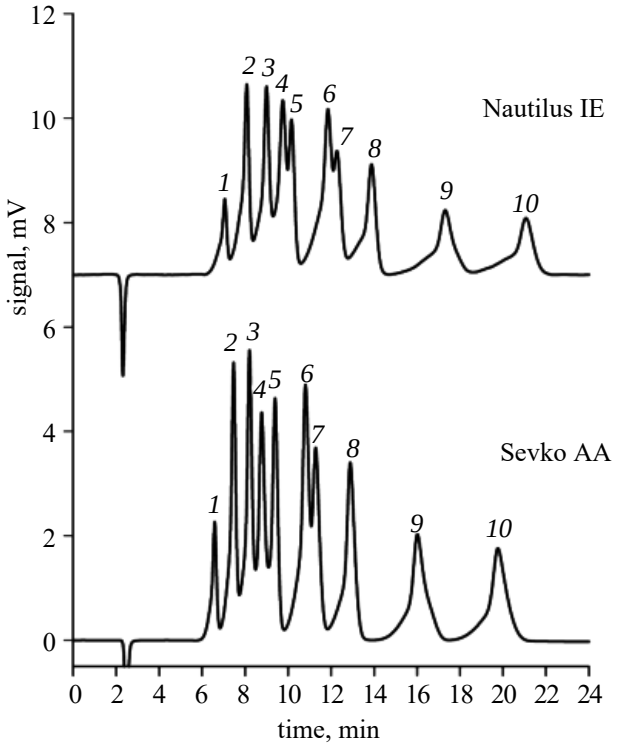

Fig. 4. Chromatogram of separation of a model mixture of monatomic alcohols with a concentration of 0.5 mg/ml: *1* – methanol, *2* – ethanol, *3* – isopropanol, *4* – *tert*-butanol, *5* – *n*-propanol, *6* – fluorobutanol, *7* – isobutanol, *8*– *n*-butanol, *9*– isopentanol, *10*– Pentanol. A refractometric detector. Columns: 200 × 4.6 mm.

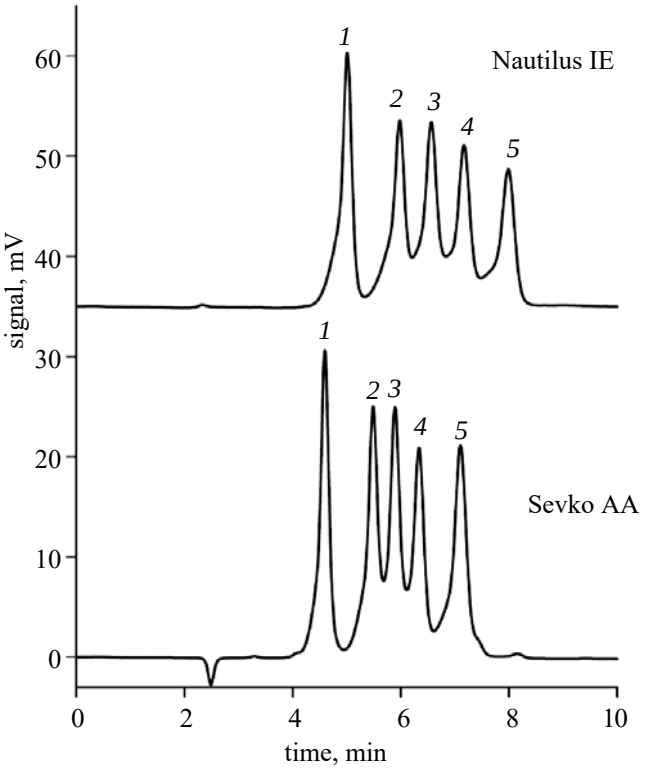

Fig. 5. Chromatogram of separation of a model mixture of polyatomic alcohols with a concentration of 1.2 mg/ml. *1* – glycerin, *2* – ethylene glycol, *3* – propanediol-1,3, *4* – butanediol-1,3, *5* – butanediol-1,4. A refractometric detector. Columns: 200 × 4.6 mm.

are retained less than disaccharides, and disaccharides, in turn, are less than monosaccharides. This pattern is true, for example, for a number of inulin < maltotriose < maltose < glucose.

It is difficult to establish other patterns of carbohydrate retention, but the resulting elution order coincides with the results of other studies. It is believed that the order of elution of monosaccharides and aldites is determined by the distribution mechanism between water bound to the sulfone group inside the ion exchange resin particles and the mobile phase outside the sorbent particles.

Sucralose – a disaccharide, is a sucrose derivative characterized by the presence of three chlorine atoms. Substitution of three hydroxyl groups with chlorine atoms in sucrose leads to a decrease in the hydrophilicity of the molecule ($\log P_{exp}$ = -0.51 [20]) and an increase in retention due to increased hydrophobic interactions with the polymer matrix of the sorbent.

Rebaudioside A is a steviol glycoside and consists of steviol and four glucose molecules attached to steviol. The presence of steviol in the rebaudioside A molecule leads to an increase in the hydrophobicity of the molecule, which leads to an increase in retention compared to polysaccharides containing a similar amount of monosaccharides.

As noted above, the Nautilus-IE column is characterized by a stronger retention of alcohols and ketones compared to the Sevko AA column. A similar situation is observed with carbohydrates and sweeteners.

When evaluating selectivity on the Nautilus-IE column, an increase in selectivity coefficients was observed for maltotriose/maltose (α = 1.72), arabinose/sucralose (α = 1.40) and sucralose/rebaudioside A pairs (α = 1.25) compared with Sevko AA, where the selectivity coefficients were 1.60, 1.29 and 1.09, respectively. However, the Sevko AA sorbent demonstrated better maltose and glucose separation selectivity (α = 2.12), whereas the Nautilus-IE sorbent had a selectivity coefficient of 1.86. This is probably due to the fact that cation exchangers have varying degrees of crosslinking, which allows larger carbohydrate molecules to diffuse more easily into the resin with fewer polymer chain crosslinking and leads to stronger retention. As for the selectivity of monosaccharide separation, it is similar for both columns. The Nautilus-IE sorbent demonstrated a higher resolution for maltotriose/maltose and maltose/glucose pairs compared to Sevko AA (Table 3).

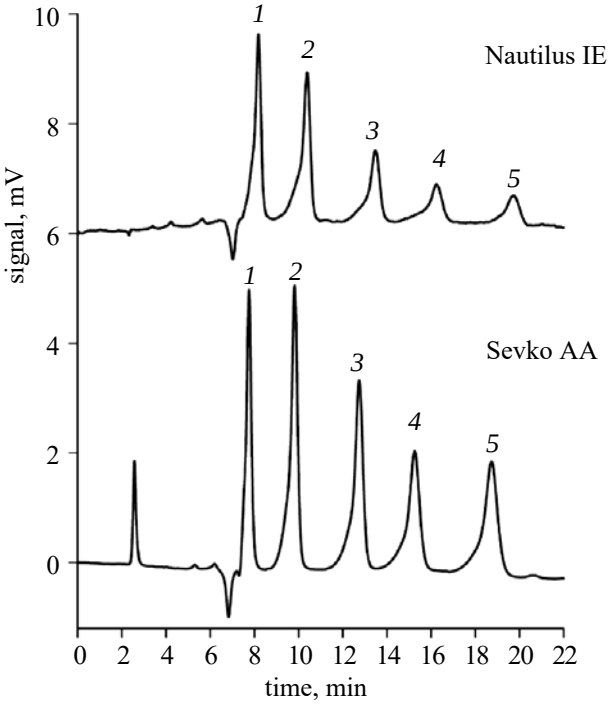

Fig. 6. Chromatogram of separation of a model mixture of ketones with a concentration of 0.5 mg/ml. *1* – acetone, *2* – methyl ethyl ketone, *3* – diethyl ketone, *4* – methyl isobutyl ketone, *5* – methyl butyl ketone. A refractometric detector. Columns: 200×4.6 mm.

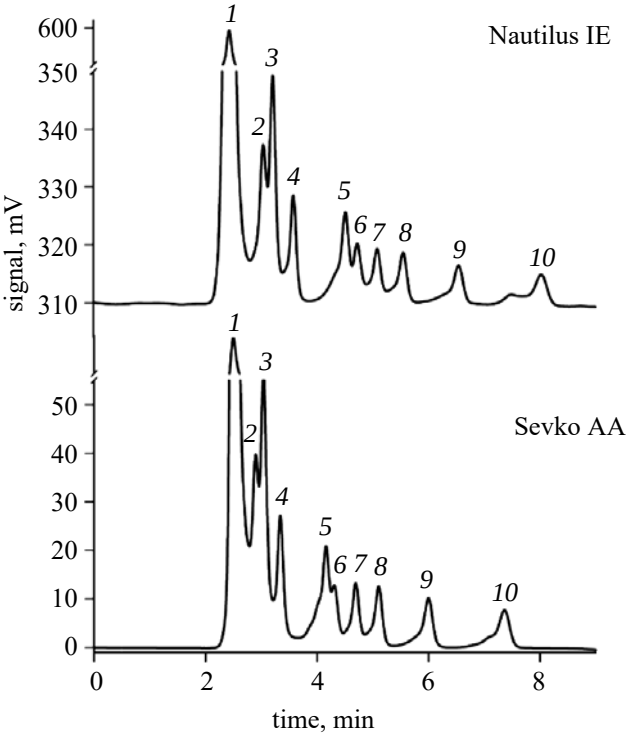

Fig. 7. Chromatogram of separation of a model mixture of acids with a concentration of 0.1 mg/ml: *1* – oxalic acid, *2* – citric acid, *3* – tartaric acid, *4* – glyoxylic acid, *5* – glycolic acid, *6* – lactic acid, *7* – formic acid, *8* – acetic acid, *9* – propionic acid, *10* – butyric acid. Spectrophotometric detector, 210 nm.

Carboxylic acids. *Monobasic acids.* The retention of monobasic carboxylic acids is proportional to their hydrophobicity (Table 1), but the electrostatic repulsion of negatively charged sorbates from similarly charged sulfogroups leads to a weaker interaction, so the resulting direct dependence $\log k'$–$\log P_{exp}$ is parallel to the dependence obtained for neutral compounds, but lower (Fig. 3). The ratio of concentrations of ionized and neutral forms of acid is determined by the dissociation constant and is equivalent to the effective charge of the solute. Thus, the retention of organic acids on sulfocation ion exchangers is determined by the sum of electrostatic and hydrophobic interactions, similar to the data of [21] for ion exchange chromatography of acids. For the Sevko AA column (Table. 1) there was a slight increase in the selectivity coefficients for glyoxylic/glycolic acid pairs (α = 1.81) and formic/lactic acid (α = 1.24), whereas for Nautilus-IE the selectivity coefficients are 1.68 and 1.14, respectively. The possibility of simultaneous separation of a mixture of ten carboxylic acids is demonstrated on Sevko AA and Nautilus-IE columns (Fig. 7).

Polybasic acids. According to the data in the table. 1 and Fig. 3 the retention patterns of polybasic and monobasic acids are similar, retention increases with increasing hydrophobicity. The exception is oxalic acid, which retains significantly less than other acids. Apparently, this is due to the low value of pK_{a1} = 1.25, which leads to a decrease in retention time due to the presence of a dissociated carboxyl group and additional repulsion of the analyte from charged sulfogroups of the cation exchanger in the eluent with a pH of ~ 2.0.

When considering the orthogonality of the dependence of the carboxylic acid retention factor on the Nautilus-IE column on the carboxylic acid retention factor on the Sevko AA column, a similar dependence is seen, as in the case of alcohols. The equation of dependence for acids:

$$k'_{\text{Nautilus-IE}} = 1.18 \times k'_{\text{Sevko AA}} + 0.08$$
$$(n = 10, r^2 = 0.998). \quad (5)$$

This may indicate an identical retention mechanism and a relatively weaker retention of carboxylic acids on the Sevko AA sorbent compared to Nautilus-IE. As mentioned above, the degree of crosslinking of the cation exchanger significantly affects the retention of organic compounds, including carboxylic acids. Thus, a sorbent with a low degree of crosslinking allows molecules to penetrate the resin more easily, which leads to an increase in retention coefficients [3, 22]. From the data in Fig. 7 and Table. 3 shows that Nautilus-IE and Sevko AA cation exchangers have similar separation efficiency and resolution in the case of monobasic carboxylic acids (formic, acetic, propionic, and butyric). For the

remaining acids, an increase in the separation efficiency and resolution on the Nautilus IE sorbent was observed. Indeed, the resolution is noticeably improved for oxalic/citric, citric/tartaric and glycolic/lactic acid pairs, whereas it is noticeably worse for Sevko AA. It is interesting to note that, despite the increase in resolution in the case of the listed acid pairs, the resolution for the lactic/formic acid pair on the Sevko AA sorbent is significantly higher compared to Nautilus IE. However, both columns show low values of *Rs* for citric/tartaric and glycolic/lactic acid pairs. Citric acid, unlike tartaric acid, is a tribasic acid, therefore, to improve separation, it is possible to change the concentration of sulfuric acid in the mobile phase, thereby changing the number of dissociated groups in the acids. Lactic and glycolic acids have similar pH values, but the presence of a methyl group in the lactic acid molecule increases its hydrophobicity. Nevertheless, low resolution values are observed for this pair of acids. To increase the selectivity of separation, the temperature of the column can be reduced and the concentration of sulfuric acid in the mobile phase can be changed.

As for the symmetry of the acid peaks, the asymmetry coefficients of both cation exchangers are close and have values below unity for all acids except oxalic acid, for which $As > 1$.

Thus, it can be concluded that the studied sorbents can be successfully used for the separation and determination of various organic compounds in the IECH regime. The obtained retention patterns of alcohols, ketones, organic acids, and carbohydrates are correct for both Sevko AA sorbent and Nautilus-IE and coincide with the elution order obtained on other sulfocation exchangers [5, 23, 24]. It is important to note that the Sevko AA sorbent showed higher efficiency and resolution in the separation of monatomic alcohols and ketones. The Nautilus-IE sorbent provides good resolution and separation efficiency for polyatomic alcohols, organic acids, and carbohydrates.

The Nautilus IE sorbent was chosen to determine the content of sugars, organic acids and alcohols in real objects. As a rule, IECHS use more voluminous columns measuring 300 × 7.8 mm. For this reason, a serial connection of two Nautilus IE speakers with lengths of 150 and 200 mm was used to improve efficiency and resolution. By increasing the efficiency of the column under isocratic conditions, 19 organic compounds of various classes, including 7 organic acids, 3 carbohydrates, and 9 alcohols, were separated in 38 minutes (Fig. 8).

Practical application. The applicability of the Nautilus IE sorbent to the analysis of real objects of complex composition is shown by the example of the determination of organic acids and alcohols in beverages such as beer and apple cider. The corresponding chromatograms obtained with two detectors are shown in Fig. 9. The use of a refractometric detector together with a spectrophotometric detector made it possible to detect chromatographic peaks of ethanol and

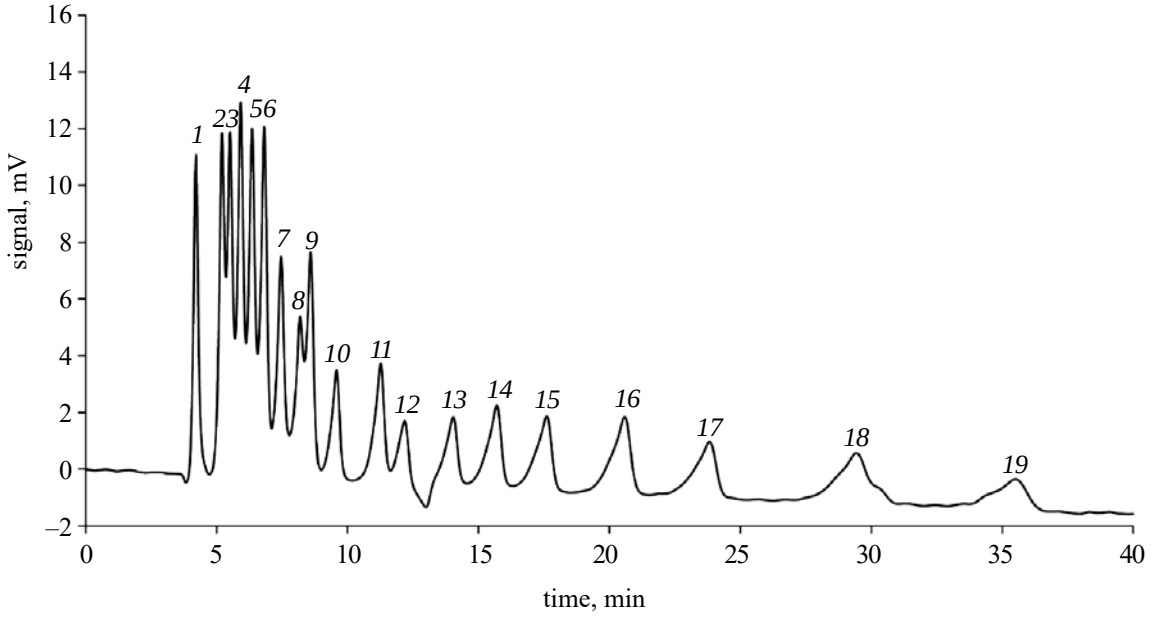

Fig. 8. Chromatogram of separation of a model mixture of organic acids, carbohydrates and alcohols with a concentration of 0.46 mg/ml. *1* – oxalic acid, *2* – citric acid, *3* – tartaric acid, *4* – glucose, *5* – fructose, *6* – arabinose, *7* – succinic acid, *8* – lactic acid-ta, *9* – glycerin, *10* – acetic acid, *11* – propionic acid, *12* – methanol, *13* – ethanol, *14* – isopropanol, *15*– *n*-propanol, *16* – fluorobutanol, *17*–*n*-butanol, *18* – isopentanol, *19* – *n*-pentanol. A refractometric detector. Series-connected Nautilus IE columns measuring 150 × 4.6 and 200 × 4.6 mm.

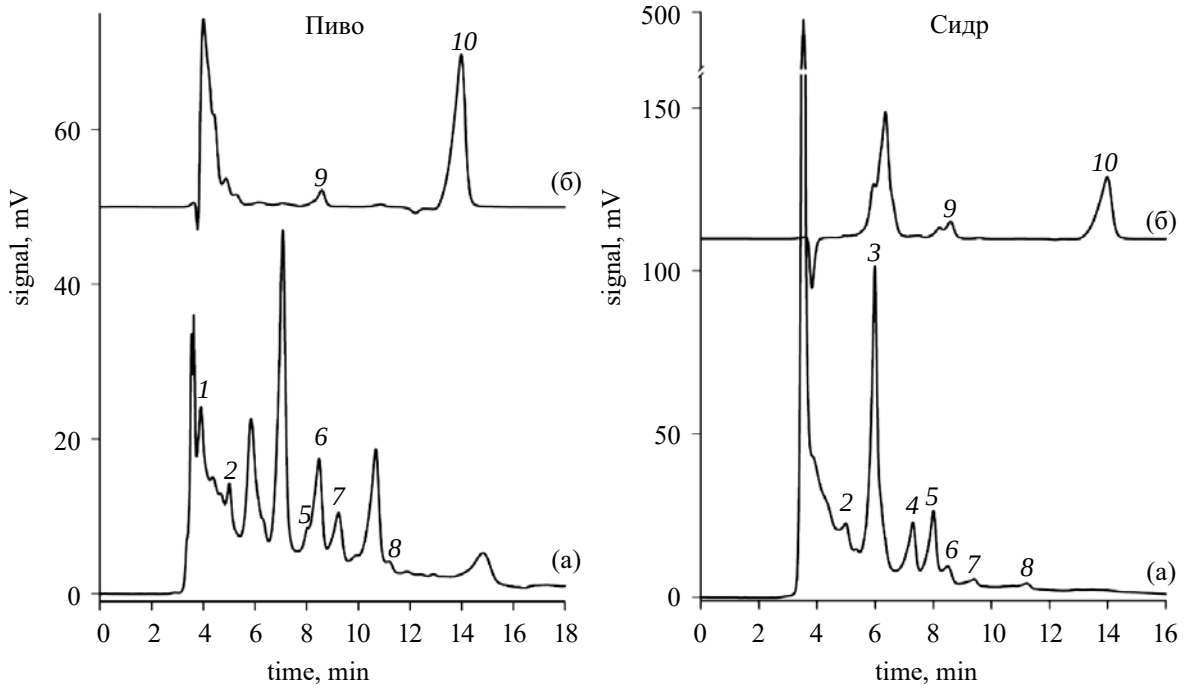

Fig. 9. Chromatograms of low-alcohol beverages. A – UV detection (210 nm), B – refractometric detection. The identified components are: *1* – oxalic acid, *2* – citric acid, *3* – malic acid, *4* – succinic acid, *5* – lactic acid, *6* – fumaric acid, *7* – acetic acid, *8* – propionic acid, *9* – glycerin, *10* – ethanol. Serially connected Nautilus IE columns measuring 150 × 4.6 and 200 × 4.6 mm.

glycerol in complex objects. As can be seen from the chromatograms, the following components were found in the composition of cider: citric, malic, succinic, lactic, fumaric, acetic, propionic acids, glycerin and ethanol. Peaks corresponding to oxalic, citric, lactic, fumaric, acetic, propionic acids, glycerin and ethanol were recorded in beer. It is important to note the use of a two-detector system when analyzing objects. The use of a refractometric detector makes it possible to clearly identify peaks of glycerol and ethanol.

* * *

A comparison of the two types of sulfocation ion exchangers showed the identity of the retention mechanism and their interchangeability for practical operation in ionoexclusive chromatography. It was found that the retention of all studied compounds ($\log k'$) linearly correlates with the values of hydrophobicity ($\log P_{exp}$). In this case, the electrostatic repulsion of organic acids from the sulfogroups of the cation exchanger shifts the $\log k'$–$\log P_{exp}$ dependences by a fixed amount proportional to the number of carboxyl groups. It has been shown that other interactions do not significantly contribute to the retention of compounds. The retention patterns found significantly simplify the selection of possible analysis objects and the identification of detectable compounds.

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CONFLICT OF INTERESTS

The authors state that they have no known financial conflicts of interest or personal relationships that could affect the work presented in this article.

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