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Acetonitrile as Tops Solvent for Liquid Chromatography and Extraction

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ABSTRACT

This article deals with acetonitrile physico-chemical properties and its mixtures with water. It covers the information about isotherms of such parameters of the acetonitrile-water binary system as density, viscosity, permeation coefficient, refraction index, optic density, a boiling point isobar. Authors suggest a generalized criterion of acetonitrile polarity. The article also discusses means of polarity assessment and eluotropic strength of mobile phases on the basis of acetonitrile mixtures with water with application of generalized criteria. Special attention is paid to the options of acetonitrile application in various extraction methods of chromatographic sample preparation. The article also reveals advantages and problems of acetonitrile application as a mobile phase modifying agent in the HPLC reversed-phase and as a hydrophilic extra-agent in liquid-liquid extraction.

Keywords: Acetonitrile; Reversed-phase high-performance liquid chromatography; Liquid-liquid extraction.

1. Introduction

This year scientific society celebrates 115th anniversary since Russian scientist Mikhail S. Tsvet invented liquid chromatography. For 50 years this method has been developing as high-performance liquid chromatography (HPLC). Due to combination of physico-chemical properties acetonitrile has taken the leading position in chromatographic analysis. This article is devoted to specific properties of this solvent and its mixtures with water.

Acetonitrile (MeCN) is an aprotonic moderately polar organic solvent. Until 1980s it had limited application in chemical and pharmaceutical technologies. The whole range of unique MeCN properties has conditioned its wide application in analytical chemistry – in optical analysis methods, liquid chromatography and various methods of sample preparation.

MeCN is applied[1-7]:

(1) for dilution of analyte samples;

(2) in extraction and sorption methods of samples concentration as a hydrophilic extra agent;

(3) as an eluent compound in reversed-phase HPLC and capillary electrophoresis.

In the study devoted to solvents Sadek P[4]. gives the following list of typical acetonitrile properties that determine active MeCN application. So, the compound

(1) obtains moderate polarity;

(2) is a moderate solvent revealing excellent solubilizing power;

(3) its application as an eluent, as a rule, is characterized by clear chromatographic solute peaks;

(4) is well mixed not only with water, but with a large number of organic solvents;

(5) unlike alcohol and ether mixtures with water MeCN mixtures with water have low viscosity;

(6) is a weak acceptor of hydrogen binding;

(7) has a low transparency value threshold in UV spectrum (especially clear acetonitrile).

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We should also add to this list MeCN mixture capacity to form two-phase liquid heterogeneous systems when cooling below – 1.32 ℃[7-12]; this fact may be used in liquid-liquid extraction (LLE).

Some negative MeCN characteristics which have been marked in several studies are as follows:

(1) phosphate buffers, especially multi-charge pH-buffers, are highly restrictedly soluble in MeCN;

(2) MeCN is not chemically inert, is slowly hydrolyzed, reacts with strong acids, for example sulfuric acid;

(3) is toxic;

(4) MeCN in high-purity form is one of the most expensive solvents applied in large amounts in sample preparation and chromatographic analysis.

In spite of the abovementioned negative parameters of acetonitrile, its positive characteristics substantially outweigh. Attempts to find proper substitution to this solvent in chromatographic analysis with reverse-phase HPLC fail.

2. Chromatographic properties of acetonitrile and its mixtures with water

Table 1 gives technical requirements towards chromatographic solvents and an expert assessment of acetonitrile conformity with these requirements on a scale from one to five[13]. To support these assessments Table 2-Table 4 describe certain quantitative characteristics of typical solvents for reversed-phase HPLC. Solvents compared in Table 2-Table 4 with MeCN appeared to be the most applicable on their physico-chemical and technical characteristics for reversed-phase HPLC realization[1-4,13].

Parameter	Requirements	Factors	Score
Boiling point	Sufficiently high	To prevent formation of vapour bubbles interfering work of pump valves and detectors; to minimize changes of eluent composition due to low-boiling component evaporation	5
Density	Sufficiently high	Denser eluents demand low pressure height over the pump entry, turbulent flow type is less typical for them	5
Viscosity	Low	In low-viscosity eluents diffusion and mass-exchange run more efficiently, pump working pressure is less	5
Absorption in UV spectrum area	Low	Visible absorption of an eluent worsens spectrophotometric detector sensitivity	5
Refraction index	Low	High refraction index worsens refractor-metric detector sensitivity	5
Chemical stability	High	In order not to use stabilizers, to avoid retention time drift	4
Chemical inertness	High	To avoid interaction with analyte, to avoid sorbent and sorbate modification	3
Purity	Lack of volatile and non-volatile admixtures	To prevent hindrances in the work of detectors and columns	5
Cost	Low	To decrease expenses for analysis	3
Miscibility with water (buffer)	High	To avoid eluent break, buffer precipitation and hindrances when detecting	5 (4)
Eluotropic strength	Sufficiently high	To reduce time for analysis	4
Solubilizing capacity	High	To prevent analyte precipitation in chromatographic tract	4
Toxicity	Low	To provide work safety	3
Flashpoint	Low	To provide safety when storing and using	3

Table 1. Expert assessment of MeCN, brand “for HPLC”, on basic physico-chemical and technical parameters

Solvents	Boiling point, °C	ρ, g / cm^3 (298 K)	n_D^{25}	η·10^3, Pa×s (298 K)	UV cutoff, nm
MeCN	81.6	0.7767	1.3416	0.341	195
Water	100	0.9971	1.3325	0.894	190
1,4-dioxane	101.3	1.0268	1.4203	1.194	225
Methanol	64.5	0.7868	1.3265	0.5445	205
IPS	82.4	0.7810	1.3752	2.073	205
THF	66.0	0.8842	1.4036	0.460	212
Ethanol	78.3	0.7850	1.3594	1.078	205

Table 2. Physical properties of solvents for reversed-phase HPLC. According to[1]

Solvents	**Relative cost**	**TLV, mg / m^3**	**Flash point, °C**	**Autoignition temperature, °C**	**Concentration limits of ignition, volumetric %**	
					lower	**upper**
MeCN	1.84	10	6	523	4	16
1,4-dioxane	2.16	10	5	300	2.0	22.5
Methanol	1.00	5	11	464	7.3	36
IPS	1.14	980	11.7	400	2.23	12.7
THF	2.37	100	-20	250	1.8	11.8
Ethanol	1.40	1000	18	392	4.3	19

Table 3. Technical properties of solvents for reversed-phase HPLC. According to [1]

Растворители	ε_r	**μ,D**	**δ_T, MPa$^{1/2}$**	**$E_T(30)$, kJ / mol**	**P'**	$\varepsilon^\circ(SiO_2)$	$\varepsilon^\circ(C_{18})$	**S**
MeCN	35.94	3.44	26.90	190.8	5.8	0.60	3.1	3.1
1,4-dioxane	78.54	1.83	52.20	264.0	10.2	-	-	0
Methanol	2.21	0.45	21.78	150.6	4.8	0.60	11.7	3.5
IPS	32.66	1.70	32.42	231.8	5.1	0.70	1.0	3.0
THF	19.13	1.66	25.30	202.5	3.9	0.55	8.3	4.2
Ethanol	7.39	1.75	20.21	156.5	4.0	0.44	3.7	4.4
MeCN	24.55	1.68	27.92	217.1	4.3	0.60	3.1	3.6

Table 4. Parameters of polarity and eluotropic srength of solvents for reversed-phase HPLC. According to[1-4]

These solvents demonstrate a range of excellent parameters, but, in general, on the amount of technical characteristics, are inferior to acetonitrile. For this very reason MeCN and its mixtures with water and buffer solutions are the most demandable as solvents for samples, hydrophilic extra agents and mobile phase components in extraction-chromatographic methods of wide range of analytical organic substances control[1-5,13].

Nature has not invented an ideal solvent, that is why, mixtures of solvents find application in analytical HPLC and LLE. Pure MeCN is not practically applied as an eluent. In reversed-phase HPLC MeCN is known to be applied as a mobile phase modificator, and water – as a dilutant. MeCN volume fraction in eluents varies in a wide range, from several volume fractions to $\varphi=0.9$ and more. Let us consider modification of basic chromatographic parameters of MeCN-water mixtures from the volume fraction of the last (φ_2) in the whole range of its concentration modifications from 0 to 1.

2.1 Density

Studies[15,16]demonstrate that density isotherms $\rho_{12}=f(\varphi_2)$ for binary solvent mixtures are adequately described by the binomial equation:

$$\rho_{12}=\rho_1+\beta\rho_1\varphi_2+(\rho_2-\beta\rho_1-\rho_1)\varphi_2^2 \quad (1)$$

where β – an empiric component, ρ_1 and ρ_2 - indicators of dilutant and modificator density respectively; φ_2 – volume fraction of a modificator.

An equation (1) restricts dependence in φ_2=0 or 1 by p values of initial mixed solvents. It has been found that in

298 K (25 ℃) β=-0.22 for "acetonitrile-water" mixture. As 3D diagram demonstrate (**Figure 1**) slightly expressed non-linear characteristic (non-additivity) from the composition for the dependence $\rho_{12}=f(\varphi_2)$. Temperature monotonously decreases with the density increase. In the interval 277-298 K these modifications may be described using linear equation (**Table 5**):

$\rho_i=a-bT_i$

(2)

With the temperature increase water-acetonitrile density decreases slightly stronger than pure water density. This effect is conditioned by the fact that the balance between homo- and hetero-intermolecular associates is shifted towards "looser" homo- and hetero-intermolecular structures. It should be noted that this effect is stronger expressed for mixtures, in which water forms hydrogenic bindings with modificators (alcohol, THF)[15,16].

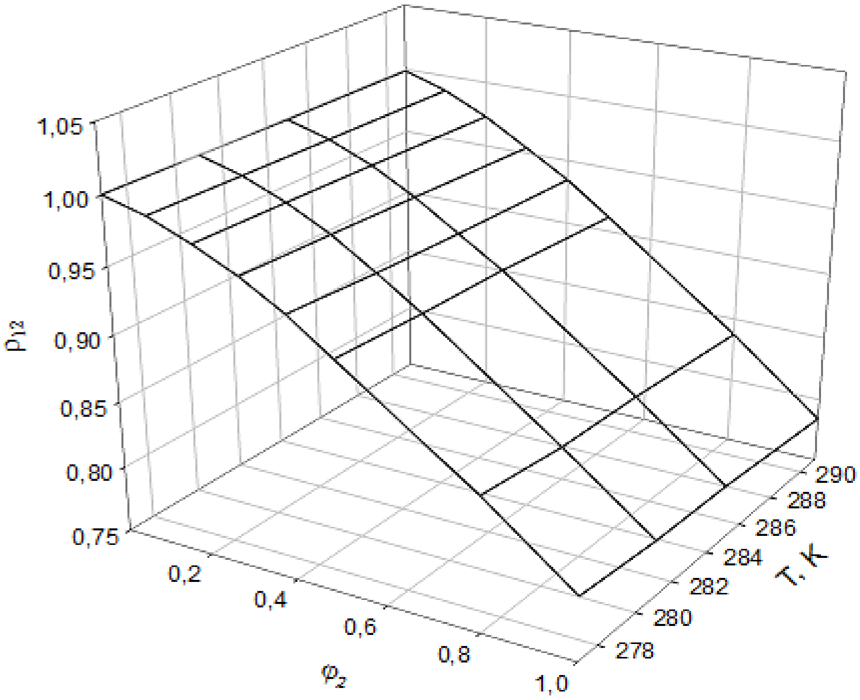

Figure 1. Dependence of the density of mixtures of MeCN-water on composition and temperature[16]

φ_2	a	$b\times10^3$
0	1.03	0.1
0.1	1.04	0.2
0.2	1.04	0.3
0.3	1.05	0.4
0.4	1.05	0.5
0.5	1.05	0.6
0.8	1.07	0.8
1	1.07	1.0

Table 5. Coefficients of equation (2) for different compositions of MeCN - water mixtures[16]

2.2 Viscosity

Dynamic coefficient of viscosity η appears to be one of the key physico-chemical values characterizing liquid properties to prevent mixing of one of its layer against another. Backpressure occurring in the chromatographic system and, firstly, in the column, depends on the solvent viscosity, tortuosity of the chromatographic tract way (a column, capillary tubes, detector cells) and on flow rate. In similar eluting rate lower-viscosity eluents demands less entry pressure. However, viscosity influences not only mechanical work of a chromatographer, but the efficiency of the chromatographic system. The lower is the eluent viscosity, the more effective is the mass exchange, the higher is the diffusion rate, and, finally, the less is the break of chromatographic peaks[3,4].

Viscosity of mixed solvents is known to depend on the composition non-linearly. This results in problems in gradient variant of HPLC. Reversed-phase eluent viscosity is often higher than pure components viscosity. Thus, viscosity of “methanol-water” mixture in the region of equi-volumetric compositions is approximately in 1.8 times more than water viscosity; viscosity of 1-propanol-water mixture taken in the same proportions has viscosity in 2.5 times more than viscosity of pure water[3,4]. As a result of such viscosity growth backpressure in the system alcohol-water increases in 2-3 times when creating 0-100% gradient. Differences in the character of $\eta_{12}=f(\varphi_2)$ dependences in water-acetonitrile mixtures and mixtures of water with other modificators are obvious on **Figure 2.** Thus, acetonitrile has unique advantages before other solvents, maximal viscosity of its mixture with water is observed in the range $\varphi_2=0.1\text{-}0.2$ and is only by 3% more than pure water viscosity.

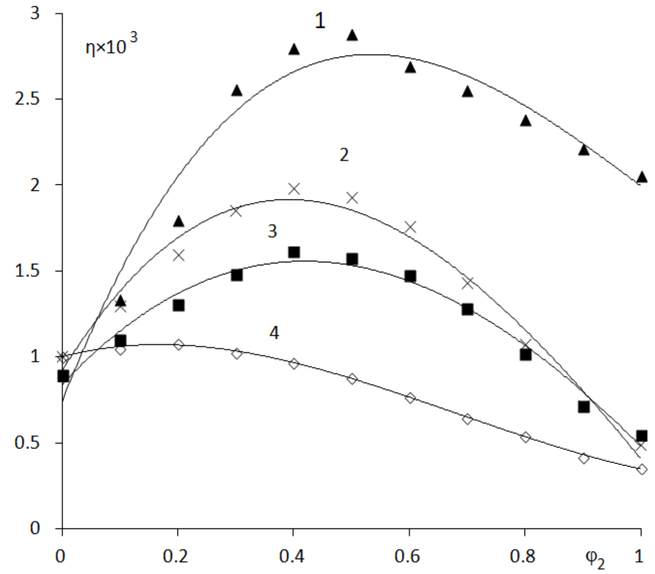

Figure 2. Experimental values of η (Pa × s) for water-solvent systems: 1) IPS, 25 ℃; 2) THF, 20 ℃; 3) methanol, 25 ℃; 4) MeCN, 20 ℃. According to the data of [17].

A rational equation (3) has been suggested [17] for approximation of dependences; it describes experimental data with high degree of approximation.

$\eta_{12}=\{\eta_1+[\eta_2(1+A+B)-\eta_1]\varphi_2\}/(1+A\varphi_2+B\varphi_2^2)$,

(3),

where η_{12} is a coefficient of dynamic viscosity of a mixture, η_1 and η_2 are diluent and modificator viscosities, A and B – empirical coefficiencies characterizing interactions of mixture components. For acetonitrile A=-1.05, B=1.45 (20 °C).

Viscosity of individual and binar solvents decreases by 4-20% with each 10 degrees (**Figure 3**), i.e. a negative effect on the work of the chromatographic system due to increased viscosity may be neutralized by thermostating in comparatively low temperatures (40-75 ℃).

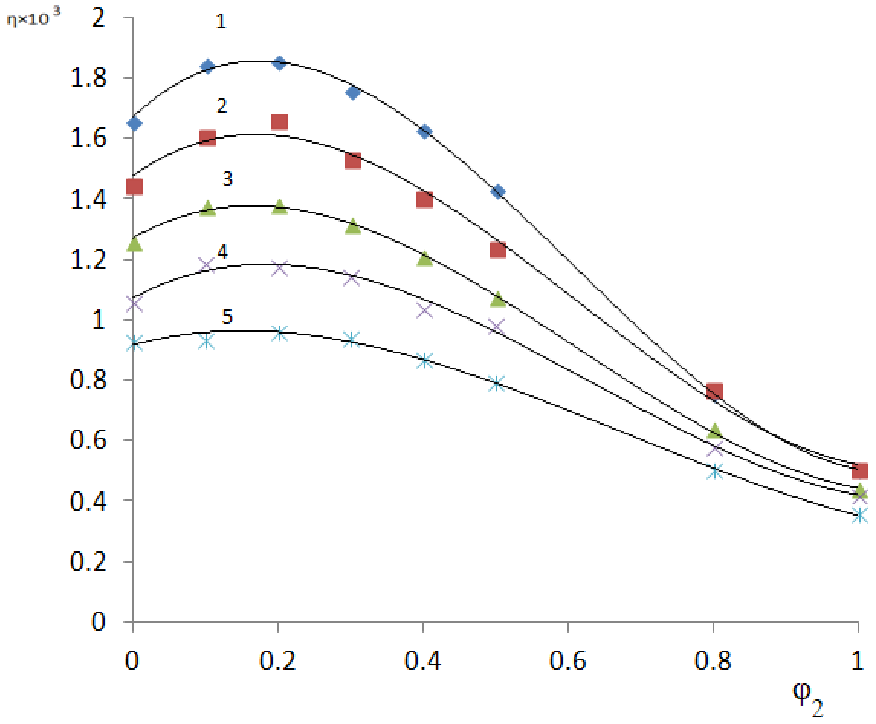

Figure 3. Dynamic viscosity (η × 103, Pa × s) of the MeCN–water system at different temperatures, 1) 3; 2) 8; 3) 13; 4) 18; 5) 23 ^{o}C. According to the data of[18]

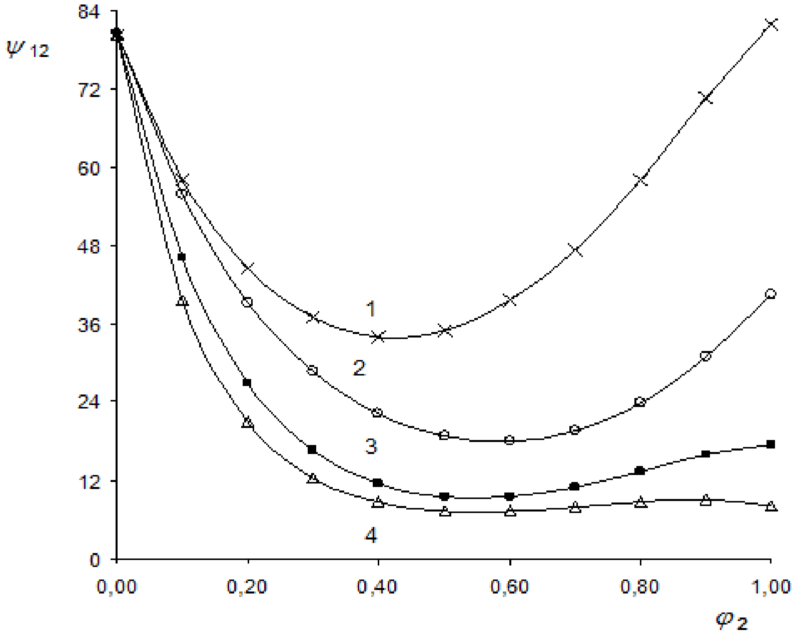

Figure 4. Isotherms of permeability ψ_{12} (m / s) reversed-phase binary systems water modifier from the volume fraction of the modifier φ_2: 1) MeCN; 2) methanol; 3) ethanol; 4) IPS. According to the data of[19]

2.3 Permeability coefficient

An interesting observation has been made in the study[19], which investigated permeability coefficient ψ equal to the ratio of the surface tension coefficient to the dynamic viscosity coefficient of binary solutions ($\psi=\sigma/\eta$, dimension m/sec). ψ_{12} for binary systems water-an organic solvent has been found to be properly approximated by a modified polinominal of the 3rd degree:

$$\psi_{12}= \psi_1+A\psi_1\varphi_2+(B\psi_2-A\psi_1-\psi_1)\varphi_2^2+(\psi_2-B\psi_2)\varphi_2^3 ,$$

(4)

where A = -3.09, B = 2.58 are the empirical coefficients for MeCN. **Figure 4** represents the obtained isotherms $\psi_{12}=f(\varphi_2)$.

A coefficient ψ characterizes a constant the liquid flow via a capillary χ and allows prognosticating distances covered by the front of the concrete solvent for a certain period of time in thin layer liquid chromatography (TLC) in the concrete porous layer:

$\chi=2k_0d_p\psi$,

(5)

where k_0 – permeability parameter depending on the structure of external pores, d_p – diameter of the sorbent particles.

Coefficient value ψ of the pure MeCN is sufficiently higher than in alcohols, i.e. in all other equal conditions a MeCN flow constant is higher than in alcohols and comparable with permeability coefficient value of pure water; its minimum is observed for equi-volumetric compositions (**Figure 4**). While for the MeCN-water mixture permeability increases again passing through minimum in $\varphi_2>0.40$, for water-alcohol mixtures with high level of alcohol permeability remains in 3-9 times lower than for pure water. Water-alcohol compounds with $\varphi_2=0.40$-80 in reversed-phase TLC conditions have too low permeability; water-acetonitrile mobile phases on the ψ parameter appear to be more preferable for this variant of chromatography.

2.4 Refraction index

Refractor-metric detectors are widely used in HPLC. They appear to be non-specific (universal) detectors[1-4]. Refractor-metric detector sensitivity is determined by the difference of refraction indexes of eluent and solvent Δn. Cuspidal functions (6) similar to those obtained for density[20] may be applied for many binary systems[20].

$n_{12}=n_1+\alpha n_1\varphi_2+(n_2 \quad -\alpha n_1-n_1)\varphi_2^2$,

(6)

where α is the empirical coefficient.

Figure 5 gives curves $n_{12}=f(\varphi_2)$ drawn for certain typical binary water-organic mixtures. An equation (3) adequately approximates isotherms $n_{12}=f(\varphi_2)$, a correlation coefficient in most cases is R>0.99. It should be fairly noted that in some systems isotherms $n_{12}=f(\varphi_2)$ are better described by the 3rd degree polynomial. That is why, the third degree polynomial (7) having the same boundary conditions as (3), namely, in $\varphi_2 = 0$ or 1 n values are equal n_1 and n_2 respectively (R=0.998) has been suggested for the isotherm $n_{12}=f(\varphi_2)$ of the most important reversed system methanol-water together with the binary polynomial (6) having relatively low coefficient of the pair correlation $r\approx0.92$.

$n_{12}=n_1+\alpha n_1\varphi_2+(bn_2 \quad -\alpha n_1-n_1)\varphi_2^2+(n_2 \quad -bn_2)\varphi_2^3$,

(7)

It is obvious that binary systems with MeCN and methanol have advantages when applying refractor metric detectors. The fact should be taken into consideration that with the temperature rise the refractor index decreases (**Figure 6**).

Figure 5. Refractive index isotherms for binary water-modifier systems, 20 °C: 1) THF; 2) IPS; 3) MeCN; 4) methanol. According to the data of[20]

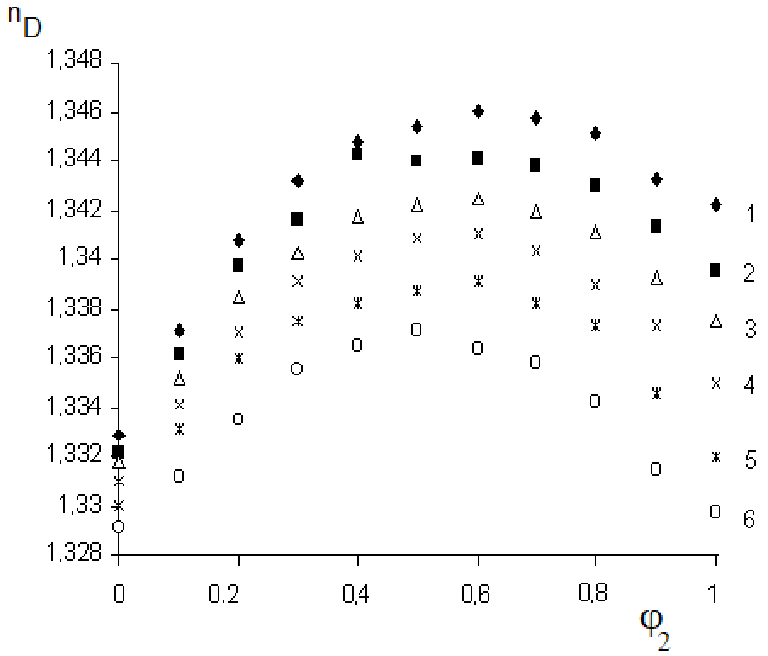

Figure 6. Isotherms of the refractive index for the binary system MeCN-water at different temperatures: 1) 25; 2) 30; 3) 35; 4) 40; 5) 45; 6) 50 °C. According to the data of[21]

2.5 Optical density

MeCN – water mixtures have high transparency in the wide band of the wave lengths (**Figure 7**)[1-4]; this allows to use an ultraviolet detector (UVD) for spectrophotometric analysis by the HPLC-UVD method and register analytical signals of various organic compounds having absorption maximums in a short-wave band of UV-spectrum (205-220 nm). The major condition is said to be as follows: MeCN should be qualified as “pure for spectrometry”, “pure for HPLC”, i.e. it should not have typical acrylonitrile and other non-saturated admixtures with conjugated multiple bonds. The study[22] demonstrates that equation (8) may be applied for binary water-organic, if consider water to be transparent in analytical wavelengths >195 nm:

$$A_{12}=(A_2-\beta)\varphi_2+\beta_{12}\varphi_2^2 \quad , \tag{8}$$

where A_{12} – is an optical density of binary solution, A_1 – is an optical density of an organic modificator, β - an empirical coefficient characterizing the binary system.

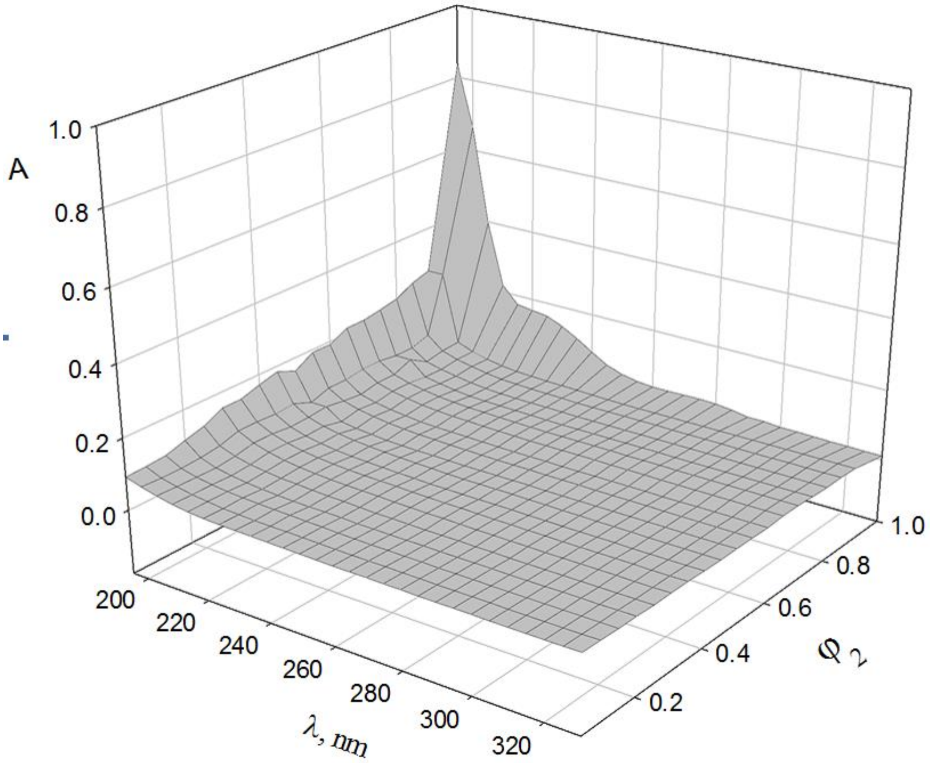

Figure 7. Absorption of MeCN-water mixtures as a function of the wavelength and the volume fraction of MeCN. The spectrum was recorded with Shimadzu UV-1800[23].

In the study[23]researchers have investigated deviation of the optical density of acetonitrile mixtures with water A_{12} from additivity. It has been shown that $A_{12}=f(\varphi_2)$ dependence is principally different in bands 195-235 nm and in λ>240 nm. Sharp nonlinearity is observed in large concentrations of a modificator in the first band, nonlinearity is comparable in the order of value with fluctuations of the optic density. It is not excluded that stochastic violations of the system’s homogeneity may be observed at nanolevel in mixtures with maximally high content of MeCN. This must result in the fact that together with absorption of radiation there exists its scattering. Existence of scattering needs extra radiation. Acetonitrile propensity to form heterogeneous phases with water is especially evidently manifested in low temperatures, which is discussed hereafter.

Chemical nature of MeCN is essentially different from chemical nature of alcohols and water. It has sufficiently high polarity comparable with methanol polarity; but, as an aprotonic solvent, MeCN does not form hydrogen binding with water, being only a slight acceptor of hydrogen binding. Structures of MeCN binary mixtures with water are still of great research interest, but remain insoluble[11]. Structures of acetonitrile solvates and methanol in mixtures with water are essentially different. Particularly, it has been determined that the mixture acetonitrile–water should be considered as, at least, the three-component system, in which two various MeCN forms may exist in solution[24]. Simultaneous existence of strong hydrogenic binding between water molecules and low-polar self-associated MeCN forms has specific impact on physical and chemical characteristics of mixtures MeCN-water.

2.6 Boiling point

To know boiling point of solvents is significant not only for the chromatograph mode control in the conditions of column thermal regulation, but for the control of extract concentration in extra agent evaporation. In the study[25] it is suggested to apply a rational function (9) passing via three most important experimental points T_1, T_2 and T_A to prognosticate boiling point of the binary system MeCN-water of selectable composition,

$$T_{12} = \frac{T_1 + [T_2(1 + A + B) - T_1]\varphi_2}{1 + A\varphi_2 + B\varphi_2^2} \tag{9}$$

In Figue 8 shows the isobar of the boiling of a mixture of MeCN – water.

Figure 8. Dependence of the boiling point of the MeCN - water mixture on the volume fraction of MeCN

2.7 Freezing point

Data on the freezing point of acetonitrile and its water solutions are different in various sources[11]. Variations are from -46 to -41 ℃. Sadly to say, but practically all results of physico-chemical parameter determination of individual and mixed solvents have a certain error, since presence of undetected or unknown admixtures is not considered in this process. Any organic solvent has signs of admixtures; their composition depends on the method of production. All organic solvents, if not specially subjected to dehydration, contain traces of moisture (φ=0.0001-0.0025). Researchers in their study[11] discuss complicated and non-linear profile of the curves of MeCN – water mixtures' freezing point. It is noted that starting with φ=0.90 of MeCN in water freezing point rapidly decreases. The minimal freezing point (-44.5 °C) is typical for the mixture containing MeCN in the amount φ=0.98. A pure acetonitrile freezing point is reported to be -43.4 ℃. Thus, MeCN freezing point may significantly differ depending on the presence of water traces and other minor admixtures. Non-typical character of freezing of MeCN-water mixtures may be due to polarity changes of a binary solvent in the MeCN concentration range φ=0.90-1. This fact is considered to be an important conclusion for its application in the reversed-phase HPLC. In the study[11] authors noted chromatographic behavior of steroids in this narrow band of compositions of MeCN-water binary mixtures typical for conditions of normal-phase liquid chromatography, where mobile phase is less polar than immobile phase. Thus, this observation directly supports the fact that polarity of the mobile phase consisting mainly of MeCN significantly changes when its concentration reaches 100%. Therefore, we can conclude that pure MeCN with low water content may function as a relatively non-polar solvent, but its polarity easily changes by adding a little amount of water.

2.8 Polarity and eluotropic strength

Polarity of solvents is understood as their ability to solvate charged or polar particles existing in it. This ability depends on all specific and non-specific interactions between molecules of a solvent and dissolved substance. It is impossible to fully consider combination of all these interactions by any physico-chemical parameter. Consequently, polarity of solvents is quantitatively more often determined by various empiric parameters. Table 4 gives data on dielectric permeability ε_r, dipolar moment μ, Hildebrand solubility parameterδ_T, Dimroth – Reichardt solvatochromic parameter $E_T(30)$, Snyder polarity P' for MeCN and other solvents for reversed-phase HPLC. These data appear to be criteria of solvents' polarity.

Drawbacks and specificity of these polarity criteria have been discussed in details in[1-3,26,27]. The criteria mentioned establish more or less similar orders of solvent polarity changes, i.e. approximate correlations are traced between various polarity scales. In addition to that, non-critical application of a concrete criterion may lead to errors in assessment of chromatographic characteristics of solvents in separate cases, since no one of them does fully consider all complex of interactions and events accompanying chromatographic process. In this regard, some authors in their work[27] suggest to apply a generalized (reductive) polarity criterion $P_{N.}$ It is obtained by generalization of P', δ_T, ε_r and $E_T(30)$ solvent parameters with all 4 generalized parameters known. P_N values are normalized in such a way that a maximal value of a polarity criterion for water is equal 100, and a minimal P_N value for a decane is equal 0. The term "reductive" supports the major meaning of a criterion – reduction of a number of variables applied in the polarity assessment. Weight coefficients of partial polarities are considered to be equal. As **Table 6** shows, acetonitrile polarity $P_N=44.66$ is actually close to a mean value.

Solvent	**P_N**	**R_L**	**Solvent**	**P_N**	**R_L**
Decane	0.00	100	Octanol-1	28.85	71.15
Isooctane	0.32	99.68	Butanone	29.03	70.97
Pentane	0.45	99.55	Pyridine	29.04	70.96
Cyclohexane	0.71	99.29	tert-Butanol	29.12	70.88
Hexane	0.85	99.15	Pentanol-1	31.25	68.75
Heptane	1.44	98.56	Benzonitrile	31.72	68.28
Triethylamine	6.24	93.76	Acetone	32.18	67.82
Carbon tetrachloride	7.29	92.71	Nitrobenzene	33.00	67.00
Carbon disulphide	8.25	91.75	Butanol-1	36.29	63.71
Diisopropyl ether	9.02	90.98	IPS	36.72	63.28
Diethyl ether	11.73	88.27	Acetic acid	38.45	61.55
Toluene	12.07	87.93	Propanol-1	39.09	60.91
Benzene	13.44	86.56	DMF	42.88	57.12
Chlorobenzene	16.65	83.35	Ethanol	43.97	56.03
Bromethane	16.72	83.28	Acetonitrile	44.66	55.34
THF	21.00	79	Nitromethane	46.72	53.28
1,4-dioxane	21.71	78.29	DMSO	48.41	51.59
Ethyl acetate	21.84	78.16	Methanol	54.34	45.66
Chloroform	22.00	78	Ethylene	62.77	37.23
Methylene chloride	23.04	76.96	Formamide	83.83	16.17
Dichloroethane	24.88	75.12	Water	100.00	0

Table 6. Generalized criteria for the polarity P_N and lipophilicity R_L of solvents. According to the data of[27,28]

Eluotropic strength of solvents is firstly determined by their polarity. In the conditions of normal-phase HPLC eluotropic strength increases with the increase of mobile phase polarity; and in the conditions of reversed-phase HPLC, on the contrary, the higher polarity of the mobile phase is, the less its eluting ability is.

Retention factor in the conditions of reversed-phase HPLC in general case non-linearly depends on the eluent composition; this dependence may be expressed by a binomial equation:

$$lnk=A\varphi_2^2+B\varphi_2+C, \quad (10)$$

where A, B and C – empirical coefficients, A has a positive sign, B – a bigger value and a negative sign, C – a natural logarithm k in pure water. Validity of the equation (10) is supported by multiple experimental data[1]. Essential deviations from this dependence may be observed in low content of a modificator ($\varphi_2<0.1$). Coefficient C does not obligatory conform with the retention factor in the pure water. Researchers in the study[1]have also investigated other methods of mathematical description of retention factor dependences on φ_2 in reversed-phase HPLC conditions. Application of non-linear dependences simplifies description of composition influence on retention in the wide band φ_2. In the narrow intervals φ_2 the retention factor k=1-10 and is adequately described by the linear equation (11),

$$\lg k=\lg k_1-S\varphi_2, \quad (11)$$

where k_1 – is the retention factor when applying water as a mobile phase; φ_2 – is a volumetric portion of an organic modificator. Coefficient C is one of the eluotropic strength measures in reversed-phase HPLC (Table 4). The drawback of the criterion S is the fact that it is not a constant parameter for this or that immobile phase and two components of chosen eluents and depends on the sorbate retention factor; moreover, the equation (11) is approximately observed only with 30% changes of the eluent composition[1]. Coefficient k_1, the same as C, in the equation (10) does not give an exact evaluation of retention in water. The equation (10) is applicable for ternary systems only if pseudo-binary systems are used, i.e. ternary mixtures, in which proportion of volumetric parts of two modificators are constant.

Additive schemes for dielectric permeability ε_r, polarity P’, Hildebrandt solubility δ_T, solvatochromic parameter E_T^N[1-3,26], as well as for generalized polarity criteria being a combination of the previous four parameters[27,28], were used to calculate isoeluotropic compositions (i.e. compositions with equal eluotropic strength) in reversed-phase HPLC. Actually, dependence ε_r and P’ on the composition of acetonitrile and methanol in water mixtures is close to linear. If rejecting relatively rapid change of E_T^N for water mixtures with most of modificators in the range φ_2=0.95-1.0, and for MeCN also in the range φ_2=0-0.05, which are conditioned by the specificity of a solvatochromic effect, E_T^N parameter is also adequately described by the linear dependence on the volumetric ratio of water. If assumption on additivity is accepted for separate polarity criteria, it may be logically expanded on the generalized criterion P_N created on their basis. In a first approximation it may be considered that parameters of sorbate retention will be approximately equal in mixtures with equal polarity[27,28]. We believe that a criterion $R_L=100-P_N$ appears to be very attractive for determination of eluotropic strength of an eluent in the conditions reversed-phase HPLC. Criterion R_L was termed a reductive lipophilic criterion[28]. Alternatively to polarity criterion, it is characterized lipophilic properties of solvents. As Table 6 demonstrates, MeCN tends to the mean of values on both – P_N parameter and R_L parameter, i.e. has a good balance between polarity and lipophilicity.

Correlation between the coefficient S and criterion R_L in the known range of solvents has the following form: $S=0.056R_L$[28]. Thus, the eluotropic strength of the binary system water-modificator will be determined by a simple linear expression convenient in the chromatographic practice:

$$R_L=R_{L2}\varphi^2, \quad (12)$$

where R_{L2} – eluotropic strength of a modificator, and φ_2 – its volumetric part. Criterion R_L is more preferable for characteristic of the eluotropic strength in the reversed-phase HPLC than criterion P_N, since we deal with direct dependence, and water parameter disappears in calculations. Criterion R_L certainly does not take into consideration influence of the pH medium if apply buffer solutions instead of water. pH parameter has an impact on ionogenous substance retention parameters (weak acids and bases) and does not practically influence non-ionogenous analyte retention[1].

3. Extraction properties of acetonitrile and its mixtures with water

Characteristics given in Table 1 may be related not only towards chromatographic, but also towards extraction properties. Since extracted analytes redistributing in the process of extraction from solid or water matrix in the extra agent in the process of sample preparation, then are included into chromatographic analysis. A sample (extract) solvent might be maximally compliant with an eluent, properly mixed with it, its absorption must not interfere detection, does not essentially influence eluotropic strength and so on[3]. Ideally, extra agent composition must be congruent with eluent composition. In this regard, acetonitrile is of great interest as a component of the extraction system.

Currently acetonitrile is widely applied as a pure hydrophilic extra agent and as an extra agent modificator in various methods of extraction: solid-phase extraction (SPE); micro-SPE; low temperature SPE; liquid-liquid extraction (LLE) with desalination and desugaring; micro-LLE; disperse micro-LLE; liquid extraction under pressure; fluid extraction, liquid-liquid extraction with partition at low temperature (LLE-PLT), extraction freezing (QuEChERS), (quick, easy, cheap, effective, rugged, safe)[5-10,12,14,29-41].

3.1 Mixability and solubilizing ability

Mixability of solvents is their important characteristic in LLE methods. If they are not mixed, then one solvent may serve as an extra agent of an analyte from another solvent. Mixability with water is a very important characteristic in practice. Based on this, solvents may be divided into hydrophobic and hydrophilic. It is known empiric mixotropic range[2], a reduced sample from it is given in **Table 7**.

Solvents	**logP**	**ClogP**
Water	-	-1.38
Acetonitrile	0.17	-0.39
Methanol	-0.27	-0.87
Ethanol	0.07	-0.24
IPS	0.38	0.07
Acetone	0.20	-0.21
Propanol-1	0.55	0.29
1,4-dioxane	-0.31	-0.39
Tetrahydrofuran	0.40	0.53
tert-Butanol	0.60	0.47
2-Butanol	0.87	0.60
2-Butanone	0.86	0.32
Cyclohexanone	1.43	0.87
tert-Amyl alcohol	1.09	1.00
1-Butanol	0.97	0.82
Ethyl acetate	0.29	0.71
Diethyl ether	0.76	0.87
Butyl acetate	1.20	1.77
Diisopropyl ether	1.40	1.49
Methylene chloride	1.01	1.25
Chloroform	1.67	1.95
1,2-Dichloroethane	1.66	1.46
1,1,2-Trichloroethane	1.89	2.05
Benzene	2.03	2.14
Toluene	2.52	2.64
Carbon tetrachloride	2.86	2.88
Cyclopentane	2.82	2.80

Cyclohexane	2.50	3.35
Hexane	3.00	3.87
Heptane	3.42	4.40

Table 7. Sampling from the empirical mixotropic series of solvents[2] and hydrophobicity criteria logP and ClogP as a measure of miscibility

Solvents located in the mixotropic range higher than tert-butanol are mixed with water and between themselves in any proportions, they may be regarded as hydrophilic. Solvents located lower than tert-butanol are hydrophobic and, as a rule, properly mixed with non-polar solvents, such as benzene or hexane. Logarith values of the distribution coefficient between water and 1-octanol (logP or ClogP) may be a convenient quantitative measure for preliminary prognosis of solvents Mixability. The latter are taken from the reference database of CambridgeSoft ChemBioOffice Ultra 14.0 Suite program (ClogP is logP determined with the help of quantum-chemical calculations on BioBite® algorithm). Hydrophilic solvents with negative or close to zero criteria (logP ≤ 0.6) are fully or partially mixed with water and between themselves, and solvents with positive criteria of hydrophobicity are unrestrictedly or partially mixed between themselves and hexane. The more the difference in logP or ClogP criteria values is, the less evident is their compatibility. There are also exceptions, e.g. benzene is properly mixed with methanol. MeCN, in the same way as IPS and THF, may be included into the range of universal solvents, as they are mixed not only with water and hydrophilic solvents, but with a lot of other hydrophobic solvents.

3.2 Solid-phase extraction

Presence of sorption or ion-exchange processes result in an opportunity to perform concentration by SPE method. The main point of this method is concentration of analyzed components due to sorption on solid support (sorbent or ion-exchange resin). SPE has become widely spread, since it appears to be a convenient method for mass analysis. A great variety of holders with sorbents realizing various types of interactions between sorbent and sorbate has been developed for SPE[34].

However, SPE application is sufficiently cost-effective, as holders or columns with sorbents are expensive, and liquid expenditures is slightly different from liquid expenditures in LLE. Acetonitrile is one of the most actively applied solvents in SPE[34].

3.3 Liquid-liquid extraction

Physical mechanism of LLE is close to the mechanism of distributive liquid chromatography, in which sorbate distribution occurs on the border of liquid mobile phase and a liquid immobile phase layer near the sorbent surface. Variation of the mobile phase composition appears to be the most available method of liquid-chromatographic process control[1-4]. Similar approach is also applied towards LLE. Thus, LLE between non-mixable water solution and a hydrophobic organic solvent, which is the most appropriate for sample preparation when analyzing by normal-phase HPLC, is applied essentially rare than reversed-phase HPLC. Addition of more hydrophilic solvent to hydrophobic one always leads to a synergetic effect – efficiency of polyfunctional analyte extraction is higher in mixed extra agents[5]. In this case acetonitrile may be used as a minor additive into a non-polar extra agent. Additional factor increasing concentration efficiency by LLE method is application of salting-out extraction[5,29,32,33] or sugaring-out extraction[29]. In this case even hydrophilic acetonitrile is demixed with water solution; this fact allows to succefully apply it as an extra agent[5,29,40]. To improve an extraction ability of a hydrophilic extra agent on the basis of MeCN towards hydrophobic compounds, it may be mixed with a less polar component (simple or complex ether, ketones) having more chemical affinity towards hydrophobic compounds, preserving, however, its ability to demix when introducing salts or carbohydrates into water solution.

Not only MeCN, but also such solvents as acetone, dioxane, propyl alcohols, 4-butyrolacton, N-methylpyrrolidone, tert-butyl alcohol, diacetone alcohol may form independent water-organic phase containing from 2 to 50% of water in the presence of salting-out agents[5]. This characteristic is applied in hydrophilic LLE. $(NH)_2SO_4$ and Li_2SO_4 have approved themselves as proper salting-out agents, though other salts, e.g. K_2HPO_4, KH_2PO_4, $MgSO_4$, NaCl, are also successfully applied. It is possible to change proportion of an organic solvent and water in two separated phases varying concentration and a type of a salting-out agent. If concentration of a salting-out agent in water solution approaches to saturation, content of an organic solvent in the water phase tends to minimum. Combination of salt application and cooling up to 4 ℃ provides formation of the heterogeneous system.

Sugaring-out extraction is primarily applied in pharmaceutical analysis and pharmaceutical technologies[41]. Glucose, fructose and saccharose and other mono- and disaccharides have been used as salting-out agents for demixing of acetonitrile phase from water phase[40,41]. Mono- and disaccharides form a jour intermolecular structures with water due to formation of hydrogen binding, which provide demixing with the acetonitrile phase. An optimal carbohydrate concentration in the extraction system is 50 g/L[41].

Application of hydrophilic extra agents leads to synergetic effect due to increase of water amount in an extra agent when performing LLE of organic compounds having both– hydrophobic and hydrophilic fragments. **Figure 9** demonstrates a diagram of dependences of coefficient D on acetonitrile content in the organic phase and phenol hydrophobicity.

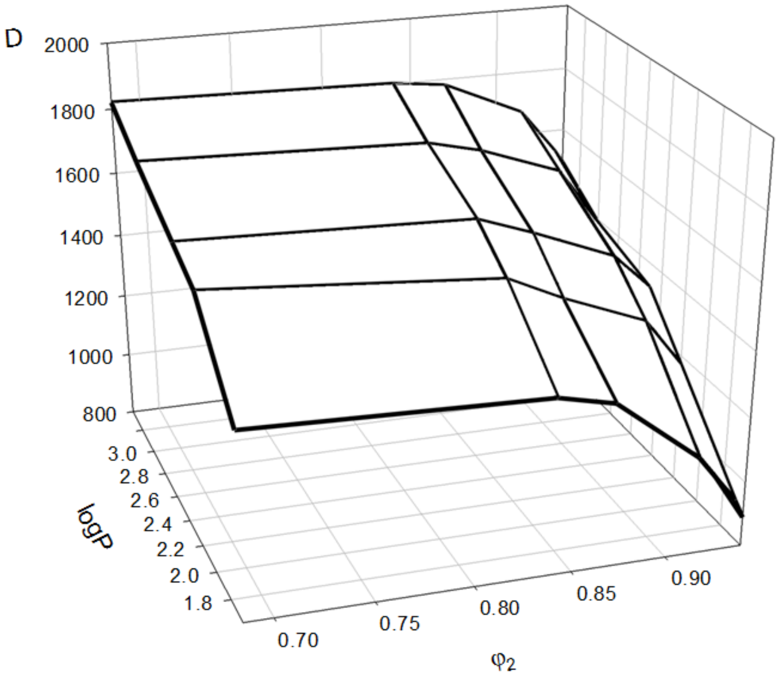

Figure 9. Dependence of the distribution coefficient of phenols on their hydrophobicity and the volume fraction of MeCN in the organic phase. Salting out $(NH_4)_2SO_4$[5]

Maximal phenol extraction was observed in 30% volume of water content in an extra agent; what is more, more hydrophobic phenols are extracted from the water-salt solutions in the water-organic phase more efficiently. On the contrary, when acetonitrile concentration approaches towards 100%, its chemical affinity towards hydroxyl-containing phenol compounds decreases and coefficient D reduces.

Azeotropic mixture of acetonitrile with water (84:16), which is formed when adding 17.5% $(NH_4)_2SO_4$ in a water sample, appears to be very attractive for hydrophilic LLE. This mixture is interesting from a practical standpoint by the fact that it is easily regenerated for multiple application. Currently, extraction of pure MeCN from azeotropic mixture with water is economically non-profitable. Application of certain ion liquids is considered to be the perspective method of the MeCN azeotropic system break[6].

Mutual solubility of acetonitrile and water strongly depends on the temperature. Data given in the studies[5,9-12,29] demonstrate that mixtures MeCN-water are demixed into two liquid phases when keeping for 30 minutes to several hours at the temperature below normal (room temperature). Phase division occur at the temperature below -1.32 ℃ and may be observed within the wide band of acetonitrile concentrations (φ_2=0.31-0.89)[5]. A range of MeCN content in mixtures with water having ability to demix is at the temperature approximately -10°C (φ_2 from 0.38 to 0.87); however, in this very case it is also possible to match solvent sample composition to be maximally close to the mobile phase content. At the temperatures ≤ -10 °C demixing into two phases occur sufficiently rapidly (≤ 30 min), this is applied for routine analysis. In these heterogeneous systems MeCN is irregularly distributed between two phases. Top phase is enriched by acetonitrile; bottom phase is enriched by water. This property of acetonitrile is used for all sample preparation including deproteinization of a raw sample, protein discharge from an organic solvent, for selective LLE of phenols, triclosan, bisphenol A out of complex mixtures, e.g. dairy products. This phenomenon may be also applied as cryomethod of removal of acetonitrile excess from biological samples and chromatographic mobile phases[5,9-14,29-31].

Formation of the two-phase heterogeneous system liquid-liquid for acetonitrile mixtures with water at low temperatures is, undoubtedly, of great practical interest in the context of application of this phenomenon for sample preparation in the chemical analysis by the reversed-phase HPLC method, since acetonitrile is a basic solvent for mobile phases in this variant of HPLC. It is of greater importance for analysis of labile BAS, metabolites discharged from biological fluids. An opportunity of acetonitrile application for realization of low temperature LLE of phenols from water samples is shown in the studies[5,8-10].

Acetonitrile has an adequate solubilizing ability for a great variety of organic analytes; however, it comparatively poorly dissolves compounds having high hydrophobicity when cooling, e.g. certain antioxidants and stabilizing phenol-type additives. That is why, to improve solubility of such analytes in the acetonitrile phase in the low temperature LLE, it is possible to apply ethylacetate and IPS as additives to acetonitrile[9]. Thus, MeCN – IPS – ethylacetate mixture (80:5:15), in which formation of the two-phase system liquid-liquid occurs for a short-time curing period (10 min) at the temperature -10 ℃, is reported to be a perspective phenol extra agent by cool LLE method. In addition to increased solubilizing ability of an acetonitrile extra agent these additives reduce its toxicity and cost.

3.4 QuEChERS method

In 2003 S.J. Lehotay, M. Annastassiades[42] suggested QuEChERS method to determine micro-quantity of pesticides in vegetables and fruits. The key point of this method is sample extraction by water acetonitrile or ethylacetate in the presence of the salting-out complex, purification by the method of disperse SPE and chromatographic analysis. Recently this method has been used to simultaneously determine not only residual pesticide content, but veterinary products, natural toxins in the food raw material with minimal costs due to application of various QuEChERS modifications at the stage of sample preparation[39,43]. In this method MeCN is used in the presence of buffering salts (sodium citrate) for component extraction. Bulk sorbents Bondesil-PSA, C18, graphitized carbon black, ion-exchanged resins and their combinations are applied to purify extracts from lipids, fats and proteins. Modern achievements in the sphere of QuEChERS development for complex matrix sample preparation include additional extracts purification by the method of disperse micro-LLE; its key point is rapid acetonitrile extract QuEChERS injection into water with an added organic solvent that is not mixed with water[43]. This results in emulsion formation, which increases efficiency of components distribution due to increased surface of the phases contact. An extracted organic phase is applied for analysis after centrifugation. Simultaneous selective chromatographic determination of a wide variety of sample components, including pesticides, veterinary preparations, growth stimulators (compounds with estrogenic activity) and mycotoxins, is possible due to this additional stage simple in performance, demanding significantly less number of organic solvents in comparison with classic extraction methods[43].

3.5 Extraction freezing

Studies[35-38]have demonstrated that acetonitrile can be applied in the mode of extraction freezing. Organic substances (analytes sought or interfering admixtures) are forced into liquid acetonitrile phase in the formation of ice out of water at the temperature -19 ÷ -20 °C. This method of sample concentration and preparation is actively applied in HPLC in the liquid-liquid extraction with partition at low temperature (LLE-PLT) method. Several ml of MeCN are added into the regrounded sample, cooled up to -20 ℃, centrifugated; an organic layer is separated, filtrated and analyzed[36-38]. This method is significantly less cost-effective than SPE, since there is no necessity to apply expensive holders with sorbents.

4. Conclusion

Pure acetonitrile and its water (buffering) mixtures obtain unique chromatographic extraction properties and have important practical application. Improvement of methods of analyte sample preparation by liquid-liquid extraction application with salting-out, sugaring, low-temperature liquid-liquid extraction, extraction freezing, QuEChERS method appear to be a perspective for chromatographic and spectroscopic analytical methods.

Author Contributions

The first author Rudakov Oleg Borisovich develops the methods of HPLC analysis of polyfunctional organic compounds, he used data on the properties of binary solvents to optimize the technical characteristics of HPLC analysis.

The second author Rudakova Lyudmila Vasilievna contributed to the development of extraction methods for sample preparation for HPLC, developed a prototype of an information and analytical system for optimizing the composition of mixed solvents for HPLC and extraction. The description of this system can be the topic of the next article.

The third author Vladimir Fedorovich Selemenev is a major expert in the field of separation of ionic compounds by chromatographic and sorption methods.

Declaration of conflict of interest

No conflict of interest was reported by the authors.

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