UNIVERSITY OF PARDUBICE

FACULTY OF CHEMICAL TECHNOLOGY

DEPARTMENT OF ANALYTICAL CHEMISTRY

UTILIZATION OF COMPLEXATION EQUILIBRIA DURING ELECTROMIGRATION SEPARATIONS OF NATURAL COMPOUNDS

DOCTORAL THESIS

AUTHOR: ING. JANA VÁŇOVÁ

SUPERVISOR: DOC. ING. JAN FISCHER, CSC.

2017

The thesis is focused on the utilization of electromigration techniques in the separation of natural substances. Capillary electrophoresis is widely used electromigration technique with many benefits, e.g. low consumption of samples and background electrolytes, fast analyses and simple instrumentation. Capillary zone electrophoresis and micellar electrokinetic chromatography are the most common used modes of capillary electrophoresis.

This thesis is divided into four sections; the first part is focused on the determination of critical micelle concentration of surfactants by different methods based on capillary electrophoresis. In the next part, the distribution coefficients of antioxidants between micelles and water phase and between liposomes and water phase are determined. The results obtained by micellar electrokinetic chromatography and liposome electrokinetic chromatography are compared with predicted values of distribution coefficients octanol/water, and octanol/buffer respectively. Next part of the thesis is focused on the optimization and separation of natural antioxidants and oligosaccharides by capillary zone electrophoresis, micellar electrokinetic chromatography and capillary zone electrophoresis coupled with mass spectrometry. In the last part, the possibility of utilization of the connection of liquid chromatography and micellar electrokinetic chromatography in 2D separations are tested. The main aim of this part is to compensate the influence of changing concentration of acetonitrile in the mobile phase, which is transferred into the capillary electrophoresis, on the retention times in micellar electrokinetic chromatography.

1. Comparison of the methods for the determination of critical micelle concentration using capillary electrophoresis

Many separation processes utilize presence of the surfactant in the separation medium. The knowledge of the critical micelle concentration of the surfactants is crucial to characterise the mechanism of the separation process. From this point of view the first part of the thesis is focused on the determination of the critical micelle concentration of sodium alkyl sulphates. The methods based on the measurement of current, retention time, retention factor, and mobility were tested. The values of critical micelle concentration obtained by different methods were compared and the differences among them were statistically evaluated.

The method based on the measurement of electric current passing through the capillary filled with background electrolyte with surfactant was described by Tickle [1]. This method is applicable only for charged surfactants. Accuracy of the method is dependent on the differences between slopes of the straight line in the region below and above the critical micelle concentration (Fig. 1A). The next method based on the measurement of retention time of neutral analytes, which interact with micelles, was introduced by Nakamura et al. [2]. Neutral analyte is moving with the electroosmotic flow through the capillary filled with surfactant at the concentration, which is lower than critical micelle concentration. Distribution equilibrium between micelles and analytes is established in presence of the micelles in the background electrolyte and cause increasing retention time of the analytes (Fig. 1B). Retention factor of the neutral analyte zone is also dependent on the concentration of the surfactant in the solution (Fig. 1C) and can be used for the determination of the critical micelle concentration too [3]. For calculation of retention factor is necessary to determine the migration time of electroosmotic flow and the migration time of the micelles. The migration time of electroosmotic flow is usually determined using low-molecular weight neutral compound, which do not interact with micelles and do not have own electrophoretic mobility. The migration time of the micelles can be determined by hydrophobic compound, which is present solely in the micelles, or by using the iterative procedure [4]. The last used method is based on the measurement of electrophoretic mobility of the compounds, witch interact with micelles (Fig. 1D). This method was introduced by Jacquier and Desbene [5].

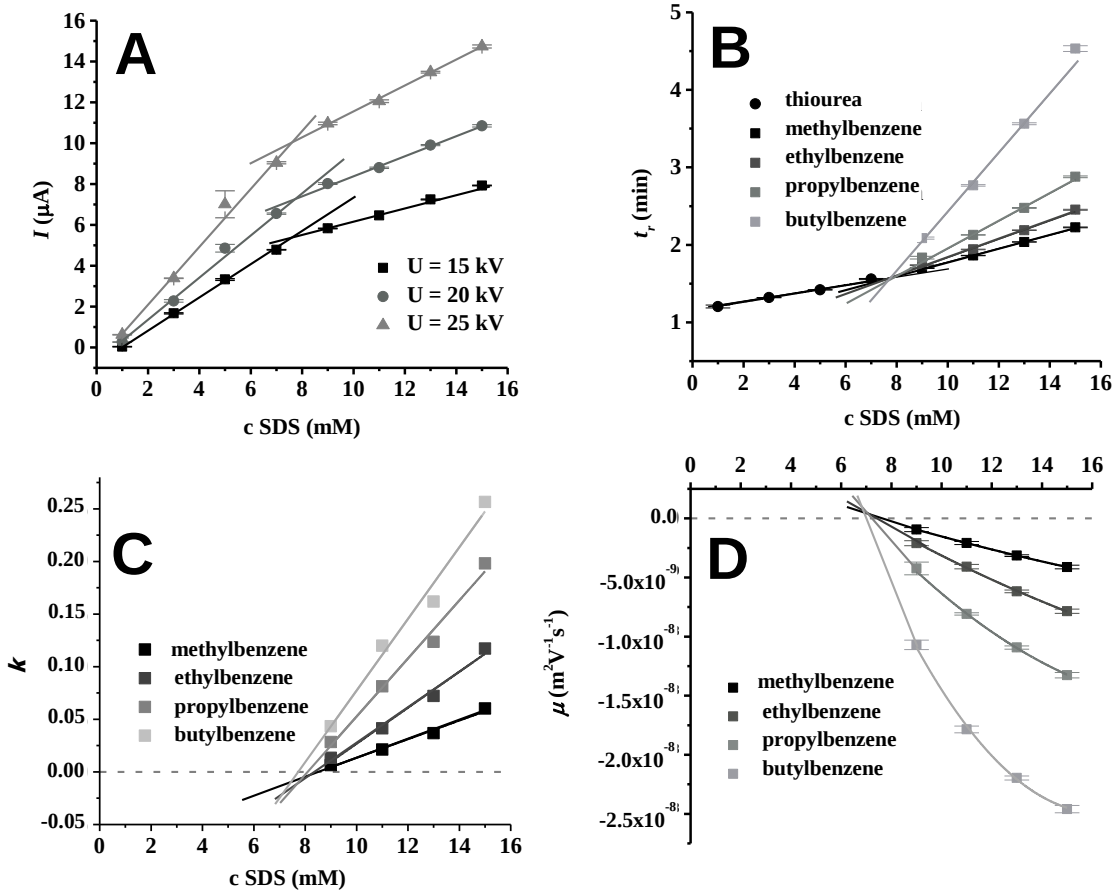

Figure Chyba! V dokumentu není žádný text v zadaném stylu.**:** The graphical representation of the used method for the determination of critical micelle concentration of sodium dodecyl sulphate in water.

A – method based on the measurement of current (applied voltage was 15; 20 and 25 kV).

B – method based on the measurement of retention times of members of homological series of alkylbenzenes (methylbenzene to butylbenzene)

C – method based on the measurement of the retention factors of the members of homological series of alkylbenzenes (methyl- to butylbenzene)

D – method based on the measurement of mobility of alkylbenzenes (methyl- to butylbenzene) Capillary: total length 37 cm, effective length 28 cm, inner diameter 50 μm. Applied voltage (for B, C, D) was 20 kV. Concentration of the sample of alkylbenzenes was 120 mg/l, sample was injected by 50 mbar for 5 s.

In Fig. 2, there are the obtained values of critical micelle concentration of sodium dodecyl sulphate in water determined by different methods. In general, the values of critical micelle concentration of sodium dodecyl sulphate obtained by the method based on the measurement of current and retention time are higher than values obtained by methods based on the measurement of retention factor and electrophoretic mobility. In the scope of the method based on the measurement of current, the values of critical micelle concentration of sodium dodecyl sulphate decreases with increasing applied voltage and standard deviation of the determination increases with increasing applied voltage.

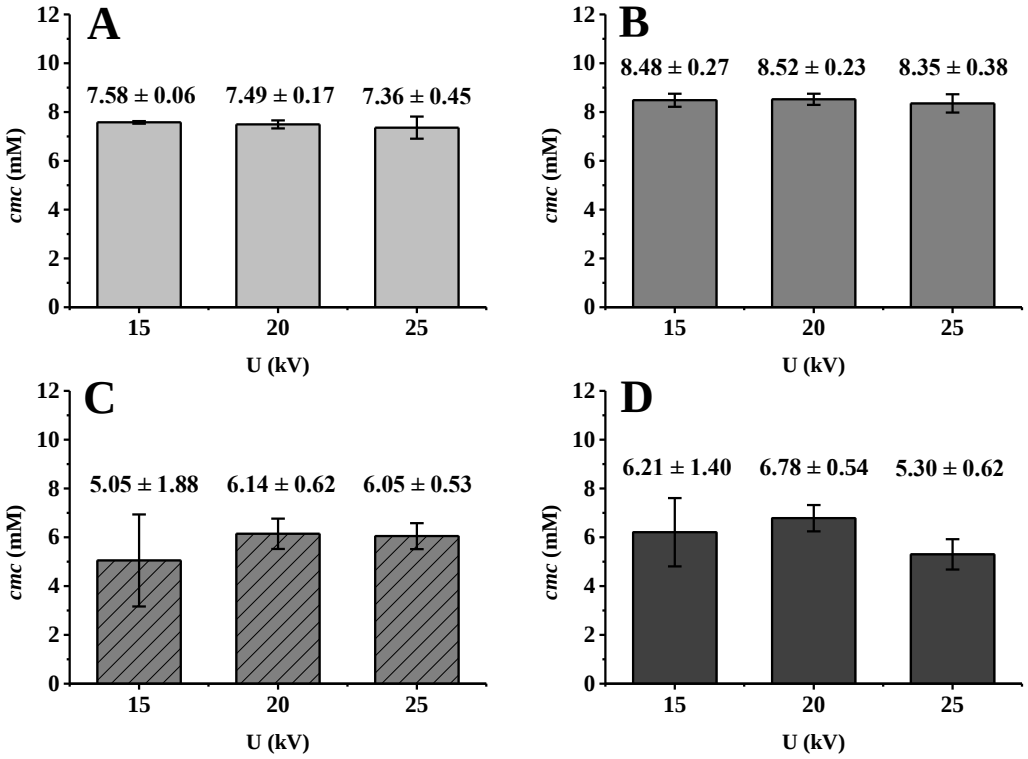

Figure 2: Comparison of the values of critical micelle concentration of sodium dodecyl sulphate in water obtained by method using capillary electrophoresis.
A – method based on the measurement of the current
B – method based on the measurement of retention time of alkylbenzenes
C – method based on the measurement of retention factor of alkylbenzenes
D – method based on the measurement of mobility of alkylbenzenes

The statistical evaluation was done using software OriginPro 9. Statistically significant differences between results obtained by using different methods were found at the significance level 0.05. The Tukey test was performed in order to find the methods with statistically different results. The results are shown in the Table 1.

Table 1: The list of the methods with significantly different results of critical micelle concentration of sodium dodecylsulphate in water.

Methods	Methods with statistically significant differences of the results of *cmc*
t_r (15 kV)	k (15 kV), k (20 kV), k (25 kV), μ_{eff} (25 kV)
t_r (20 kV)	k (15 kV), k (20 kV), k (25 kV), μ_{eff} (15 kV), μ_{eff} (25 kV)
t_r (25 kV)	μ_{eff} (25 kV)
k (15 kV)	I (15 kV), I (20 kV), I (25 kV)

The next part of the work was focused on the effect of the concentration and composition of the sample of alkylbenzenes and injections into the capillary on the value of

critical micelle concentration of sodium dodecylsulphate in water and in borate buffer. There were found the statistical significant differences between the results. The composition of the sample and time of the injection are the significant factors.

Finally, influence of the concentration of dodecanol in the solution of sodium dodecylsulphate on the value of critical micelle concentration was tested. The traces of dodecanol can be present in the commercially available sodium dodecyl sulphate, which was the motivation for the study. There were found significant differences (level of significance $\alpha = 0.5$) between results for solutions with 0, 1, 3, 5 % of added dodecanol but there wasn´t observed the dependence between the concentration of added dodecanol and value of critical micelle concentration of sodium dodecyl sulphate.

In conclusion, the different methods used capillary electrophoresis for determination of critical micelle concentration of surfactants were tested, specifically method based on the measurement of current, conductivity, retention time, retention factor and mobility. Higher standard deviations were observed in the methods based on the measurement retention factors and mobilities, which could be given by difficulties process to get the result (mobility of the micelles, non-linear dependency of mobility of alkylbenzenes on the concentration of sodium dodecyl sulphate). The method based on the measurement of current is the simplest and the fastest method for the determination of critical micelle concentration in water solutions, but this method is limited by higher concentration of organic modifier in the solution. For that cases, the method based on the measurement of retention time of alkylbenzenes is better.

2. Comparison of distribution coefficients octanol-water, micelle-aqueous phase, liposome-aqueous phase of selected antioxidants

Antioxidants are secondary metabolites of plants, which can protect an organism from the effect of free radicals. Antioxidants are able to inhibit oxidative damages and to improve the immune function of the organism [6]. *In vivo* effects of these compounds are dependent on their lipophilicity and hydrophilicity, which govern membrane and protein interactions. In general, the hydrophobicity or lipophilicity of compounds can be expressed by the distribution constant between a polar and a non-polar phase [7]. The octanol-water partition coefficient and its logarithm, log $P_{o/w}$, is commonly used for description of the hydrophobicity or lipophilicity of compounds. To get a better understanding of the distribution of compounds between lipophilic and hydrophilic phases, micelle or liposome-aqueous partition coefficients, which are dependent on van der Waals and hydrogen donor/acceptor interactions between the compounds and the lipophilic membrane, can be determined [8].

Liposome electrokinetic chromatography (LEKC) and micellar electrokinetic chromatography (MEKC) were used for the determination of distribution coefficients of selected natural antioxidants. In LEKC and MEKC, the retention factor *k* is given by equation [9]:

$$k = K_D \cdot \frac{V_{psp}}{V_{aq}} = K_D \cdot \Phi \tag{1}$$

where K_D is distribution coefficient, V_{psp} is the volume of pseudostationary phase and V_{aq} is volume of aqua phase. Ratio V_{psp}/V_{aq} is called phase ratio, Φ, which is given by equation:

$$\Phi = \frac{V_{psp}}{V_{aq}} = \frac{\nu_{spec,vol} \cdot M \cdot (c_{PAL} - cmc)}{1 - \left(\nu_{spec,vol} \cdot M \cdot (c_{PAL} - cmc)\right)} \tag{2}$$

where $\nu_{spec,vol}$ is parcial specific volume of micelles or liposomes, *M* is molar mass, c_{PAL} is the concentration of the surfactant or phospholipids and *cmc* is the critical micelle concentration of surfactant (or critical aggregation concentration of the phospholipids in the case of LEKC).

Retention factor was calculated from effective electrophoretic mobility of antioxidant (μ_{eff}) in LEKC or MEKC mode, effective electrophoretic mobility of antioxidant in CZE mode (μ_0), and electrophoretic mobility of pseudostationary phase (μ_{psp}):

$$k = \frac{\mu_{eff} - \mu_0}{\mu_{psp} - \mu_{eff}} \quad (3)$$

Electrophoretic mobility of pseudostationary phase was calculated by iterative procedure using homological series of alkylbenzoates [10,11].

Effective electrophoretic mobility was calculated by equation:

$$\mu_{eff} = \frac{l_t \cdot l_d}{U}\left(\frac{1}{t} - \frac{1}{t_0}\right) \quad (4)$$

where l_t is total length of capillary, l_d is the length of the capillary from the beginning to the detection window, U is applied voltage, t is migration/retention time of the analyte and t_0 is migration time of the marker of electroosmotic flow.

The distribution coefficients of antioxidants were determined between liposomes contained 1-palmitoyl-2-oleyl-*sn*-glycero-3-fosfocholin (POPC) and 1-palmitoyl-2-oleyl-*sn*-glycero-3-fosfo-L-serin (POPS) 80:20 mol%, 1-palmitoyl-2-oleyl-*sn*-glycero-3-fosfocholin and 1-palmitoyl-2-oleyl-*sn*-glycero-3-fosfo-(1´-rac-glycerol) (POPG) 80:20 mol%, micelles of sodium dodecylsulphate and phosphate buffer at pH 7.4 and ionic strength 20 mM. In the table 2, there are the values of pKa predicted using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2015 ACD/Labs), and log $P_{o/w}$ values for neutral compounds and log $D_{pH7.4}$ for ionisable compounds predicted by ACD/Labs Percepta Platform – PhysChem Module.

Table 2: Values of pKa, log $P_{o/w}$, log $D_{pH7.4}$ of studied compounds predicted by ACD/Labs software.

compound	pKa	$log\ P_{o/w}$	$log\ D_{pH\ 7.4}$
4-hydroxybenzoic acid	4.57 ± 0.10	1.42	-1.15
salicylic acid	3.01 ± 0.10	2.06	-0.77
protocatechuic acid	4.45 ± 0.10	1.16	-1.86
gallic acid	4.33 ± 0.10	0.91	-2.30
vanillic acid	4.45 ± 0.10	1.33	-1.46
syringic acid	4.33 ± 0.10	1.13	-1.63
p-coumaric acid	4.65 ± 0.10	1.88	-1.32
caffeic acid	4.58 ± 0.10	1.42	-1.74
ferulic acid	4.58 ± 0.10	1.64	-1.38
sinapic acid	4.53 ± 0.10	1.29	-2.02
chlorogenic acid	3.91 ± 0.50	-0.36	-3.91
(-)-epicatechin	9.54 ± 0.10	0.49	0.56
(+)-catechin	9.54 ± 0.10	0.49	0.56
flavone	-	3.56	3.42
7-hydroxyflavon	7.02 ± 0.40	3.32	2.76
luteolin	6.50 ± 0.40	2.40	1.12
rutin	6.17 ± 0.40	1.76	-1.75

biochanin A	6.50 ± 0.20	3.14	1.97
isoquercitrin	6.17 ± 0.40	1.75	-1.19
kaempferol	6.34 ± 0.40	2.05	0.81
myricetin	6.30 ± 0.40	2.11	0.26
quercetin	6.31 ± 0.40	2.08	0.58
morin	6.30 ± 0.40	1.61	0.34
naringin	7.17 ± 0.40	2.73	-0.05
naringenin	7.52 ± 0.40	3.19	2.22
hesperetin	7.49 ± 0.40	2.90	2.25
hesperidin	7.15 ± 0.40	1.78	-0.26
esculin	7.00 ± 0.20	-1.52	-1.92
4-hydroxycoumarin	4.50 ± 1.00	1.60	-1.72
6,7-dihydroxycoumarin	8.60 ± 1.00	0.98	1.30

The values of distribution coefficients of selected antioxidants determined by micellar electrokinetic chromatography and liposome electrokinetic chromatography are listed in the table 3.

Table 3: Determined values of log K_D (POPC/POPS), log K_D (POPC/POPG), and log K_D (SDS).

compound	*log* K_D (POPC/POPS)	*log* K_D (POPC/POPG)	*log* K_D (SDS)
gallic acid	1.19	1.37	2.30
4-hydroxybenzoic acid	--	2.13	2.21
protocatechuic acid	2.07	1.14	1.97
salicylic acid	2.20	2.54	2.68
syringic acid	1.76	1.85	2.32
vanillic acid	2.20	1.69	2.34
caffeic acid	0.65	--	2.18
chlorogenic acid	2.48	2.49	1.98
ferulic acid	1.29	0.79	2.33
p-coumaric acid	1.05	1.74	2.22
sinapic acid	1.39	1.86	2.42
(+)-catechin	2.43	2.22	--
(-)-epicatechin	2.57	2.50	--
7-hydroxyflavone	3.15	3.42	3.10
biochanin A	4.12	4.62	3.25
flavone	3.40	3.47	--
isoquercitrin	2.46	2.20	2.45
luteolin	3.96	3.72	3.00
rutin	1.88	0.71	2.59
kaempferol	3.97	3.20	3.11
morin	2.88	1.81	2.41
myricetin	3.45	3.61	2.65

quercetin	4.19	4.30	--
hesperetin	3.39	3.65	2.60
hesperidin	--	--	--
naringenin	3.79	3.77	2.60
naringin	--	--	--
4-hydroxycoumarin	1.78	1.46	2.29
6,7-dihydroxycoumarin	3.40	2.41	2.23
esculin	2.47	1.55	2.09

In the work, micellar electrokinetic chromatography and liposome electrokinetic chromatography were used for the determination of micellar/aqueous and liposome/aqueous distribution constants. SDS micelles were used as the pseudostationary phase in micellar electrokientic chromatography and liposomes comprising 80:20 mol% POPC/POPS or 80:20 mol% POPC/POPG were used as the pseudostationary phase in liposome electrokinetic chromatography. In order to compare the distribution constants obtained by MEKC and LEKC, pseudostationary phases of similar phase rations were utilised. The distribution constants were calculated from the phase ratio and the retention factors, and the K_D values were compared with predicted values of octanol/water or octanol/aqueous phase constants and distribution constants of the selected antioxidants. The results showed that the distribution constants of phenolic acids and flavonoid glycosides are, in general, lower than the distribution constants of the flavonoids. Obviously, the type of polar head groups in the phospholipids or surfactants has a considerable effect on the distribution constants of the phenolic acids and flavonoids. The data include a stronger positive correlation between the two studied liposome systems than between the liposome/SDS systems.

3. Application of MEKC, CZE and CZE/MS for the separation of natural antioxidants and oligosaccharides

This part of the work is focused on the analysis of natural antioxidants and oligosaccharides by micellar electrokinetic chromatography and capillary zone electrophoresis with UV/Vis detector or mass spectrometry detector.

Bioactive compounds from the berry or flowers of elderflowers have strong antioxidant, anti-carcinogenic, antimicrobial and anti-inflammatory properties [12]. Natural antioxidants can be determined by micellar electrokinetic chromatography. This method allows separation of the mixture of charged and non-charged analytes and high resolution, low consumption of sample and short time of the analyses are the main benefits of this method [13]. Micellar electrokinetic chromatography can be modified by cyclodextrins which are able to improve selectivity between positional isomers, functional groups, homologues and enantiomers [14]. During the analysis by MEKC with cyclodextrin in the background electrolyte, the analyte is distributed among three phases - aqueous phase, micelles and cyclodextrins. One of the possibilities to modified micellar electrokinetic chromatography is to add another surfactant into the background electrolyte.

Oligosaccharides are compounds involved in the process of glycosylation, which is important for controlling of different processes in the organism (communication between the cells, recognition, protein solubility, enzyme protection, adhesion, and proliferation) [15]. The molecules of oligosaccharides lack the chromophore, hence the derivatization with 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS), 2-aminopyridine (2-AP), 2-aminobenzamide (2-AB), 2-aminobenzoic acid (2-AA) was used.

The influence of the composition of background electrolyte (type and concentration of the micelles, composition and concentration of mixed micelles, addition of β-cyclodextrines) on the analysis of natural antioxidants was tested. The best result was obtained in the 25 mM borate buffer pH 9.10 with 36 mM sodium decyl sulphate. The quantitative analyses of the composition of three series of elderflower infusions were done. The first series was represented by tea bags bought in the local market, the second series was represented by mixture made from the content of the tea bags and the third series was represented by air-dried elderflower from Veltruby (June 2013). The results of quantitative analyses are in the table 4.

Table 4: The results of the quantitative analysis of the samples of elderflower infusions.

Analyte	*c* (mg/g) tea bags	*c* (mg/g) mixture of tea bags	*c* (mg/g) air-dried elderflower
gallic acid	0.19 ± 0.04	0.06 ± 0.04	c
neochlorogenic acid	5.15 ± 0.57	5.21 ± 0.62	4.02 ± 0.31
p-hydroxybenzoic acid	0.32 ± 0.05	0.46 ± 0.06	0.18 ± 0.10
chlorogenic acid	11.04 ± 0.05	12.44 ± 1.37	6.50 ± 0.66
cryptochlorogenic acid	a	a	a
caffeic acid	0.28 ± 0.06	0.32 ± 0.04	0.20 ± 0.09
p-coumaric acid	c	c	c
ferulic acid	0.33 ± 0.05	0.43 ± 0.25	0.18 ± 0.06
isoquercitrin	1.64 ± 0.33	1.80 ± 0.30	0.66 ± 0.31
rutin	17.45 ± 1.01	21.59 ± 1.95	12.83 ± 0.74
hesperidin	c	c	c
kaempferol-3-*O*-rutinoside	3.20 ± 0.18	1.34 ± 0.29	3.27 ± 0.21
isorhamnetin-3-*O*-rutinoside	b	b	b
isorhamnetin-3-*O*-glukoside	0.89 ± 0.07	4.20 ± 0.30	0.69 ± 0.20
quercetin	c	c	c
naringenin	0.91 ± 0.16	1.08 ± 0.09	0.61 ± 0.11
hesperetin	d	d	d
kaempferol	d	d	d
a – the sum of cryptochlorogenic acid and neochlorogenic acid was determined **b – the sum of isorhamnetin-3-*O*-rutinosid a kaempferol-3-*O*-rutinosid was determined** **c –the concentration of the analyte is lower than limit of quantification** **d – the concentration of the analyte is lower than limit of detection**			

CZE and CZE/MS analysis of oligosaccharides included the optimization of composition of the background electrolyte, derivatization agent, length of the capillary, cover of the capillary (poly dimethyl acrylamide, polyvinyl alcohol), and applied voltage. In the Fig. 3, there are three analyses of ANTS derivative oligosaccharides in background electrolyte with different composition. The short end of the capillary was used for the analysis and the origin time of the analysis (about 20 min) was shorten to 1.2 min.

The connection of the capillary electrophoresis and mass spectrometry was also optimized. The influence of the position of the separation capillary, the geometry of the end of separation capillary in the ion source, the composition of the sheath liquid and flow rate. The final separation of the oligosaccharides is show in the Fig. 4.

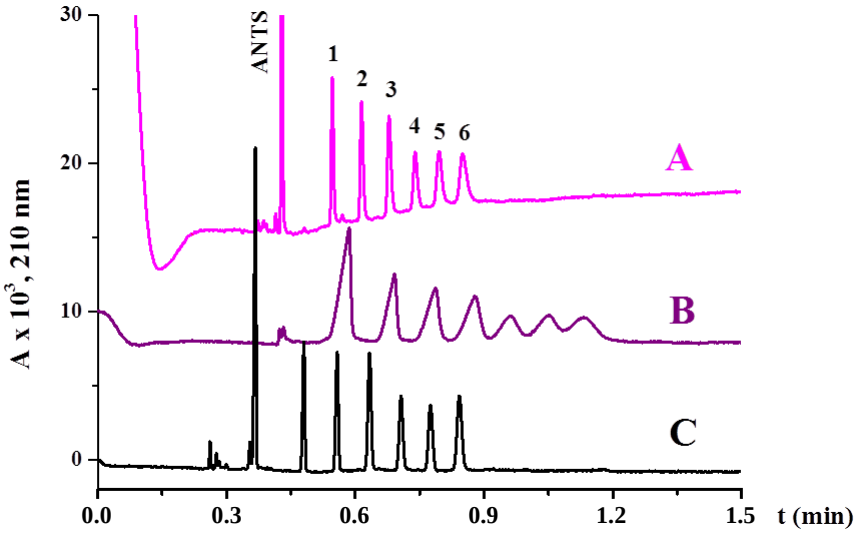

Figure 3: Comparison of the separation of ANTS derivate of oligosaccharides. A – BGE: 50 mM $HCOONH_4$ + HCOOH, pH 3.00; **B** – BGE: 50 mM HCOOH; **C** – BGE: 50 mM NaH_2PO_4 + H_3PO_4, pH 2.50. Effective length of the capillary was 8 cm, inner diameter of the capillary 50 µm. Applied voltage was 25 kV, temperature 25 °C. 1 – maltosa, 2 – maltotriosa, 3 – maltotetrosa, 5 – maltopentosa, 6 – maltoheptosa.

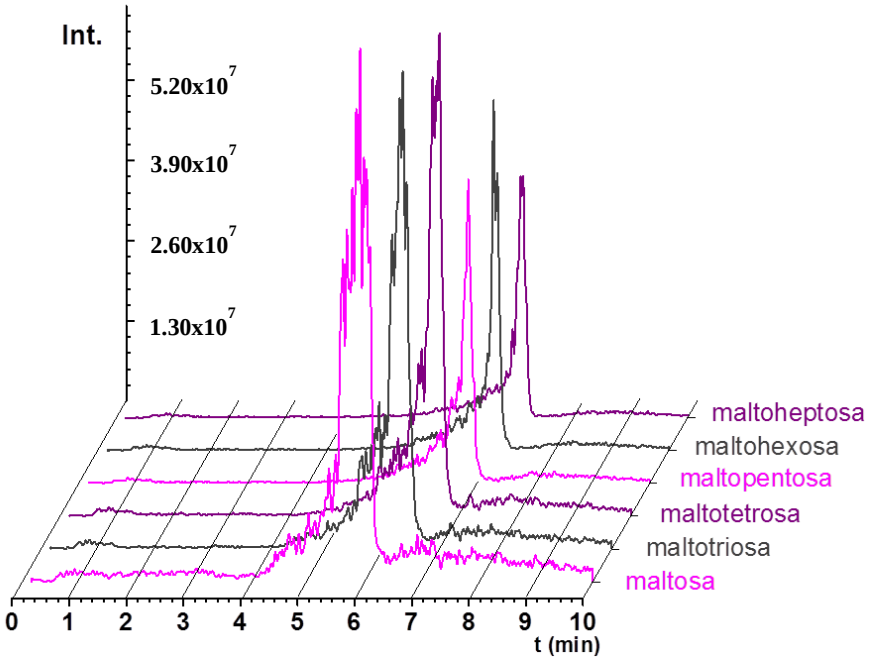

Figure 4: CE/MS separation of oligosaccharides. BGE: 400 mM HCOOH, separation capillary was 80 cm length with inner diameter 50 µm. The end of the capillary was sharpened in the angle 50°. The position of the end of the separation capillary was 0.4 mm from the sprayer tip. Separation schedule: in 0 min pressure 950 mbar was applied, in 1 min -25 kV and pressure 25 mbar were set. Flow rate of sheath liquid H_2O/CH_3OH/HCOOH (5 ml : 5 ml : 30 µl) was 2 µl/min. Entrance potential was -10 V, collision energy was -10 V, voltage in the ion source was -4500 V, declusteration potential -100 V, and scan rate was 1000 Da/s.

The conditions for the separation of natural antioxidants in elderflower infusions were optimized. Optimization included influence of the type and concentration of surfactant, β-cyclodextrins, and using of mixed micelles for the analysis. From the calibration line, the limit of detection and limit of quantification were calculated and the concentrations of the natural antioxidants in the three types of sample of elderflower infusions were calculated. The highest content of antioxidants was found in the sample represented by mixture of twenty tea bags, the lowest contents of antioxidants was determined in the air-dried elderflower from Veltruby.

The analyses of oligosaccharides were performed by the capillary zone electrophoresis with UV/Vis detection and with mass spectrometry detection. The different derivative agents were tested and also different conditions (including type, length, position and geometry of the separation capillary, composition and flow rate of the sheath liquid, composition of the background electrolyte, applied voltage). Finally, the separation of oligosaccharides by CE/MS in seven minutes was achieved.

4. Multidimensional separation

Multidimensional separations are based on the combination of separation techniques which are based on the different separation mechanism. In this work, the natural antioxidants were analysed by two-dimensional separation implemented by liquid chromatography with gradient elution in the first dimension and micellar electrokinetic chromatography in the second dimension. Changing concentration of organic solvent in the mobile phase in liquid chromatography in the first dimension causes change in the retention time of the analytes in the second dimension. This effect of the gradient profile of aqueous-organic mobile phase on the analysis in the second dimension can be compensate by using background electrolytes with increasing concentration of micelles of surfactant as well as the increasing length of alkyl chains in the surfactant molecule.

The critical micelles concentration of selected surfactants, specifically sodium octyl sulphate to sodium tetradecyl sulphate, was determined in dependency on the concentration of acetonitrile in the solution. The critical micelle concentration decreases with increasing length of the alkyl chain in surfactant molecule and slightly increases in background electrolyte with addition of acetonitrile in comparison with the background electrolyte without acetonitrile (Fig. 5).

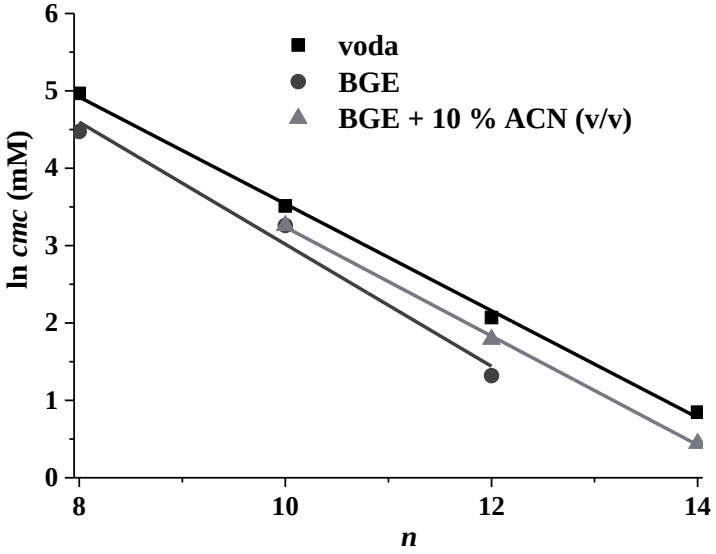

Figure 5: Dependency of ln *cmc* on the number of carbon in alkyl chain of sodium alkyl sulphate (*n*).

During the LC separations in the first dimension the concentration of acetonitrile growth from 1 % to 30 % (v/v). The tested compounds eluted into 12 min, the fractions were collected every 15 s (volume of the fraction was 100 µl). The change of the concentration of acetonitrile was compensated by composition of the background electrolyte in the second

dimension. The concentration of sodium decyl sulphate which is needed to compensate the influence of changing concentration of acetonitrile is shown in the Fig. 6.

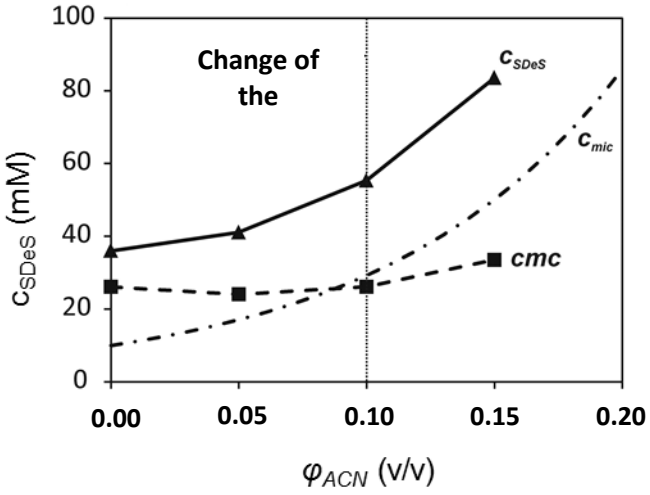

Figure 6: Dependence of the concentration of sodium decyl sulphate on the concentration of acetonitrile in the background electrolyte.

Increasing concentration of sodium decyl sulphate in the background electrolyte causes increasing current and thereby decreasing efficiency. To minimalize this phenomena sodium decyl sulphate was replaced by sodium dodecyl sulphate at the concentration 10 % acetonitrile in effluent. The concentration of sodium dodecyl sulphate used for the compensation of concentration in acetonitrile is shown in Fig. 7.

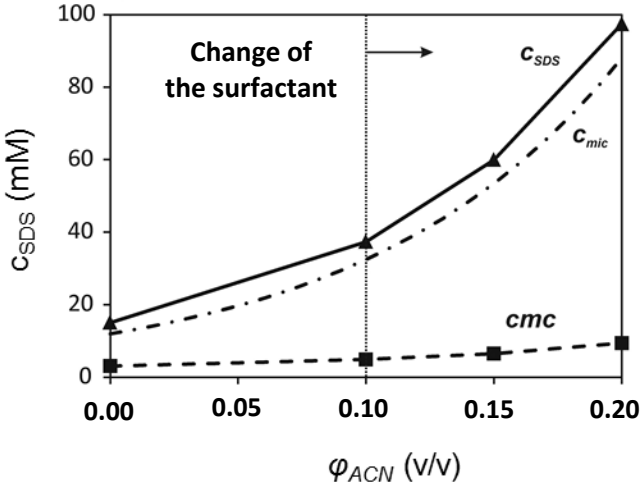

Figure 7: Dependence of the concentration of sodium dodecyl sulphate on the concentration of acetonitrile in the background electrolyte.

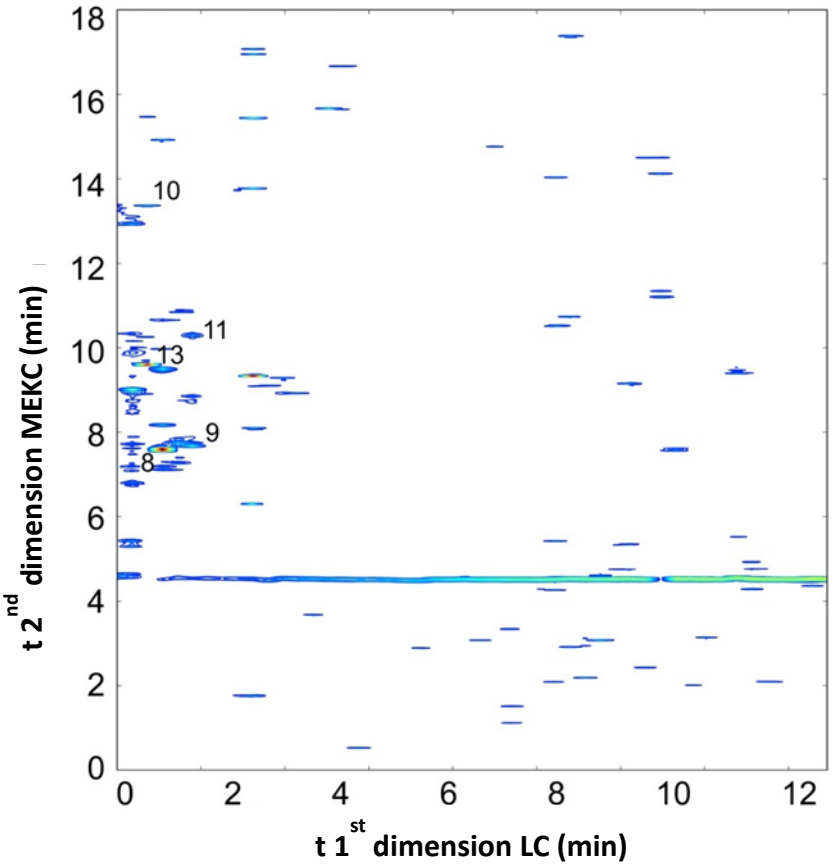

Figure 8: 2D LC-MEKC analyse of elderflowers infusion with variable concentration and type of surfactant in the second MEKC dimension. Background electrolyte 25 mM borate buffer, pH 9.00, concentration of surfactant was selected according to the Fig. 6 and 7. Capillary was 48 (40) cm length with inner diameter 50 μm and detection window 150 μm. Applied voltage 20 kV, temperature 25 °C, detection 214 and 254 nm. Gradient profile in LC in the first dimension: 0 min 1% B (A – water with 0.2 % (v/v); B – acetonitrile), 15 min – 30 % B. Temperature 40 °C. 8 – rutin, 9 – naringenin, 10 – gallic acid, 11 – caffeic acid, 13 – chlorogenic acid.

The two-dimensional method combining liquid chromatography with linear increasing concentration of acetonitrile in mobile phase and micellar electrokinetic chromatography in off-line setup was developed. Using optimized pseudostationary phase gradients, successful correction for shift was accomplished, yielding an approximately constant migration window. Sodium decyl sulphate in concentrations from 36 mM to 123 mM and sodium dodecyl sulphate in concentrations from 37 mM to 97 mM was used for compensation of the acetonitrile concentration in the range of 1 – 20 % (v/v). The utility of the method was verified by the analysis of natural antioxidants in elderflower water infusion.

[1] Tickle D. C., Okafo G. N., Camilleri P., Jones R. F. D., Kirby A. J., *Analytical Chemistry* 66 (1994) 4121-4126.

[2] Nakamura H., Sano A., Matsuura K., *Analytical Science* 14 (1998) 379-382.

[3] Terabe S., Otsuka K., Ichikawa K., Tsuchiya A., Ando T., *Analytical Chemistry* 56 (1984) 111.

[4] Míková M., *Diplomová práce* (2007) Pardubice.

[5] Jacquire J. C., Desbène P. L., *Journal of Chromatography A* 718 (1995) 167-175.

[6] Jáč P., Polášek M., & Pospíšilová M. (2006). Recent trends in the determination of polyphenols by electromigration methods. *J. Pharm. Biomed. Anal., 40,* 805-814.

[7] Rothwell J. A., Day A. J., & Morgan M. R. A. (2005). Experimental determination of octanol-water partition coefficients of quercetin and related flavonoids. *J. Agric. Food Chem., 53,* 4355-4360.

[8] Wiedmer S. K., Kulovesi P., & Riekkola M.-L. (2008). Liposome electrokinetic capillary chromatography in the study of analyte-phospholipid membrane interactions. Application to pesticides and related compounds. *J. Sep. Sci.*, *31*, 2714-2721.

[9] Terabe S., *Handbook of Capillary and Microchip Electrophoresis and Associated Microtechniques,* kapitola 3: Micellar electrokinetic chromatography, str. 109 – 130, 2008.

[10] M.M. Bushey, J.W. Jorgenson, *Analytical Chemistry* 61 (1989) 491.

[11] Wiedmer S. K., Lokajová J., Riekkola M.-L., *Journal of Separation Science* 33 (2010) 394-409.

[12] Nile S. H., Park S. W., *Nutrition* 30 (2014) 134-144.

[13] Delgado-Zamarreno M. M., González-Masa I., Sánchez-Pérez A., Carabias Martínez R., *Food Chemistry* 100 (2007) 1722-1727.

[14] Singh M., Sharma R., Banerjee U. C., *Biotechnology Advances* 20 (2002) 341-359.

[15] Rustighu I., Campa C., Rossi M., Semeraro S., Vetere A., Gamini A., *Electrophroresis* 30 (2009) 2632-2639.

Publications in foreign and international reviewed journals (IF journals)

Váňová J., Česla P., Češlová L., Fischer J., Development of micellar electrokinetic chromatographic method for determination of polyphenols in elder infusions. *Chemické listy* 3 (2014) S192-S196. ISSN 0009-2770, impact factor 0,272.

Váňová J., Česla P., Fischer J., CZE and MEKC separation of phenolic compounds. *Chemické listy* 107 (2013) S465-S467. ISSN 0009-2770, impact factor 0,196.

Publications in other reviewed scientific journals

Váňová J., Liimatta L. J., Česla P., Wiedmer S., Determination of distribution constants of antioxidants by electrokinetic chromatography. Cogent Chemistry 3 (2017). https://doi.org/10.1080/23312009.2017.1385173

Česla P., Dinisová P., Váňová J., Češlová L., Fischer J., Characterization of Sambucus nigra L. infusions using capillary electrophoresis and liquid chromatography-tandem mass spektrometry. *Instrumental methods for the analysis and identification of bioactive molecules* (2014) 165-188. ISBN 978-0-8412-2976-1.

Česla P., Váňová J., Fischer J., Elektrokinetické chromatografické metody. Analýza organických látek (2014) 189-198. ISBN 978-80-260-7085-6.

Lectures at foreign conferences or at important international conferences held in the Czech Republic

Česla P., Křenková J., Váňová J., Vaňková N., Fischer J., Development of microfluidic interface for on-line two-dimensional liquid chromatorgaphy-capillary electrophoresis. 30th International Symposium on Chromatography, Salzburg, 14. – 18. 9. 2014.

Váňová J., Česla P., Češlová L., Fischer J., Development of micellar electrokinetic chromatographic method for determination of polyphenols in elder infusions. 21st Young investigators´ seminar on analytical Chemistry – YISAC 2014, Pardubice, 25. – 28. 6. 2014.

Váňová J., Liimatta L. J., Česla P., Wiedmer S., Determination of distribution constants of antioxidants between liposomes, alkylsulfate micelles, octanol and water using electrokinetic chromatography. *CECE 2016: 13th International interdisciplinary meeting on bioanalysis* (2016) 62-65 (WoS). ISBN 978-80-904959-4-4. CECE 2016: 13th International interdisciplinary meeting on bioanalysis, Brno, 17. – 19. 10. 2016.

Foreign conference posters or at important international conferences held in the Czech Republic

Váňová J., Česla P., Fischer J., Jandera P., Optimization of MEKC separation selectivity of phenolic compounds using homological series of anionic surfactants. *CECE 2012: 9th International Interdisciplinary Meeting on Bioanalysis* (2012) 193-199. ISBN 978-80-904959-1-3. CECE 2012: 9th International Interdisciplinary Meeting on Bioanalysis, Brno, 31. 10. – 2. 11. 2012.

Česla P., Váňová J., Vaňková N., Hlavová K., Křenková J., Fischer J., Orthogonality of liquid chromatography and capillary electrophoresis in two-dimensional separation of oligosaccharides. 41st International Symposium on High Performance Liquid Phase Separations and Related Techniques, New Orleans, 11. – 15. 5. 2014.

Váňová J., Česla P., Hlavová K., Fischer J., Optimization of separation of oligosaccharides using capillary electrophoresis. 30th International Symposium on Chromatography, Salzburg, 14. – 18. 9. 2014.

Váňová J., Dinisová P., Česlová L., Fischer J., Česla P., Characterization of Sambucus nigra L. infusions using micellar electrokinetic capillary chromatography and liquid chromatography-tandem mass spektrometry. 41st International symposium on high performance liquid phase separations and related techniques, New Orleans, 11. – 15. 5. 2014.

Váňová J., Česla P., Hlavová K., Fischer J., capillary electrophoretic separation of fluorescently labelled oligosaccharides. CECE 2014: 11th International interdisciplinary meeting on bioanalysis (2014) 443-446 (WoS). ISBN 978-80-904959-2-0. CECE 2014: 11th International interdisciplinary meeting on bioanalysis, Brno, 20. – 22. 10. 2014

Váňová J., Česla P., Píchová K., Nováková Z., Fischer J., Fast in-source CE/MS separation of biologically active compounds. 42th Sympousium on high performance liquid phase separations and related techniques, HPLC 2015, Ženeva, 21. – 25. 2015.

Váňová J., Česla P., Šimánek V., Křenková J., Fischer J., Development of capillary separation systém for complex characterization of glycomic samples. 23rd International symposium on electro and liquid phase separation techniques, Minneapolis, 18. – 23. 9. 2016.

Wiedmer S., Váňová J., Česla P., Comparison of liposome- and octanol-water distribution constants of antioxidants. 23rd International symposium on electro and liquid phase separation techniques, Minneapolis, 18. – 23. 9. 2016.

Domestic conference lectures

Váňová J., Česla P., Fischer J., Využití tenzidů v MEKC separaci herbicidů. XLVI. *Seminář o tenzidech a detergentech: sborník přednášek* (2013) 27-35. ISBN 978-80-7395-698-1. XLVI. Seminář o tenzidech a detergentech, Strážnice, 20. 10. – 1. 11. 2013.

Váňová J., Česla P., Fischer J., Využití homologické řady alkylsíranů sodných pro MEKC separace přírodních antioxidantů. *Monitorování cizorodých látek v životním prostředí XV., sborník příspěvků ze semináře* (2013) 165-174. ISBN 978-80-7395-683-7. Monitorování cizorodých látek v životním prostředí XV., Ovčárna pod Pradědem, 10. - 12. 4. 2013

Váňová J., Česla P., Fischer J., Využití směsných micel v MEKC separaci přírodních antioxidantů. XLVII. Seminář o tenzidech a detergentech, Jetřichovice, 5. – 7. 11. 2014.

Váňová J., Česla P., Fischer J., Vliv cyklodextrinů na separaci vybraných přírodních antioxidantů pomocí micelární elektrokinetické chromatografie. *Monitorování cizorodých látek v životním prostředí XVI., sborník příspěvků ze semináře* (2014) 123-131. Monitorování cizorodých látek v životním prostředí XVI., Ovčárna pod Pradědem, 9. – 11. 4. 2014.

Váňová J., Česla P., Fischer J., Stanovení kritické micelární koncentrace tenzidů pomocí kapilární elektroforézy. *XLVIII. Seminář o tenzidech a detergentech: sborník přednášek* (2015) 63-71. XLVIII. Seminář o tenzidech a detergentech, Velké Bílovice, 4. – 6. 11. 2015.

Váňová J., Česla P., Fischer J., Využití β-cyklodextrinů při separaci vybraných isomerů fenolických kyselin a flavonoidů pomocí MEKC. *Monitorování cizorodých látek v životním prostředí XVII: sborník příspěvků ze semináře* (2015) 129-139. ISBN 978-80-7395-926-5. Monitorování cizorodých látek v životním prostředí XVII, Ovčárna pod Pradědem, 8. – 10. 4. 2015.

Other contributions in conference papers

Česla P., Váňová J., Hlavová K., Fischer J., Separation of inorganic anions by capillary zone electrophoresis employing sulfonated dyes as probes for indirect UV detection. *Sensing in Electroanalysis* 7 (2012) 433-442. ISBN 978-80-7395-563-2. 8th Seminar/Workshop on Sensing in Electroanalysis, Pardubice, 14. 11. – 17. 11. 2012.

Váňová J., Česla P., Fischer J., Jandera P., Optimization of MEKC separation selectivity of phenolic compounds using homological series of anionic surfactants. *CECE 2012: 9th International Interdisciplinary Meeting on Bioanalysis* (2012) 193-199 (WoS). ISBN 978-80-904959-1-3. CECE 2012: 9th International Interdisciplinary Meeting on Bioanalysis, Brno, 31. 10. – 2. 11. 2012.

Česla P., Váňová J., Fischer J., Jandera P., Optimization of gradient conditions in two-dimensional liquid chromatography-micellar electrokinetic capillary chromatography separations. *HPLC 2013 Book of Abstracts* 1 (569-569) 2013. 39th International Symposium on high performance liquid phase separations and related techniques.

Váňová J., Česla P., Fischer J., Využití tenzidů v MEKC separaci herbicidů. XLVI. *Seminář o tenzidech a detergentech: sborník přednášek* (2013) 27-35. ISBN 978-80-7395-698-1. XLVI. Seminář o tenzidech a detergentech, Strážnice, 20. 10. – 1. 11. 2013.

Váňová J., Česla P., Fischer J., Využití homologické řady alkylsíranů sodných pro MEKC separace přírodních antioxidantů. *Monitorování cizorodých látek v životním prostředí XV., sborník příspěvků ze semináře* (2013) 165-174. ISBN 978-80-7395-683-7. Monitorování cizorodých látek v životním prostředí XV., Ovčárna pod Pradědem, 10. - 12. 4. 2013

Česla P., Křenková J., Václavek T., Váňová J., Vaňková N., Fischer J., Modelling of microfluidic flow-gating interface for two-dimensional liquid chromatography-capillary electrophoresis. *CECE 2014: 11th International Interdisciplinary Meeting on Bioanalysis* (2014) 158-161 (WoS). CECE 2014: 11th International Interdisciplinary Meeting on Bioanalysis, Brno, 20. – 22. 10. 2014.

Váňová J., Česla P., Fischer J., Využití směsných micel v MEKC separaci přírodních antioxidantů. XLVII. *Seminář o tenzidech a detergentech* 1 (2014) 43-50. ISBN 978-80-7395-836-7. XLVII. Seminář o tenzidech a detergentech, Jetřichovice, 5. – 7. 11. 2014.

Váňová J., Česla P., Hlavová K., Fischer J., capillary electrophoretic separation of fluorescently labelled oligosaccharides. CECE 2014: 11th International interdisciplinary meeting on bioanalysis (2014) 443-446 (WoS). ISBN 978-80-904959-2-0. CECE 2014: 11th International interdisciplinary meeting on bioanalysis, Brno, 20. – 22. 10. 2014.

Váňová J., Česla P., Fischer J., Vliv cyklodextrinů na separaci vybraných přírodních antioxidantů pomocí micelární elektrokinetické chromatografie. *Monitorování cizorodých látek v životním prostředí XVI., sborník příspěvků ze semináře* (2014) 123-131. Monitorování cizorodých látek v životním prostředí XVI., Ovčárna pod Pradědem, 9. – 11. 4. 2014.

Česla P., Váňová J., Vaňková N., Nováková Z., Křenková J., Fischer J., Optimization of fraction transfer proces in on-line 3D LC-CZE and its influence on peak capacity. *22nd International symposium on electro- and liquid phase-separation techniques (ITP 2015):*

book of abstracts. 22nd International symposium on electro- and liquid phase-separation techniques (ITP 2015) and 8th nordic separation science symposium (NoSSS2015).

Česla P., Vaňková N., Váňová J., Nováková Z., Fischer J., Towards complex characterization on glycomic samples using combination of HILIC with CZE in microfluidic system. *Applied natural science 2015: konference proceedings* (2015). ISBN 978-80-8105-723-6. Applied natural science 2015, Jasná, 30. 9. – 2. 10. 2015.

Váňová J., Česla P., Fischer J., Optimization of sheath-flow CE/MS separation of oligosaccharides. *CECE 2015: 12th International interdisciplinary meeting on bioanalysis* (2015) 212-213 (WoS). ISBN 978-80-904959-3-7. CECE 2015: 12th International interdisciplinary meeting on bioanalysis, Brno, 21. – 23. 9. 2015.

Váňová J., Česla P., Fischer J., Stanovení kritické micelární koncentrace tenzidů pomocí kapilární elektroforézy. *XLVIII. Seminář o tenzidech a detergentech: sborník přednášek* (2015) 63-71. XLVIII. Seminář o tenzidech a detergentech, Velké Bílovice, 4. – 6. 11. 2015.

Váňová J., Česla P., Fischer J., Využití β-cyklodextrinů při separaci vybraných isomerů fenolických kyselin a flavonoidů pomocí MEKC. *Monitorování cizorodých látek v životním prostředí XVII: sborník příspěvků ze semináře* (2015) 129-139. ISBN 978-80-7395-926-5. Monitorování cizorodých látek v životním prostředí XVII, Ovčárna pod Pradědem, 8. – 10. 4. 2015.

Česla P., Vaňková N., Váňová J., Optimization of gradient separation conditions in two-dimensional liquid phase system. *CECE 2016: 13th International interdisciplinary meeting on bioanalysis* (2016) 95-97 (WoS). ISBN 978-80-904959-4-4. CECE 2016: 13th International interdisciplinary meeting on bioanalysis, Brno, 17. – 19. 10. 2016.

Váňová J., Liimatta L. J., Česla P., Wiedmer S., Determination of distribution constants of antioxidants between liposomes, alkylsulfate micelles, octanol and water using electrokinetic chromatography. *CECE 2016: 13th International interdisciplinary meeting on bioanalysis* (2016) 62-65 (WoS). ISBN 978-80-904959-4-4. CECE 2016: 13th International interdisciplinary meeting on bioanalysis, Brno, 17. – 19. 10. 2016.