FACULTY OF CHEMISTRY
University of Lodz

UNIVERSITY OF LODZ

Author's review of his research

(annex 2b)

PhD Paweł Kubalczyk

Department of Environmental Chemistry,
Faculty of Chemistry,
University of Lodz

LODZ 2017

1. Name and surname:

Paweł Kubalczyk

2. Education, scientific diploma and degrees:

- 1996, MSc degree, Faculty of Mathematics, Physics and Chemistry, University of Lodz, MSc dissertation: „Crystal structure determination of N-(3,4-dichloro-phenetyl)acetamideisopropylammonium chloride by X-ray diffraction analysis".
  Supervisor: Prof. dr hab. Mieczysław Grabowski
- 2003, PhD degree, Faculty of Physics and Chemistry, University of Lodz,
  PhD dissertation: „High performance capillary electrophoresis of 2-*S*-pyridinium derivatives of hydrophilic thiols".
  Supervisor: Prof. dr hab. Edward Bald

3. Employment:

- 1996-2003, assistant in the Department of Chemical Technology and Environmental Protection, Faculty of Chemistry, University of Lodz,
- since 2003, adjunct in the Department of Environmental Chemistry, Faculty of Chemistry, University of Lodz.

4. Scientific achievements:

- Number of publications – 31
- Number of publications before PhD degree – 2
- Number of publications after PhD degree – 29, including:
  - original papers included in Journal Citation Reports (JCR) – 21
  - original papers not included in JCR – 7
  - review articles included in JCR – 2
  - review articles not included in JCR – 1
- Number of publications comprising the scientific achievements – 10
- Impact Factor (IF):
  - according to publication's year – 53,868

- IF for publications comprising the scientific achievements:
  - according to publication's year – 27,158
- Number of citations (Web of Science):
  - sum – 245
  - without self-citations – 213

Hirsch index – 8

- Number of citations (Scopus):
  - sum – 261
  - without self-citations – 223

Hirsch index – 8

5. Indication of achievement resulting from article 16 section 2 of the Act on Academic Degrees and Titles and about Degrees and Titles in the Field of Fine Arts of March 14, 2003 (Dz. U. No 65/2003, item 595, with subsequent amendments):

5.1. Title of scientific achievement:

Capillary electrophoresis of biological samples with the use of on-line sample stacking techniques

5.2. List of publications comprising the scientific achievement:

Publications included in scientific achievement were sorted in accordance with discussion order. Corresponding author was indicated with asterisk.

H1 **Kubalczyk P.**, Bald E.* (2006)
Transient pseudo-isotachophoretic stacking in analysis of plasma for homocysteine by capillary zone electrophoresis
Analytical and Bioanalytical Chemistry, 384, 1181-1185. $IF_{(2006)}$ 2,695
My contribution is related to: (•) plan and carry out all the experiments, (•) describe and interpret the results, (•) discuss with co-author on the research results, (•) the manuscript preparation. I declare my contribution to be equal to 80%.

H2 **Kubalczyk P.**, Bald E.* (2008)
Method for determination of total cysteamine in human plasma by high performance capillary electrophoresis with acetonitrile stacking
Electrophoresis, 29, 3636-3640. $IF_{(2008)}$ 3,609
My contribution is related to: (•) plan and carry out all the experiments, (•) describe and interpret the results, (•) discuss with co-author on the research results, (•) the manuscript preparation. I declare my contribution to be equal to 80%.

H3 **Kubalczyk P.**, Bald E.* (2009)
Analysis of orange juice for total cysteine and glutathione content by CZE with UV-absorption detection
Electrophoresis, 30, 2280-2283. $IF_{(2009)}$ 3,509
My contribution is related to: (•) plan and carry out all the experiments, (•) describe and interpret the results, (•) discuss with co-author on the research results, (•) the manuscript preparation. I declare my contribution to be equal to 80%.

H4 **Kubalczyk P.***, Chwatko G., Bald E. (2014)
Capillary electrophoresis determination of tiopronin in human urine after derivatization with 2-chloro-1-methylquinolinium tetrafluoroborate
Current Analytical Chemistry, 10, 375-380. $IF_{(2014)}$ 1,194
My contribution is related to: (•) plan and carry out all the experiments, (•) describe and interpret the results, (•) discuss with co-author on the research results, (•) the manuscript preparation, (•) corresponding with the editor of the journal. I declare my contribution to be equal to 75%.

H5 **Kubalczyk P.***, Bald E., Furmaniak P., Głowacki R. (2014)
Simultaneous determination of total homocysteine and cysteine in human plasma by capillary zone electrophoresis with pH-mediated sample stacking
Analytical Methods, 6, 4138-4143. $IF_{(2014)}$ 1,938
My contribution is related to: (•) plan and carry out all the experiments, (•) describe and interpret the results, (•) discuss with co-author on the research results, (•) the manuscript preparation, (•) corresponding with the editor of the journal. I declare my contribution to be equal to 75%.

H6 Furmaniak P., **Kubalczyk P.***, Głowacki R. (2014)
Determination of homocysteine thiolactone in urine by field amplified sample injection and sweeping MEKC method with UV detection
Journal of Chromatography B, 961 36-41. $IF_{(2014)}$ 2,694
My contribution is related to: (•) plan and supervise all of the experiments, (•) discuss with co-authors on the research results, (•) participate in the manuscript preparation, (•) corresponding with the editor of the journal. I declare my contribution to be equal to 35%.

H7 **Kubalczyk P.***, Borowczyk K., Chwatko G., Głowacki P. (2015)
Simple micellar electrokinetic chromatography method for the determination of hydrogen sulfide in hen tissues
Electrophoresis, 36 1028-1032. $IF_{(2015)}$ 3,028
My contribution is related to: (•) plan and carry out all the experiments, (•) describe and interpret the results, (•) discuss with co-authors on the research results, (•) participate in the manuscript preparation, (•) corresponding with the editor of the journal. I declare my contribution to be equal to 70%.

H8 Borowczyk K., Krawczyk M., **Kubalczyk P.**, Chwatko G.* (2015)
Determination of lipoic acid in biological samples
Bioanalysis, 7, 1785-1798. $IF_{(2015)}$ 3,003
My contribution is related to: (•) prepare review of the literature, (•) participate in chapters 1, 2, 3 and 4 preparation, (•) the manuscript correction. I declare my contribution to be equal to 30%.

H9 **Kubalczyk P.***, Chwatko G., Głowacki R. (2016)
Fast and simple MEKC sweeping method for determination of thiosulfate in urine Electrophoresis, 37, 1155–1160. $IF_{(2016)}$ 2,744
My contribution is related to: (•) plan and carry out all the experiments, (•) describe and interpret the results, (•) discuss with co-authors on the research results, (•) participate in the manuscript preparation, (•) corresponding with the editor of the journal. I declare my contribution to be equal to 75%.

H10 **Kubalczyk P.***, Głowacki R. (2017)
Determination of lipoic acid in human urine by capillary zone electrophoresis
Electrophoresis, DOI: 10.1002/elps.201700002. $IF_{(2016)}$ 2,744
My contribution is related to: (•) plan and carry out all the experiments, (•) describe and interpret the results, (•) discuss with co-author on the research results, (•) participate in the manuscript preparation, (•) corresponding with the editor of the journal. I declare my contribution to be equal to 85%.

5.3. Description of the scientific aim and results achieved

Abbreviations

ACN – acetonitrile

BCPB – 1-benzyl-2-chloropyridinium bromide

BGE – background electrolyte

CMQT – 2-chloro-1-methylquinolinium tetrafluoroborate

CASH – cysteamine

Cys – cysteine

CysGly - cysteinylglycine

CZE – capillary zone electrophoresis

DHLA – dihydrolipoic acid

FASI – field amplified sample injection

GSH – glutathione

Hcy – homocysteine

HTL – homocysteine thiolactone

LA – lipoic acid

LOD – limit of determination

LOQ – limit of quantification

MEKC – micellar electrokinetic chromatography

MTQ – 1-methyl-2-thioquinolone

TBP – tributylphosphine

TCEP – tris(2-carboxyethyl)phosphine

TP – tiopronin, (N-2-mercaptopropionylglycine)

*tp*ITP – transient-pseudo isotachophoresis

Introduction

Throughout analytical laboratories electromigration techniques are very often utilized for separation and determination of different compounds in complicated matrices. These techniques are characterized by strong analytical potential which mainly results from good resolution, short separation time, high selectivity, relatively high sensitivity and susceptibility to miniaturization. Electrophoresis is defined as differential movement of ions under electric field in the separation medium. Arne Tiselius in 1937, for the first time used electrophoresis as a separation technique. Among electromigration techniques the most commonly used in analytical laboratories, a primary place is held by gel electrophoresis and capillary electrophoresis (CE). Gel electrophoresis is mainly used for separation of biological macro-compounds such as proteins and nucleic acids. Unfortunately, this technique is characterized by several drawbacks, namely long analysis time, low efficiency as well as time consuming gel visualization. On the other hand modern CE is analytical technique that allows the relatively fast and efficient separation of compounds in very small volume of a sample. CE combines advantages offered by two separation techniques: conventional gel electrophoresis and high performance liquid chromatography (HPLC) [1].

The most commonly known drawback of CE is inferior limit of detection, especially when compared to HPLC. Introduction of larger sample volume into the capillary is not good solution, because bigger amount of the sample causes broadening of the bands, efficiency loss and significantly deteriorated resolution. Several attempts in order to improve low sensitivity in CE have been made, including application of sophisticated capillaries and high sensitivity detection cells, but unfortunately these technical solutions could only help to a limited extent. Since the use of high sensitivity fluorescence detector or mass spectrometer allow for significant lowering of quantification and detection limits, thus it undoubtedly seems to be a very good solution. However, this is related to the additional purchase of expensive apparatus. Derivatization reaction during sample preparation is also frequently utilized. Derivatives formed in this reaction are characterized by different detection or electrophoretic properties resulting from the chromophore, fluorophore or electrophorus incorporation into the structure of analyte.

Sample stacking techniques realized directly inside the capillary are now very popular among other sample concentration methods utilized in order to lower detection limit in CE. Stacking is usually based on changes in electrophoretic velocities of analytes due to local differences in the strength of electric field. Analytes concentration can be obtained by long injection of low conductivity sample solution and then concentration of the analyte in a narrow zone using chromatographic and/or electrophoretic effects. Manipulation of the sample or BGE

conductivity is not related to the modification of commercial CE apparatus. Additionally, stacking and separation take place inside the same capillary what prevent from loss of analyte [2, 3]. Several techniques of analytes concentration in measuring system have been developed in recent years. These techniques can be generally classified into two groups, according to the mechanism of sample components separation. Techniques based on capillary zone electrophoresis (CZE), predominantly used to charged analytes, are included in the first group, whereas second group constitute methods which utilize micellar electrokinetic chromatography (MEKC) for the separation of neutral particles. Increasing number of papers prove that there is still interest to use techniques involving analyte concentration in measuring system [3-6].

The main challenge in biological sample CE analysis is the choice of a correct way of sample preparation. Biological samples usually contain small amounts of different compounds in a very complicated matrix. Key problems in biological sample preparation procedures come from a large number of individual compounds and a low concentration of exogenous or endogenous compounds of interest. Important problem is the presence of proteins in biological samples because they can modify the inner capillary wall, change its properties or even completely clog capillary. In the case of CE, numerous sample preparation procedures including extraction, derivatization and deproteinization are utilized. All of these sample preparation steps can significantly influence sample conductivity. Adequate match of sample and BGE conductivities seems to be essential for separation efficiency and resolution. Too high sample conductivity relative to BGE conductivity causes undesirable band broadening and loss of sensitivity.

My experiments aimed at the development of a new, simple, fast and sensitive CE methods for the determination of exogenous or endogenous analytes in biological samples, with the use of in-capillary sample stacking techniques..

Sample preparation

Samples such as plasma and urine are the best known biological matrices and therefore appears as the most commonly analyzed human fluids. The matrix composition of these fluids is almost constant and normal reference values of several compounds recognized as markers of different diseases are well known. Therefore, both urine and plasma are significant sources of valuable information about the conditions of living organisms. Unfortunately, analysis of biological samples is not easy due to complex matrices and low (trace) concentration of analytes [6]. It has been well known that the complication of sample preparation procedure decreases with the degree of sample fluidity. In this case, body fluids (urine, plasma, natural juices, surface water, municipal and industrial waste water) are the easiest to handle whereas

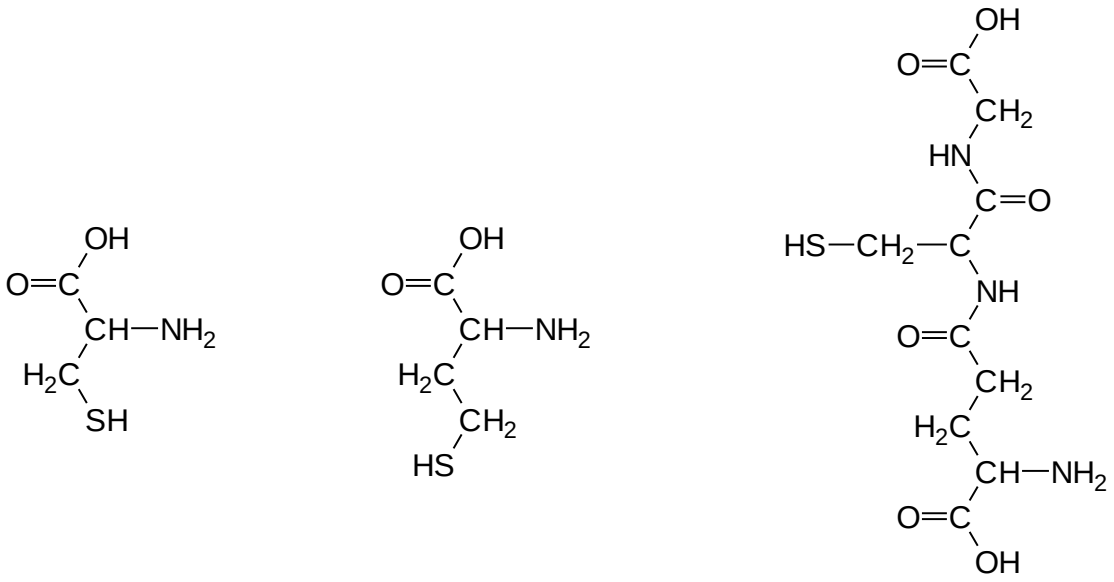
solid samples (stomach, liver, heart, brain, hair, nail) are the most difficult. Plasma, urine as well as solid tissues are very complex mixtures that can also contain proteins and inorganic salts. From an analytical point of view, proteins are the most troublesome because they can impede the analysis and modify properties of electrophoretic columns, what often leads to clog of capillary. Sample preparation is one of the most difficult and thus very important step during development of a new electrophoretic method. Moreover, it is one of the most time-consuming step of the analytical procedure and constitutes the main source of the total error [6]. Filtration and ultrafiltration for mechanical removal of high molecular weight compounds are used. However, the most commonly used techniques of protein precipitation involve the addition of chemical agents such as acids (trichloroacetic acid, perchloric acid, 5-sulfosalicilic acid), salts or organic solvents (acetone, acetonitrile, acetonitrile/ammonia) [6, 7, 8]. The use of chemical agents causes changes in the solubility of proteins and their precipitation out in solution. During my experiments, protein precipitation step was necessary for plasma [**H1**, **H2, H5**] and hen tissues (stomach, liver and heart) [**H7**] preparation. Perchloric acid as the deproteinization agent was used followed by sample centrifugation and further supernatant CE analysis. It is commonly known that normal urine and fruit juices do not contain proteins. Thus, in the case of urine [**H4**, **H6**, **H9**, **H10**] and orange juice [**H3**] samples the protein precipitation step was not included in sample preparation procedure.

The complication of real (biological) sample preparation procedure decreases with the degree of sample fluidity. One of my study concerns determination of hydrogen sulfide in solid samples from animals (hen tissues) [**H7**]. In the first step of sample preparation procedure, homogenization of a tissue was carried out. The homogenization parameters of hen tissues (stomach, liver and heart) were optimized during experiments. It is commonly known that pKa of H_2S at 37°C is 6.84°C [9], thus homogenates prepared at pH 6 would contain free sulfides predominantly as the neutral species (H_2S), which readily volatilize during the process of sample preparation. Under alkaline conditions (pH 9), almost all sulfides (>90%) should be present in the anionic form (HS^-) and should remain in solution, allowing their determination. Consequently to prevent against loss of analyte, all tissue homogenates were prepared in pH 9 0,2 mol/L phosphate solution.

Plasma, like most biological samples, contains salts, mainly sodium chloride at a concentration of about 1%, which causes band spreading in capillary electrophoresis due to the low field strength especially when the sample volume exceeds 1-2% of the capillary volume. In order to adjust sample conductivity different treatments such as mixing a sample with organic solvent or liquid-liquid extraction/microextraction resulting in matrix simplification and salts removal are commonly used. In order to significantly decrease sample conductivity I utilized

the mixing of the sample with acetonitrile (ACN) in procedures for homocysteine (Hcy) and cysteamine (CASH) in plasma [**H1**, **H2**], tiopronin (TP) in urine [**H4**] and cysteine (Cys) and glutathione (GSH) in orange juice [**H3**] determinations. During development of above mentioned methods the volume ratio of ACN to sample was checked. I noticed that sample mixed with ACN in the ratio of 1:2 (v/v) was optimal and is consistent with previously published experiments [10]. In my methodologies for homocysteine thiolactone (HTL) [**H6**] and lipoic acid (LA) [**H10**] determination in urine conductivity of the sample was effectively corrected by removal of inorganic salts by selective liquid–liquid extraction. Extraction of HTL from urine samples was performed with chloroform–methanol mixture (2:1; v/v), whereas extraction of LA from urine samples was carried out with acetone. Since HTL in slightly alkaline conditions (pH 8) does not possess a charge (the pKa of its amino group is 6.67 [11]), it could be extracted into organic phase. After centrifugation the chloroform layer was transferred to polypropylene vial followed by evaporation to dryness. The residue was reconstituted in HCl (0,0075 mol/L, pH 5,5) in order to allow electrokinetic injection (charge required on HTL) into the capillary.

Thiols became the most popular compounds in my studies. Thiols are very reactive compounds due to the presence of sulfhydryl group in their molecules, play significant role in several biological and pharmacological processes and exhibit antioxidant properties. Cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) are considered to be the most important biological thiols (Fig.1).



Cysteine Homocysteine Glutathione

Figure 1. Structures of main biologically important thiol amino acids.

Cys and Hcy are relatively small molecules differing in structure by the presence of an additional methylene group in the Hcy molecule. GSH (γ-glutamylcysteinylglycine) is tripeptyde consisted with γ-glutamyl acid, glycine and Cys which is the source of sulfhydryl group. All natural biological thiols such as Hcy, Cys and GSH are the products of the amino acid and sulfur metabolism of the methionine typically obtained from diet. Hcy plays a main role in methionine metabolism because it determines synthesis of other amino acids and additionally is the precursor for re-methylation to methionine. Hcy is transformed through the transsulfuration pathway to Cys that is the precursor for GSH synthesis [12, 13].

Lipoic acid (LA) is another disulfide-containing natural compound and is also known as 6,8-dithioctic acid [14]. LA is rapidly absorbed from the organism's diet, transported, taken up by cells and reduced to dihydrolipoic acid (DHLA) in various tissues [15]. LA together with DHLA form an ideal antioxidant couple. LA is able to recycle other natural antioxidants, especially is capable of reducing the oxidized forms of vitamin C, α-tocopherol, GSH, and Q10 coenzyme [16]. LA has been often used for prevention and treatment of various diseases associated with oxidative stress, including diabetes, cardiovascular diseases, neurodegenerative disorders, HIV infection, and cancer [17]. Most problems related to sampling, handling, deproteinization, extraction, derivatization and procedures for LA determination in various samples were in details described with my contribution in review paper [**H8**]. It should be emphasized that topics presented in this paper although concerning the LA determination, are universal and are also applicable for determination of other sulfhydryl group containing compounds in biological samples.

Cysteamine (CASH) and N-(2-mercaptopropionyl)-glycine (TP) are thiols commonly used as drugs in the treatment of many diseases. CASH appears to be a promising treatment for cystinosis. It depletes cystine from cystinotic cells and is used for treatment of children with nephropathic cystinosis [18], a rare autosomal recessive disease characterized by an excessive intra-lysosomal cystine accumulation in some organs (e.g. cornea, kidney, muscle). In affected children, cystine accumulation is also responsible for delayed complications such as hypothyroidism, retinopathy, myopathy, pancreatic dysfunction and dementia [19]. The thiol group in CASH makes it a potential antioxidant in oxidative stress conditions such as after radiotherapy [20]. Tiopronin (TP) is a synthetic thiol drug used in clinical applications for the treatment of cystinuria, rheumatoid arthritis, hepatic disorders as well as a mucolytic in respiratory disorders [21].

Thiols can easily be ionized on their functional (amino or carboxylic) groups and are thus possible to effectively separate in an electric field by using electromigration techniques, usually CZE or MEKC [6]. In biological systems, thiols are either free or bound to proteins.

The free thiols can be further divided into reduced or oxidized forms. However, the most commonly determined parameter is total thiol content, that is the combination of free reduced, free oxidized, and protein-bound thiols. Total thiols are measured in properly prepared sample treated on the beginning with reducing agent, thereby releasing the thiols from proteins and reducing the free oxidized forms. In order to determine total thiols for reduction of disulfide linkages I used commonly known reductive agents such as tributylphosphine (TBP) for CASH in plasma [**H2]** and tris(2-carboxyethyl)phosphine (TCEP) for Hcy in plasma [**H1**, **H5**], TP and LA in urine [**H4**, **H10**] as well as Cys and GSH in orange juice [**H3**].

Thiols, thiosulfates and sulfides do not possess structural properties (suitable chromophores) necessary for the production of signals compatible with common as UV absorbance detector. Two practical solutions could be implemented, direct detection at low wavelengths of the UV region or derivatization of an analyte. In the majority of procedures for determination of compounds containing -SH group advantage of the last one has been commonly taken. The only exceptions are aromatic thiols which absorb UV radiation in the range of 250-280 nm [22, 23]. The two peptide bonds in the GSH molecule barely allow direct UV detection in the range of 190-220 nm. The simpler thiols which slightly absorb below 200 nm can be analyzed only after derivatization [8]. The derivatization reaction is accompanied by bathochromic shift from reagent maximum to that of derivative. The absorption maximum shift is analytically advantageous because in general the higher detection wavelength the fewer potentially interfering compounds. Thanks to this phenomenon, it is possible to apply a large excess of derivatization reagent in order to drive the derivatization reaction to completion (in real world sample) and avoid appearance of peak of unreacted reagent on the electropherogram. Additionally, the derivative obtained during reaction is usually characterized by higher stability when compared with unprocessed analyte. In the case of thiols, derivatization reaction also protect very reactive -SH group against undesirable oxidation reactions to disulfides, which could occur before and during sample analysis. Other reason for derivatization concerns incorporation of a suitable charge into the analytes [24]. Finally modified analyte is obtained which possesses physico-chemical properties compatible with actual analytical technique. It should be noted that reported by some authors limit of detection (LOD) is not the lowest derivatizable concentration but is obtained by extrapolation of observed signal at much higher concentration or after dilution of yet derivatized compound. This way of presentation of LOD ignores poorer derivatization efficiency at low analyte concentration. It is well known that derivatization of subnanomolar concentrations of analyte is not possible with the use of most derivatization reagents.

Halogensulfonylbenzofurazan reagents, monobromobimane and onium salts are very popular derivatization reagents with activated halide. Maleimides, dialdehydes, quinones, enons and aziridines are also frequently used for determination of thiols [25, 26]. Two onium salts, 1-benzyl-2-chloropyridinium bromide (BCBP) and 2-chloro-1-methylquinolinium tetrafluoroborate (CMQT) were utilized in my experiments. These reagents were synthesized in this laboratory according to previously described procedures, BCPB by quaternization of 2-chloropyridine with benzyl bromide [27] and CMQT by quaternization of 2-chloroquinoline with trimethyloxonium tetrafluoroborate [28]. The derivatization scheme takes advantage of great susceptibility of the pyridinium/quinolinium molecule at 2 position to nucleophilic displacement and the high nucleophilicity of the thiol group (Fig.2). Most of compounds in the center of my attention besides -SH group also contain carboxylic and amino groups. It was proved that this group of derivatization reagents in aqueous solution selectivity react with thiols. Additionally during thiol derivatization with the use of CMQT or BCPB the only one derivative is formed.

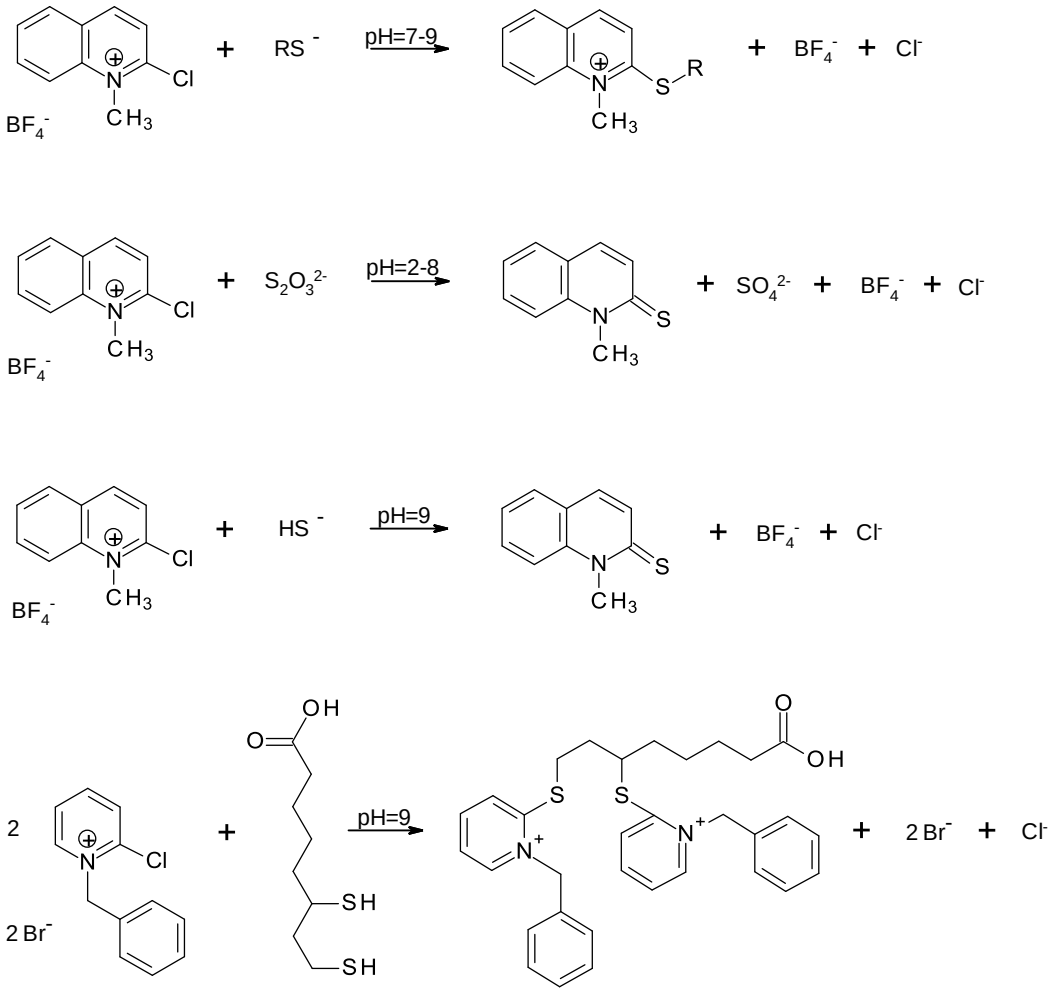


Figure 2. Reaction schemes of thiols, thiosulfate and sulfides with CMQT and dihydrolipoic acid with BCPB.

Derivatization reaction was conducted to introduce to the compound structure not only chromophore [**H1**-**H5**, **H7**-**H10**], but also permanent positive charge on quaternary nitrogen atom of the pyridinium or quinolinium moiety regardless of pH of BGE [**H1**-**H5**, **H10**]. Depending on the presence of ionizable amine and carboxylic groups in the compound structure, the net charge of the derivative as a whole changes with changing pH of the environment (Table 1). For example, the net positive charge of quinolinium derivatives of Hcy and Cys approaches +2 in strongly acidic conditions owing to protonation of the amine group and ion suppression of the carboxylic group. The net positive charge shows a dropping trend to 0 with pH increase. It should be emphasized, that the derivatization reaction with the use of CMQT or BCPB reagents, leads to the formation of a stable thioethers and blocks the labile thiol group.

Table 1. Optimized parameters of sample preparation procedures.

Derivative	**Sample preparation procedure**	**Derivative charge**	**Literature**
Hcy-CMQT	0.2 mol/L phosphate buffer, pH 7.6; reduction: 0.5 mol/L TCEP, temp. 25°C; time 30 min; derivatization: 0.1 mol/L CMQT, time 4 min	from +2 to 0	[**H1**, **H5**]
Cys-CMQT	2 mol/L Tris/HCl, pH 7.6; reduction: 0.5 mol/L TCEP, temp. 25°C; time 15 min; derivatization: 0.1 mol/L CMQT, time 5 min 0.2 mol/L phosphate buffer, pH 7.6; reduction: 0.5 mol/L TCEP, temp. 25°C; time 30 min; derivatization: 0.1 mol/L CMQT, time 4 min	from +2 to 0	[**H3**] [**H5**]
GSH-CMQT	2 mol/L Tris/HCl, pH 7.6; reduction: 0.5 mol/L TCEP, temp. 25°C; time 15 min; derivatization: 0.1 mol/L CMQT, time 5 min	from +2 to -1	[**H3**]
CASH-CMQT	2 mol/L Tris/HCl, pH 7.6; reduction: 2 mol/L TBP, temp. 60°C; time 30 min; derivatization: 0.1 mol/L CMQT, time 4 min	from +2 to +1	[**H2**]
TP-CMQT	0.2 mol/L phosphate buffer, pH 7.6; reduction: 0.1 mol/L TCEP, temp. 25°C; time 5 min; derivatization: 0.1 mol/L CMQT, time 4 min	from +1 to 0	[**H4**]
MTQ (H_2S + CMQT)	0.2 mol/L phosphate solution, pH 9; homogenization: mechanical (6000 rpm), time 3 min; derivatization: 0.1 mol/L CMQT, time 3 min	0	[**H7**]
MTQ ($S_2O_3^{2-}$ + CMQT)	0.2 mol/L phosphate buffer, pH 2; derivatization: 0.1 mol/L CMQT, time 3 min	0	[**H9**]
LA-BCPB	extraction: with acetone (1:9, v/v), time 2 min; 0.025 mol/L phosphate buffer, pH 9; reduction: 0.25 mol/L TCEP, temp. 25°C; time 5 min, derivatization: 0.1 mol/L BCPB, time 15 min	from +2 to +1	[**H10**]

In the case of electromigration methods, derivatization reaction can be implemented either before the separation of sample components (pre-column derivatization) or immediately after their separation (post-column derivatization). The derivatization methods can be also divided in pre-capillary, on-capillary and post-capillary derivatization. On-capillary derivatization can be realized on the capillary inlet (at-inlet), in the middle of the capillary in permeating zones (zone-passing) or on entire length of the capillary (throughout-capillary) [23]. In the developed procedures [**H1-H5**, **H7**, **H9**, **H10**], pre-separation derivatization reaction was successfully utilized. 2-S-quinolinium [**H1-H5**, **H7**, **H9**] and 2-S-pyridinium [**H10**] derivatives of determined compounds were characterized by favorable spectroscopic and electrophoretic properties. Formed derivatives exhibit bathochromic shift from reagent maximum to that of derivative (Fig.3). Bathochromic shift values ranged from 20 to 40 nm regarding to derivatization reagent and an analyte. Due to this phenomena, high derivatization reagent excess needed for biological samples could be applied. Possibility to measure the peaks at significantly higher wavelength than that of reagent maximum resulted in absence of the derivatization reagent signal on the electropherogram.

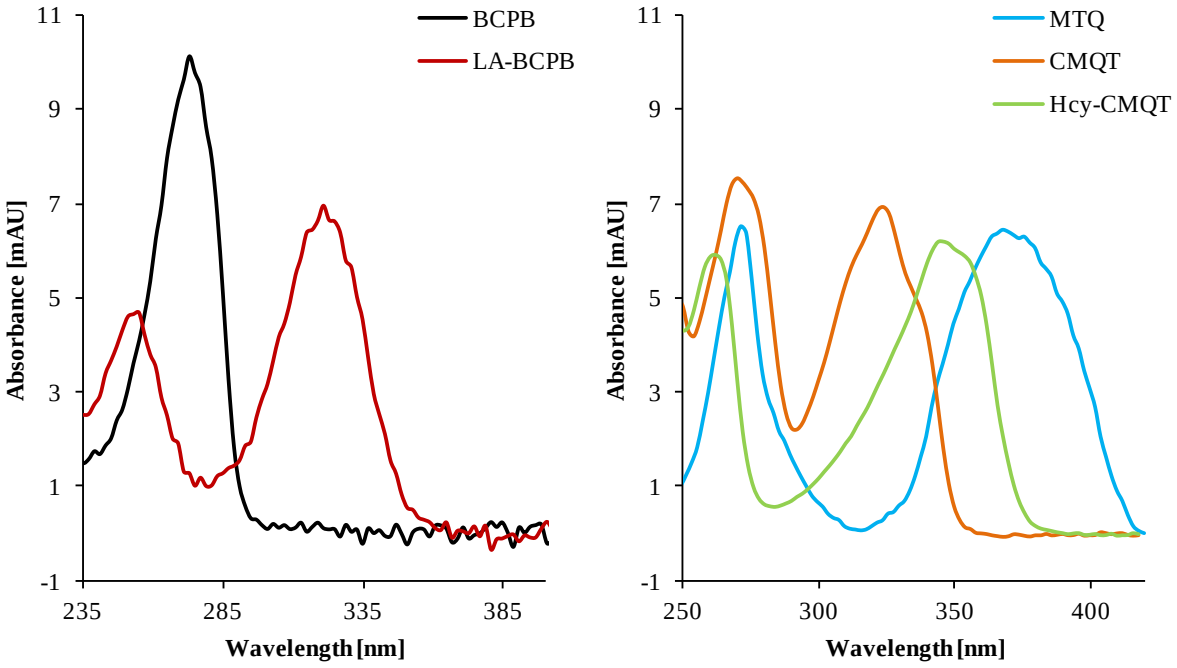


Figure 3. Spectra comparison of derivatization reagents (CMQT and BCPB) and their derivatives.

The derivatization reaction yield was optimized in terms of temperature, pH, reagent excess and time. Optimal reaction pH for derivatization of Hcy, Cys, GSH, CASH and TP with CMQT occurred in the pH 7.6 [**H1-H5**], for derivatization of hydrogen sulfide with CMQT in pH 9 [**H7**], while for derivatization of thiosulfates with CMQT in the wide pH range between 2-8 [**H9**]. The derivatization reagent (CMQT) reacts with the thiosulfate to form stable

derivative 1-methyl-2-thioquinolone (MTQ). Despite the fact that normal human urine should not contain hydrogen sulfide which in alkaline conditions also reacts with CMQT to form MTQ, we strongly recommend to provide derivatization reaction at pH 2. Optimal reaction pH for derivatization of LA with BCPB occurred in the pH 9 [**H10**]. I confirmed that in room temperature CMQT (in fivefold excess) reacts quickly (3-5 min) with Hcy, Cys, GSH, CASH, TP, hydrogen sulfide and thiosulfates, while BCPB (in fivefold excess) reacts with LA at 25°C but in much longer time (15 min).

Optimization of electrophoretic separation conditions and on-capillary stacking

Electromigration techniques, including CE, have several advantages over HPLC which is commonly used throughout separation techniques. High resolution and efficiency, relatively short analysis time and small amount of chemicals needed are commonly cited advantages of CE. Unfortunately, the most known drawback of electromigration techniques concerns low concentration sensitivity. During my experiments CE apparatus equipped with UV-Vis diode-array detector, known of its low sensitivity, was utilized. The most of analytes in the center of my interest are present in biological fluids at very low concentration. Thus, I decided to implement, besides derivatization reaction, some of very popular in-line preconcentration techniques. Analytes concentration can be obtained by long injection of sample zone into the capillary and then concentration of the analyte in a narrow zone using some electrophoretic effects. Manipulation of the sample or BGE conductivity is not related to the modification of commercial CE apparatus. Different stacking techniques, such as transient-pseudo isotachophoresis (*tp*ITP) [**H1**-**H4**], pH-mediated sample stacking [**H5**], field amplified sample injection (FASI) [**H10**], sweeping MEKC [**H7**, **H9**] and field amplified sample injection and sweeping MEKC [**H6**] were used during my investigations.

Preconcentration and separation processes are directly related, occur in the same place and time or directly one after the other. Therefore, conditions of sample components preconcentration and separation were simultaneously optimized. Several parameters were considered for optimization, including type, concentrations and pH values of the BGE, organic solvent addition as well as temperature during separation. BGE concentration (ionic strength) used is limited by capillary dimensions (length, inner diameter), the thermostating system efficiency and the applied voltage as well. Selection of the appropriate BGE concentration was not easy because it also influenced the analytes stacking. Optimal BGE/sample solution conductivity ratio used was crucial to generate higher electric field in the sample zone and to promote analytes concentration on the BGE/sample boundary. Buffer pH was selected in order to obtain the most favorable charge to mass ratio of derivative. Therefore, low pH of BGE (0.2-

0.25 mol/L Tris/HCl, pH 2.0-2.1 or 0.1 mol/L lithium acetate, pH 4.75) was selected for efficient separation of quinolinium derivatives of Hcy, Cys and CASH in plasma [**H1**, **H2**, **H5**], Cys and GSH in fruit juices [**H3**] and TP in urine [**H4**]. Determination of LA in the form of pyridinium derivative was performed in 0.05 mol/L borate buffer at pH 9 [**H10**]. BGEs mentioned above were used without any addition of organic modifiers which caused decrease in resolution and lower sample stacking efficiency. After derivatization reaction of hydrogen sulfide or thiosulfate with CMQT the same derivative 1-methyl-2-thioquinolone (MTQ) is formed which does not possess charge. Thus MEKC technique, invented for separation of neutral compounds, has been chosen for determination of thiosulfate in urine [**H9**] and hydrogen sulfide in hen tissues [**H8**]. The most effective separation was obtained in alkaline (pH 8) 0.05-0.055 mol/L phosphate solutions with the addition of 0.035-0.040 mol/L SDS and 25-26% ACN).

Appropriate selection of the separation capillary was not without significance. Only uncoated fused-silica capillaries with an inlet to detector length of 52-64.5 cm and different inner diameter of 50-100 μm were served as a separation columns. The capillary length and inner diameter were selected in order to decrease current generated and limit Joule heating. Effective heat removal can be obtained for capillaries of small diameter in which high ratio of surface to volume is assured. Hence, high voltage can be applied and consequently higher resolution and shorter analysis time are achieved.

Transient-pseudo isotachophoresis (*tp*ITP)

This kind of analytes concentration inside the capillary is also called acetonitrile-salts stacking. It was used for the first time for electrophoretical analysis of biological samples by Shihabi [10, 29, 30]. Biological samples contain high concentration of salts (more than 1%), mainly sodium chloride. Historically, isotachophoresis (ITP) is one of the oldest forms of electrophoresis and is particularly useful for preconcentration as it allows minor sample components to be stacked in the presence of a large concentrations of other ions [31]. Shihabi observed that separations from serum samples deproteinized with ACN were highly efficient and found that the presence of sodium chloride was not detrimental but actually a key requirement of this form of stacking. In this technique sample (e.g. plasma) is mixed with organic solvent in the proper volume ratio. Next, the large volume of sample (up to 30% of the capillary volume) is introduced into the capillary. The probable stacking mechanism is proposed to be transient pseudo-isotachophoresis (Fig.4). Under ITP conditions all analytes move at the same velocity when the steady state is achieved, however, the speed is dictated by the mobility of the leading ion and its concentration. A terminating ion supplies high field

strength and is critical to keep the analyte in the band and prevents its diffusion. ACN can act as a pseudo-terminating ion (origin of technique name) by supplying the high field strength needed to accelerate migration of the analytes without being an ion itself.

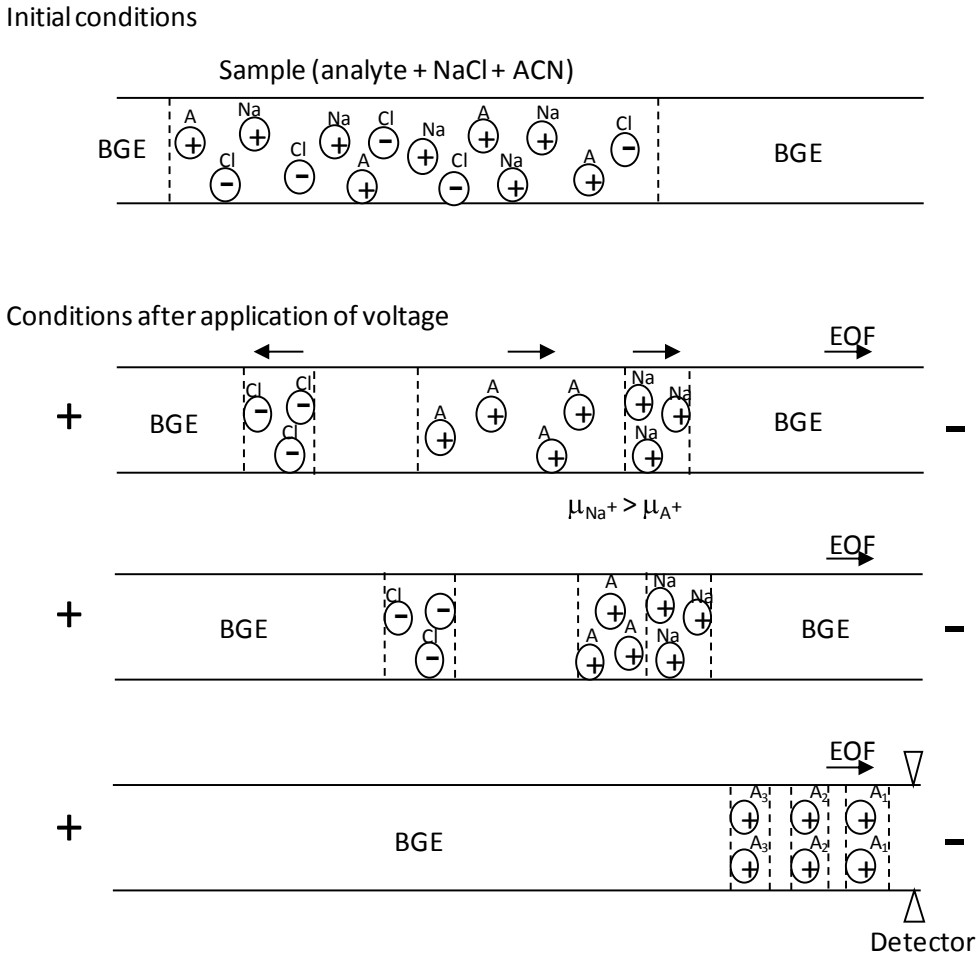


Figure 4. Transient-pseudo isotachophoresis mechanism [32].

As soon as the voltage was turned on, the small cations (ions of salts) moved rapidly due to their high mobility and the presence of low conductivity organic solvent. Next they decelerate on the boundary between the sample and BGE. Two regions of different field strength are formed, high field one in ACN and low field one in a salt. At this time the analytes are accelerated in the direction of appropriate electrode and undergo stacking after they reach low field strength zone. Although the processes involved are not fully understood, but the rational for calling this type of stacking pseudo-transient isotachophoresis is that small inorganic cations act as leading ions, while ACN acts as pseudo-terminator.

*tp*ITP mechanism was used by me in some procedures for stacking Hcy and CASH in plasma [**H1**, **H2**], TP in urine [**H4**] as well as Cys and GSH in orange juice [**H3**].

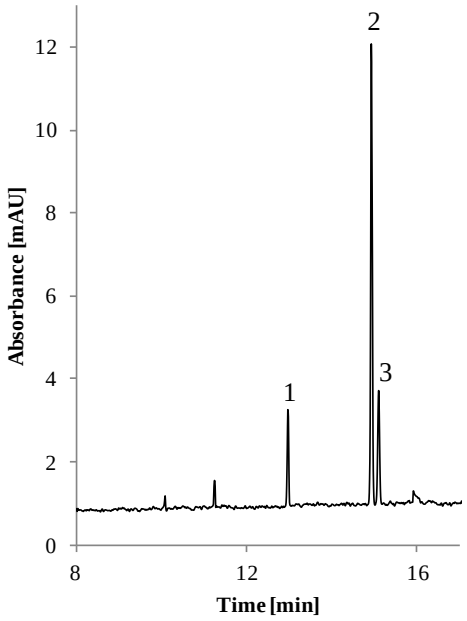


*Figure 5. Electropherogram of urine sample obtained under transient-pseudo isotachophoresis conditions. Peaks: 1 - CMQT, 2 - Cys-CMQT, 3 - TP-CMQT (40 μmol/L). Electrophoretic conditions: fused-silica capillary (l=56 cm, 50 μm), BGE (0.25 mol/L Tris/HCl buffer, pH 2), voltage 30 kV, analytical wavelength λ=350 nm, temperature 25°C, injection volume (20 nl). Modified Fig.2 in [**H4**].*

ACN as low conductivity organic solvent was used during these experiments. Appropriate sample to ACN volume ratio (for all matrices) as well as amount of salt in the sample sufficient to generate stacking were tested. I confirmed that the optimal sample to ACN volume ratio is equal 1:2 (v/v). Increased salt concentrations in the sample only resulted in higher current, but not influenced height or area of the peak. Next, the sample amount higher than 1% of capillary volume was hydrodynamically injected into the capillary. Due to significant differences in the sample composition (plasma, urine, neat fruit juice) volumes of introduced sample were optimized individually during each method development. Finally, sample injection volume ranged from 40 nl to 60 nl (4%-6% of capillary volume). The sample matrix as well as the capillary length were the main factors affecting current and thus Joule heating generated inside the capillary. Larger sample volumes resulted in analytical signals broadening, significantly decrease resolution and led to unstable or lack of current.

The new CE method with acetonitrile-salts stacking (*tp*ITP) was successfully applied for determination of total Cys and GSH in fresh orange juices and commercial orange beverages [**H3**]. To the best of our knowledge, only two papers describing the methods for determination of Cys and GSH in juices (one of them in Chinese) with the use of electromigration technique have been reported in the literature at that time [33, 34]. Measured concentrations were summarized in Table 2.

*Table 2. Total Cys and GSH profiles of fruit juices. Prepared on the basis of Table 2 in [**H3**].*

Juice origin	Measured ± SD [μmol/L]	
	Cysteine	**Glutathione**
Orange juices		
Garden	1.8 ± 0.1	11.6 ± 0.2
Tymbark	1.6 ± 0.1	16.6 ± 0.1
Fortuna	2.1 ± 0.1	22.7 ± 0.3
Cappy	2.1 ± 0.1	23.4 ± 0.3
Neat orange juice	6.5 ± 0.2	44.8 ± 0.2
Grape juice		
Garden	Not detected	Not detected
Apple juice		
Fortuna	Not detected	Not detected

pH-mediated sample stacking

Biological sample is often a solution of high conductivity and thus very difficult to analyze by electromigration techniques. Low conductivity of the sample for its further stacking inside the capillary can be obtained by efficient pH manipulation of subsequently introduced solutions. New method was developed for simultaneous determination of total Hcy and Cys in human plasma [**H5**]. Relatively long fused-silica capillary (l=91.5 cm, L=100 cm, 75 μm) and BGE consisted of 0.1 mol/L lithium acetate (pH 4.75) have been chosen during method optimization in order to inject sample volume as large as possible and apply maximum voltage. In pH-mediated sample stacking, invented in Lunte's laboratory [35], in the case of cations (2-S-quinolinium derivatives of Hcy and Cys), a large volume of sample is electrokinetically injected into a capillary (20 kV and 60 s were used). Simultaneously, anions of BGE (acetate) that fills the capillary migrate in the opposite direction, which is towards the capillary inlet, displacing the anions of strong acid present in the plasma sample. When a sample injection is accomplished, the sample zone is composed exclusively of 2-S-quinolinium derivatives of Hcy and Cys and BGE anions. Next, a large zone (20 kV, 96 s) of strong acid (0.1 mol/L HCl) is electrokinetically injected into the capillary. H^+ ions from the acid titrate acetate anions in the sample zone, the conductivity of which significantly decreases and as a result a strong electric field appears and rapid growth in the ion migration velocity is observed. When ionic analytes reach the boundary between the sample zone and the BGE, the electric field strength decreases, the migration velocities are decreased and analytes undergo stacking. Next, the separation proceeds according to the CZE mechanism. This simple, accurate and reliable method allows for efficient determination of Hcy, Cys as well as cysteinylglycine (CysGly) in human plasma (Fig.6).

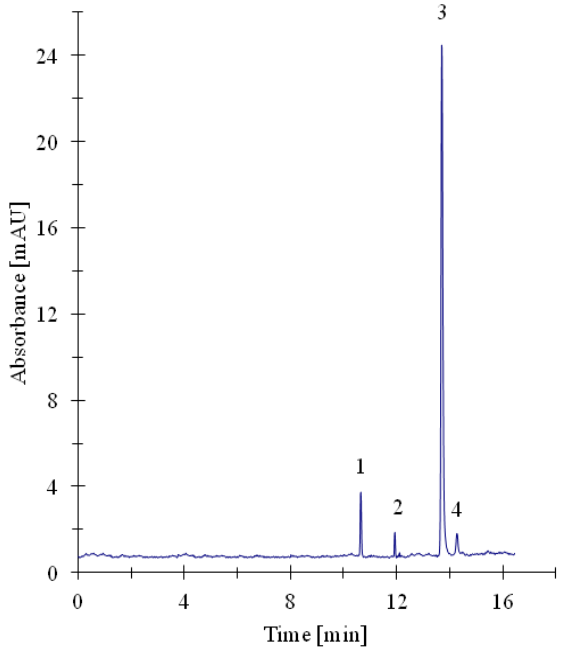


*Figure 6. Representative electropherogram of plasma sample obtained under pH-mediated sample stacking conditions. Peaks: 1 - CMQT, 2 - Hcy-CMQT (6.48 μmol/L), 3 - Cys-CMQT (159.7 μmol/L), 4 - CysGly-CMQT. Electrophoretic conditions: fused-silica capillary (l=91.5 cm, 75 μm), BGE (0,1 mol/L lithium acetate buffer, pH 4.75), voltage 30 kV, analytical wavelength λ=355 nm, temperature 25°C, sample injection (20 kV, 60 s). Modified Fig.2 in [**H5**].*

It should be emphasized, that at that time to the best of our knowledge there was no capillary electrophoresis procedure with pH-mediated sample stacking for simultaneous determination of Hcy and Cys in body fluids. The method was used for determination of Hcy in plasma samples from five apparently healthy volunteers (three men and two women) [**H5**]. The total Hcy concentrations determined are in good agreement with Hcy levels reported in literature and also with those established with the use of my other work using *tp*ITP stacking [**H1**]. Total Hcy content determined in plasma by both procedures [**H1** and **H5**] are summarized in Table 3.

*Table 3. Total Hcy content in human plasma determined by two CE methods [**H1**, **H5**].*

Sample number	Sex (M, F)	Hcy content ± SD [μmol/L]	Precision RSD[a] [%]
*Transient-pseudo isotachophoresis stacking [**H1**]*			
1	F	5.87 ± 0.23	3.4
2	M	6.83 ± 0.21	2.8
3	M	7.90 ± 0.22	2.6
4	M	9.88 ± 0.30	2.8
*pH-mediated sample stacking [**H5**]*			
1	M	4.95 ± 0.18	3.6
2	M	5.62 ± 0.24	4.2
3	M	6.48 ± 0.19	2.9
4	F	7.95 ± 0.28	3.5
5	F	8.56 ± 0.24	2.8

a) n = 4

Field amplified sample injection (FASI)

Electrokinetic sample injection with electric field amplification is considered as one of the simplest technique for sample stacking inside the CE capillary. This kind of sample preconcentration technique was utilized for LA determination in urine and described in the paper [**H10**]. FASI is characterized by important advantage, an analyte ions are electrokinetically injected into the capillary as a result of two combined phenomena, i.e. electrophoresis and electroosmosis. As a result cations from the sample, including 2-S-pyridinium derivative of LA, are mainly introduced to the capillary and migrate in the same direction as EOF. To the best of our knowledge there are only three other CE methods allowing LA determination in different matrices [36-38], but unfortunately there is no procedure for LA determination in urine. LA content was measured in urine samples from volunteers (collected 1 hour after the treatment) who received single 100 mg dose of LA. Urine concentrations of LA varied from 6.53 to 73.66 66 μmol/L. Obtained analytical results for LA concentrations in urine were also adjusted for creatinine (Crn) to correct for variable dilutions. Crn is a by-product of muscle metabolism, and it is normally released into the urine at a constant rate, which is a characteristic that makes it useful for gauging overall urine "dilution". Optimized electrophoretic procedure allowed to efficiently separate components of very complicated urine matrix and to determine LA as 2-S-pyridinium derivative. Under these conditions the LA derivative migrated as a well separated peak after 2.8 min (Fig.7).

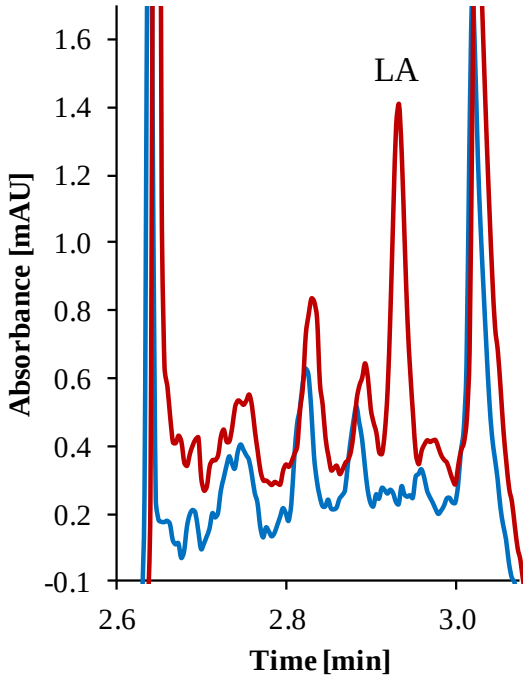


*Figure 7. Electropherograms of urine (blue line) and urine from donor after oral administration of LA (red line). Electrophoretic conditions: fused-silica capillary (l=51.5 cm, 75 μm), BGE (0.05 mol/L borate buffer, pH 9), voltage 20 kV, analytical wavelength λ=322 nm, temperature 20°C, sample injection (5 kV, 25 s). Modified Fig.2 in [**H10**].*

Sweeping MEKC

Thiosulfates as well as hydrogen sulfide were also identified during my experiments. These compounds, just like thiols, do not possess structural properties enabling their effective UV-Vis detection. Thus, derivatization reaction with the use of CMQT was utilized (Fig.2). CMQT reacts rapidly with hydrogen sulfide (pH 9) or with thiosulfates (pH 2-8) to form stable derivative 1-methyl-2-thioquinolone (MTQ), which do not possess charge at the whole pH range. Since MEKC was developed by Terabe for the separation of neutral compounds which all migrate according to EOF [39], this technique was used in my studies for determination of MTQ. Sweeping is one of many methods enabling the concentration of analytes in MEKC. The concentration efficiency depends on the analytes affinity to the micelles, i.e. the higher affinity, the higher concentration level [40]. In sweeping phenomena, under electric field micelles permeate into a sample zone and sweep analytes into a narrow zone (Fig.8). Sweeping MEKC technique was successfully utilized for the determination of hydrogen sulfide in hen tissues [**H7**] and thiosulfates in urine [**H9**].

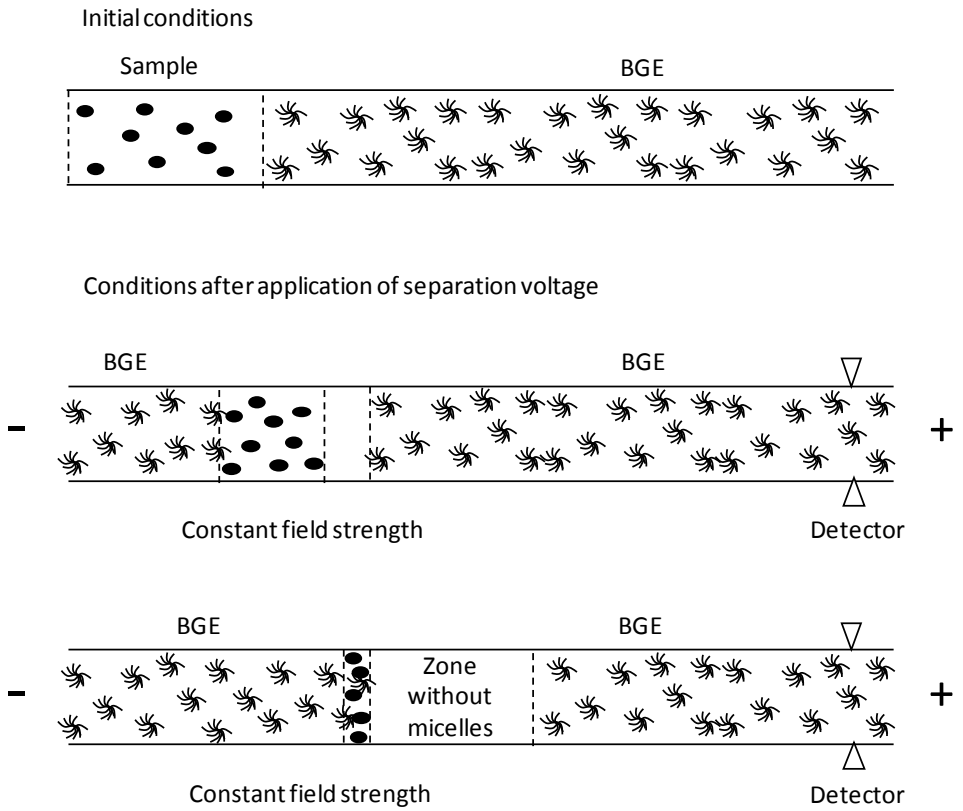


Figure 8. Sweeping mechanism [41].

Optimized analytical procedure consists of four essential steps: (1) before introduction of sample the capillary was flushed with appropriate electrolyte (hydrogen sulfide determination: 0.05 mol/L, pH 8 phosphate buffer with the addition of 0.04 mol/L SDS and 26% ACN; thiosulfates determination: 0.055 mol/L, pH 8 phosphate buffer with the addition of 0.035 mol/l SDS and 25% ACN); (2) the sample was hydrodynamically injected into the capillary at the anode end (hydrogen sulfide determination: 50 mbar for 50 s; thiosulfates determination: 50 mbar for 30 s); (3) when the separation voltage was applied (hydrogen sulfide determination: 18 kV; thiosulfates determination: 20.5 kV), analytes were swept into a narrow zone at the border between the BGE and the sample zone; (4) analytes were separated according to MEKC mechanism. Final electrophoretic conditions allow successfully separate matrix components (hen tissues, urine) and determine hydrogen sulfide and thiosulfates as MTQ derivative after 7.56 min and 10.1 min (Fig.9 and Fig.10), respectively. Tissue concentrations of hydrogen sulfide varied from 0.129 to 0.292 μmol/g depending on the kind of tissue studied. Urine concentrations of thiosulfate varied from 3.10 to 39.15 μmol/L and from 0.45 to 1.92 mmol/mol creatinine (Crn) after normalization against Crn.

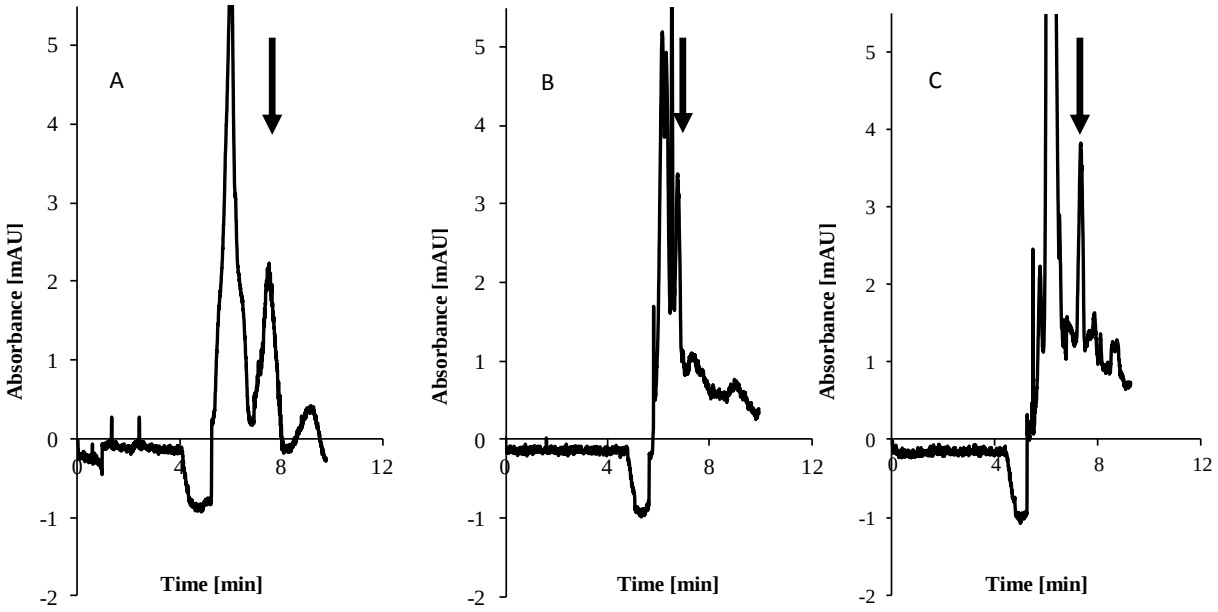


*Figure 9. Electropherograms of homogenized hen tissues: A: liver (0.22 μmol/g); B: stomach (0.19 μmol/g); C: heart (0.29 μmol/g). Electrophoretic conditions: fused-silica capillary (l=41.5 cm, 75 μm), BGE (0.05 mol/L phosphate buffer, pH 8 with the addition of 0.04 mol/L SDS and 26% ACN), voltage 18 kV, analytical wavelength λ=375 nm, temperature 30°C, sample injection (50 mbar, 50 s). Modified Fig.3 in [**H7**].*

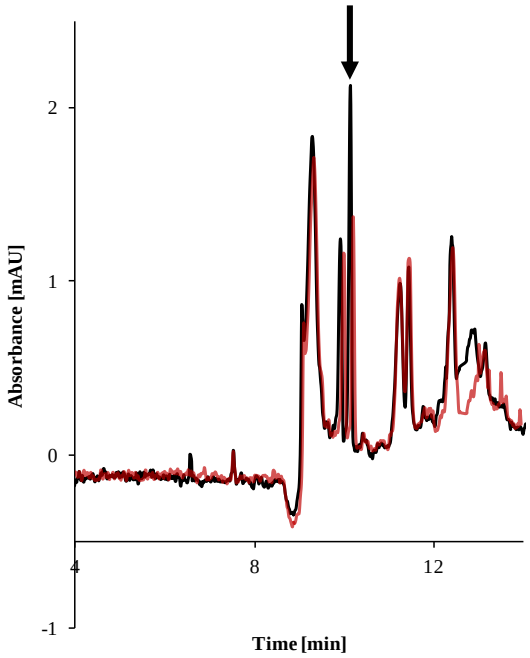


*Figure 10. Electropherograms of urine (red line) and urine spiked with 8 μmol/L of thiosulfate (black line). Electrophoretic conditions: fused-silica capillary (l=51.5 cm, 75 μm), BGE (0.055 mol/L phosphate buffer, pH 8 with the addition of 0.035 mol/L SDS and 25% ACN), voltage 20.5 kV, analytical wavelength λ=375 nm, temperature 25°C, sample injection (50 mbar, 30 s). Modified Fig.3 in [**H9**].*

We proposed new procedure [**H7**] which was the first capillary electrophoresis UV–Vis method allowing hydrogen sulfide determination in animal tissues. Besides our second

methodology [**H9**], there was only one other capillary electrophoresis UV-Vis method for the determination of thiosulfates in human [42].

Field amplified sample injection and sweeping MEKC

Other CE methodology developed during my studies utilizing two different mechanisms of analytes concentration inside the capillary. Field amplified sample injection (FASI) and sweeping MEKC (FASI-sweeping MEKC) was performed. It provided better sensitivity enhancement than the stacking with the use of the only one of above mentioned methods (Fig.11). HTL unlike previously described compounds at pH lower than 6.67 possess positive charge on amine group. Since HTL exhibit absorption of UV-Vis radiation it can be directly detected at 240 nm. Presented method was applied for HTL determination in human urine [**H6**].

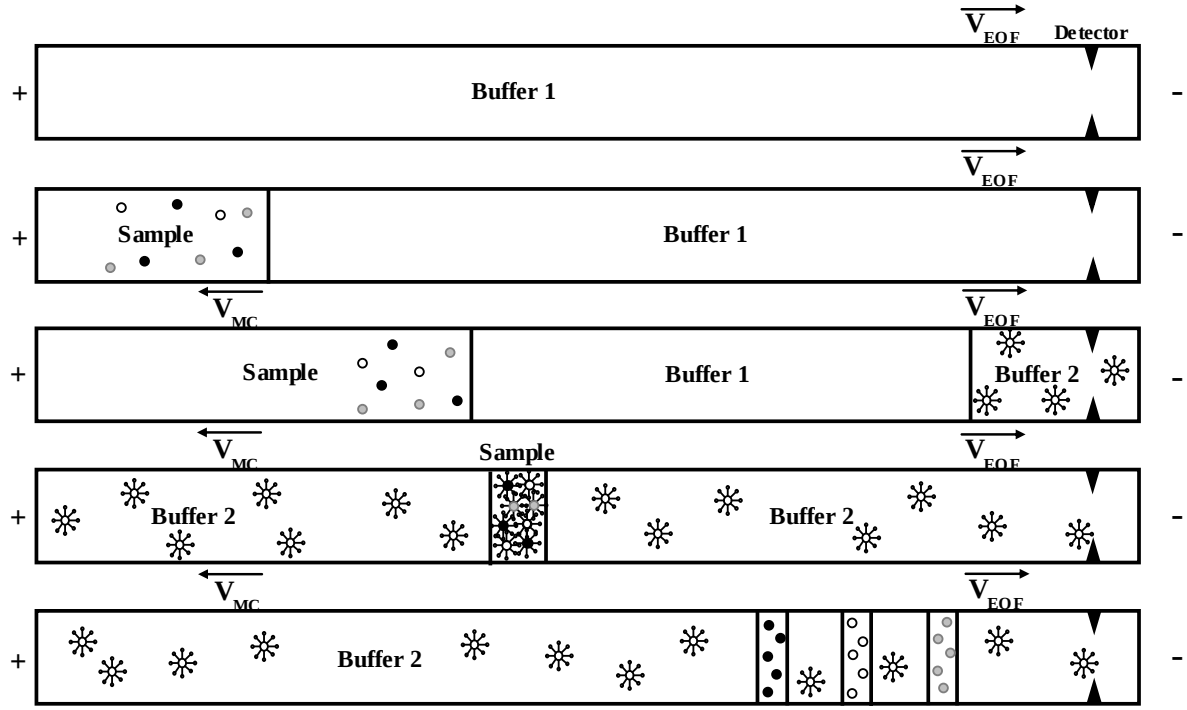


*Figure 11. Proposed mechanism of field amplified sample injection and sweeping MEKC of HTL. Modified Fig.2 in [**H6**].*

In this stacking method we used two different buffer systems. Initially, (a) the capillary was flushed with 100 mmol/L phosphate buffer, pH 6 (Buffer 1); (b) next, the sample in 0.0075 mol/L HCl (pH 5.5) was electrokinetically injected (15 kV for 20 s) into the capillary at the anode end. HTL shows in sample solution (pH 5.5) higher mobility than in Buffer 1 (pH 6). As a result, when HTL reaches the boundary between the sample zone and the Buffer 1, the migration velocity is decreased and analyte undergo stacking; (c) the buffer vials at the capillary ends were changed to these filled with 100 mmol/L phosphate buffer (pH 4.75) containing 15% ACN and 100 mmol/L SDS (Buffer 2); Taking into account EOF migration, the electrolyte pH was selected in order to HTL migration velocity slightly exceeded velocity

of micelles; (d) when the separation voltage of 20 kV was applied, the negatively charged SDS micelles entered into the capillary at the cathodic end. At the nearly retained EOF, micelles permeate into a sample zone sweeping and concentrating the analytes into a narrow zone at the border between the Buffer 2 zone and the sample zone; (e) finally, the analytes were separated according to MEKC using Buffer 2 and voltage of 20 kV. Presented procedure [**H6**] was implemented for determination of HTL in urine samples obtained from 15 healthy volunteers (Fig.12). Measured HTL content in urine varied from 0.129 μmol/L to 0.229 μmol/L (average concentration 0.170 ± 0.029 μmol/L). These HTL values are similar to the values obtained previously by HPLC-OPA derivatization methods [43, 44]. The identity of HTL peak was confirmed by its comigration with an authentic HTL, by comparison of its UV spectrum as well as by its signal disappearance after NaOH addition to the urine sample (HTL hydrolysis to Hcy).

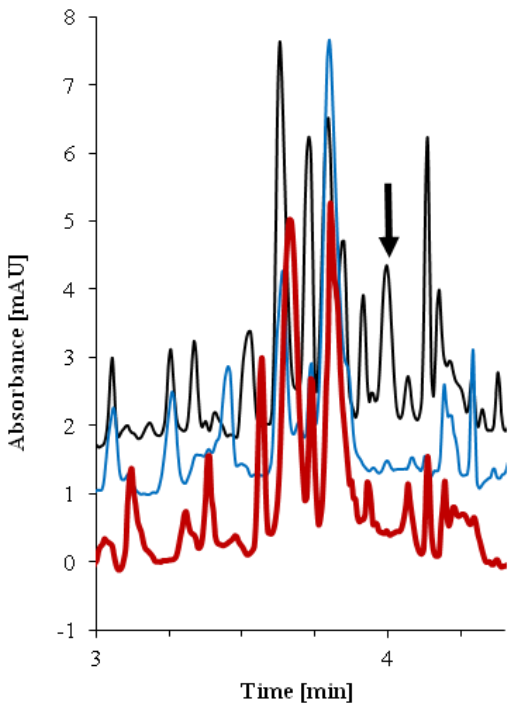


*Figure 12. Electropherograms of urine sample (blue line), urine spiked with 1 μmol/L of HTL (black line) and urine treated with 0.1 mol/L NaOH (red line). Electrophoretic conditions: fused-silica capillary (l=51.5 cm, 50 μm), BGE1 (0.1 mol/L phosphate buffer, pH 6), BGE2 (0.1 mol/L phosphate buffer, pH 4.75 with the addition of 0.1 mol/L SDS and 15% ACN), voltage 20 kV, analytical wavelength λ=240 nm, temperature 25°C, sample injection (15 kV, 20 s). Modified Fig.3 in [**H6**].*

The method is fast (HTL migration time short than 4 min), simple and reproducible. There was no need of analyte derivatization, whereas analytes preconcentration inside the capillary and direct UV detection were utilized. To the best of our knowledge this methodology was the first application of CE for determination of HTL in urine.

Methods validation and comparison of sensitivity enhancement factors

All presented methods were validated according to the criteria for biological sample analysis [45] under optimized experimental conditions. The linearity, limit of detection (LOD) and limit quantification (LOQ), precision expressed as relative standard deviation (RSD), accuracy expressed as recovery, and specificity were evaluated during validation protocol of the methods. Since all presented procedures involve sample stacking step, thus some calculations were performed to establish efficiency of an analyte concentration in the sample. LOD and LOQ are defined as signal to noise ratio of 3 and 6, respectively. Comparison of validation parameters for individual methods was presented in Table 4.

Table 4. Validation parameters for developed methods.

Matrix	Analyte	Linearity [μmol/L]	RSD [%]	Recovery [%]	LOD [μmol/L]	LOQ [μmol/L]	Literature
Plasma	Hcy	5.0 - 80	1.7 - 6.7	99.4 - 103.2	1.0	3.0	[**H1**]
Plasma	CASH	2.5 - 20	2.8 - 5.4	95.0 - 99.7	0.8	2.5	[**H2**]
Fruit juices	Cys GSH	2.5 - 30	3.8 - 6.7 1.1 - 3.6	94.4 - 105.2 94.7 - 107.7	1.0	2.5	[**H3**]
Urine	TP	5.0 - 160	1.2 - 7.1	96.8 - 101.2	1.5	5.0	[**H4**]
Plasma	Hcy Cys	2.0 - 20 20 - 300	2.5 - 8.0 1.1 - 1.8	98.8 - 103.7 99.0 - 100.6	0.7	2.0	[**H5**]
Urine	HTL	0.1 -1.0	3.2 - 6.1	94.7 - 101.8	0.09	0.1	[**H6**]
Tissues	H_2S	0.15 - 2.0 [a)]	2.4 - 6.3	95.7 - 110.8	0.05 [a)]	0.12 [a)]	[**H7**]
Urine	$S_2O_3^{2-}$	4.0 - 64	1.6 - 5.4	98.1 - 102.7	2.0	4.0	[**H9**]
Urine	LA	2.5 - 80	1.1 - 8.4	95.4 - 100.3	1.2	2.5	[**H10**]

a) concentration in [μmol/g]

There are several methods to express the efficiency of an analyte concentration in the sample. It could be for instance conveniently determined by comparing peak areas/heights obtained when following a conventional (short) injection with peak areas/heights obtained as a result of the method involving concentration. This kind of measure, known as sensitivity enhancement factor (SEF), is very simple, reliable and common. The SEF values reported in literature are different, usually vary from dozen to hundreds but sometimes are higher than several thousands. SEF mainly depends on a sample origin, procedure of sample preparation as well as stacking method. The SEF values calculated for my methods obviously differ from

each other but not so much (from 21.1 to 69.6). It is because biological samples with comparable complexity of matrices were analyzed and also similar steps in sample preparation procedure were utilized. The corresponding comparison is presented in Table 5.

Table 5. Comparison of sensitivity enhancement factor (SEF) calculated for developed methods.

Method	**Stacking mechanism**	**SEF**
Determination of Hcy in plasma [**H1**]	Stacking by transient pseudo-isotachophoresis (*tp*ITP)	50.5
Determination of CASH in plasma [**H2**]	Stacking by transient pseudo-isotachophoresis (*tp*ITP)	43.3
Determination of Cys and GSH in orange juice [**H3**]	Stacking by transient pseudo-isotachophoresis (*tp*ITP)	45.1
Determination of TP in urine [**H4**]	Stacking by transient pseudo-isotachophoresis (*tp*ITP)	39.6
Determination of Hcy and Cys in plasma [**H5**]	pH-mediated sample stacking	55.8
Determination of HTL in urine [**H6**]	Field amplified sample injection (FASI) and sweeping MEKC	69.6
Determination of hydrogen sulfide in hen tissues [**H7**]	Sweeping MEKC	51.3
Determination of thiosulfate in urine [**H9**]	Sweeping MEKC	59.5
Determination of LA in urine [**H10**]	Field amplified sample injection (FASI)	21.1

Main achievements

I have developed and applied new electrophoretic methods which utilize on-line sample stacking techniques for analysis of biological samples:

- the method for determination of homocysteine in plasma based on CZE and stacking by transient-pseudo isotachophoresis [**H1**],
- the method for determination of cysteamine in plasma based on CZE and stacking by transient-pseudo isotachophoresis [**H2**],
- the method for determination of cysteine and glutathione in orange juice based on CZE and stacking by transient-pseudo isotachophoresis [**H3**],
- the method for determination of tiopronin in urine based on CZE and stacking by transient-pseudo isotachophoresis [**H4**],

- the method for determination of homocysteine and cysteine in plasma based on CZE and pH-mediated sample stacking [**H5**],
- the method for determination of homocysteine thiolactone in urine based on field amplified sample injection and sweeping MEKC [**H6**],
- the method for determination of hydrogen sulfide in hen tissues based on sweeping MEKC [**H7**],
- the method for determination of thiosulfate in urine based on sweeping MEKC [**H9**],
- the method for determination of lipoic acid in urine based on CZE and field amplified sample injection [**H10**].

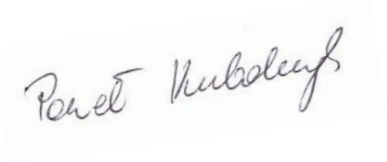
Bearing in mind the use of capillary electrophoresis for analysis of biological samples that have very complicated matrices, I have optimized listed below procedures:

- for plasma preparation for determination of homocysteine [**H1**, **H5**], cysteine [**H5**] and cysteamine [**H2**],
- for urine preparation for determination of tiopronin [**H4**], homocysteine thiolactone [**H6**], thiosulfate [**H9**] and lipoic acid [**H10**] contents,
- for solid tissues preparation for determination of hydrogen sulfide [**H7**],
- for orange juice preparation for determination of cysteine and glutathione [**H3**] levels.

6. Brief presentation of other achievements

My other scientific achievements consist of 11 original and 2 review articles as well as 6 chapters in monographs (3 in English and 3 in Polish). I'm also coauthor of dictionary entitled "Chromatografia i techniki elektromigracyjne", prepared under editorship of Z. Witkiewicz and E. Śliwka. The majority of my scientific achievements have been presented on 54 international and national scientific conferences. The subject of my other scientific achievements concerned development of a new electrophoretic or chromatographic methods for the determination of compounds which possess -SH group in biological samples. Different sample preconcentration techniques in these analytical procedures were implemented. I have received seven times, in years 1998, 1999, 2007, 2009, 2011, 2012, 2015, first degree team award from the Rector of the University of Lodz. In 2013 and 2016 I was a member of the organizing committee of the national chromatographic conference "Zastosowanie technik chromatograficznych w analizie środowiskowej i klinicznej". In the years 2012-2016 I acted as the secretary of the Faculty Electoral Commission, and since 2008 to the present I am a member of the Faculty Committee for Diploma Exams. I have also actively participated in the popularization of science, inter alia through the lecture for upper secondary school students in

the *Akademia Ciekawej Chemii*, an educational program organized annually by the Faculty of Chemistry of the University of Lodz. A detailed list of other achievements is provided in the Appendix 3.



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