Brazilian Journal of Analytical Chemistry
2023, Volume 10, Issue 39, pp 17-51
doi: 10.30744/brjac.2179-3425.RV-13-2022

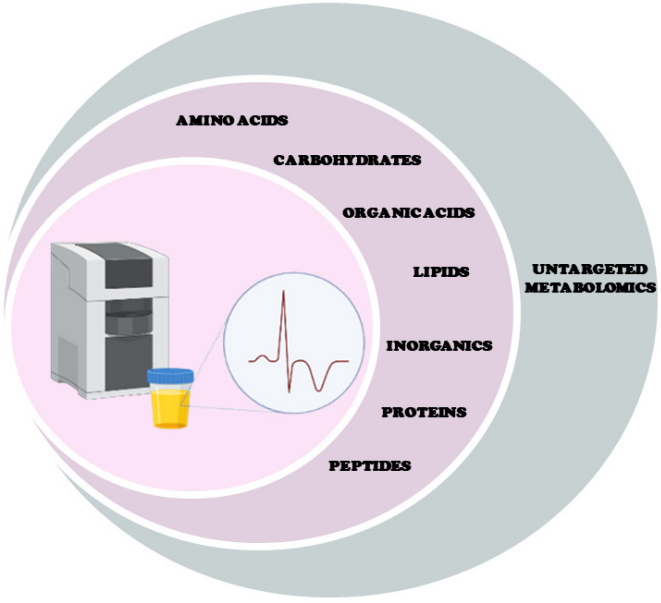
BrJAC

REVIEW

Capillary Electrophoresis Applied to Human Urine Analysis for Clinical Diagnosis: New Trends and Perspectives

Olívia Brito de Oliveira Moreira[1,2], Jéssica Cordeiro Queiroz de Souza[1,2], João Marcos Beraldo Candido[1,2], Maria Patrícia do Nascimento[1,2], Eduarda Alves Penna[1,3], Paula Rocha Chellini[1,3], Marcone Augusto Leal de Oliveira[1,2,4]

[1]Grupo de Química Analítica e Quimiometria. Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora (UFJF), 36036-900, Juiz de Fora-MG, Brasil
[2]Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora (UFJF), 36026-900, Juiz de Fora-MG, Brasil
[3]Faculdade de Farmácia, Universidade Federal de Juiz de Fora (UFJF), 36026-900, Juiz de Fora, MG, Brasil
[4]Instituto Nacional de Ciência e Tecnologia de Bionalítica – INCTBio, Instituto de Química, Universidade Estadual de Campinas (UNICAMP), 13083-970, Campinas, SP, Brasil



Capillary electrophoresis (CE) is an electromigration-based separation technique that emerged in the early 90s and has been evolving since then. Over recent decades, the association of CE with new technologies and detections strategies is enhancing its intrinsic mightiness. Today, CE is a part of the main high-throughput analytical platform used for medical-related applications, together with traditional gas chromatography, high-performance liquid chromatography, and fingerprinting spectroscopic methods. New strategies for assessing human disorders involve a thorough analysis of bodily fluids through said instrumentation. In this context, human urine is considered an excellent alternative, for being rich in information and easily collected with minimal inconvenience for the doners. Overall, since it is an ultrafiltrate of the human bloodstream, urine composition should be changed if any condition jeopardizes human homeostasis. Thus, monitoring the levels of biomarkers in urine by an advantageous technique such as CE can be an interesting choice for diagnostic and other clinical purposes. In this review, we will be commenting on the new tendencies and

Cite: Moreira, O. B. O.; de Souza, J. C. Q.; Candido, J. M. B.; do Nascimento, M. P.; Penna, E. A.; Chellini, P. R.; de Oliveira, M. A. L. Capillary Electrophoresis Applied to Human Urine Analysis for Clinical Diagnosis: New Trends and Perspectives. *Braz. J. Anal. Chem.*, 2023, *10* (39), pp 17-51. http://dx.doi.org/10.30744/brjac.2179-3425.RV-13-2022

Submitted 22 February 2022, Resubmitted 11 April 2022, Accepted 25 April 2022, Available online 25 May 2022.

technologies applied to human urine analysis by CE over the last five years. We will be starting by commenting the applications onto target groups of molecules followed by addressing the CE feasibility for the determination of general chemical profiles through untargeted omics approaches, and finishing with the perspectives on the subject.

Keywords: Human urine, Diagnosis, Capillary Electrophoresis, Quantitative methods, Metabolomics

INTRODUCTION

Urine is a rich bodily fluid explored by scientists and physicians as a source of information about human health. It is a sterile, amber-colored solution generated by the kidneys as a result of ultrafiltration of our bloodstream.[1–3] As being constituted of water-soluble waste products of metabolic process, over the last four decades, more than 3000 metabolites have been defined in human urine. Basically, it is an aqueous solution that contains high concentrations of urea, inorganic salts, creatinine, ammonia, organic acids, proteins, peptides, enzymes, toxins, and other molecules.[1,2,4–6]

Variations in the concentrations of these substances and the emergence of other biochemicals can occur due to several external and intrinsic factors. In addition, urine samples are noninvasively and easily obtainable with minimal inconveniences for the donor and can be collected in large amounts. Therefore, it is indeed a good mediator for monitoring human homeostasis and is considered an interesting specimen for providing accurate and cost-effective diagnostic screening data for monitoring multi-systems infections or multiple metabolic disorders.[1,3–5]

Concerning the analytical platform, if the aim is to detect variations in human biochemicals under conditions of interest, robust instrumental and statistical methods are in need. For the analysis of urinary metabolites (small molecules <1.5 kDa), reference protocols consider Nuclear Magnetic Resonance (NMR), Gas Chromatography coupled with Mass Spectrometry (GC-MS), and Liquid Chromatography coupled with Mass Spectrometry or tandem Mass Spectrometry (LC-MS and LC-MS/MS, respectively).[5,7] Within this context, Capillary Electrophoresis (CE) was introduced in this particular field as a complementary separation technique. Back in 2006, Ramautar *et al.* commented in their review paper[8] that, at the time, only a few authors applied this technique towards wide-ranging metabolite analysis, even though it was created in the late 80s.[8,9] Ten years later, CE became highly noticeable as an analytical choice for complex biological samples and has been an interesting object of discussion of specialists.[6,10–12]

CE, in a general way, is an electromigration separation technique based on the differential migration of charged species through the application of an electric field into a pH-controlled electrolyte that fills in a capillary tube.[9,13–15] From a comprehensive point-of-view, this electromigration technique provides simultaneous analysis of neutral, solvated ions, and ionizable species. It is important to highlight that CE present different modes with different separation potentialities.[14] Within this context, for instance, when neutral species are considered, together with ionic species, is possible to achieve successful separation by using micellar electrokinetic chromatography (MEKC), microemulsion electrokinetic chromatography (MEEKC), or capillary electrochromatography (CEC). In this sense, like the classical separation techniques, CE offers high efficiency and precision with some additional advantageous features[13,16] (Figure 1), which makes it an excellent choice for the analysis of complex biological samples such as urine.

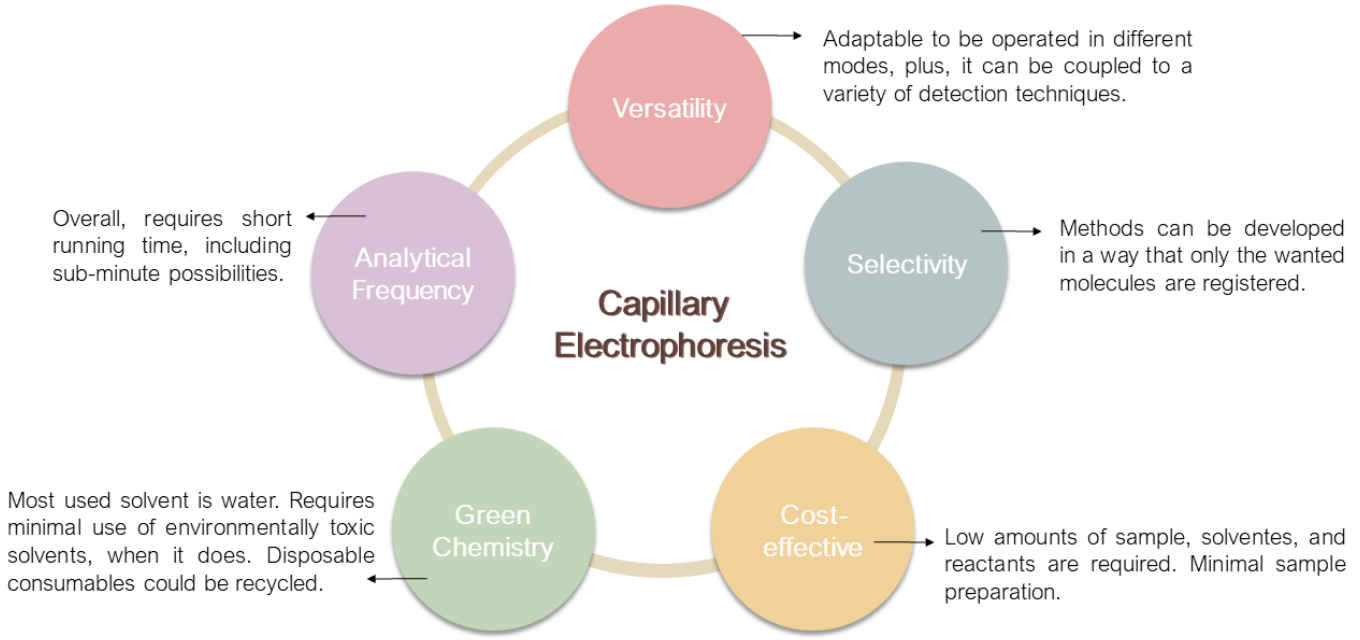


Figure 1. Cycle diagram on capillary electrophoresis advantageous features for any field of application.

In this context, several CE specialists have already published a substantial series of papers discussing the applicability of the technique to the analysis of complex samples considering omics-based approaches, especially within the last ten years.[6,10–12] In this work, we will discuss these comprehensive strategies, as well as the studies that explored the selectivity and versatility mightiness of CE for segmented analysis of major structural chemicals of the human body. To be more thorough, we will be commenting on methods, instrumentation, innovations, and perspectives of a compilation of studies focused on analyzing these biomolecules that might indicate any human disorder (Figure 2). A selection of studies from the last 5 years (2017-2021) was primarily considered, since a lot of compilations have already been published, aiming to access the progress of this particular field.

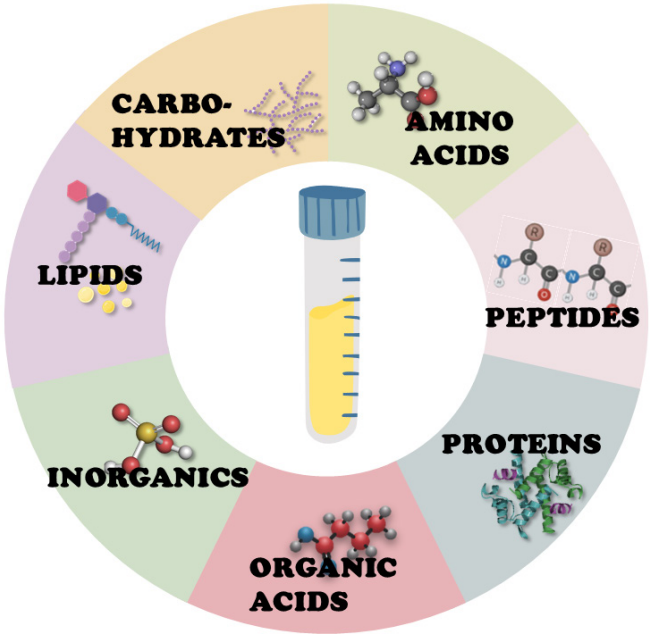


Figure 2. Schematic diagram of the main structural biochemicals accessed in human urine focused on the discussion of this review.

Organic acids

The presence of organic acids (OAs) as intermediates or final products in a broad range of biochemical metabolism pathways in the human body makes these compounds promising candidates for biomarkers in the assessment of patients diagnosed with organic acidurias (Organic Acid Disorders, OADs).[17,18] Considering all the different kinds of bodily fluids, urine is the matrix of best-choice for analysis of short-chain OAs, since the concentration of these compounds in urine is considerably higher than in blood, in addition to being an easier-to-collect biological fluid.[19] Thus, by accessing the urinary OAs, it is possible to infer the diagnosis of several metabolic disorders since more than 250 OAs can be excreted in the urine.[20]

For the specific determination of low-weighed OAs in urine, GC is the separation technique traditionally used.[21] However, CE could be a good alternative for these analyzes, especially due to all the advantages mentioned in the previous section. Studies dedicated to the use of capillary electrophoresis for targeted analysis of carboxylic acids in human urine were compilated and will be discussed in this section.

Most authors describe different methods using CE for the determination of OA in human urine aiming to figure out an auxiliary diagnosis method for OADs by identifying a specific metabolite. The enantiomers of D, L-2-hydroxyglutaric acid (D, L-2-HG) were studied in the urine of children diagnosed with 2-hydroxyglutaric aciduria by using a CE-ESI-MS/MS.[22] The authors developed and validated a complete capillary filling method (CFM) for each one of the enantiomers by using vancomycin chloride (VC) as a non-volatile chiral selector. In the CFM technique, the sample is dissolved in the background electrolyte (BGE) and is introduced into the capillary to fill it entirely.

The aforementioned strategy associated with the triple quadrupole (QqQ) MS/MS technology required neither derivatization nor the addition of extra modifiers in the running BGE. Optimization steps were necessary to avoid the possible adsorption of VC into the inner silica of the separation capillary and specially to avoid the introduction of VC into the ESI. UV-VIS detection at 200 nm was the setup for the determination of creatinine, used as normalization parameter for the concentration of OAs.[22] Dual detection (UV-MS) has been successfully used in several studies to monitor creatinine without matrix interference and also without compromising the analytical frequency of the method, as highlighted by the authors.[23]

About that, a simple flow injection analysis (FIA) system with both capacitively coupled contactless conductivity detector (C^4D) and paired emitter detector diodes (PEDD) was applied for simultaneous and direct quantification of urea and creatinine.[24] All the analyses were carried out in a hybrid lab-made cost-effective device built by coupling the proposed FIA system with a lab-made online gas diffusion (GD) unit equipped with in-house C^4D and PEDD detectors. With an analytical frequency of 31 samples per hour for urea and 66 samples per hour for creatinine, they achieve a high-throughput method with statistic metrics comparable to classical techniques. That study illustrates how similar methods considering C^4D, Laser-Induced Fluorescence (LIF), or any diode-based detectors or even lab-made simpler devices could be a good alternative to quantify the urine major components used as normalization parameters for minor molecules without jeopardizing the determination of the analytes.

Selective screening and determination of glutaric acid (GA), 3-methyl glutaric acid (3-CH_3-GA), and 3-hydroxy-3-methyl glutaric acid (3-OH-3-CH_3-GA) were the focused analytes for the diagnosis of different glutaric acid acidurias. Using a CE coupled to a single quadrupole (Q) MS system, the three analytes were quantified using a standard addition method through a selected ion monitoring (SIM) detection mode, enhancing method selectivity. The optimized protocol was applied for examining urine from affected newborns, as a result, the levels of OAs evaluated were found to be higher than what is considered normal for this group.[25]

Diode array detection (DAD) was used in a study dedicated to the determination of homogentisic acid (HGA) in individuals diagnosed with alkaptonuria.[26] The optimization process was carried out through a 2^4 two-level full factorial design (FFD) with the variable response to the resolution. A follow-up experimental design was pursued to complete the resolution optimization. Through the optimized conditions, the higher levels of HGA found in alkaptonuria patients were successfully confirmed. According to the authors, this method has a better analytical frequency than similar analytical protocols.

Another interesting approach using two capillaries with different internal diameters was thought out by another research group for the analysis of orotic acid, a biomarker for inborn metabolic inconsistencies.[27] According to the authors, this approach can decrease separation times, so the analyte was detected by UV within 2 minutes with no sample preparation. However, another study published in 1996 accomplished a shorter migration time for the same analyte using Micellar Electrokinetic Chromatography (MEKC) with reverse electroosmotic flow (EOF).[28] On the other hand, the two-capillary setup could be an alternative to be explored for other screening studies or for reducing migration time that already considers reverse EOF, and/or when voltage can no longer be used to shorten time to avoid coelution.

Two distinct studies were dedicated to CE-C^4D methods. Ethylmalonic acid was the target for the first study whose aim was to validate a direct quantification method.[29] The group achieved the separation within 4 minutes with a limit of quantification (LOQ) of about 0.5 mg L^{-1} which presented to be sensible enough to analyze real samples with just dilution and filtration handling. A homemade C^4D with a mini detection cell was the center of a second study focused on the quantification of oxalate and citrate in random urine. In this method, a solid-phase extraction (SPE) purification procedure was necessary to achieve good efficiency with minimal matrix interference. The author reached a sensitivity of about 250 mg L^{-1}, pretty remarkable when compared to similar studies, according to the authors.[30]

Overall, regarding the sample preparation, for the majority of the analysis just filtration and simple dilution in deionized water were sufficient. It is a good aspect when we compare the protocol for analysis of complex matrices by other separation techniques, which in general requires centrifugation, protein precipitation, derivatization, among others. About the statistics and validation parameters, the evaluation of the linearity of the calibration curves was based overall only on the value of the regression coefficient (R^2), an argument that is considered insufficient given the statistical significance of the adjusted regression by the least-squares method.[31,32]

About the carboxylic acids themselves, we observe that this class has a wide range of carbonic chains, so the method development is highly specific depending on the analyte feature. We believe that the best way to start developing methods for OAs determination is to do so by deeply studying the ionization profile of each molecule and the physical-chemical proprieties to choose the most propitious condition, since we observe that the optimum ionization could be achieved anywhere within the pH scale and its structure could enable the detection through UV, MS, C^4D, LIF, etc. Table I summarizes the instrumental specifics of all the studies aforementioned.

Table I. Analytical methods applied to the analysis of organic acids in human urine samples by CE

Analyte	Application	Sample preparation	Analytical platform	CE experimental conditions	Detector parameters	Ref.
D, L-2-HG	2-Hydroxyglutaric aciduria	Dilution and filtration	CZE-ESI-QqQ	BGE: 50 mmol L^{-1} AA and 25 mmol L^{-1} VC (pH 4.5). PAM coated capillary: 50 µm i.d. and 60 cm total length. 150 mbar x 15 s, -25 kV, 25 ºC.	SHL: (50:49.5:0.5%, v/v/v) MeOH:H_2O:NH_4OH, 6 µL min^{-1}. Capillary voltage: -25 kV. Drying gas: 200 ºC, 10 L min^{-1}, 10 psi. TIC; SRM (*m/z* 147.1 [M-H])	22
GA, 3-CH_3-GA, 3-OH-3-CH_3-GA	Glutaric acid acidurias	Filtration and dilution	CZE-ESI-Q	BGE: 20 mmol L^{-1} AA, 10% MeOH (pH 9.1) adjusted with ammonia 1 M. Fused-silica capillary, 75 µm i.d. and 100 cm total length. 30 mbar x 5s, -12 kV, 25 ºC.	SHL: 25% formic acid in 50% (v/v) 2-propanol-water, 0.4 L min^{-1}. Capillary voltage: 3.6 kV. Fragmentor voltage: 75 V. Drying gas: 210 °C, 6 L min^{-1}, 16 psi. SIM mode $[M-H]^-$ *m/z* 131.1 (GA); *m/z* 145.1 (3-CH_3-GA) and *m/z* 161.1 (3-OH-3-CH_3-GA).	25
HGA	Alkaptonuria	Dilution and filtration	CZE-UV	BGE: 45 mmol L^{-1} phosphate buffer (pH 7.0). Fused silica capillary, 75 µm i.d. and 46 cm effective length. 60 mbar x 6 s, 22 kV, 25 ºC.	190 nm	26
Orotic Acid	Orotic Acidurias	None	CZE-UV	BGE: 100 mmol L^{-1} phosphate buffer (pH 2.2). Fused silica capillary, 50 and 100 µm i.d. and 8.4 effective lengths. 50 mbar x 5 s, 5 kV, 25 ºC.	214 nm	27
Ethylmalonic acid	Ethylmalonic aciduria	Dilution and filtration	CZE-C^4D	BGE: 50 mmol L^{-1} MES and 0.13 mmol L^{-1} CTAB (pH 6.5) adjusted with Tris. Fused silica capillary, 50 µm i.d. and 52 cm effective length. 100 mbar x 10 s, -28 kV, 25 ºC.	100% gain and -18 dB voltage.	29
Oxalic and citric acids	Random	SPE purification and dilution	CZE-DAD-C^4D	BGE: 10 mmol L^{-1} MES, 10 mmol L^{-1} His, and 50 µmol L^{-1} CTAB in 10% MeOH (pH 5.82). Bare fused-silica capillary, 50 µm i.d., 40 cm effective length to C^4D detector, and 41.5 cm to DAD detector window. 50 mbar x 10 s, -25 kV; 25 ºC.	Excitation voltage, 20.8 V; oscillation frequency: 29 kHz; gain, 100 times. DAD: 232 nm.	30

3-CH_3-GA: 3-methyl glutaric acid; 3-OH-3-CH_3-GA: 3-hydroxy-3-methyl glutaric acid; AA: ammonium acetate; BGE: background electrolyte; C^4D: capacitively coupled contactless conductivity detector; CTAB: hexadecyltrimethylammonium bromide; CZE: capillary zone electrophoresis; DAD: diode array detector; D, L-2-HG: D, L-2-hydroxyglutaric acid; ESI: electrospray ionization; GA: glutaric acid; His: L-Histidine; i.d.: internal diameter; HGA: homogentisic acid; MeOH: methanol; MES: 2-(*N*-morpholino) ethane sulfonic acid; PAM: Polyacrylamide; Q: single-quadrupole mass spectrometer; QqQ: triple-quadrupole mass spectrometer; SIM: selected ion monitoring; SHL: sheath liquid; SPE: solid-phase extraction; SRM: selected reaction monitoring; Tris: tris hydroxymethyl aminomethane; TIC: total ion chromatogram; VC: vancomycin chloride; UV: ultraviolet.

Inorganics

In normal human urine, there are cations such as sodium, potassium, ammonium, calcium, and magnesium, besides anions such as chloride, sulfate, and phosphate.[1,33] Those inorganic ions are an important part of the human urine composition. The detection of any deviation from the standard level of each one of them could be an indicator of diseases and organs dysfunction. For example, when the bodily acid-base equilibrium needs to be restored as a result of an abnormal activity that has compromised human homeostasis, the level of sodium salts and other ions effectively changes. Therefore, monitoring the levels of inorganics in urine is a good way to access health-related conditions.[3,34,35]

This topic aims to show how CE has been used in the last 5 years for the analysis of inorganic ions in urine. However, in this period only 3 articles were found, as most of the works addressing this topic as innovative were published between 1987 and 2010. Thus, to better discuss the CE for the analysis of inorganic ions in urine, the time of search has been increased to 10 years. We believe though, that given the intrinsic capability of CE to analyze charged inorganic compounds, this technique could be a good alternative and should be better explored.

The most recent publication is a 2019 study dedicated to simultaneously determining chloride, sulfate, sulfite, nitrate, ammonium, potassium, sodium, calcium, magnesium, cyanide, and thiosulfate in urine.[36] By using CFM and C^{4}D detection, they successfully separated all the ions within 80 s (Figure 3b). As commented in the previous section, in the CFM method the samples are mixed with BGE and injected into the capillary tube to fill it entirely. The capillary is then immersed in BGE at both ends, as is normally done, and a negative voltage was applied, therefore, anions and cations move through the capillary in opposite directions being detected at different times. Cations migrate from the positive electrode (anode) towards the negative electrode (cathode) passing through the C^{4}D detector whereas the anions migrate in the opposite direction. The discontinuity in the signal separates the ions generating a raw electropherogram, which is automatically translated by the detection system[36] (Figure 3).

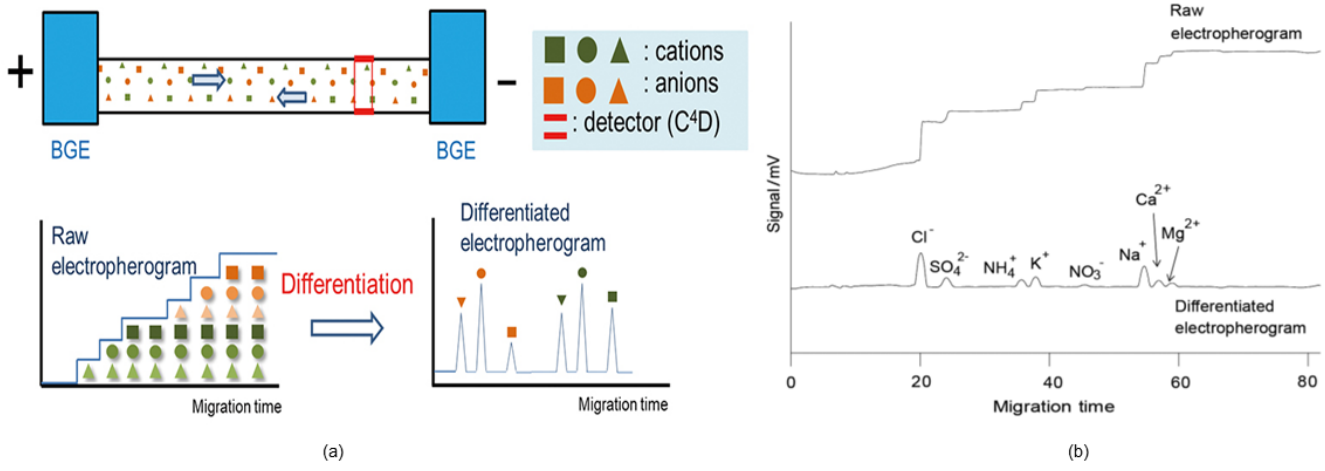


Figure 3. Schematic overview of the CFM with C^{4}D coupling (a) and resulting electropherograms of simultaneous analysis of cations and anions (b). Migration time is expressed in seconds. Source: Yamamoto et al., 2019, https://doi.org/10.2116/analsci.18P422 (free access)[36].

Previously, the simultaneous determination of anions (sulfate, sulfite, and chloride) with remarkable selectivity was achieved for another group by using strong acidic BGE under reverse polarity with direct UV detection.[37] They added a dual co-ion probe system containing 1,5-naphthalenedisulfonic acid disodium salt hydrate (NDS) and 1,3(6,7)-naphthalenetrisulfonic acid trisodium salt hydrate (NTS) and to the BGE to enable UV detection. Moreover, the samples were treated with formaldehyde to form a

hydroxymethylsulfonate adduct (UV transparent) to prevent oxidation of sulfite to sulfate under ambient conditions.[37]

In terms of detection mode, C^4D, LIF, and both direct and indirect UV were the standouts for inorganic ion determination by CE over the last ten years, with the latter being the differential of CE when compared to other UV-coupled techniques. However, that was not the strategy adopted by the determination of thiosulfate, analyzed for being a biomarker of exposure to hydrogen sulfide gas. As thiosulfate does not have sufficient radiation absorption in the UV-Vis region, derivatization with 2-chloro-1-methylquinolinium tetrafluoroborate (CMQT) at pH 2 was performed. The obtained product, 1-methyl-2-thioquinolone (MQT), was then detected at 375 nm.[38] Cyanide, considered the center of a toxicological study, was also online derivatized with naphthalene-2,3-dicarboxaldehyde (NDA) and a primary amine (glycine) to produce a fluorescent derivative, which was then detected by the LIF detector.[39] As they used electrokinetically injections via the flow gated mode, a limit of detection (LOD) of 4.0 nmol L^{-1} was achieved. In both studies, the authors comment that the derivatization option, alongside the adopted CE mode strategies, has shortened the total time of analysis.

Overall, Capillary Zone Electrophoresis (CZE) is the highlighted strategy, since charged inorganics are the basic species for electromigration techniques, except for thiosulfate, which after derivatization a neutral compound is obtained. Thus, MEKC was used for its separation utilizing sodium dodecyl sulfate (SDS) as an anionic surfactant. This work also uses sweeping as an online pre-concentration procedure. This technique adds additives to BGE, such as “partitioning of the analytes between the pseudo-stationary phase and the surrounding phase which promotes separation of analytes from the matrix”.[38]

Since these species have low absorptivity, in general, indirect UV detection seems to be the best option, considering that UV lamps are intrinsic to most CE equipment. Nevertheless, conductivity and laser-induced approaches are also in hand for species such as inorganic ions. A general concern about the analysis of ions in urine is sample handling. To eliminate matrix interferences and assess the free ions, organic compounds might have to be discarded. Other than that, ionic strength correction might also be considered to avoid problems during analysis and consequently underestimation or overestimation of quantitative responses. The experimental information from the studies discussed in this section is summarized in Table II.

Table II. Analytical methods applied to the analysis of inorganic ions in human urine samples by CE

Analyte	Application	Sample preparation	Analytical platform	CE experimental conditions	Detector parameters	Ref.
Cl^-, SO_4^{2-}, SO_2^- NO_3^-, NH_4^+, K^+, Na^+ Mg^{2+}, Ca^{2+}	Random	Diluted x 100 with water and 2 times with double-concentrated BGE followed by PVDF membrane filtration.	CZE-C^4D	BGE: 30 mmol L^{-1} malic acid, 100 mmol L^{-1} DDAPS, 3 mmol L^{-1} 18-crown-6-ether, and 18 mmol L^{-1} His (pH 3.6). Fused silica capillary, 25 µm i.d., and 20 cm effective length for cations, and 15 cm for anions. 3.45 kPa x 2 min 30 kV, 25 °C.	Low pass: 2 Hz. Frequency: 800 kHz. Amplitude: 70%. Headstage gain ON.	36
Cl^-, SO_4^{2-}, SO_2^-	Confirmatory testing of cystic fibrosis, sulfite oxidase deficiency, urolithiasis, and chronic/acute renal failure.	Dilution in perchlorate (150 mmol L^{-1}), 2% (v/v) formaldehyde, and 20 mmol L^{-1} formic acid buffer (pH 2.6). Vortex mixing. Centrifugation.	CZE-indirect-UV	BGE: 5 mmol L^{-1} NDS, 5 mmol L^{-1} NTS in 0.4 mol L^{-1} formic acid (pH 2.0). Uncoated fused silica capillary 25 µm i.d. and 50 cm effective length. 0.5 psi x 10 s; - 30 kV; 25 °C.	DAD: 214 nm	37
Thiosulfate	Biomarker for exposure to hydrogen sulfide gas.	Derivatization with 0.2 mol L^{-1} phosphate buffer (pH 2) and CMQT. Vortex mixing and centrifugation.	MEKC-sweeping-UV	BGE: 0.055 mol L^{-1} phosphate buffer (pH 8), 0.035 mol L^{-1} SDS, and 25% ACN. Fused silica capillary tube 75 µm i.d. and 51.5 cm effective length). 50 mbar x 30 s; + 20.5 k; 25 °C.	DAD: 375 nm	38
Cyanide	Investigation of the effect of smoking.	Dilution with 15 mmol L^{-1} NaOH and derivatization with 8.0 mmol L^{-1} NDA in DMF/water (50/50 v/v), 80.0 mmol L^{-1} glycines in BGE (pH 9.2), 5.0 mmol L^{-1} EDTA and KCN in BGE or urine (95/5 v/v) by mixing at room temperature.	CZE-LIF	BGE: 20 mmol L^{-1} tetraborate (pH 9.2). Fused silica capillary, 10 µm i.d. and 10 cm effective length. Reaction capillary tube: 30 cm and 100 µm i.d. Total flow rate for derivatization reaction: 800 nL min^{-1}. -5kV x 0.5 s, -23 kV; 25 °C.	LIF excitation (491 nm) and emission (520 nm).	39

ACN: acetonitrile; BGE: background electrolyte; C^4D: capacitively coupled contactless conductivity detector; CMQT: 2-chloro-1-methylquinolinium tetrafluoroborate; CZE: capillary zone electrophoresis; DAD: diode array detector; DDAPS: N-dodecyl-N, N-dimethyl-3-ammonio-1-propanesulfonate; DMF: dimethylformamide; EDTA: ethylenediaminetetraacetic acid; His: L-Histidine; i.d.: internal diameter; KCN: potassium cyanide; LIF: Laser-Induced Fluorescence; MEKC: micellar electrokinetic chromatography; NDA: naphthalene-2,3-dicarboxaldehyde; NDS: 1,5-naphthalenedisulfonic acid disodium salt hydrate; NTS: 1,3(6,7)-naphthalenetrisulfonic acid trisodium salt hydrate; PVDF: polyvinylidene difluoride; SDS: sodium dodecylsulfate; UV: ultravioleta.

Carbohydrates

Carbohydrates are biomolecules made exclusively out of carbon, hydrogen, and oxygen with chains of variable lengths and hydrogen-oxygen ratio at 2:1, obtained from an endogenous or exogenous source.[40] The presence of any of them in the urine may reflect dietary carbohydrate intake, but it may also be an indicative of a carbohydrate metabolism disorder. Therefore, precise analysis methods are needed. Carbohydrate analysis by CE, for separation and identification is very arduous,[41] restricting the number of articles on the subject. Therefore, for better comparative analysis the research range was expanded.

Galactose was the targeted analyte to develop a CE-LIF method to diagnose galactosemia. Although there are several methods for diagnosis, they usually require blood as sample and take some time to get to the results which are not always reliable. Through the application of the newly-developed method, eight main carbohydrates were simultaneously separated, those being, maltose, lactose, D-mannose, D-glucose, D-ribose, D-xylose, L-arabinose, and D-galactose, in that order of migration. The separation method relies on the tendency of borate to form complexes with cis-oriented hydroxyl groups, allowing them to be separated within 10 minutes, reducing the total analysis time by 30 minutes compared to standard methods without it. Thus, according to the authors, the CE-LIF method has proved to be superior to other techniques so far, as it is a fast and high-performance technique with less-invasive sample requirements.[42]

CE-LIF was also the setup to determine urine oligosaccharide patterns. The biochemical screening of oligosaccharides is traditionally done by thin-layer chromatography on silica gel plates. Thus, CE-LIF has also provided a shorter analysis time and more sensible and selective analysis. However, the developed method was still not capable of detecting nonreducing glycocompounds in urine, such as the ones responsible for aspartylglucosaminuria or Schindler's disease, which can be detected by ion-exchange chromatography.[43]

In a 2021 study, a technology that utilizes high-throughput single-domain antibody-based prostate-specific antigen (PSA) selective capture, followed by preconcentration and capillary gel electrophoresis (CGE) coupled to LIF was applied to high-resolution N-glycan profile determination in prostate cancer (PCa) patients.[44] Basically, the urinary PSA is captured and then the N-glycans are enzymatically released for free analysis. Due to this higher resolution of the method, which proved to be superior to those previously validated, it allowed having a full panel of N-glycans. Since changes in the N-glycosome occur during tumor genesis, they are essential biomarkers for the diagnosis of cancer.

In the 2014 study dedicated to the same analysis,[45] the diagnosis of PSA glycoforms was achieved by a DNA-sequencer-assisted fluorophore-assisted carbohydrate electrophoresis technology, a pre-established protocol[46] that promoted an analysis considered at the time an improvement compared to molecular techniques. The N-glycosylation profiling demonstrated differences between benign prostate hyperplasia (BPH) and PCa. According to the authors, these changes were a promising discovery of new biomarkers back then. Some progress has already been made, as the above-mentioned recent study, but it is something that is still worth being thoroughly investigated.

CE-ESI-QTOF-MS system was another setup applied for carbohydrate analysis. According to the authors and previously mentioned, carbohydrates are one of the hardest compounds to be separated and structurally identified. Despite that, the micro-and nano-ESI ion sources improved the level of sensitivity of the CE/ESI-MS, promoting higher analyte concentration, lower spraying potential, and closer positioning of the sprayer to the orifice of the MS and significant improvement in ionic transfer the MS. They used a copper-coated micro sprayer interface for online sheathless capillary electrophoresis electrospray that promoted an excellent solution for the aggressive highly alkaline media required in the direct polarity CE for separation of carbohydrates. Thus, their method allowed the analysis of a complex mixture of O-glycosylated sialylated amino acids from urine, with the elution of disaccharide (Gal-Gal) in 3.94 minutes and proved to be sensitive for a complex mixture of glycoconjugates.[41]

A recent study evaluated the creatinine normalized urinary glycosaminoglycans (GAGs) content, molecular weight distribution, and disaccharide composition through CE-ESI-Orbitrap.[47] In addition, the ten most abundant GAGs were obtained. The sheath liquid composition used was the same as BGE to

provide reproducible separations and optimal spray stability, which allowed the detection and separation of GAGs at low concentrations to determine various GAG compositions in urine. According to the authors, GAGs profile of healthy individuals was obtained, however, a small number of nondiverse individuals was examined, so they did not come to any definitive conclusion.

Overall, compared to the biomolecules discussed in the two previous sections, carbohydrates have undoubtedly the most extensive sample preparation protocols so far, involving denaturation, derivatization, purification, pre-concentration, and other massive handlings. These laborious preparations will may also be considered for HPLC and other techniques given the complexity of these compounds. Alongside with the difficulty in detecting these large molecules in simple CE operation modes, it may explain why CE has not been considered. However, as commented before, a lot has been developing in the analytical platform field, so the analysis of carbohydrates and large biochemicals might be an opportunity for innovation and improvement of CE and the other traditional methods. The publications discussed in this section are summarized in Table III.

Lipids

Lipids are a diverse group of biomolecules including fat, hormones, and fat-soluble compounds.[49] Belonging to the constitution of lipids, fatty acids (FAs) are aliphatic linear monocarboxylic acids with long hydrocarbon chains, being a source of energy and the major components of triglycerides, phospholipids, and cell membranes. Excessive levels of FAs can result from altered metabolism, gene expression and as part of the production of biologically active substances, therefore, lipidurias are always a clinically significant sign.[4,50–52]

However, very few CE-based methods applied to lipid analysis exist in the literature, especially in urine.[53] The same is true for the analysis of free FAs in biological samples. According to Poinsot *et al.* (2019) from the instrumental perspective, the main obstacle for analyzing lipids remains their insolubility in water and surfactant properties, where lipid-silica wall interaction is not rare.[54] Even so, some attempts were discussed in their review but only one study used urine as a sample, involving phospholipids investigation by capillary electrochromatography (CEC) coupled to MS.[55]

Over the last five years, we found some studies that considered the analysis of steroids. Steroids are polycyclic compounds that are naturally occurring or synthetic. Despite the fact that their structures are different from other lipids, they are considered one of the major parts of the class.[56] The detection of steroids in urine means an overproduction of these biochemicals, which usually indicates metabolic disorders, pregnancy, bone disease, or cancer.[57]

Recently, a simple CZE-UV method was optimized for quantification of testosterone and epitestosterone, its stereoisomer, considering that the ratio between them should be 1/1 in healthy people that never taken the hormone. For human health in general, low levels of testosterone might cause depression, osteoporosis, and fat increase.[58] Methods such as this could be an alternative for monitoring the hormone in urine. An interesting approach to steroid analysis considering the limitations above-mentioned together with the absence of charge in these molecules was the development of a reverse-polarity sweeping (RP-sweeping) method. The mechanism works through the suppression of EOF with acid, consequently, the analytes are “swept” and attached to micelles migration towards the UV detector, in that case. The BGE was optimized considering the impact of organic modifiers since surfactants had to be added to help solubilize the analytes in water and so avoid its precipitation. After optimization and validation, the method was applied to monitor five steroids simultaneously, mitotane and its main metabolite (DDA) (Figure 4) in the urine of adrenocortical carcinoma patients under treatment.[59]

Table III. Analytical methods applied to the analysis of carbohydrates in human urine samples by CE

Analyte	Application	Sample preparation	Analytical platform	CE experimental conditions	Detector parameters	Ref.
Maltose, lactose, D-mannose, D-glucose, D-ribose, D-xylose, L-arabinose, and D-galactose	Galactosemia	Double centrifugation followed by reductive amination[48] and dilution.	CE-LIF	BGE: 130 mmol L^{-1} borate buffer at (pH 10.2). Fused silica capillary, 20 µm i.d. and 20 cm effective length. 0.5 psi x 5 s, 25 kV.	LIF excitation (488 nm) and emission (520 nm).	42
Oligosaccharide	Screening of oligosaccharidosis and related diseases	Pre-concentration followed by derivatization with 200 mmol L^{-1} ATPS in acetic acid and 1 mmol L^{-1} sodium cyanoborohydride in THF. Dilution.	CE-LIF	BGE: 25 mmol L^{-1} sodium acetate buffer with 0.4% of polyethylene oxide (pH 4.75). Neutrally coated N-CHO capillary, 50 µm i.d. and 50 cm effective length. 0.5 psi x 3 s, 30 kV, 20 °C.	LIF excitation (488 nm).	43
N-glycan profile	Prostate cancer	Denaturation and digestion followed by derivatization with ATPS and a three-step resuspension.	CGE-LIF	HR-NCHO separation gel buffer in 40 cm and 20 cm effective length of bare fused silica capillary tubes 50 cm and 30 cm total length, 50 µm i.d. Three-step injection: 3.0 psi x 5 s water, 1.0 kV x 1 s sample, 1.0 kV x 1 s bracketing standard, 30 kV, 30 °C.	LIF excitation (488 nm) and emission (520 nm).	44
Gal, GalNAc, GlcNAc, Man	Schindler´s disease	n. m.	CE-ESI-QTOF	BGE: 50 mmol L^{-1} AA with 32% ammonia (pH 12.0). TSP fused-silica capillary, 50 µm i.d. 125 cm total length. 0.5 psi x 8 s, 25 kV, 20 °C.	ESI: - 1.2 kV. drying gas (80 °C, 5 L h^{-1}), 40 V.	41
GAGs	Random	Vortex mixing is followed by lyophilization and purification.	CE-ESI-Orbitrap	BGE: 50 mmol L^{-1} AA in 70% MeOH. Cation-coated capillary. Two-step injection: 950 mbar x 9 s sample and 10 mbar x 10 s BGE. -30 kV.	SHL: vide BGE. ESI: -1.9 kV.	47
N-glycan profiles	Prostate Cancer	Desalination with 10 mmol L^{-1} AA (pH 5.0) and 40 mU *Arthrobacter ureafaciens*-2,3/6/8-sialidase. Dilution.	Multi capillary electrophoresis-based sequencer	Commercial BGE. Capillary array filled with polyacrylamide linear polymer. 36 cm, 1.2 kV x 16 s, 15 kV, 60 °C.[46]	n. m.	45

AA: ammonium acetate; APTS, 8-aminopyrene-1,3,6-trisulfonic acid; BGE: background electrolyte; CE: capillary electrophoresis; CGE: capillary gel electrophoresis; ESI: electrospray ionization; GAGs: Glycosaminoglycans; Gal: galactose; GalNAc: N-acetyl galactosamine; GlcNAc: N-acetyl glucosamine; i.d.: internal diameter; LIF: Laser-Induced Fluorescence; Man: mannose; MeOH: methanol; n. m.: not mentioned; QTOF: quadrupole time-of-flight mass spectrometer; SHL: sheath liquid; THF: tetrahydrofuran; TSP: standard polyimide-coated capillary.

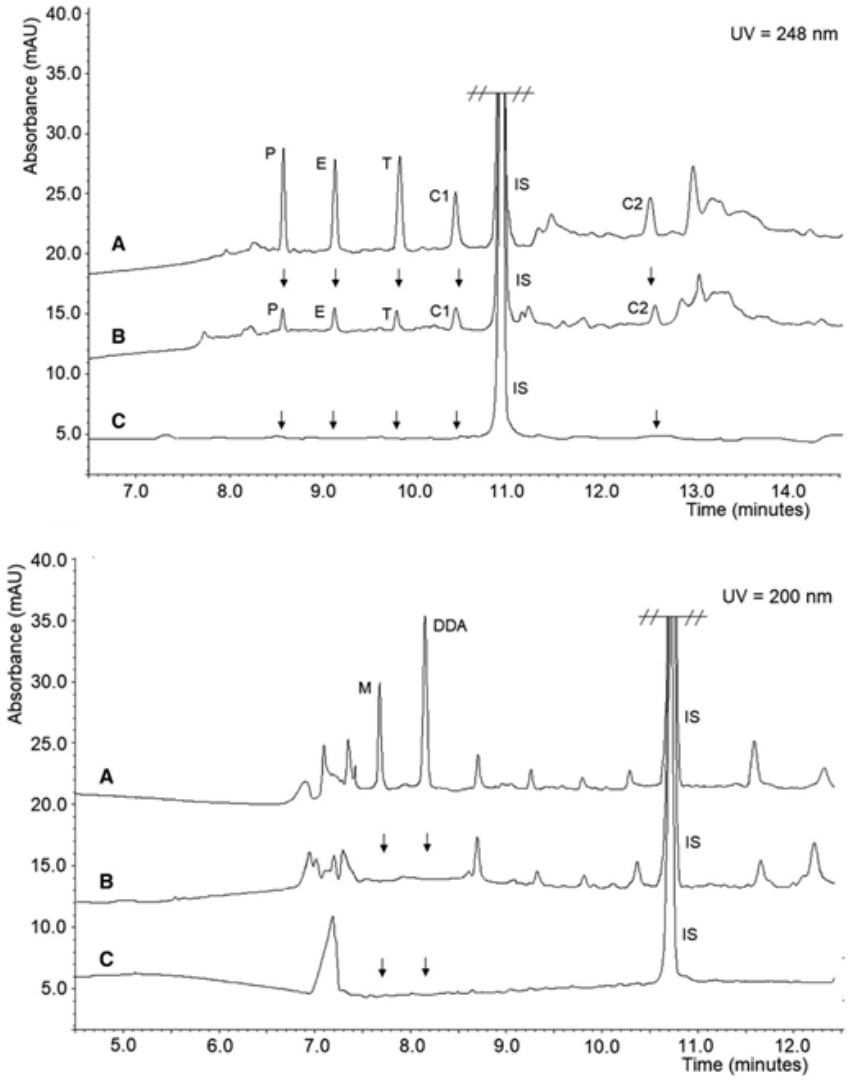


Figure 4. Representative electropherograms of (A) spiked urine, (B) urine samples, and (C) blank. At 248 nm are register the steroids (P, progesterone; E, epitestosterone; T, testosterone; C1, corticosterone; C2, cortisol). At 200 nm M, mitotane and DDA (1,1-(o,p'-dichlorodiphenyl) acetic acid). [Reprinted with permission from: Pieckowski, M.; Kowalski, P.; Olędzka, I.; Miękus-Purwin, N.; Plenis, A.; Roszkowska, A.; Bączek, T. Simultaneous determination of mitotane, its metabolite, and five steroid hormones in urine samples by capillary electrophoresis using β-CD2SDS1 complexes as hydrophobic compounds solubilizers. *Electrophoresis* **2021**, 1–8. https://doi.org/10.1002/elps.202100250. License granted by John Wiley and Sons, on February 14, 2022.]

Other methods employed for the analysis of liposoluble vitamins, steroids, and random lipids in a variety of matrices are also discussed in the previously mentioned review.[54] In fact, a few studies explored the quantitation of these molecules in urine by CE or any other technique, so we believe there is an opportunity to develop new reliable procedures to do so. Even though liposoluble species are minor in urine, that is exactly why we should have more studies considering screening approaches for lipids and fatty acids, for example, to investigate possible lipidurias, since the unexpected presence of a compound from this class will probably indicate some health-related issue. The publications discussed in this section are summarized in Table IV.

Table IV. Analytical methods applied to the analysis of lipid structures in human urine samples by CE

Analyte	Application	Sample preparation	Analytical platform	CE experimental conditions	Detector parameters	Ref.
Testosterone and epistosterone	Random	Hydrolysis with 1.0 mol L^{-1} acetate buffer (pH 5.15) and 10000 U of β-glucuronidase. Centrifugation and SPE purification.	CZE-UV	BGE: 15 mmol L^{-1} acetate buffer (pH 4.74). Fused silica capillary 75 µm i.d. 3.43 x 10^3 Pa x 10 s, 25 kV, 25 °C.	242 nm	58
Progesterone, testosterone, epitestosterone, cortisol, and corticosterone	Patients with inoperable adrenocortical carcinoma treated by mitotane administration.	Filtration, vortex mixing with acid addition, precipitation, and resuspension.	RP-sweeping-UV	BGE: 100 mmol L^{-1} SDS, 25% ACN, 25 mmol L^{-1}, phosphate buffer (pH 2.5), and 7 mmol L^{-1} β-cyclodextrin. Uncoated fused-silica capillary 75 µm i.d. 50 cm effective length, 0.5 psi x 0.5 s, -25 kV, 25 °C.	248 nm	59
Phospholipids	Random	Lyophilization and dissolution.	CEC-ESI-MS-IonTrap	BGE: 2-propanol/ACN/50 mmol L^{-1} NH_4OH (60:30:10, v/v) (pH 9). OT-CEC column, 50 um, 30 cm. Injection via siphoning for 15 s at 10 cm height. 18 kV.	SHL: 80:20 MeOH/water with 0.05% ammonium hydroxide at 500 nL min^{-1} ESI: negative mode, 2.2 kV.	55

ACN: acetonitrile; BGE: background electrolyte; CEC: capillary electrochromatography; CZE: capillary zone electrophoresis; ESI: electrospray ionization; i.d.: internal diameter; OT-CEC: open tubular capillary electrophoresis; MS: mass spectrometry; RP: reverse-polarity; SHL: sheath liquid; SPE: solid-phase extraction; UV: ultraviolet.

Amino Acids

Proteinogenic amino acids are the monomers that constitute peptides and proteins, represented by 20 compounds (Figure 5). Although non-protein amino acids do not participate in the protein composition, they perform important functions in human metabolisms alongside proteinogenic amino acids, such as being intermediaries in metabolic pathways and precursors in biomolecules syntheses (for example hormones and neurotransmitters).[60] Therefore, these compounds can be considered biomarkers for medical applications, being an important subject to be studied to achieve suitable disease diagnosis and monitoring, among further relevant health-related information. In this context, CE could be used for this purpose, due to the advantages aforementioned.

Many articles were found talking about amino acid analysis. The most recent review published in 2021 reported the analysis of amino acids by CE over the last twenty years considering foods and medical applications.[61] Two others reviews brought the determination of nonprotein amino acids in biological samples and foods,[62] and analysis of amino acids and related compounds, both using CE technique.[63] Lastly, a series of ten reviews reported the developments of every two years on the analysis of amino acids using CE, considering the detection technique, the type of application, some enantiomeric separations, and instrumentation improvements.[64–72] In this section, some studies involving the determination of amino acids in human urine by CE produced in the last five years will be discussed.

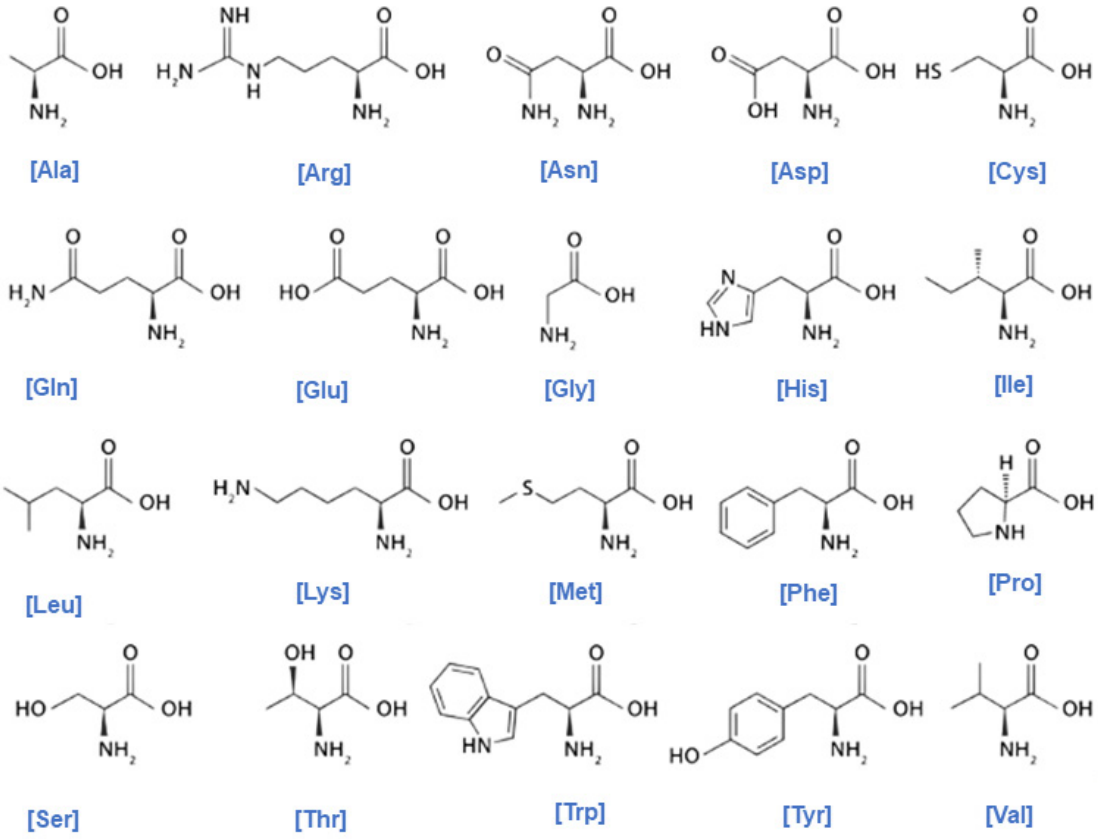


Figure 5. The 20 proteinogenic amino acids: alanine (Ala), arginine (Arg), (Asn) asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr) and valine (Val).

CE-UV methods were described by three research groups with different aims.[73–75] In the first work, Arg, Tyr, and another 9 neurochemicals related to sports fatigue in human urine were simultaneously determined.[73] As being a neurotransmitter precursor, the amino acid Arg was the biomarker for sports fatigue in athletes as determined by a simple CZE-UV method. The samples were collected at three stages: under relaxation, before and after exercise. The authors affirm that this result can be applied to validate metabolic indicators during exercise training.

In a second work, a CE-UV method for Cys and homocysteine (Hys) determination in human urine and plasma was developed and the analytes were derivatized using 1,1'thiocarbonyldiimidazole followed by the pre-concentration step, a chloroform-acetonitrile liquid-liquid extraction (LLE).[75] Considering the good analytical frequency of the optimized method, the derivatization procedure was better than using indirect UV detection. Along with the new approach of pH-mediated stacking combined with field-amplified sample stacking, the authors achieved a significative increase in sensitivity, reproducibility, plus, this clean-up approach reduced the matrix influence. A dynamic coating with surfactants was necessary due to the acid character of the derivatives that reverse the EOF towards the cathode, that is, the same direction of analytes. So, a mixture of cation surfactant, hexadecyltrimethylammonium bromide (CTAB), and an anionic surfactant, SDS, was used in the BGE system.

The third study describes a simple on-column derivatization CZE-UV method for the identification of sarcosine (Sar) as a biomarker for PCa.[74] In this context, a metal-coded hydrogel magnetic molecularly imprinted polymer (Hydro-MeC-MMIP) was coupled to an on-column derivatization CE to promote Sar cleanup and preconcentration in human urine, and phthalic anhydride was used as a derivatization agent. The MMIP showed high selectivity towards Sar and the on-column derivatization CE promoted a significant peak resolution of the analyte and the other 12 amino acids analyzed simultaneously. The authors state that the method was simple and low-cost for quantifying Sar in urine and to study its potential to be a biomarker for PCa.

Clean-up procedures or derivatization steps in sample treatment were also employed in other studies that used contact conductivity (CC)[76] and LIF[77] detectors. A microchip electrophoresis (MCE) method that combines isotachophoresis (ITP), as sample clean-up, and CZE as separation mode, was developed for the determination of Hys in urine, using CC detection.[76] A solid-phase microextraction (SPME) pre-treatment was necessary to remove chloride and sulfate before injection. The combination of these techniques enhanced the trace quantification of Hys. The use of LIF detection was reported in a flow-gated MECK method coupled to sample alternate injections for profile amino acids determination.[77] The analytes were treated through fluorogenic derivatization with NDA followed by dilution aiming to avoid detector saturation. The amino acids were quantified by the one-point standard addition method and the results showed that Gly and His concentration were higher than other analytes.

Changing the detector system, some authors introduced the use of MS with just dilution sample treatment. A CE-MS method for the separation and quantification of 27 amino acids present in the urine of children with vesicoureteral reflux was performed on an ion trap mass analyzer in positive ion mode.[78] The method optimization was carried out considering the interface system, CE parameters, and MS conditions. A pH-stacking procedure was used to increase sensitivity and injection volumes without affecting the resolution. Except for Asp, Ala, Cys, Ile, and citrulline (Cit), all other amino acids were successfully quantified in urine. The authors stated that it is a promising approach for targeted analysis of amino acids.

Using an MS/MS system, 20 amino acids in the human urine of inflammatory bowel disease (IBD) patients were determined through a positive ionization mode with no sample treatment.[79] As a result, there were significant differences in concentrations of Gly, Val, Cys, Gln, Asn, His, and Arg between 10 healthy individuals and 13 IBD patients under thiopurine treatment. When compared with a UHPLC-MS method, the new approach has a favorable performance and is suitable for studying amino acids in urine considering the advantages previously commented in the introduction section. In another study, a target analysis of cardiovascular disease (CVDs) biomarkers candidates was performed by the CE-MS/MS method, aiming at the determination of carnitine (Car) and trimethylamine-N-oxide in the urine of healthy individuals and

infarcted patients. The authors demonstrate that the proposed method is considered efficient for the targeted metabolomics of CVDs. Given the experimental cut-off, healthy individuals had smaller amounts of Car, compared to those with CVDs.[23]

To improve the sample throughput, a multisegmented injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS) for target analysis of Phe in human urine and plasma was developed using a TOF mass spectrometer in positive and negative ionization modes.[80] The samples were collected from 22 classic phenylketonuria (PKU) patients with different disease severity, ages, and dietary. The samples were injected at 100 mbar for 5s between alternating spacer BGE for the 40s, depicting a 7-sample serial injection. A comparative study between plasma and urine confirmed that Phe concentration in plasma was strongly correlated with its concentration in urine, besides determining several metabolites associated with poor adherence to Phe restricted diets, mostly present in older PKU patients. Within this context, urine becomes a more convenient and non-invasive biological sample for detecting and monitoring the disease in routine analysis. The publications discussed in this section are summarized in Table V.

Table V. Analytical methods applied to the analysis of amino acids in human urine samples by CE

Analyte	Application	Sample Preparation	Analytical platform	CE experimental conditions	Detector parameters	Ref.
Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val, Car, Orn, Cit, GABA, 4Hyp	Vesicoureteral reflux	Dilution	CE-ESI-IonTrap	BGE: 0.80 mol L^{-1} formic acid, 15% MeOH (pH 1.96); Uncoated fused-silica capillary: 50 µm i.d. and 85 cm total length. 0.5 psi x 9 s (12.5% NH_4OH), 0.6 psi x 20 s (sample), + 30 kV, 20 ºC.	SHL: 0.50% formic acid in 60% (v/v) MeOH/H_2O, 5 µL min^{-1}. + 4.5 kV, drying gas 200 ºC, 5 L min^{-1}, 8 psig.	78
Cys, Hys	Kidney diseases	Derivatization and LLE	CE-UV	BGE: 0.1 mol L^{-1} H_3PO_4 with 30 mmol L^{-1} TEA (pH 2), 25 µmol L^{-1} CTAB, 2.5 µmol L^{-1} SDS, and 2.5% PEG-600 (v/v). Undeactivated silica capillary: 50 µm i.d. 23.5 cm effective length. -17 kV x 30 s (0.5 M KOH), 50 mbar x 45 s (sample), -17 kV, 30 ºC.	285 nm	75
Ala, Arg, Asn, Asp, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr, Val, Tau	Random	Fluorogenic derivatization and dilution	Flow-gated-MECK-LIF	BGE: 40 mmol L^{-1} tetraborate mol L^{-1} HP-β-CD. Capillary: 10 µm i.d., 10 cm effective length. -5 kV x 0.3 s, - 22 kV.	442 nm (excitation) 485 nm (emission)	77
Hys	Random	Dilution and SPME	MCE-ITP-CZE-CC	BGE: n. m. (pH 9.8). PMMA microchip with coupled-channels.	n. m.	76
Car	Cardiovascular diseases	PPT followed by dried extract solubilization and dilution.	CZE-ESI-QqQ	BGE: 0.10 mol L^{-1} formic acid (pH 2.4). Fused-silica capillary: 50 µm i.d., 92 cm total length. 5000 Pa x 15 s, + 25 kV, 20 ºC.	SHL: (70:30, v/v) MeOH: H_2O with 0.05% (v/v) formic acid, 5 µL min^{-1}. +4.5 kV, drying gas (150 ºC, 7 L min^{-1}), 3 psi. SRM (Car *m/z* 162.1 → 43.3).	23
Arg, Tyr	Sport fatigue	Centrifugation and dilution.	CE-UV	BGE: 12.5 mmol L^{-1} $Na_2B_4O_7$ (pH 9.9). Uncoated sílica capillary: 75 µm i.d., 365 µm o.d., 50 cm effective length. 0.5 psi x 20 s, + 25 kV, 25 ºC.	200 nm	73
Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val	Inflammatory Bowel Disease	Dilution and filtration	CZE-ESI-QqQ	BGE: 500 mmol L^{-1} formic acid. Fused-silica capillary: 50 µm i.d., 300 µm o.d., 90 cm total length. 50 mbar x 10 s, + 30 kV.	SHL: (50:50, v/v) MeOH/H_2O with 5 mmol L^{-1} AA, 8 µL min^{-1}, +4 kV, drying gas 300 ºC, 10.0 L min^{-1}, 10 psi. MRM.	79
Phe	Phenylketonuria	Centrifugation and dilution	MSI-CE-ESI-TOF	BGE (+): 1 mmol L^{-1} formic acid, 15% ACN (pH 1.8). BGE (-): 50 mmol L^{-1} ammonium bicarbonate (pH 8.5); Uncoated fuse-silica capillary: 50 µm i.d., 120 cm total length. 100 mbar x 5 s, between alternating spacer BGE 100 mbar x 40 s, + 30 kV; 25 ºC.	SHL (+): 60% MeOH with 0.1% formic acid, 10 µL min^{-1}. SHL (-): (50:50, v/v) MeOH:H_2O. Drying gas 300 ºC, 8.0 L min^{-1}, 10 psi. Phe *m/z* 166.1.	80
Sar	Prostate cancer	Derivatization on-column	CE-DAD	BGE: 40 mmol L^{-1} borate buffer (pH 9.37). Fused-silica capillary: 75 µm i.d., 52.8 cm effective length. 1000 mbar x 2 min (NaOH 0.1 M), 1000 mbar x 2 min (water), 1000 mbar x 2 min (BGE), 40 mbar x 4 s (phthalic anhydride), 100 mbar x 5 (sample), +21 kV x 12 min (BGE), + 21 kV.	200 nm	74

4Hyp: trans-4-hydroxyproline; AA: ammonium acetate; ACN: acetonitrile; BGE: background electrolyte; CC: contact conductivity; Car: carnosine; CE: capillary electrophoresis; Cit: citrulline; CTAB: hexadecyltrimethylammonium bromide; CZE: capillary zone electrophoresis; DAD: diode array detector; ESI: electrospray ionization; GABA: γ-aminobutyric acid; Hys: homocysteíne; HP-β-CD: hydroxypropyl-beta-cyclodextrin; i.d.: internal diameter; ITP: isotachophoresis; LIF: laser-induced fluorescence; LLE: liquid-liquid extraction; MCE: microchip electrophoresis; MEKC: micellar electrokinetic chromatography; MeOH: metanol; MRM: multiple-reaction monitoring; MSI: multisegmented injection; n. m.: not mentioned; o.d.: outer diameter; Orn: ornithinemonohydrochloride; PEG: polyethylene glycol; PMMA: poly(methylmethacrylate); PPT: protein precipitation treatment; QqQ: triple-quadrupole mass spectrometer; Sar: sarcosine; SPME: solid-phase microextraction; SDS: sodium dodecyl sulfate; SHL: sheath liquid; SRM: selected reaction monitoring; Tau: taurine; TEA: triethanolamine; TOF: time-of-flight mass spectrometer; UV: ultraviolet.

Peptides and Proteins

For basic contextualization, peptides are formed when two out of the twenty existing proteogenic amino acids unite through a covalent bond (peptide bond), thereby forming a dipeptide. When these amino acids form combinations with themselves in a characteristic linear sequence, it is considered a polypeptide. After that, these polypeptide chains interact to generate larges biomolecules, the proteins. Some proteins could contain two or more polypeptide chains, those being exclusive synthetized with amino acids or not, as the case of conjugated proteins, constituted also by lipids, carbohydrates, and metals.[81,82]

Normally, the total volume of urine collected throughout one day for a healthy person may contain approximately 150 mg of protein. Among them, albumin and immunoglobulin are in higher concentrations. Through a variety of analytical routines, some scientists have found about 2000 proteins in human urine over the years. Thus, it is well known that proteins are important biomarkers. For example, proteinuria is primarily indicative of glomerular diseases and other kidney-related disturbers. Not only that, but even cardiovascular diseases, cancerogenic activity, and viral infection can interfere with the normal levels of proteins present in human urine.[83]

Despite being macromolecules, CE-MS can be considered a reliable setup for getting information about intact proteins, such as protein quality and degradation products and pathways.[84] Therefore, a few studies were published considering target approaches for peptides and proteins using CE. The analysis of peptides and proteins in urine by CE has become more expressive over the last decade. In a few published reviews are found insightful discussions about related works.[83,85] Concerning specific peptide analysis, urinary peptides related to cardiovascular conditions[86] and chronic kidney diseases (CKD)[87] and are being vastly investigated through CE-MS as shown in the respective reviews.

About the recently-developed methods for dipeptides, concentrations of some free propyl compounds in unhydrolyzed urine were found to be biomarkers for some collagen-associated diseases, like bone turnover and osteoporosis.[88] A flow-gated CE-LIF method was developed for the simultaneous determination of six free prolyl peptides from collagen degradation, in particular, the prolyl hydroxyproline (Pro-Hyp) and total 4-hydroxyproline (Hyp). The sample treatment was performed by blocking the primary amine, with no cleanup step, followed by fluorogenic derivatization, which allowed a direct injection in a flow-gated system. As highlighted by the authors, the analytical throughput was improved with the analytes separated in 30 seconds, and the procedure proved to be suitable for rapid analyses of prolyl compounds.

For the analysis of free proteins, CE-MS was the chosen setup. Concerning PCa, two works focused on detecting the PSA, as opposed to the recent study that analyzed the N-glycome freed from urinary PSA,[44] discussed in the carbohydrates section. Aiming to investigate the profile of PSA proteoforms in the urine sample, an intact protein assay by CE-ESI-MS/MS was performed.[89] The PSA was captured by using anti-PSA beads with no further sample treatment. A total glycosylation profile was also appraised to achieve some complementary information. This process was suitable to assess the intact protein (cleaved PSA) and glycosylation profile in the same procedure, showing the relevance of the intact protein analysis in a bottom-up approach.[89] In this context, another study employs an in-depth high-performance PSA Glycomics Assay (PGA) by CE-MS in urine samples dedicated to identifying potential new biomarkers for differentiating the levels of PCa (Figure 6).[90]

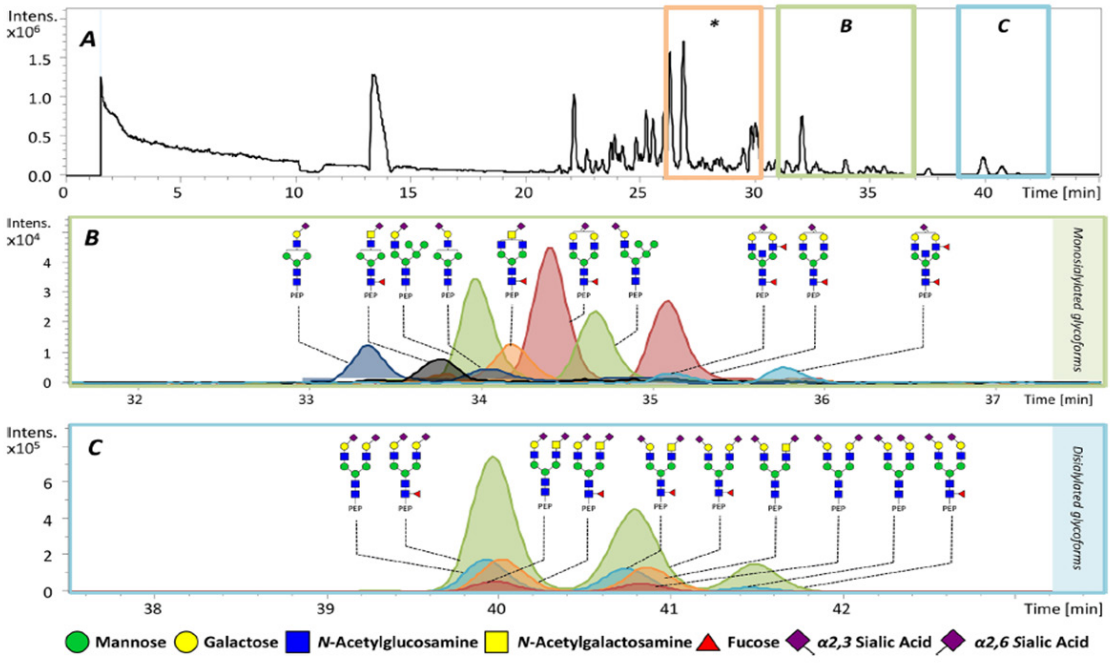


Figure 6. Representative electropherogram for a tryptic digest of PSA (A). Extracted ion electropherograms (EIC) for monosialylated N-glycopeptides (B) and disialylated N-glycopeptides (C). Glycopeptides are represented individually by each color. [Reprinted with permission from: Kammeijer, G. S. M.; Nouta, J.; de La Rosette, J. J. M. C. H.; de Reijke, T. M.; Wuhrer, M. An In-Depth Glycosylation Assay for Urinary Prostate-Specific Antigen. *Anal. Chem*. **2018**, *90* (7), 4414–4421. https://doi.org/10.1021/acs.analchem.7b04281 License granted by the American Chemical Society, on February 15, 2022. Further permission related to this figure should be directed to the ACS].

With a different approach, the PSA was captured from urine, followed by an in-solution digestions and glycopeptides analysis. The results established multiple glycoforms of PSA, and the authors state, as previously commented, that PSA glycosylation can be used as a diagnosis for PCa in the initial stage, besides the differentiation of aggressive and nonaggressive disease.[90]

Untargeted analysis of peptides is also being pursued. Peptidomics is the analysis of all bioactive peptide content within any organism, as proteomics is the study of protein alteration.[82,91] In this context, a standardized peptide screening procedure was conducted according to ISO13485 yielding quality control by using a P/ACE MDQ CE coupled to ESI-microTOF developed in the early 2000s, together with the standardized urinary sample treatment involving dilution with urea, ammonium hydroxide, and SDS followed by ultrafiltration, desalinization, and lyophilization. Finally, the resulting extract is resuspended with HPLC-grade water before injection.[92–94] Basically, this approach follows the traditional workflow of omics-based analysis. After the definition of the biological problem that will be elucidated, the selected samples are collected and treated according to the defined handling protocol followed by the instrumentational analysis, data processing, and, finally, statical treatment are performed usually applying multivariate analysis (MVA) until it achieves a response with biological meaning (Figure 7).[91,95]

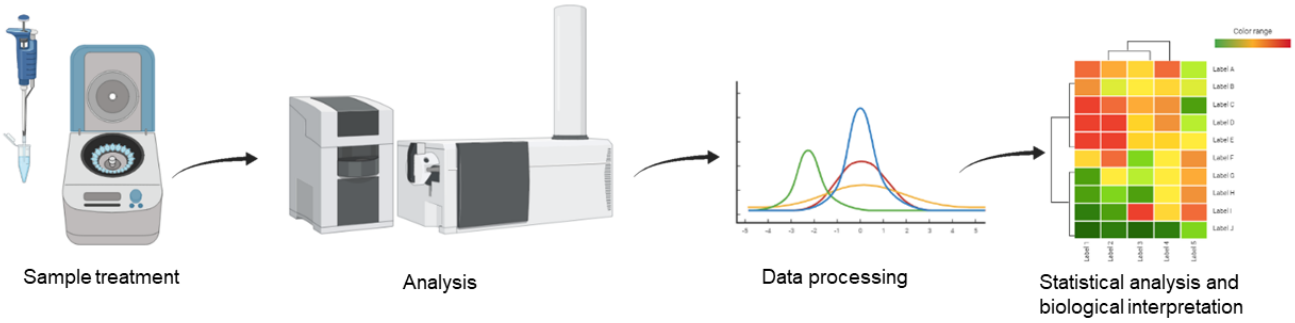


Figure 7. Schematic representation of the general workflow of omics-based protocols.

Recently, through said workflow, a urinary peptide profile was the goal for biomarker determination of Sars-Cov-2 infection patients. Nearly 300 urine samples were analyzed aiming to relate the severity and progression of the disease, according to WHO stages, to the 50-peptide urinary peptides (COV 50) and potential cofounders identified through the CE-MS/MS system and statistical treatment.[96] The same analytical protocol was applied to urine donated by 53 patients with different stages of Sars-Cov-2 infection. In this study, 593 were found to be associated with the disease severity. The authors also compared these peptides with kidney disease or heart failure.[97]

Over the last five years, we have found that this standard workflow was also the chosen course of action for urinary biomarkers access related to Lupus Nephritis,[98] cholangiocarcinoma[99] periprosthetic joint infection,[100] liver fibrosis,[101] PCa,[102] lupus erythematosus,[103] kidney evaluation,[104] detection of solid tumors,[105] and CKD.[106] For CKD in special, the National Kidney Foundation established a urinary proteomics-based classifier (CKD273) where peptides are quantified.[107] Through the standardized method, CE-MS is today one of the main platforms employed for that quantification.[108,109]

As commented before, a few specialists have been promoting the feasibility of CE for proteomics-driven studies as well in a series of review papers.[82,110–113] As Chen *et al.* (2021) highlight, CE-MS can achieve highly efficient separations of mixtures of peptides, proteoforms, and intact protein complexes.[113] The full proteome analysis by CE-MS exhibits significant growth. The evolution of the technique and new technologies facilitated the generation of a large number of comparable data sets, which, according to Latosinka et al. (2019), provides an opportunity for the development of multiple CE-MS-based biomarker panels describing complex diseases for use in diagnosis, prognosis, and treatment.[111,113] The last-mentioned review[111] covers the main studies involving proteomics and peptidomics by CE-MS in urine and other biological samples over the last two decades. The publications discussed in this section are summarized in Table VI.

Table VI. Analytical methods applied to the analysis of peptides and proteins in human urine samples by CE

Analyte	Application	Sample Preparation	Analytical platform	CE experimental conditions	Detector parameters	Ref.
Pro-Hyp, Pro-Pro, Pro-Gly, Pro-Leu, Hyp, and Pro	Bone turnover and osteoporosis	Primary amines blockage followed by fluorogenic derivatization with NBD-F.	Flow-gated CE-LIF	BGE: 40 mmol L^{-1} tetraborate, 40 mmol L^{-1} cholate, 40 mmol L^{-1} deoxycholate. Fused silica capillary: 10 µm i.d., 10 cm effective length, -5 kV x 0.3 s, -25 KV, room temperature.	492 nm (excitation), 520 nm (emission)	88
PSA	Prostate cancer	PSA capture with anti-PSA beads, centrifugation, beads washing, extraction. Reconstitution of a final extract with sodium bicarbonate.[90]	CE-ESI-QqTOF	BGE: 10% acetic acid (v/v, 1.74 mol L^{-1}, pH 2.3). Bare-fused silica capillary: 30 µm i.d., 90 cm total length, 150 µm o.d. 1 psi x 60 s (sample), 0.5 psi x 25 s (BGE), 20 kV.	Capillary voltage between -1.1 kV and -1.3 kV. Drying gas:150 ºC, 1.2 L min^{-1}.	90
PSA	Prostate cancer	PSA capture with anti-PSA beads, centrifugation, beads washing, extraction. Reconstitution of final extract with sodium bicarbonate.[90]	CE-nanoESI-QqTOF	BGE: 20% acetic acid (v/v, 3.49 mol L^{-1}, pH 2.3). Bare-fused silica capillary: 30 µm i.d., 91 cm total length, 150 µm o.d. 100 psi x 4 min (BGE), 8.8 psi x 35 s (sample), -20 kV.	Capillary voltage – 1.35 kV, drying gas: 100 ºC, 1.2 L min^{-1}.	89
Peptidomic profile. (COV50)[96]	COVID-19[96,97], Lupus Nephritis[98], cholangiocarcinoma[99], periprosthetic joint infection[100], liver fibrosis[101], prostate cancer[102] lupus erythematosus[103], kidney evaluation[104], detection of solid tumors[105], and chronic kidney diseases[106]	Dilution with urea, NH_4OH, and SDS and ultrafiltration followed by desalinization and lyophilization. Re-suspension with water.[92]	CE-ESI-microTOF	BGE: 20% ACN in water with 0.94% formic acid. Fused silica capillary: 50 µm i.d. 95 cm 2 psi x 99 s, 30 kV, 35 ºC.[93]	ESI: Capillary voltage between -4.0 kV and -4.5 kV. *m/z* range: 350 – 3000.[93,94,114] Other conditions not mentioned.	96,97, 99-106

ACN: acetonitrile; BGE: background electrolyte; CE: capillary electrophoresis; ESI: electrospray ionization; Hyp: 4-hydroxyproline; i.d.: internal diameter; LIF: laser-induced fluorescence; Q: quadrupole mass spectrometer; NBD-F: 4-fluoro-7-nitro-2,1,3-benzoxadiazole; o.d.: outer diameter; PSA: prostate-specific antigen; SDS: sodium dodecyl sulfate; TOF: time-of-flight mass spectrometer.

Untargeted metabolomics

The primary goal of omic sciences is to gain a complete understanding of cellular function and biological changes. Metabolomics, a term introduced in 2011, is considered the comprehensive and quantitative analysis of low-molecular weight compounds within a biological system. It provides a reading of the physiological status of any organism and thus was rapidly embraced by the science community.[91,115,116] The untargeted metabolomics is based on the qualitative analysis of as many metabolites as possible.[91] For that reason, as mentioned in the previous section is important to use high-performance analytical instrumentation.

CE coupled to MS is a well-established and mature platform for comprehensive analysis, but it still is underappreciated compared to LC-MS systems, one of the reasons is the challenging reproducibility. On that matter, a paper published in 2020 is described a multi-laboratory trial for accessing reproducible data in CE-MS based metabolomics.[117] The aim was to get migration-time reproducibility and determine the most suitable approach for metabolite annotation by comparing relative migration time (RMT) versus electrophoretic mobility (μeff) in standardized BGE. Seventeen laboratories with 20 distinct CE-MS systems participated. By using only BGE composition as a common parameter, 3.1% of total variation on the μeff was achieved, thus, as the authors commented, this so-called Metabo-ring study invigorates the use of CE-MS.

Recently, a few review papers have been commenting on how CE-MS associated with chemometrics is being applied to metabolomics.[6,11,12,118–121] Some authors discuss the simple strategies that are being used for pre-concentration proceedings, for enhancing sensitivity and molecular coverage.[119] Some new approaches will also be discussed in this section. Overall, considering the analytical proceedings, for CE-MS based metabolomics two readings are primarily done, one using acid BGE in positive mode followed by adaptations to negative mode with alkaline BGE.

We have found some authors employing multisegmented injection (MSI) coupled with high-resolution MS/MS protocol aiming to enhance analytical frequency, sensitivity, and mass coverage. The most recent study applied this method for the discovery of biomarkers for the diagnosis and prognosis of pediatric inflammatory bowel disease (IBD).[122] Using both acid and alkaline media with coaxial sheath liquid and tandem MS in positive and negative mode, 122 urinary metabolites were found. The levels of some organic acids, inorganics salts and glucuronides were found to be distorted in IBD infant patients. As a follow-up, a new target protocol using the same system was used to monitor this compound. A similar MSI-CZE-ESI-QTOF method has already been applied two years previously to monitor PKU patients aiming a targeted assay of Phe, this study was a comment on amino acid section.[80]

A convectional CZE-ESI-TOF was used to colorectal cancer urinary profile (CRC).[123] The method used, similar to the protocol aforementioned, was previously applied in a salivary profile study.[124] A different feature of the method is a commercial cationic coated capillary that enables reverse EOF for anionic species[125]. The researchers identified and quantified 154 metabolites, including glycolysis, amino acids, polyamine, and products from tricarboxylic acid (TCA) and urea cycles. In a follow-up study, new samples were analyzed corroborating the previous result where 132 significant metabolites were again identified.[126] Through the same platform and similar screening method, another research group selected glycine and ethanolamine as biomarkers for acute kidney injury (AKI).[127] Finally, similar CZE-ESI-TOF routines were successfully employed for biomarkers discovery of rheumatoid arthritis,[128] gut microbiota on uremic solutes,[129] and in large-scale cohort study.[130]

A novel online two-phase electroextraction (EE) was developed recently and applied to random human urine for demonstration.[131] The system enables rapid sample extraction and it is even more interesting for volume-limited cases. The analysis through the EE-CE-ESI-microTOF optimized method result in the detection of 122 putative metabolites.

A feasibility verification routine was performed using CE-ESI-QqQ as the main untargeted and targeted platform of the sub-5 kDa urine metabolome of specific cancer patients.[132] For the untargeted analysis, ESI was achieved by a bevelled needle tip geometry with a flow-through microvial previously developed and

applied as a robust interface for other purposes.[133] With this distinguished apparatus, they have identified over 400 distinct metabolites for patients.

Overall, we observed a pattern within the optimized CE-MS methods. Basically, in order to enhance *m/z* coverage, a full screening of cations was done by using acidic BGE, normally achieved with formic acid at pH 2 or near, applying positive high separation voltage and analysis in positive mode. As a complement, the screening of anions is done with alkaline BGE, around pH 8.5, negative separation voltage, and negative mode detection, with or without the use of sheath liquid in both acquisition modes. Slight variations were found concerning total capillary length, MS acquisition conditions (capillary, nozzle, and fragmentor voltage), and drying gas flow, pressure, and temperature in the ESI source. Minimal sample preparation was carried out among all studies, we believe that the point is not to lose information as a consequence of sample overhandling. The publications discussed in this section are summarized in Table VII.

Table VII. Analytical methods applied to the analysis of untargeted metabolomics in human urine samples by CE

Application	Sample Preparation	Analytical platform	CE experimental conditions	Detector parameters	Ref.
Pediatric Inflammatory Bowel Disease	Dilution	MSI-CE-ESI-QTOF	BGE (+): 1 mol L^{-1} formic acid 15% ACN (pH 1.8). BGE (-): 50 mmol L^{-1} ammonium bicarbonate (pH 8.50). Uncoated fused silica capillary. 50 µm i.d. 110 cm total length. 30 kV, 25 °C. MSI: sample 100 mbar x 5 s followed by BGE 100 mbar x 40 s.	SHL (+): 60% (v/v) MeOH with 0.1% (v/v) formic acid. SHL (-): 50% (v/v) MeOH. ESI: 3500 V, nozzle voltage: 2000 V. Drying gas: 16 L min^{-1}, 200 °C. Sheath gas: 3.5 L min^{-1}, 200 °C. Nebulizer pressure: 8 psig. Fragmentor: 120 V. *m/z* range 50–1700.	122
Colorectal cancer	Centrifugation, dilution, and filtration	CE-ESI-TOF	BGE (+): 1 mol L^{-1} formic acid. Capillary (+): Fused silica 50 µm i.d. 95 cm total length. 50 mbar x 5 s, 30 kV, 20 °C. BGE (-): 50 mmol L^{-1} AA (pH 8.50). Capillary column coated with cationic polymer 50 µm i.d. 105 cm total length. 50 m bar x 30 s, -30 kV, 20 °C, tray temperature kept below 5 °C.[124]	SHL (+): 50 % (v/v) MeOH/water at 10 µl min^{-1} ESI (+): 4000 V. Drying gas: 300 °C. Nebulizer pressure: 7 psig. Fragmentor: 75 V. *m/z* range 50 – 1000. SHL (-): 5 mmol L^{-1} AA in 50% (v/v) MeOH/water, 1:100 splitter at 10 µl min^{-1}. ESI (-): 3500 V. Drying gas: 300 °C. 7 psig. Fragmentor: 100 V. *m/z* range 50 – 1000.[124]	123
Acute kidney Injury	Dilution, ultrafiltration and centrifugation	CE-ESI-TOF	BGE (+): 1 mol L^{-1} formic acid. Fused-silica capillary column 50 µm i.d. 100 cm total length. 5 kPa x 3 s, 30 kV. BGE (-): 50 mmol L^{-1} AA (pH 8.5). Capillary coated with cationic polymer. 50 kPa x 30 s, -30 kV.	SHL (+): 50% (v/v) MeOH/water at 10 L min^{-1}. SHL (-): 5 mmol L^{-1} AA in 50% (v/v) MeOH/water at 10 L min^{-1}.	127
Rheumatoid arthritis	Dilution with water and organic solvent. Centrifugation and filtration. Filtrate resuspended in water.	CE-ESI-QTOF	BGE (+):1 mol L^{-1} formic acid. Fused-silica capillary column 50 µm i.d. 80 cm total length. 50 mbar x 10 s, 27 kV. BGE (-): Commercial solution (H3302-1022) and 50 mmol L^{-1} AA (pH 8.5). 50 mbar x 6 s.[134]	SHL: commercial solution (H3301-1020, HMT) at 10 µl min^{-1}. ESI (+) 4000 V. Drying gas: 300 °C at 7 L min^{-1}, 5 psig. Fragmentor: 80 V. ESI (-). 3500 V. Drying gas: 300 °C at 7 L min^{-1}, 5 psig. Fragmentor: 125 V. *m/z* range 50 – 1000.[134]	128
Gut microbiota	Extraction with methanol	CE-ESI-TOF	BGE (+): 1 mol L^{-1} formic acid. Fused-silica capillary column 50 µm i.d. 100 cm total length. 50 mbar x 3 s, 30 kV, 20 °C. BGE (-): 50 mmol L^{-1} AA (pH 8.50). Capillary column coated with cationic polymer: 50 µm i.d. 100 cm total length. 50 mbar x 30 s, -30 kV.[135,136]	SHL (+): 5 mmol L^{-1} AA in 50% (v/v) MeOH/water at 10 µL min^{-1}. ESI (+): 4000 V. Drying gas: 300 °C, 10 µL min^{-1}. *m/z* range: 70 – 1027. ESI (-): 3500 V. [135,136]	129
Random	Dilution with water and EtOAc	EE-CE-ESI-microTOF	BGE: 1 mol L^{-1} formic acid. Bared fused silica capillary 50 µm i.d. 80 cm total length.	SHL: 1 mol L^{-1} formic acid in 50% (v/v) MeOH/water at 15 µL min^{-1}. Make-up liquid for EE: 1 mol L^{-1} formic acid at 50 µL min^{-1}	131
Prostate and bladder cancer	none	CE-ESI-QqQ	BGE (-): 2% (v/v) formic acid, 50% (v/v) MeOH and 48% (v/v) water, Fused silica capillary coated with cationic polymer trimethoxysilylpropyl polyethyleneimine-HCl in isopropanol (50% v/v). 1 psi x 10 s, -30 kV.	*m/z* range 50 – 850. Other conditions are not mentioned.	132

AA: ammonium acetate; ACN: acetonitrile; BGE: background electrolyte; CE: capillary electrophoresis; EE: electroextraction; ESI: electrospray ionization; EtOAc: ethyl acetate; i.d.: internal diameter; MeOH: methanol; MSI: multisegmented injection; QqQ: triple-quadrupole mass spectrometer; QTOF: quadrupole time-of-flight mass spectrometer; SHL: sheath liquid; TOF: time-of-flight mass spectrometer.

OVERVIEW AND PERSPECTIVES

Throughout this compilation of recent studies involving urinary identification of specific molecules and comprehensive profiles by CE, we came across some insights and tendencies of novel and traditional methods application. Comparing all the chemical classes discussed in this review, we noticed that the main adopted strategies have some similarities considering sample treatment, on the other hand, CE was explored in a variety of modes. BGE composition for instance has used at the extremes of the pH scale within a unique class of compounds. Typically, the same protocol is used in a variety of cases because what really matters is the most comprehensive response possible. In this context, we put together a mind-map containing all the most common sample handling and analytical procedures for the analysis of specific molecules and groups of molecules considering their respective singularities (Figure 8).

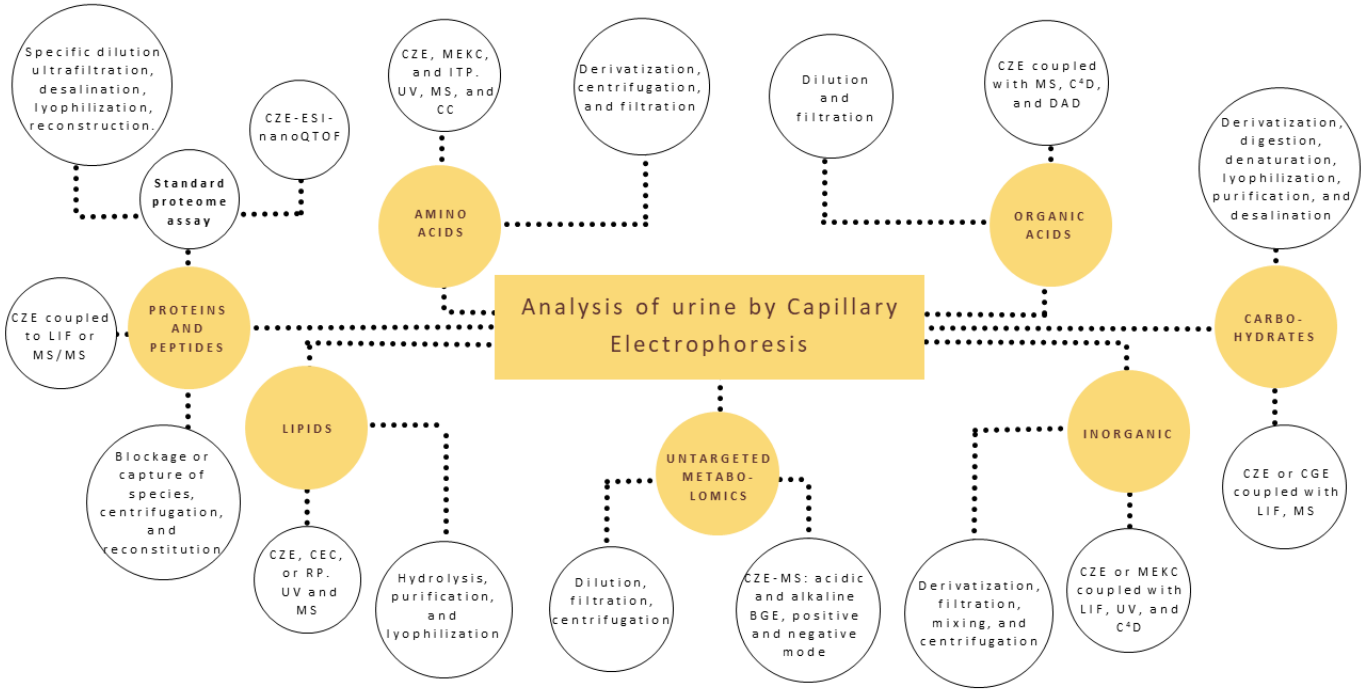


Figure 8. Mind-map of information gathered about sample preparation, CE modes, and detection techniques used for the urinary analysis of amino acids, organic acids, carbohydrates, inorganics, lipids, proteins, peptides, and untargeted metabolomics.

About the technique itself, we have noticed a growth in the number of publications pursuing CE method development in the past two decades but it is still underappreciated when compared to traditional instrumentation, especially HPLC. For the specific analysis of organic acids and inorganic species, we believe CE could already be more explored given its intrinsic potential to separate these compounds with much lower cost and reduced residue generation. We have shown that CE-MS at most is already being introduced for direct clinical analysis, so, we believe that going further, with the rapid advance of miniaturization devices, 3D printing, portable detectors possibilities, the potential of CE is in hand to be applied in multi-segmented facilities.

Conflicts of interest

The authors declare no financial nor management relationship conflicts.

Acknowledgements

This work was supported by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil" (CAPES) – Finance Code 001; "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPQ: 424032/2018-0, 303867/2020-5, Schorlaship: 142502/2020-0); "Financiadora de Estudos e Projetos" (Finep) (CT-INFRA 01/2013-REF 0633/13); RQ-MG (CEX.RED- 00010-14); "Instituto Nacional de Ciência e Tecnologia Bioanalítica" (INCTBio) (FAPESP 2014/50867-3, CNPq 465389/2014-7, 401256/2020-0); "Fundação de Amparo à Pesquisa do Estado de Minas Gerais" (FAPEMIG) (APQ-00585-21).

REFERENCES

(1) Sarigul, N.; Korkmaz, F.; Kurultak, İ. A New Artificial Urine Protocol to Better Imitate Human Urine. *Sci. Rep.* **2019**, *9* (1), 1–11. https://doi.org/10.1038/s41598-019-56693-4

(2) Bouatra, S.; Aziat, F.; Mandal, R.; Guo, A. C.; Wilson, M. R.; Knox, C.; Bjorndahl, T. C.; Krishnamurthy, R.; Saleem, F.; Liu, P.; Dame, Z. T.; Poelzer, J.; Huynh, J.; Yallou, F. S.; Psychogios, N.; Dong, E.; Bogumil, R.; Roehring, C.; Wishart, D. S. The Human Urine Metabolome. *PLoS One* **2013**, *8* (9). https://doi.org/10.1371/journal.pone.0073076

(3) Rowe, S. *Fundamentals of Urine and Body Fluid Analysis [Book Review]*; 2005; Vol. 26.

(4) Ridley, J. W. *Fundamentals of the Study of Urine and Body Fluids*; 2018. https://doi.org/10.1007/978-3-319-78417-5

(5) Thévenot, E. A.; Roux, A.; Xu, Y.; Ezan, E.; Junot, C. Analysis of the Human Adult Urinary Metabolome Variations with Age, Body Mass Index, and Gender by Implementing a Comprehensive Workflow for Univariate and OPLS Statistical Analyses. *J. Proteome Res.* **2015**, *14* (8), 3322–3335. https://doi.org/10.1021/acs.jproteome.5b00354

(6) Helena, H.; Ivona, V.; Roman, Ř.; František, F. Current Applications of Capillary Electrophoresis-Mass Spectrometry for the Analysis of Biologically Important Analytes in Urine (2017 to Mid-2021): A Review. *J. Sep. Sci.* **2022**, *45* (1), 305–324. https://doi.org/10.1002/jssc.202100621

(7) Canuto, G. A. B.; Costa, J. L. da; Cruz, P. L. R. da; Souza, A. R. L. de; Faccio, A. T.; Klassen, A.; Rodrigues, K. T.; Tavares, M. F. M. Metabolomics: Definitions, State-of-the-Art and Representative Applications. *Quim. Nova* **2018**, *41* (1), 75-91. http://dx.doi.org/10.21577/0100-4042.20170134

(8) Ramautar, R.; Demirci, A.; Jong, G. J. d. Capillary Electrophoresis in Metabolomics. *TrAC - Trends Anal. Chem.* **2006**, *25* (5), 455–466. https://doi.org/10.1016/j.trac.2006.02.004

(9) Vaz, F. S.; Oliveira, C. L. M. De; Oliveira, M. A. L. De. Fundamentos de Eletroforese Capilar: Uma Abordagem por Animações. *Quím. Nov.* **2015**, *38* (5), 732–737. http://dx.doi.org/10.5935/0100-4042.20150065

(10) Gałęzowska, G.; Cieszynska-Semenowicz, M.; Okrągła, E.; Szychowska, K.; Wolska, L. Progress in Analytical Techniques for Determination of Urine Components. *Sep. Purif. Rev.* **2017**, *46* (4), 305–318. https://doi.org/10.1080/15422119.2017.1281826

(11) Maier, T. V.; Schmitt-Kopplin, P. Capillary Electrophoresis in Metabolomics. In: Schmitt-Kopplin. P. (Ed) *Capillary Electrophoresis. Methods in Molecular Biology*, **2016**, *1483*. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-6403-1_21

(12) Ramautar, R. *Capillary Electrophoresis-Mass Spectrometry for Clinical Metabolomics*, 1st ed.; Elsevier Inc., **2016**, *74*. https://doi.org/10.1016/bs.acc.2015.12.002

(13) Oliveira, M. A. L. de; Soares, D. do C.; Tostes, G. S.; Guimarães, M. do C.; Vaz, F. A. S. Optimization of an Alternative Methodology for Simultaneous Analysis of Nitrite and Nitrate in Water from Urban Stream by Capillary Electrophoresis under Direct UV Detection. *Am. J. Anal. Chem.* **2012**, *03* (07), 484–490. https://doi.org/10.4236/ajac.2012.37064

(14) Da Silva, J. A. F.; Coltro, W. K. T.; Carrilho, E.; Tavares, M. F. M. Terminology for Analytical Capillary Electromigration Techniques. *Quim. Nova* **2007**, *30* (3), 740–744. https://doi.org/10.1590/s0100-40422007000300040

(15) Riekkola, M. L.; Jönsson, J. Å.; Smith, R. M. Terminology for Analytical Capillary Electromigration Techniques: (IUPAC Recommendations 2003). *Pure Appl. Chem.* **2004**, *76* (2), 443–451. https://doi.org/10.1351/pac200476020443
(16) Souza, J. C. Q. De; Moreira, O. B. O.; Oliveira, M. A. L.; Chellini, P. R. Potencialidades da Eletroforese Capilar de Zona em Análises de Fármacos. **2020**.
(17) Vaidyanathan, K.; Narayanan, M. P.; Vasudevan, D. M. Organic Acidurias: An Updated Review. *Indian J. Clin. Biochem.* **2011**, *26* (4), 319–325. https://doi.org/10.1007/s12291-011-0134-2
(18) Gallagher, R. C.; Pollard, L.; Scott, A. I.; Huguenin, S.; Goodman, S.; Sun, Q. Laboratory Analysis of Organic Acids, 2018 Update: A Technical Standard of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* **2018**, *20* (7), 683–691. https://doi.org/10.1038/gim.2018.45
(19) Galli, V.; García, A.; Saavedra, L.; Barbas, C. Capillary Electrophoresis for Short-Chain Organic Acids and Inorganic Anions in Different Samples. *Electrophoresis* **2003**, *24* (12–13), 1951–1981. https://doi.org/10.1002/elps.200305473
(20) Duez, P.; Kumps, A.; Mardens, Y. GC-MS Profiling of Urinary Organic Acids Evaluated as a Quantitative Method. *Clin. Chem.* **1996**, *42* (10), 1609–1615. https://doi.org/10.1093/clinchem/42.10.1609
(21) Kuhara, T. Gas Chromatographic-Mass Spectrometric Urinary Metabolome Analysis to Study Mutations of Inborn Errors of Metabolism. *Mass Spectrom. Rev.* **2005**, *24* (6), 814–827. https://doi.org/10.1002/mas.20038
(22) Švidrnoch, M.; Přibylka, A.; Bekárek, V.; Ševčík, J.; Smolka, V.; Maier, V. Enantioseparation of D,L-2-Hydroxyglutaric Acid by Capillary Electrophoresis with Tandem Mass Spectrometry—Fast and Efficient Tool for D- and L-2-Hydroxyglutaracidurias Diagnosis. *J. Chromatogr. A* **2016**, *1467*, 383–390. https://doi.org/10.1016/j.chroma.2016.05.095
(23) Cieslarova, Z.; Magaldi, M.; Barros, L. A.; do Lago, C. L.; Oliveira, D. R.; Fonseca, F. A. H.; Izar, M. C.; Lopes, A. S.; Tavares, M. F. M.; Klassen, A. Capillary Electrophoresis with Dual Diode Array Detection and Tandem Mass Spectrometry to Access Cardiovascular Biomarkers Candidates in Human Urine: Trimethylamine-N-Oxide and L-Carnitine. *J. Chromatogr. A* **2019**, *1583*, 136–142. https://doi.org/10.1016/j.chroma.2018.10.005
(24) Chaneam, S.; Kaewyai, K.; Mantim, T.; Chaisuksant, R.; Wilairat, P.; Nacapricha, D. Simultaneous and Direct Determination of Urea and Creatinine in Human Urine Using a Cost-Effective Flow Injection System Equipped with in-House Contactless Conductivity Detector and LED Colorimeter. *Anal. Chim. Acta* **2019**, *1073*, 54–61. https://doi.org/10.1016/j.aca.2019.05.003
(25) Fernández-Bravo, J.; de Andrés, F.; Zougagh, M.; Ríos, Á. Selective Screening of Glutaric Acid Acidurias by Capillary Electrophoresis-Mass Spectrometry. *J. Pharm. Biomed. Anal.* **2017**, *145*, 40–45. https://doi.org/10.1016/j.jpba.2017.06.037
(26) Öztekin, N.; Balta, G. S.; Cansever, M. Ş. Determination of Homogentisic Acid in Urine for Diagnosis of Alcaptonuria: Capillary Electrophoretic Method Optimization Using Experimental Design. *Biomed. Chromatogr.* **2018**, *32* (7). https://doi.org/10.1002/bmc.4216
(27) Šebestová, A.; Petr, J. Determination of Orotic Acid in Human Urine Using a Combination of Two Capillaries with Different Internal Diameters. *Chem. Pap.* **2020**, *74* (7), 2375–2379. https://doi.org/10.1007/s11696-020-01076-7
(28) Milofsky, R.; Spaeth, S. Determination of Orotic Acid in Aqueous Solutions by Micellar Electrokinetic Capillary Chromatography Using Sensitized Lanthanide-Ion Luminescence Detection. *Chromatographia* **1996**, *42* (1–2), 12–16. https://doi.org/10.1007/BF02271048
(29) Özçelik, S.; Öztekin, N.; Kıykım, E.; Cansever, M. Ş.; Aktuğlu-Zeybek, A. Ç. Capillary Electrophoresis with Capacitively Coupled Contactless Conductivity Detection for the Determination of Urinary Ethylmalonic Acid for the Diagnosis of Ethylmalonic Aciduria. *J. Sep. Sci.* **2020**, *43* (7), 1365–1371. https://doi.org/10.1002/jssc.201901044
(30) Zhao, S.; Yin, D.; Du, H.; Tian, X.; Chen, Y.; Zhang, W.; Yu, A.; Zhang, S. Determination of Oxalate and Citrate in Urine by Capillary Electrophoresis Using Solid-Phase Extraction and Capacitively Coupled Contactless Conductivity Based on an Improved Mini-Cell. *J. Sep. Sci.* **2018**, *41* (12). https://doi.org/10.1002/jssc.201701432

(31) Danzer, K.; Currie, L. A. Guideline for Calibration in Analytical Chemistry— Part 1. Fundamentals and Single Component Calibration. *Pure Appl. Chem.* **1998**, *70* (4), 993–1014. https://doi.org/10.1351/pac199870040993
(32) Pimentel, M. F.; Neto, B. B. Calibração: Uma Revisão para Químicos Analíticos. *Quim. Nova*. **1996**, *19* (3), 268-277.
(33) Chutipongtanate, S.; Thongboonkerd, V. Systematic comparisons of artificial urine formulas for in vitro cellular study. *Anal. Biochem*. **2010**, *402* (1), 110-112. http://dx.doi.org/10.1016/j.ab.2010.03.031
(34) Tanyanyiwa, J.; Rainelli, A.; Hauser, P. C.; Wan, Q. J.; Kub, P. Determination of Major Inorganic Ions in Blood Serum and Urine by Capillary Electrophoresis with Contactless Conductivity Detection. **2004**, *525*, 11–16. https://doi.org/10.1016/j.aca.2004.08.002
(35) Ghaderinezhad, F.; Koydemir, H. C.; Tseng, D.; Karinca, D. Sensing of electrolytes in urine using a miniaturized paper-based device. *Sci. Rep*. **2020**, *10*, 13620. https://doi.org/10.1038/s41598-020-70456-6
(36) Yamamoto, S.; Fujiwara, H.; Maruyama, K.; Tanaka, Y.; Kinoshita, M.; Suzuki, S. Simultaneous Determination of Inorganic Anions and Cations in Water and Biological Samples by Capillary Electrophoresis with a Capacitive Coupled Contactless Conductivity Detector Using Capillary Filling Method. *Anal. Sci.* **2019**, *35* (3) 295-300. https://doi.org/10.2116/analsci.18P422
(37) Macedo, A. N. De; Jiwa, I. M. Y.; Macri, J.; Belostotsky, V.; Hill, S.; Britz-mckibbin, P. Strong Anion Determination in Biological Fluids by Capillary Electrophoresis for Clinical Diagnostics. *Anal. Chem.* **2013**, 85, 22, 11112–11120. https://doi.org/10.1021/ac402975q
(38) Kubalczyk, P.; Chwatko, G.; Głowacki, R. Fast and Simple MEKC Sweeping Method for Determination of Thiosulfate in Urine. *Electrophoresis* **2016**, 1155–1160. https://doi.org/10.1002/elps.201500411.
(39) Zhang, Q.; Maddukuri, N.; Gong, M. A Direct and Rapid Method to Determine Cyanide in Urine by Capillary Electrophoresis. *J. Chromatogr. A* **2015**, *1414*, 158–162. https://doi.org/10.1016/j.chroma.2015.08.050
(40) OpenStax. Carbohydrates. https://openstax.org/ (Accessed on 2022-05-13).
(41) Zamfir, A. D.; Dinca, N.; Sisu, E.; Peter-Katalinic, J. Copper-Coated Microsprayer Interface for on-Line Sheathless Capillary Electrophoresis Electrospray Mass Spectrometry of Carbohydrates. *J. Sep. Sci*. **2006**, *29* (3), 414–422. https://doi.org/10.1002/jssc.200500374
(42) Easley, C. J.; Ji, L.; Presto, K. B.; Jellum, E.; Landers, J. P.; Ferrance, J. P. Capillary Electrophoresis with Laser-Induced Fluorescence Detection for Laboratory Diagnosis of Galactosemia. *J. Chromatogr. A* **2003**, *1004*, 29–37. https://doi.org/10.1016/S0021-9673(03)00767-2
(43) Casado, M.; Altimira, L.; Montero, R. A Capillary Electrophoresis Procedure for the Screening of Oligosaccharidoses and Related Diseases. *Anal. Bioanal. Chem*. **2014**, *406*, 4337–4343. https://doi.org/10.1007/s00216-014-7832-6
(44) Reider, B.; Gacsi, E.; Jankovics, H.; Vonderviszt, F.; Szarvas, T.; Guttman, A.; Jarvas, G. Integrated workflow for urinary prostate specific antigen *N*-glycosylation analysis using sdAb partitioning and downstream capillary electrophoresis separation. *Anal. Chim. Acta* **2021**, *1184*, 338892. https://doi.org/10.1016/j.aca.2021.338892
(45) Vermassen, T.; Praet, C. Van; Vanderschaeghe, D.; Maenhout, T.; Lumen, N.; Callewaert, N.; Hoebeke, P.; Belle, S. Van; Rottey, S. Capillary Electrophoresis of Urinary Prostate Glycoproteins Assists in the Diagnosis. *Electrophoresis* **2014**, *35* (7), 1017–1024. https://doi.org/10.1002/elps.201300332
(46) Laroy, W.; Contreras, R.; Callewaert, N. Glycome Mapping on DNA Sequencing Equipment. *Nat. Protoc.* **2006**, *1* (1), 397–405. https://doi.org/10.1038/nprot.2006.60
(47) Han, X.; Sanderson, P.; Nesheiwat, S.; Lin, L.; Zhang, F.; Amster, I. J.; Linhardt, R. J. Structural Analysis of Urinary Glycosaminoglycans from Healthy Human Subjects. *Glycobiology* **2020**, *30* (3), 143–151. https://doi.org/10.1093/glycob/cwz088
(48) Jin, L. J.; Li, S. F. Y. Screening of Carbohydrates in Urine by Capillary Electrophoresis. *Electrophoresis* **1999**, *20* (17), 3450–3454. https://doi.org/10.1002/(SICI)1522-2683(19991101)20:17<3450::AID-ELPS3450>3.0.CO;2-G

(49) Thompson, T. E. *Lipid*. Available at: https://www.britannica.com/science/lipid [Accessed on Jan. 2022].
(50) De Oliveira, M. A. L.; Porto, B. L. S.; Faria, I. D. L.; De Oliveira, P. L.; De Castro Barra, P. M.; Castro, R. J. C.; Sato, R. T. 20 Years of Fatty Acid Analysis By Capillary Electrophoresis. *Molecules* **2014**, *19* (9), 14094–14113. https://doi.org/10.3390/molecules190914094
(51) Nagy, K.; Tiuca, I.-D. Importance of Fatty Acids in Physiopathology of Human Body. *Fat. Acids* **2017**, 3–22. https://doi.org/10.5772/67407
(52) Calder, P. C. Functional Roles of Fatty Acids and Their Effects on Human Health. *J. Parenter. Enter. Nutr.* **2015**, *39*, 18S-32S. https://doi.org/10.1177/0148607115595980
(53) Phillips, T. M. Recent Advances in CE and Microchip-CE in Clinical Applications: 2014 to Mid-2017. *Electrophoresis* **2018**, *39* (1), 126–135. https://doi.org/10.1002/elps.201700283
(54) Poinsot, V.; Ta, H. Y.; Meang, V. O.; Perquis, L.; Gavard, P.; Pipy, B.; Couderc, F. A Digest of Capillary Electrophoretic Methods Applied to Lipid Analyzes. *Electrophoresis* **2019**, *40* (1), 190–211. https://doi.org/10.1002/elps.201800264
(55) Jang, R.; Kim, K. H.; Zaidi, S. A.; Cheong, W. J.; Moon, M. H. Analysis of Phospholipids Using an Open-Tubular Capillary Column with a Monolithic Layer of Molecularly Imprinted Polymer in Capillary Electrochromatography-Electrospray Ionization-Tandem Mass Spectrometry. *Electrophoresis* **2011**, *32* (16), 2167–2173. https://doi.org/10.1002/elps.201100205
(56) Fellah, S. El; Sirén, H. Recent Developments in Capillary Electrophoresis of Steroids and Sterols. *J. Biomed. Res. Pract.* **2018**, *2* (1), 1–21. http://hdl.handle.net/10138/309706
(57) Flor, S.; Lucangioli, S.; Contin, M.; Tripodi, V. Simultaneous Determination of Nine Endogenous Steroids in Human Urine by Polymeric-Mixed Micelle Capillary Electrophoresis. *Electrophoresis* **2010**, *31* (19), 3305–3313. https://doi.org/10.1002/elps.201000096
(58) Du, B.; Zhang, J.; Dong, Y.; Wang, J.; Lei, L.; Shi, R. Determination of Testosterone/Epitestosterone Concentration Ratio in Human Urine by Capillary Electrophoresis. *Steroids* **2020**, *161*, 108691. https://doi.org/10.1016/j.steroids.2020.108691
(59) Pieckowski, M.; Kowalski, P.; Olędzka, I.; Miękus-Purwin, N.; Plenis, A.; Roszkowska, A.; Bączek, T. Simultaneous determination of mitotane, its metabolite, and five steroid hormones in urine samples by capillary electrophoresis using β-CD2SDS1 complexes as hydrophobic compounds solubilizers. *Electrophoresis* **2021**, 1–8. https://doi.org/10.1002/elps.202100250
(60) Burtis, C. A.; Bruns, D. E. *Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics*, 7th ed.; Elsevier, **2014**.
(61) Ta, H. Y.; Collin, F.; Perquis, L.; Poinsot, V.; Ong-Meang, V.; Couderc, F. Twenty Years of Amino Acid Determination Using Capillary Electrophoresis: A Review. *Anal. Chim. Acta* **2021**, *1174*. https://doi.org/10.1016/j.aca.2021.338233
(62) Pérez-Míguez, R.; Salido-Fortuna, S.; Castro-Puyana, M.; Marina, M. L. Advances in the Determination of Nonprotein Amino Acids in Foods and Biological Samples by Capillary Electrophoresis. *Crit. Rev. Anal. Chem.* **2019**, *49* (5), 459–475. https://doi.org/10.1080/10408347.2018.1546113
(63) Denoroy, L.; Parrot, S. Analysis of Amino Acids and Related Compounds by Capillary Electrophoresis. *Sep. Purif. Rev.* **2017**, *46* (2), 108–151. https://doi.org/10.1080/15422119.2016.1212378
(64) Poinsot, V.; Ong-Meang, V.; Ric, A.; Gavard, P.; Perquis, L.; Couderc, F. Recent Advances in Amino Acid Analysis by Capillary Electromigration Methods: June 2015–May 2017. *Electrophoresis* **2018**, *39* (1), 190–208. https://doi.org/10.1002/elps.201700270
(65) Poinsot, V.; Ong-Meang, V.; Gavard, P.; Couderc, F. Recent Advances in Amino Acid Analysis by Capillary Electromigration Methods, 2013-2015. *Electrophoresis* **2016**, *37* (1), 142–161. https://doi.org/10.1002/elps.201500302
(66) Poinsot, V.; Ong-Meang, V.; Gavard, P.; Couderc, F. Recent Advances in Amino Acid Analysis by Capillary Electromigration Methods, 2011-2013. *Electrophoresis* **2014**, *35* (1), 50–68. https://doi.org/10.1002/elps.201300306

(67) Prata, C.; Bonnafous, P.; Fraysse, N.; Treilhou, M.; Poinsot, V.; Couderc, F. Recent Advances in Amino Acid Analysis by Capillary Electrophoresis. *Electrophoresis* **2001**, *22*, 4129–4138. https://doi.org/10.1002/elps.201100360
(68) Poinsot, V.; Gavard, P.; Feurer, B.; Couderc, F. Recent Advances in Amino Acid Analysis by CE. *Electrophoresis* **2010**, *31* (1), 105–121. https://doi.org/10.1002/elps.200900399
(69) Poinsot, V.; Rodat, A.; Gavard, P.; Feurer, B.; Couderc, F. Recent Advances in Amino Acid Analysis by CE. *Electrophoresis* **2008**, *29* (1), 207–223. https://doi.org/10.1002/elps.200700482
(70) Poinsot, V.; Lacroix, M.; Maury, D.; Chataigne, G.; Feurer, B.; Couderc, F. Recent Advances in Amino Acid Analysis by Capillary Electrophoresis. *Electrophoresis* **2006**, *27* (1), 176–194. https://doi.org/10.1002/elps.200500512
(71) Poinsot, V.; Bayle, C.; Couderc, F. Recent Advances in Amino Acid Analysis by Capillary Electrophoresis. *Electrophoresis* **2003**, *24* (22–23), 4047–4062. https://doi.org/10.1002/elps.200305692
(72) Smith, J. T. Recent Advancements in Amino Acid Analysis Using Capillary Electrophoresis. *Electrophoresis* **1999**, *20* (15–16), 3078–3083. https://doi.org/10.1002/(sici)1522-2683(19991001)20:15/16<3078::aid-elps3078>3.0.co;2-j
(73) Ma, S.; Xu, Z.; Ren, J. Analysis of Neurochemicals by Capillary Electrophoresis in Athletes' Urine and a Pilot Study of Their Changes Responding to Sport Fatigue. *Anal. Methods* **2019**, *11* (20), 2712–2719. https://doi.org/10.1039/c9ay00457b
(74) Ramezani, Z.; Safdarian, M.; Ghadiri, A. A. Metal-Coded Hydrogel Magnetic Molecularly Imprinted Polymer for Preconcentration and Cleanup of Sarcosine: Determination in Urine; Coupled to on-Column Capillary Electrophoresis. *Talanta* **2021**, *230*, 122309. https://doi.org/10.1016/j.talanta.2021.122309
(75) Ivanov, A. V.; Bulgakova, P. O.; Virus, E. D.; Kruglova, M. P.; Alexandrin, V. V. evich; Gadieva, V. A.; Luzyanin, B. P.; Kushlinskii, N. E. evich; Fedoseev, A. N.; Kubatiev, A. A. Capillary Electrophoresis Coupled with Chloroform-Acetonitrile Extraction for Rapid and Highly Selective Determination of Cysteine and Homocysteine Levels in Human Blood Plasma and Urine. *Electrophoresis* **2017**, *38* (20), 2646–2653. https://doi.org/10.1002/elps.201700133
(76) Troska, P.; Mandzakova, A.; Hradski, J.; Sevcik, J.; Masar, M. Determination of Homocysteine in Urine and Saliva by Microchip Electrophoresis. *Hungarian J. Ind. Chem.* **2018**, *46* (1), 43–46. https://doi.org/10.1515/hjic-2018-0010
(77) Zhu, Q.; Zhang, Q.; Zhang, N.; Gong, M. Alternate Injections Coupled with Flow-Gated Capillary Electrophoresis for Rapid and Accurate Quantitative Analysis of Urine Samples. *Anal. Chim. Acta* **2017**, *978*, 55–60. https://doi.org/10.1016/j.aca.2017.04.044
(78) Rodrigues, K. T.; Mekahli, D.; Tavares, M. F. M.; van Schepdael, A. Development and Validation of a CE-MS Method for the Targeted Assessment of Amino Acids in Urine. *Electrophoresis* **2016**, *37* (7–8), 1039–1047. https://doi.org/10.1002/elps.201500534
(79) Piestansky, J.; Matuskova, M.; Cizmarova, I.; Majerova, P.; Kovac, A.; Mikus, P. Ultrasensitive Determination of Serotonin in Human Urine by a Two Dimensional Capillary Isotachophoresis-Capillary Zone Electrophoresis Hyphenated with Tandem Mass Spectrometry. *J. Chromatogr. A* **2021**, *1648*, 462190. https://doi.org/10.1016/j.chroma.2021.462190
(80) Wild, J.; Shanmuganathan, M.; Hayashi, M.; Potter, M.; Britz-Mckibbin, P. Metabolomics for Improved Treatment Monitoring of Phenylketonuria: Urinary Biomarkers for Non-Invasive Assessment of Dietary Adherence and Nutritional Deficiencies. *Analyst* **2019**, *144* (22), 6595–6608. https://doi.org/10.1039/c9an01642b
(81) Nelson, D. L.; Cox, M. M. *Principios de Bioquímica de Lehninger*, 6th ed.; Fraga, S. de, Ed.; Artmed Editora LTDA, 2014. https://doi.org/10.2307/j.ctvk8vxxm.7
(82) Štěpánová, S.; Kašička, V. Recent Developments and Applications of Capillary and Microchip Electrophoresis in Proteomic and Peptidomic Analyses. *J. Sep. Sci.* **2016**, *39* (1), 198–211. https://doi.org/10.1002/jssc.201500973
(83) Aitekenov, S.; Gaipov, A.; Bukasov, R. Review: Detection and Quantification of Proteins in Human Urine. *Talanta* **2021**, *223* (P1), 121718. https://doi.org/10.1016/j.talanta.2020.121718

(84) Haselberg, R.; de Jong, G. J.; Somsen, G. W. CE-MS for the Analysis of Intact Proteins 2010-2012. *Electrophoresis* **2013**, *34* (1), 99–112. https://doi.org/10.1002/elps.201200439
(85) Mikšík, I. Coupling of CE-MS for Protein and Peptide Analysis. *J. Sep. Sci.* **2019**, *42* (1), 385–397. https://doi.org/10.1002/jssc.201800817
(86) Latosinska, A.; Siwy, J.; Faguer, S.; Beige, J.; Mischak, H.; Schanstra, J. P. Value of Urine Peptides in Assessing Kidney and Cardiovascular Disease. *Proteomics - Clin. Appl.* **2021**, *15* (1), 1–14. https://doi.org/10.1002/prca.202000027
(87) Pontillo, C.; Mischak, H. Urinary Peptide-Based Classifier CKD273: Towards Clinical Application in Chronic Kidney Disease. *Clin. Kidney J.* **2017**, *10* (2), 192–201. https://doi.org/10.1093/ckj/sfx002
(88) Zhang, N.; Zhu, Q.; Gong, M. Rapid Determination of Free Prolyl Dipeptides and 4-Hydroxyproline in Urine Using Flow-Gated Capillary Electrophoresis. *Anal. Bioanal. Chem.* **2017**, *409* (30), 7077–7085. https://doi.org/10.1007/s00216-017-0666-2
(89) Moran, A. B.; Domínguez-Vega, E.; Nouta, J.; Pongracz, T.; de Reijke, T. M.; Wuhrer, M.; Lageveen-Kammeijer, G. S. M. Profiling the Proteoforms of Urinary Prostate-Specific Antigen by Capillary Electrophoresis – Mass Spectrometry. *J. Proteomics* **2021**, *238*, 0–9. https://doi.org/10.1016/j.jprot.2021.104148
(90) Kammeijer, G. S. M.; Nouta, J.; De La Rosette, J. J. M. C. H.; De Reijke, T. M.; Wuhrer, M. An In-Depth Glycosylation Assay for Urinary Prostate-Specific Antigen. *Anal. Chem.* **2018**, *90* (7), 4414–4421. https://doi.org/10.1021/acs.analchem.7b04281
(91) Canuto, G. A. B.; Da Costa, J. L.; Da Cruz, P. L. R.; De Souza, A. R. L.; Faccio, A. T.; Klassen, A.; Rodrigues, K. T.; Tavares, M. F. M. Metabolômica: Definições, Estado-Da-Arte E Aplicações Representativas. *Quim. Nova* **2018**, *41* (1), 75–91. https://doi.org/10.21577/0100-4042.20170134
(92) Theodorescu, D.; Wittke, S.; Ross, M. M.; Walden, M.; Conaway, M.; Just, I.; Mischak, H.; Frierson, H. F. Discovery and Validation of New Protein Biomarkers for Urothelial Cancer: A Prospective Analysis. *Lancet Oncol.* **2006**, *7* (3), 230–240. https://doi.org/10.1016/S1470-2045(06)70584-8
(93) Theodorescu, D.; Fliser, D.; Wittke, S.; Mischak, H.; Krebs, R.; Walden, M.; Ross, M.; Eltze, E.; Bettendorf, O.; Wulfing, C.; Semjonow, A. Pilot Study of Capillary Electrophoresis Coupled to Mass Spectrometry as a Tool to Define Potential Prostate Cancer Biomarkers in Urine. *Electrophoresis* **2005**, *26* (14), 2797–2808. https://doi.org/10.1002/elps.200400208
(94) Mischak, H.; Vlahou, A.; Ioannidis, J. P. A. Technical Aspects and Inter-Laboratory Variability in Native Peptide Profiling: The CE-MS Experience. *Clin. Biochem.* **2013**, *46* (6), 432–443. https://doi.org/10.1016/j.clinbiochem.2012.09.025
(95) Lima, N. M.; Fernandes, B. L. M.; Alves, G. F.; de Souza, J. C. Q.; Siqueira, M. M.; Patrícia do Nascimento, M.; Moreira, O. B. O.; Sussulini, A.; de Oliveira, M. A. L. Mass Spectrometry Applied to Diagnosis, Prognosis, and Therapeutic Targets Identification for the Novel Coronavirus SARS-CoV-2: A Review. *Anal. Chim. Acta* **2022**, *1195*. https://doi.org/10.1016/j.aca.2021.339385
(96) Wendt, R.; Thijs, L.; Kalbitz, S.; Mischak, H.; Siwy, J.; Raad, J.; Metzger, J.; Neuhaus, B.; Leyen, H. von der; Dudoignon, E.; Mebazaa, A.; Spasovski, G.; Milenkova, M.; Canevska-Talevska, A.; Czerwieńska, B.; Wiecek, A.; Peters, B.; Nilsson, Å.; Schwab, M.; Rothfuss, K.; Lübbert, C.; Staessen, J. A.; Beige, J. A Urinary Peptidomic Profile Predicts Outcome in SARS-CoV-2-Infected Patients. *eClinicalMedicine* **2021**, *36*, 6–7. https://doi.org/10.1016/j.eclinm.2021.100883
(97) Siwy, J.; Wendt, R.; Albalat, A.; He, T.; Mischak, H.; Mullen, W.; Latosinska, A.; Lübbert, C.; Kalbitz, S.; Mebazaa, A.; Peters, B.; Stegmayr, B.; Spasovski, G.; Wiech, T.; Staessen, J. A.; Wolf, J.; Beige, J. CD99 and Polymeric Immunoglobulin Receptor Peptides Deregulation in Critical COVID-19: A Potential Link to Molecular Pathophysiology? *Proteomics* **2021**, *21* (20), 1–8. https://doi.org/10.1002/pmic.202100133
(98) Tailliar, M.; Schanstra, J. P.; Dierckx, T.; Breuil, B.; Hanouna, G.; Charles, N.; Bascands, J. L.; Dussol, B.; Vazi, A.; Chiche, L.; Siwy, J.; Faguer, S.; Daniel, L.; Daugas, E.; Jourde-chiche, N. Urinary Peptides as Potential Non-invasive Biomarkers for Lupus Nephritis: Results of the Peptidu-lup Study. *J. Clin. Med.* **2021**, *10* (8). https://doi.org/10.3390/jcm10081690

(99) Voigtländer, T.; Metzger, J.; Husi, H.; Kirstein, M. M.; Pejchinovski, M.; Latosinska, A.; Frantzi, M.; Mullen, W.; Book, T.; Mischak, H.; Manns, M. P. Bile and Urine Peptide Marker Profiles: Access Keys to Molecular Pathways and Biological Processes in Cholangiocarcinoma. *J. Biomed. Sci.* **2020**, *27* (1), 1–14. https://doi.org/10.1186/s12929-019-0599-5

(100) Omar, M.; Windhagen, H.; Krettek, C.; Ettinger, M. Noninvasive Diagnostic of Periprosthetic Joint Infection by Urinary Peptide Markers: A Preliminary Study. *J. Orthop. Res.* **2021**, *39* (2), 339–347. https://doi.org/10.1002/jor.24913

(101) Bannaga, A. S.; Metzger, J.; Kyrou, I.; Voigtländer, T.; Book, T.; Melgarejo, J.; Latosinska, A.; Pejchinovski, M.; Staessen, J. A.; Mischak, H.; Manns, M. P.; Arasaradnam, R. P. Discovery, Validation and Sequencing of Urinary Peptides for Diagnosis of Liver Fibrosis—A Multicentre Study. *EBioMedicine* **2020**, *62*. https://doi.org/10.1016/j.ebiom.2020.103083

(102) Frantzi, M.; Gomez Gomez, E.; Blanca Pedregosa, A.; Valero Rosa, J.; Latosinska, A.; Culig, Z.; Merseburger, A. S.; Luque, R. M.; Requena Tapia, M. J.; Mischak, H.; Carrasco Valiente, J. CE–MS-Based Urinary Biomarkers to Distinguish Non-Significant from Significant Prostate Cancer. *Br. J. Cancer* **2019**, *120* (12), 1120–1128. https://doi.org/10.1038/s41416-019-0472-z

(103) Pejchinovski, M.; Siwy, J.; Mullen, W.; Mischak, H.; Petri, M. A.; Burkly, L. C.; Wei, R. Urine Peptidomic Biomarkers for Diagnosis of Patients with Systematic Lupus Erythematosus. *Lupus* **2018**, *27* (1), 6–16. https://doi.org/10.1177/0961203317707827

(104) Magalhães, P.; Pontillo, C.; Pejchinovski, M.; Siwy, J.; Krochmal, M.; Makridakis, M.; Carrick, E.; Klein, J.; Mullen, W.; Jankowski, J.; Vlahou, A.; Mischak, H.; Schanstra, J. P.; Zürbig, P.; Pape, L. Comparison of Urine and Plasma Peptidome Indicates Selectivity in Renal Peptide Handling. *Proteomics - Clin. Appl.* **2018**, *12* (5), 1–9. https://doi.org/10.1002/prca.201700163

(105) Belczacka, I.; Latosinska, A.; Siwy, J.; Metzger, J.; Merseburger, A. S.; Mischak, H.; Vlahou, A.; Frantzi, M.; Jankowski, V. Urinary CE-MS Peptide Marker Pattern for Detection of Solid Tumors. *Sci. Rep.* **2018**, *8* (1), 1–11. https://doi.org/10.1038/s41598-018-23585-y

(106) Siwy, J.; Zürbig, P.; Argiles, A.; Beige, J.; Haubitz, M.; Jankowski, J.; Julian, B. A.; Linde, P. G.; Marx, D.; Mischak, H.; Mullen, W.; Novak, J.; Ortiz, A.; Persson, F.; Pontillo, C.; Rossing, P.; Rupprecht, H.; Schanstra, J. P.; Vlahou, A.; Vanholder, R. Noninvasive Diagnosis of Chronic Kidney Diseases Using Urinary Proteome Analysis. *Nephrol. Dial. Transplant.* **2017**, *32* (12), 2079–2089. https://doi.org/10.1093/ndt/gfw337

(107) Argilés, À.; Siwy, J.; Duranton, F.; Gayrard, N.; Dakna, M.; Lundin, U.; Osaba, L.; Delles, C.; Mourad, G.; Weinberger, K. M.; Mischak, H. CKD273, a New Proteomics Classifier Assessing CKD and Its Prognosis. *PLoS One* **2013**, *8* (5). https://doi.org/10.1371/journal.pone.0062837

(108) Rodríguez-Ortiz, M. E.; Pontillo, C.; Rodríguez, M.; Zürbig, P.; Mischak, H.; Ortiz, A. Novel Urinary Biomarkers For Improved Prediction Of Progressive Egfr Loss In Early Chronic Kidney Disease Stages And In High Risk Individuals Without Chronic Kidney Disease. *Sci. Rep.* **2018**, *8* (1), 1–11. https://doi.org/10.1038/s41598-018-34386-8

(109) Zürbig, P.; Mischak, H.; Menne, J.; Haller, H. CKD273 Enables Efficient Prediction of Diabetic Nephropathy in Nonalbuminuric Patients. *Diabetes Care* **2019**, *42* (1), e4–e5. https://doi.org/10.2337/dc18-1322

(110) Štěpánová, S.; Kašička, V. Recent Developments and Applications of Capillary and Microchip Electrophoresis in Proteomics and Peptidomics (2015–Mid 2018). *J. Sep. Sci.* **2019**, *42* (1), 398–414. https://doi.org/10.1002/jssc.201801090

(111) Latosinska, A.; Siwy, J.; Mischak, H.; Frantzi, M. Peptidomics and Proteomics Based on CE-MS as a Robust Tool in Clinical Application: The Past, the Present, and the Future. *Electrophoresis* **2019**, *40* (18–19), 2294–2308. https://doi.org/10.1002/elps.201900091

(112) Persson, F.; Rossing, P. Urinary Proteomics and Precision Medicine for Chronic Kidney Disease: Current Status and Future Perspectives. *Proteomics - Clin. Appl.* **2019**, *13* (2), 1–6. https://doi.org/10.1002/prca.201800176

(113) Chen, D.; McCool, E. N.; Yang, Z.; Shen, X.; Lubeckyj, R. A.; Xu, T.; Wang, Q.; Sun, L. Recent Advances (2019–2021) of Capillary Electrophoresis-Mass Spectrometry for Multilevel Proteomics. *Mass Spectrom. Rev.* **2021**, 1–26. https://doi.org/10.1002/mas.21714

(114) Klein, J.; Papadopoulos, T.; Mischak, H.; Mullen, W. Comparison of CE-MS/MS and LC-MS/MS Sequencing Demonstrates Significant Complementarity in Natural Peptide Identification in Human Urine. *Electrophoresis* **2014**, *35* (7), 1060–1064. https://doi.org/10.1002/elps.201300327

(115) Ramautar, R.; Berger, R.; van der Greef, J.; Hankemeier, T. Human Metabolomics: Strategies to Understand Biology. *Curr. Opin. Chem. Biol.* **2013**, *17* (5), 841–846. https://doi.org/10.1016/j.cbpa.2013.06.015

(116) Kell, D. B.; Oliver, S. G. The Metabolome 18 Years on: A Concept Comes of Age. *Metabolomics* **2016**, *12* (10), 1–8. https://doi.org/10.1007/s11306-016-1108-4

(117) Drouin, N.; Van Mever, M.; Zhang, W.; Tobolkina, E.; Ferre, S.; Servais, A. C.; Gou, M. J.; Nyssen, L.; Fillet, M.; Lageveen-Kammeijer, G. S. M.; Nouta, J.; Chetwynd, A. J.; Lynch, I.; Thorn, J. A.; Meixner, J.; Lößner, C.; Taverna, M.; Liu, S.; Tran, N. T.; Francois, Y.; Lechner, A.; Nehmé, R.; Al Hamoui Dit Banni, G.; Nasreddine, R.; Colas, C.; Lindner, H. H.; Faserl, K.; Neusüß, C.; Nelke, M.; Lämmerer, S.; Perrin, C.; Bich-Muracciole, C.; Barbas, C.; Gonzálvez, Á. L.; Guttman, A.; Szigeti, M.; Britz-Mckibbin, P.; Kroezen, Z.; Shanmuganathan, M.; Nemes, P.; Portero, E. P.; Hankemeier, T.; Codesido, S.; González-Ruiz, V.; Rudaz, S.; Ramautar, R. Capillary Electrophoresis-Mass Spectrometry at Trial by Metabo-Ring: Effective Electrophoretic Mobility for Reproducible and Robust Compound Annotation. *Anal. Chem.* **2020**, *92* (20), 14103–14112. https://doi.org/10.1021/acs.analchem.0c03129

(118) Zhang, W.; Hankemeier, T.; Ramautar, R. Next-Generation Capillary Electrophoresis–Mass Spectrometry Approaches in Metabolomics. *Curr. Opin. Biotechnol.* **2017**, *43*, 1–7. https://doi.org/10.1016/j.copbio.2016.07.002

(119) Sastre Toraño, J.; Ramautar, R.; de Jong, G. Advances in Capillary Electrophoresis for the Life Sciences. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2019**, *1118–1119* (April 2019), 116–136. https://doi.org/10.1016/j.jchromb.2019.04.020

(120) López-Gonzálvez, Á.; Godzien, J.; García, A.; Barbas, C. Capillary Electrophoresis Mass Spectrometry as a Tool for Untargeted Metabolomics. *Methods Mol. Biol.* **2019**, *1978*, 55–77. https://doi.org/10.1007/978-1-4939-9236-2_5

(121) Buko, A. Capillary Electrophoresis Mass Spectrometry Based Metabolomics. *J. Appl. Bioanal.* **2017**, *3* (1), 5–20. https://doi.org/10.17145/jab.17.002

(122) Yamamoto, M.; Shanmuganathan, M.; Hart, L.; Pai, N.; Britz-Mckibbin, P. Urinary Metabolites Enable Differential Diagnosis and Therapeutic Monitoring of Pediatric Inflammatory Bowel Disease. *Metabolites* **2021**, *11* (4). https://doi.org/10.3390/metabo11040245

(123) Udo, R.; Katsumata, K.; Kuwabara, H.; Enomoto, M.; Ishizaki, T.; Sunamura, M.; Nagakawa, Y.; Soya, R.; Sugimoto, M.; Tsuchida, A. Urinary Charged Metabolite Profiling of Colorectal Cancer Using Capillary Electrophoresis-Mass Spectrometry. *Sci. Rep.* **2020**, *10* (1), 1–10. https://doi.org/10.1038/s41598-020-78038-2

(124) Ishikawa, S.; Sugimoto, M.; Kitabatake, K.; Sugano, A.; Nakamura, M.; Kaneko, M.; Ota, S.; Hiwatari, K.; Enomoto, A.; Soga, T.; Tomita, M.; Iino, M. Identification of Salivary Metabolomic Biomarkers for Oral Cancer Screening. *Sci. Rep.* **2016**, *6*, 2–8. https://doi.org/10.1038/srep31520

(125) Cosmosil®. Column for Capillary Electrophoresis COSMO(+)Capillary. Available at: https://winlab.com.au/Chromatography/Cosmosil/COSMOSIL_Winlab_Capillary.pdf [Accessed on Jan. 2022].

(126) Sakurai, T.; Katsumata, K.; Udo, R.; Tago, T.; Kasahara, K.; Mazaki, J.; et al. Validation of Urinary Charged Metabolite Profiles in Colorectal Cancer Using Capillary Electrophoresis-Mass Spectrometry. *Metabolites* **2022**, *12* (1), 59. https://doi.org/10.3390/metabo12010059

(127) Saito, R.; Hirayama, A.; Akiba, A.; Kamei, Y.; Kato, Y.; Ikeda, S.; Kwan, B.; Pu, M.; Natarajan, L.; Shinjo, H.; Akiyama, S.; Tomita, M.; Soga, T.; Maruyama, S. Urinary Metabolome Analyses of Patients with Acute Kidney Injury Using Capillary Electrophoresis-Mass Spectrometry. *Metabolites* **2021**, *11* (10). https://doi.org/10.3390/metabo11100671

(128) Sasaki, C.; Hiraishi, T.; Oku, T.; Okuma, K.; Suzumura, K.; Hashimoto, M.; Ito, H.; Aramori, I.; Hirayama, Y. Metabolomic Approach to the Exploration of Biomarkers Associated with Disease Activity in Rheumatoid Arthritis. *PLoS One* **2019**, *14* (7), 1–13. https://doi.org/10.1371/journal.pone.0219400

(129) Mishima, E.; Fukuda, S.; Mukawa, C.; Yuri, A.; Kanemitsu, Y.; Matsumoto, Y.; Akiyama, Y.; Fukuda, N. N.; Tsukamoto, H.; Asaji, K.; Shima, H.; Kikuchi, K.; Suzuki, C.; Suzuki, T.; Tomioka, Y.; Soga, T.; Ito, S.; Abe, T. Evaluation of the Impact of Gut Microbiota on Uremic Solute Accumulation by a CE-TOFMS–Based Metabolomics Approach. *Kidney Int.* **2017**, *92* (3), 634–645. https://doi.org/10.1016/j.kint.2017.02.011

(130) Ishibashi, Y.; Harada, S.; Takeuchi, A.; Iida, M.; Kurihara, A.; Kato, S.; Kuwabara, K.; Hirata, A.; Shibuki, T.; Okamura, T.; Sugiyama, D.; Sato, A.; Amano, K.; Hirayama, A.; Sugimoto, M.; Soga, T.; Tomita, M.; Takebayashi, T. Reliability of Urinary Charged Metabolite Concentrations in a Large-Scale Cohort Study Using Capillary Electrophoresis-Mass Spectrometry. *Sci. Rep.* **2021**, *11* (1), 1–9. https://doi.org/10.1038/s41598-021-86600-9

(131) Oedit, A.; Hankemeier, T.; Lindenburg, P. W. On-Line Coupling of Two-Phase Microelectroextraction to Capillary Electrophoresis – Mass Spectrometry for Metabolomics Analyses. *Microchem. J*. **2021**, *162*. https://doi.org/10.1016/j.microc.2020.105741

(132) MacLennan, M. S.; Kok, M. G. M.; Soliman, L.; So, A.; Hurtado-Coll, A.; Chen, D. D. Y. Capillary Electrophoresis-Mass Spectrometry for Targeted and Untargeted Analysis of the Sub-5 kDa Urine Metabolome of Patients with Prostate or Bladder Cancer: A Feasibility Study. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2018**, *1074–1075*, 79–85. https://doi.org/10.1016/j.jchromb.2018.01.007

(133) Maxwell, E. J.; Zhong, X.; Zhang, H.; Van Zeijl, N.; Chen, D. D. Y. Decoupling CE and ESI for a More Robust Interface with MS. *Electrophoresis* **2010**, *31* (7), 1130–1137. https://doi.org/10.1002/elps.200900517

(134) Kami, K.; Fujimori, T.; Sato, H.; Sato, M.; Yamamoto, H.; Ohashi, Y.; Sugiyama, N.; Ishihama, Y.; Onozuka, H.; Ochiai, A.; Esumi, H.; Soga, T.; Tomita, M. Metabolomic Profiling of Lung and Prostate Tumor Tissues by Capillary Electrophoresis Time-of-Flight Mass Spectrometry. *Metabolomics* **2013**, *9* (2), 444–453. https://doi.org/10.1007/s11306-012-0452-2

(135) Mishima, E.; Fukuda, S.; Shima, H.; Hirayama, A.; Akiyama, Y.; Takeuchi, Y.; Fukuda, N. N.; Suzuki, T.; Suzuki, C.; Yuri, A.; Kikuchi, K.; Tomioka, Y.; Ito, S.; Soga, T.; Abe, T. Alteration of the Intestinal Environment by Lubiprostone Is Associated with Amelioration of Adenine-Induced CKD. *J. Am. Soc. Nephrol.* **2015**, *26* (8), 1787–1794. https://doi.org/10.1681/ASN.2014060530

(136) Soga, T.; Ohashi, Y.; Ueno, Y.; Naraoka, H.; Tomita, M.; Nishioka, T. Quantitative Metabolome Analysis Using Capillary Electrophoresis Mass Spectrometry. *J. Proteome Res.* **2003**, *2* (5), 488–494. https://doi.org/10.1021/pr034020m